American Journal of Engineering Research (AJER)
e-ISSN: 2320-0847 p-ISSN : 2320-0936
Volume-12, Issue-9, pp-07-22
www.ajer.org
Research Paper Open Access

Effectof using Bacillus sphaericus, Bacillus pasteurii,and Bacillus subtilis on durability of ferrocement laminates

Mariam Farouk Ghazy[1],Alsaeed Abdelsalam Maaty[2] and GhadaRagab Mohamed[3]

[1,2]*Prof, Structural Engineering Dept., Faculty of Engineering, Tanta University, Tanta, Egypt*
[3]*MSc, Student, Faculty of Engineering, Tanta University, Tanta, Egypt*

ABSTRACT :*The durability of concrete structures is often compromised by the formation of cracks, which can lead to reduced service life and increased maintenance costs. This study employed Microbial Induced Calcium Precipitation (MICP) as a smart and eco-friendly approach to produce bio-based durable materials. In this investigation three different types of bacteria: Bacillus sphaericus (SpM1), Bacillus pasteurii (PaM1), and Bacillus subtilis (SuM1) were used at 0.5% content by cement weight and with 0.25% calcium lactate as a nutrient. Physical, mechanical, and durability performance were conducted for cement mortar specimens. Moreover, the performance of ferrocement laminates with dimensions 150× 300× 30 mm was studied. Setting times, flow, rate of water absorption, coefficient of permeability, compressive strength, flexural strength at different ages (7, 28, 56 and 90 days), restoration of compressive strength and flexural strength against preloading (up to 50% of maximum capacities at different ages), and residual compressive strength after exposure to 1.5% sulphuric acid were the main responses taken into consideration. The test results of cement mortar revealed that all different types of bacteria have an impact on its performance. A reduction of about (30%-70%) and (49.8%-86.5%) in rate of water absorption and permeability coefficient, respectively were recorded in compared to mortar specimens without bacteria. Whilst the improvement in compressive and flexural strengths of about (28% and 13%) at early ages and (9% and 49%) at later ages, respectively. The test results of the restoration of compressive and flexural strengths proved that MICP by utilizing bacteria can improve the durability performance of the mortar specimens by achieving to (90.6% and 75.5%) of their original compressive and flexural strengths at early ages (7-28 days) and (92% and 76.8%) at later ages (28-56 days), respectively. On the other hand ,the ferrocement laminates with bacteria restored up to 107.4% of its original loads at 90 days. SEM analysis confirmed that mortar samples have a denser structure with fewer voids, attributed to the MICP process. Furthermore, the performance of ferrocement laminates incorporating bacteria at different ages exhibited significant improvement, with respect to maximum capacity and toughness.*
This innovative microbial self-healing approach holds great potential for the continuous repair of microcracks in concrete, resulting in improved durability and reduced maintenance costs thus providing insight into its applications and prospects in reinforced concrete structures.

KEYWORDS: *Bacillus sphaericus, bacillus pasteurii, bacillus subtilis, ferrocement laminates, restoration of strength, acidic exposure.*

Date of Submission: 01-09-2023 Date of acceptance: 08-09-2023

I. INTRODUCTION

Concrete, being the most commonly utilized construction material worldwide, is produced in excess of 6 million cubic meters each year due to its impressive compressive strength, casting properties, and relatively economical cost [**1**]. However, concrete does possess a drawback in terms of its limited tensile strength and relatively low resistance to cracking [**2**]. Cracks have a considerable impact on the durability of concrete, as they can arise easily due to external loads or volumetric changes induced by temperature variations or shrinkage [**3**]. The presence of cracks in concrete allows various detrimental substances such as water, gases, salts, acids, and other agents to penetrate the matrix, leading to an accelerated degradation and corrosion process. Consequently, the service life of the structure is shortened [**4**]. It is worth noting that the maintenance of concrete structures has a significant impact on community budgets and the environment [**5**]. In addition, conventional repair methods

have certain drawbacks [**6**]. These include operational limitations during reconstruction, the challenge of dealing with the varying thermal expansion coefficients between the existing concrete matrix and the added repair material, as well as potential environmental hazards [**7**]. Developing bio-based self-healing concrete aims to address durability concerns associated with cracking. Consequently, researchers are actively exploring various self-healing techniques [**8**].

Bio cementation is a natural process where certain bacterial species can deposit calcium carbonate ($CaCO_3$) [**9**]. This unique phenomenon has shown great promise as a binder for protecting and consolidating construction materials [**10**]. By leveraging the bacterial remediation technique, it is possible to preserve historical structures effectively [**11**]. Many researchers have explored the use of bio cementation to improve the durability of cementitious materials and restore buildings [**12**]. Compared to conventional treatments, this microbial technique has several advantages. For instance, the thermal expansion properties of the calcite produced by the bacteria are like those of concrete surfaces [**13**]. Additionally, bio cementation is commercially viable, eco-friendly, and has a self-healing tendency. Microbial self-healing of concrete typically involves two metabolic pathways, which involve either urea hydrolysis facilitated by uratolytic bacteria or respiration performed by non-ureolytic bacteria [**14**]. Ureolytic bacteria, specifically Bacillus sphaericus and Bacillus pasteurii, have garnered significant attention in the realm of bio-cementation techniques [**15**]. Both Bacillus sphaericus and Bacillus pasteurii are widely found in soil and aquatic environments [**16**]. These Gram-positive, aerobic, rod-shaped bacteria are non-pathogenic. They exhibit urease activity and are capable of thriving in highly alkaline conditions [**17**]. These bacteria can bio transform urea into carbonate and ammonium compounds. The ammonia produced because of this biotransformation raises the pH level, which triggers the precipitation of calcite within the micro-cracks. This process effectively seals the cracks [**18**]. Incorporating sustainable and eco-friendly biomaterials in the construction of building structures can serve as a viable alternative to traditional chemicals. This approach minimizes the potential environmental and health hazards associated with conventional materials [**19**]. Previous research conducted in our laboratory has successfully improved the durability of bio-concrete by incorporating Egyptian strains of bacillus subtilis and bacillus megaterium. Building upon this achievement, the current study endeavors to develop an alternative sustainable bio-concrete utilizing ureolytic bacteria. The newly developed bio-concrete's physico-mechanical properties were assessed at various stages of curing. The characterization of the new bio-concrete involved evaluating its healing capabilities, load deflection of bacterial-reinforced laminates, restoration of mechanical properties, and durability.

This study focuses on the impact of microbial induced calcite precipitation on enhancing the physical and mechanical properties of cement mortar. Additionally, internal crack remediation with a 50% reload of the samples was performed. This technique demonstrated superior results in healing micro cracks, resulting in increased durability and reduced maintenance materials.

II. EXPERIMENTAL WORK

a. MATERIAL

Cement: Ordinary Portland (CEMI 42.5N) satisfies the requirements of (EN197-1/2011) [**20**]and Egyptian Standard Specifications (ES 4756-1/2013) [**21**].

Fine aggregate: Medium well-graded sand of fineness modulus 2.2 used for mortar complies theEgyptian Standards (ES 1109-2008) requirements[**22**].

Water: fresh tap water was used for mixing and curing of the test specimens with w/c ratio 0.47 for mixing.

Bacteria: Bacillus sphaericus DSM 396,Bacillus pasteurii DSM 33and Bacillus subtilis DSM 1088 were purchased from the Microbiological Resources Centre (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Calcium lactate: Calcium lactate is a white crystalline salt made by the action of lactic acid on calcium carbonate and its chemical formula is $C_6H_{10}CaO_6$. Pure Calcium lactate was used as a nutrient obtained from Oxford Laboratory, Mumbai, India. $CaC_6H_{10}O_6$ is converted into $CaCO_3$ as presented in Eq.(**1**), (**2**),(**3**)and (**4**) [**3**]:

$$C_6H_{10}O_6CaCO_3+CO_2+5H_2O \longrightarrow \quad (1)$$

$$CO_2+H_2O \;\; H_2CO_3 \longrightarrow \quad (2)$$

$$2OH+ H_2CO_3CO_3 +2H_2O \longrightarrow \quad (3)$$

$$Ca^2 + CO_3 \;\; CaCO_3\downarrow \longrightarrow \quad (4)$$

Reinforcement: Expanded wire mesh was used for ferro cementlaminates and had strips weighing 700 g/m^2, with short and long way pitches measuring 10 mm and 20 mm, respectively. The strands of the mesh had a thickness of 0.55 mm and a width of 0.6 mm. As shown in **Fig. 1**.

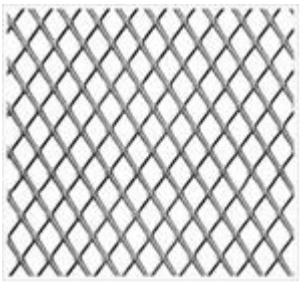


Fig. 1. Expanded wire mesh.

b. CULTIVATION AND SUSPENSION OF BACTERIA

1) Three differential types of bacteria were used in this work (Bacillus sphaericus DSM 396, Bacillus pasteurii DSM 33, and Bacillus subtilis DSM 1088). The pH for these bacteria can sustain from 7 to 9.
2) All bacteria were cultured in "Luria-Bertani" (LB) broth medium containing, (5g meat Extract, 5g peptone , 15g agar and 20 g/L filter-sterilized urea) [**23**].
3) PH was adjusted to 7.5 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. All microbiological assays were prepared at Faculty of Science, University of Kafr-Elsheikh, Egypt.
4) Different types of bacteria were cultured in liquid media containing (1000.0 ml Distilled water, 5.0g Peptone, 3.0g Meat extract, and 15.0g Agar). Adjust pH to 7.2 for all Bacillus and the addition of 10.0 mg of manganese sulfate ($MnSO_4$ x H_2O) is recommended for sporulation [**24**]. Before addition to the cement mortar, bacterial cultures were incubated for 7 days to ensure sporulation then washed by repeated 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 1x10^8 Cell/mL, used in concentrations including 0.5% by the cement weight.

c. SPECIMEN PREPARATION AND CURING

Mortar with Bacteria is prepared by using Portland cement mixed with solution of bacteria 0.5% by wt. All batches were weighed. Cement, sand, and calcium lactate (if applicable in bacterial mortar) were mixed using mechanical mixer (rotary mixer 60 liters capacity) for five minutes without water. Bacteria (if applicable) were added to the mixing water. Then, water and bacteria were added to the mixture and the mixing process continued for five minutes. Mixing ratios are given in **Table 1**.Finally, three layers of fresh mortar were being poured into molds and each layer was compacted by using the vibrating Table for 30 sat laboratory temperature. One of the most significant steps performedfor self-healing mortar is curing. The specimens were demolished after one day from casting and bacterial samples and control were cured under wet cloth by tap water until the specified test date.

.

Table 1: Proportions of mortar mixes (ratio by weight)

Mix ID	Type of bacteria	Sand/ Cement	Water/ Cement	Bacteria/ Cement	Calcium Lactate/ Cement ratio by weight
M0	-	3:1	0.47	0.0	0.0
SpM1	Bacillus sphaericus			0.5%	0.25%
PaM1	Bacillus pasteurii				
SuM1	Bacillus subtilis				

d. TEST PROCEDURES

i. SETTING TIMES AND FLOW TESTS

Initial and final setting time tests were carried out according to ASTM C 403 [**25**]. Due to rapid setting time of some mixes, measured penetration distances every 5 min or less at room temperature (about 25 °C) and flow table was used to measure the flow in accordance with ASTM C 1437-07 [**26**].

ii. RATE OF WATER ABSORPTION AND CAPILLARY PERMEABILITY TESTS

Mortar samples were dried in an oven at 110°C for 24 hours and then cooled as per ASTM C 1585[**27**] after 7, 28 ,56 and 90 days of moist curing. The sides of the mortar samples were covered with epoxy resin to allow the flow of water in one direction. The end of the samples was sealed with tightly attached plastic sheet and protected in position by an elastic band. The initial mass of the samples was taken after which they were kept partly immersed in a depth of 5mm in water. The readings were started with the initial mass of the sample after 2 hours from first contact with water, the samples were removed, and excess water was blotted off using paper towel and then weighed. For 7, 28, 56 and 90 days, the readings were taken at selected times after first contact with water (typically 1, 5, 10, 20, 30, 60, 110 and 120 min), the samples were removed, excess water was blotted off using paper towel and then weighed. The rate of water absorption (I, $m/s^{0.5}$) as per the equation [**28**]:-

$I=\Delta m / (a.d)$ ...Eq. (**5**)

Where: I: the rate of water absorption ($m/s^{0.5}$), Δm :The gain in mass (kg/s)

a :exposed area of the specimen (m^2), d :density of water, t :the time elapsed (s)

For capillary permeability tests is considered as a measure of Capillary Action of water and in this study, it has been measured in accordance with the ASTM C642[**29**]. This was measured by determining the rate of water uptake by dry mortar in a period of 24 h. The mortar samples were dried at 110°C in an oven for 24 h until they reached to constant weight and then cooled. The sides of the samples were covered with epoxy resin and were placed partly immersed in water to a depth of 5mm at one end, and at the other end a tightly attached plastic was secured in position by an elastic band. The amount of water absorbed during 24 h was calculated for concrete samples after 7, 28, 56 and 90 days of curing under wet cloth of tap water, The coefficient of water absorption (Ka, m^2/s), as per the Eq. [**30**]: -

$Ka= (Q/A)^{2.} (1/ t)$. …Eq. (**6**)

Where:K :capillary permeability coefficient (m^2/s), Q :the amount of water absorbed (m^3), A :the area of the specimen in contact with water (m^2) andt :the time elapsed (s).

iii. MECHANICAL STRENGTH TESTS

The mechanical properties in this study were mainly: compressive and flexural strengths at different ages (7 ,28 ,56 ,90 days).Three specimen cubes (70×70×70 mm) from each mixture were tested ateachage for compressive test. Prisms with dimensions (40 x 40 x 160 mm) were prepared to measure flexural strengths according to EN 196-1:2016 [**31**] using digital hydraulic compression testing machine with 300 kN capacities. Moreover, the flexural strength on ferro cement was measured using Reinforced-Laminates with dimensions (300 x 150 x 30 mm) [**3**] , and the test was carried out using the Universal Testing Machine of 300 kN capacities. **Fig. 2** shows the tests setup.

a) **compression**

b) flexure

Fig. 2. The test setup of mortar samples.

iv. RESTORATION OF COMPRESSIVE STRENGTH TEST

Samples were loaded after 7, 28, 56 days from casting date with 50% of failure load at 7, 28, 56 days simultaneously. Samples were kept moisturized by wet cloth. After The Curing Period, Samples were tested to determine the compressive strength until failure at 28, 56, 90 days.

v. RESTORATION OF FLEXURAL STRENGTH TEST

Samples were loaded after 28 and 56 days from casting date with half of failure load at 28 and 56 days simultaneously. Samples were kept moisturized by wet cloth. They were tested to determine the flexural strength until failure at 56 and 90 days. Average for three tested specimens for each age were taken.

vi. ACID RESISTANCE TEST

The samples were taken after a month from the date of casting and immersed in sulfuric acid (H_2SO_4)solution with 1.5% concentration. Three tested specimens for each age were taken to determinate the compressive strength. **Fig. 3**shows the tests setup.

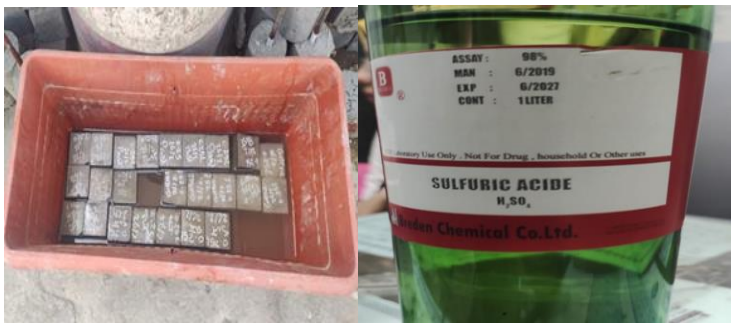


Fig. 3. Test samples and sulfuric acid.

V. FLEXURAL STRENGTH AND RESTORATION OF FERROCEMENT LAMINATES TESTS

Reinforced- Laminates were loaded and tested until failure after 28, 56, 90 days of curing. Deflection of mid-point for each Reinforced-Lamina and its maximum load were measured[**1**]. Reinforced-Laminates were loaded with half failure load after 28 and 56 days from casting to assess flexural strength restoration. During the testing time, all samples were cured after that, samples were reloaded and examined for flexural strength restoration after 56 and 90 days.The toughness of Ferrocement can be considered as their energy absorption capacity, which is usually characterized by some portion of the area under the load deflection curve obtained during flexure test (ACI 544, 1988) [**32**].

III. RESULTS AND DISCUSSION

All mixes were formulated to investigate the impact of different types of Bacteria on mortar properties. The test results are given in **Table 2**.

Table (2): Test results of experimental mortar mixes at different ages

Property	Results				Relative results (%)			
	Mix ID				Mix ID			
	M0	SpM1	PaM1	SuM1	M0	SpM1	PaM1	SuM1
Setting time (min)					**Compared with control mix (M0)**			
Initial	262	213	201	210	100	81	77	80
Final	500	498	357	446	100	100	71	40
Flow %					**Compared with control mix (M0)**			
-	27.00	26.50	26.00	26.50	100	98	96	98
Rate of water absorption×10^{-7} ($m/s^{0.5}$)					**Compared with control mix (M0)**			
7 days	2.06	1.37	1.44	1.37	100	67	70	67
28 days	1.92	1.10	1.03	1.23	100	57	54	64
56 days	1.51	0.89	0.69	0.96	100	59	46	64
90 days	1.37	0.41	0.41	0.82	100	30	30	60
Coefficient of permeability (m^2/s)					**Compared with control mix (M0)**			
7 days	259.92	70.51	101.53	130.41	100	27	39	50
28 days	176.01	27.1	52.15	76.26	100	15	30	43
56 days	130.41	27.1	17.63	36.55	100	21	14	28
90 days	62.30	17.63	16.24	25.38	100	28	26	41
Compressive strength (MPa)					**Compared with control mix (M0)**			
7 days	17.45	22.41	19.95	19.66	100	128	114	113
28 days	23.88	29.59	28.69	28.37	100	124	120	119
56 days	29.59	34.49	36.19	40.75	100	117	122	138
90 days	35.67	38.23	33.98	37.48	100	107	95	105
Restoration of compressive strength (MPa)					**Compared with original strengths**			

7-28 days	20.4	26.8	25.93	25.14	85	91	90	89
28-56 days	24.79	31.1	33.29	30.93	84	90	92	91
56-90 days	28.49	33.71	35.85	32.37	80	88	88	86

Table (2): Continuetest results of experimental mortar mixes at different ages

Flexural strength (MPa)					**Compared with control mix (M0)**			
28 days	6.94	7.69	7.55	7.64	100	111	109	110
56 days	8.64	12.91	12.83	11.23	100	149	148	130
90 days	9.21	13.28	13.55	11.78	100	144	147	128
Restoration of flexural strength (MPa)					**Compared with original strengths**			
28-56 days	5.98	9.75	9.46	7.91	69	76	74	70
56-90 days	6.05	10.2	10.18	8.36	66	77	75	71
Residual compressive strength after exposure to 1.5% sulfuric acid (MPa)					**Compared with control mix (M0)**			
28 days	15.16	19.16	19.36	16.69	100	126	128	110
56 days	12.43	16.86	16.84	14.02	100	136	135	113
90 days	9.12	15.51	14.73	11.08	100	170	162	121

a. mortar samples

i. SETTING TIME

The incorporation of bacteria resulted in reducing the initial and final setting times in compared with control mix (M0). This may be due to nutrition of bacteria added or the bacteria solution[**33**]. The obtained results from the initial and final setting times of bacterial and control mortar are shown in **Fig.4**. This means that adding bacteria and calcium lactate to cement mortar play an important role in accelerating the final and initial setting time this is also agree with Xu and Wang[**16**].

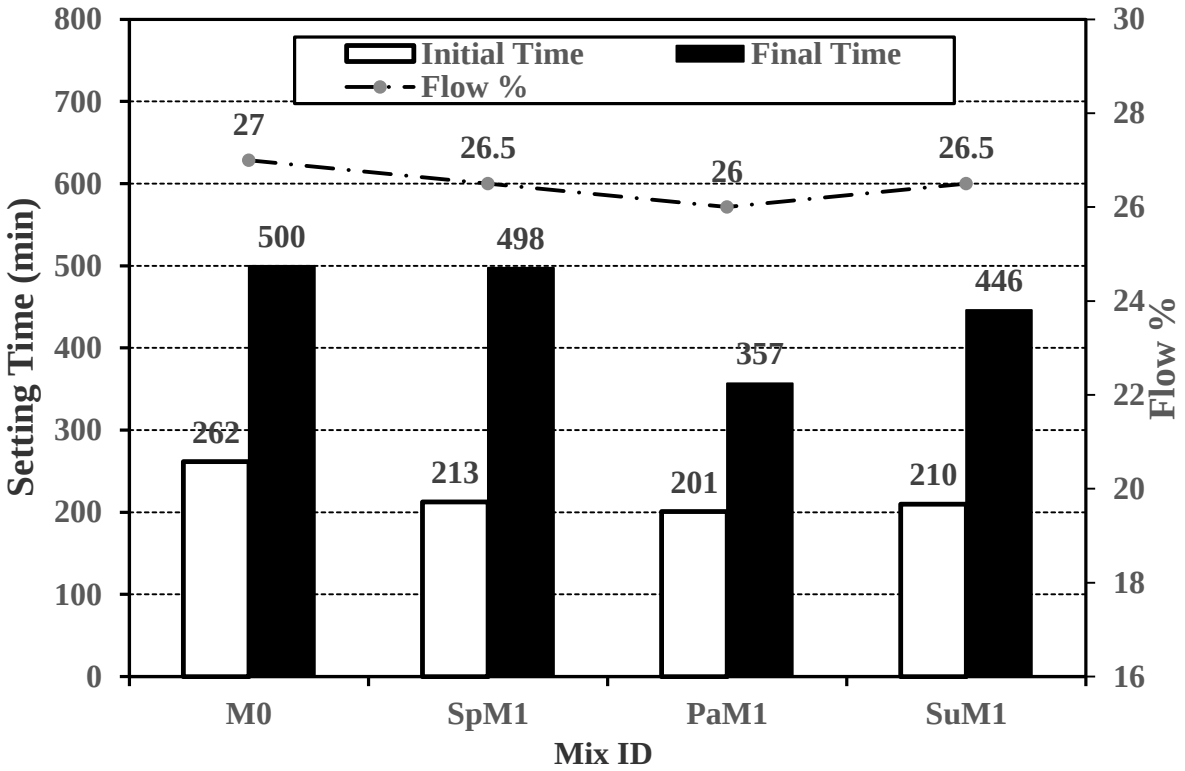


Fig. 4. Initial and final setting times of bacterial and control cement mortar

ii. FLOW

The flow test results of different mortarsare shown in **Fig.4**. The flow of mortar mixes is affected by adding bacteria to the mortar. The control mix showed the highest flow percentage value, then this value gradually decreased by using different types of bacteria and the same percentage.

iii. RATE OF WATER ABSORPTION

The rate of water absorption of different mortar mixes is shown in **Fig. 5**. The rate of water absorption for bacterial mortar specimens decreased compared to control specimens for all ages. Results for 7, 28, 56, 90 days at selected times after first contact with water. Microbial Induced CalcitePrecipitation (MICP) is responsible for filling up the pores in mortar and hence decreasing water absorption of bacterial mortar specimens. This agrees with the results of investigation conducted by [**34**],using bacillus pasteurii induce reduction in water absorption which could in turn increase durability of concrete structures.

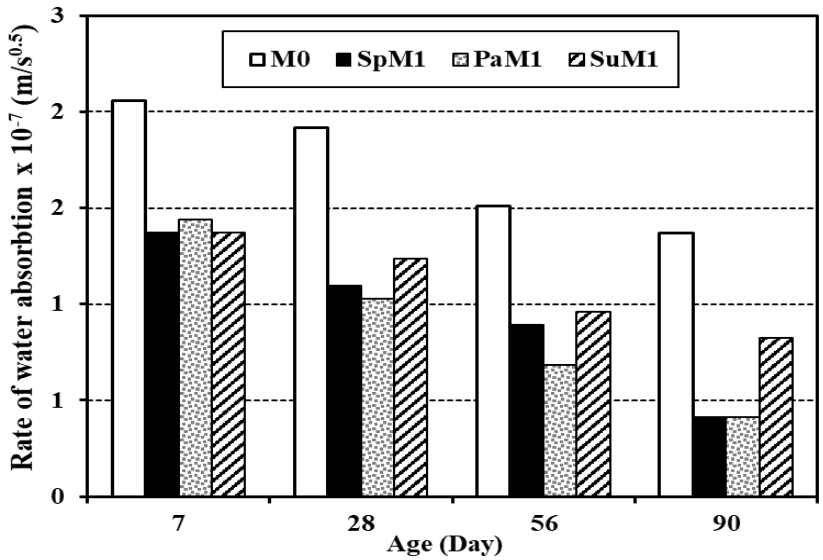


Fig. 5. Rate of water absorption for bacterial and control mortar.

iv. PERMEABILITY COEFFICIENT

The effect of bacteria on permeabilityof the mortar was studied after 24 hours. permeabilitydecreased in all bacterial mortar specimens as shown in **Fig.6.** At the age 90 days, permeability of SpM1 and PaM1 samples became 28%, and 26% comparing to control samples, respectively. This proves that metabolic activities by bacteria lead to fill mortar voids by bacterial precipitation, which decreases permeability coefficient, and this is agree with Chen[**35**].

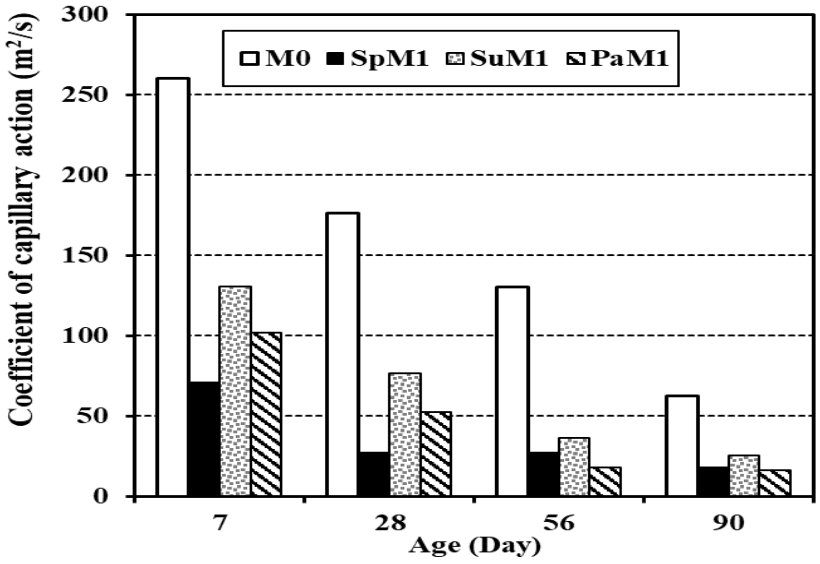


Fig. 6. Permeability coefficient of bacteria and control mortar specimens.

v. COMPRESSIVE STRENGTH

Results of compressive strength test revealed that there is an increase in the strength for bacterial mortar compared to control mortar Calcite Precipitation Induced by bacteria fill the pores in the microstructure, which resist loads significantly and hence compressive strength increased, compared to control mortar. **Fig.7** shows the improvement of the compressive strength of the bacterial mortar comparing with the control mortar. Also, illustrate that compressive strength of bacterial samples developed earlier than that of control samples due to filling pores of bacterial precipitation. This improvement reached the highest values in the age of 28 days, which is the main value for the design criteria this is also According to Wu et al. [**36**].

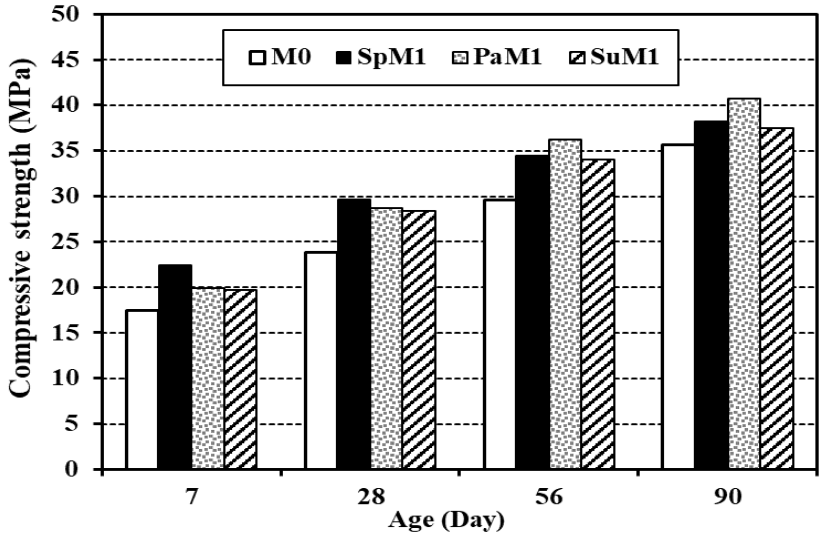


Fig.7. Compressive strength for bacterial and control mortar specimens.

vi. FLEXURAL STRENGTH

Results of flexural strength test revealed that there is an increase in the strength for the bacterial mortar compared to the control mortar. At the age of 28 days, the flexural strength values of SpM1 and PaM1 were 111% and 109% of flexural strength comparing with control mortar, respectively. At the age of 56 days, the flexural strength value of SPM1and PaM1 were 149% and 148% of flexural strength comparing with control mortar, respectively. At the age of 90 days, the flexural strength value of SpM1 and PaM1 were 144% and 147% of flexural strength comparing with control mortar, respectively. Microbial Induced Calcite Precipitation (MICP) is responsible for filling up the pores in mortar. According to Ahmed et al. [**1**] the treated samples showed improved physical-mechanical properties as a result of calcite deposition facilitated by Egyptian strains of bacillus subtilis. **Fig.8** shows the improvement of the flexural strength of the bacterial mortar over the control mortar.

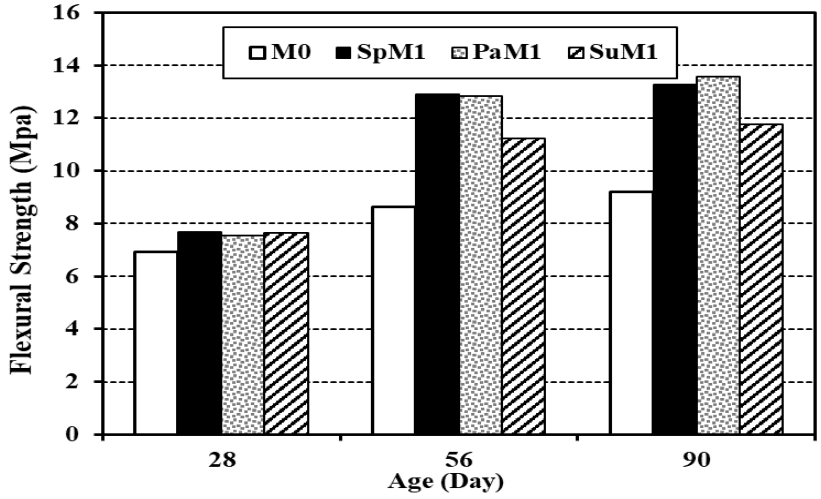


Fig. 8. Flexural strength of mortar specimens.

vii. RESTORATION OF COMPRESSIVE STRENGTH

Samples were loaded after 7,28 and 56 days from casting date with half of failure load at 7, 28 and 56 days then reloaded at 28,56 and 90 days until failure simultaneously. Results of compressive strength test revealed that there is an increase in strength for all bacterial mortar when compared to the original samples at the age of 28, 56 and 90 days as shown in **Fig.9**. Restored samples were compared to original samples.

At the age of 28 days, the compressive strength value of control, SpM1, PaM1, and SuM1 were 85.4%, 90.5%, 90.4%, 88.6%, compared to compressive strength of unloaded samples.

At the age of 56 days, the restored compressive strength value of control, SpM1, PaM1 and SuM1 were 83.8%, 90.2%, 92.0% and 91.0% compared to compressive strength of unloaded samples.

At the age of 90 days, the restored compressive strength value of control, SpM1, PaM1 and SuM1 were 79.9%, 88.2%, 88.0% and 86.3% compared to compressive strength of unloaded samples.

Increasing in compressive strength value of bacterial mortar specimens such as SpM1, PaM1and SuM1 and decreasing in compressive strength values of control mortar specimen assure that self-healing in that of mortar occurred. Bacterial Samples compressive strength restored more compressive strength than control samples. Compressive strength values of control mortar specimen decreased. This means that bacteria could restore the mortar mechanical properties to its original state.

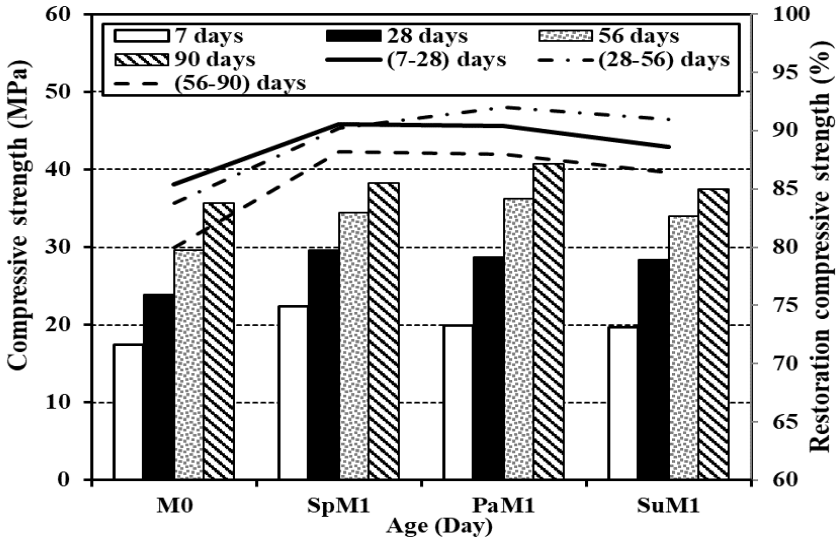


Fig. 9. Improvement percentage of compressive strength.

viii. RESTORATION OF FLEXURAL STRENGTH

Samples were loaded after, 28 and 56 days from casting date with half of failure load at, 28 and 56days then reloaded at 56 and 90 days until failure simultaneously [**1**]. Results of Flexural Strength test revealed that there is an increase in strength for all bacterial mortar when compared to the original samples at the age of 56 and 90 days as shown in **Fig.10**. Restored samples were compared to original samples. At the age of 56 days, the flexural strength value of control, SpM1, PaM1 and SuM1 were 69%, 76%, 74% and 70% compared to Flexural Strength of unloaded samples.

At the age of 90 days, the restored Flexural Strength value of control, SpM1, PaM1 and SuM1 were 66%, 77%, 75% and 71% compared to flexural strength of unloaded samples. Increasing in flexural strength value of bacterial mortar specimens such as SPM1 and decreasing in flexural strength values of control mortar specimen assure that self-healing in that of mortar occurred. Bacterial Samples flexural strength restored more flexural strength than control samples. Flexural strength values of control mortar specimen decreased. This means that bacteria could restore the mortar mechanical properties to its original state.

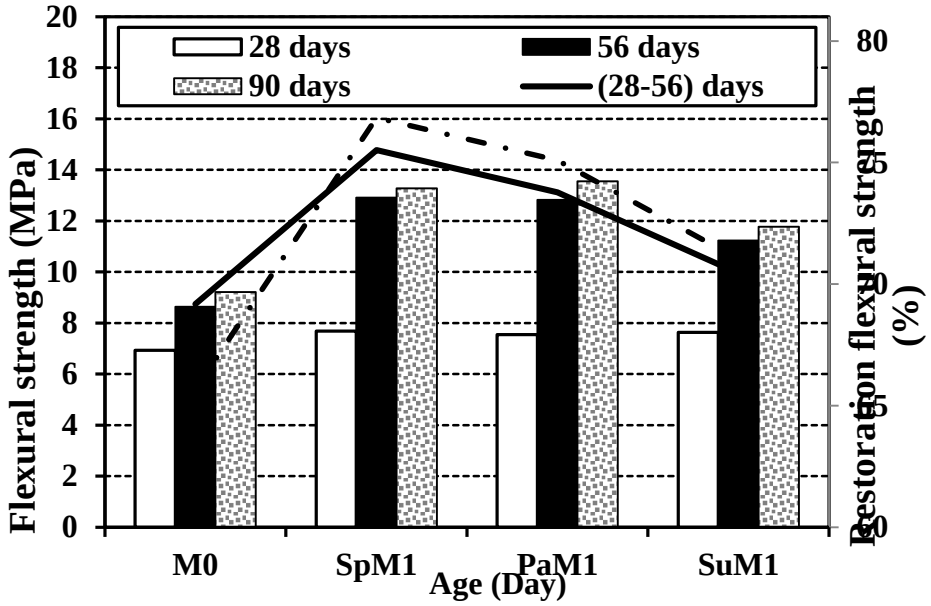


Fig. 10. Improvement percentage of flexural strength of restored samples compared to original samples (Prisms).

ix. SULFURIC ACID RESISTANCE

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 shown in **Fig. 11**. At the age of 28 days all bacterial mortar increased such as SpM1, PaM1 and SuM1 was 126%, 128%, and 110% respectively when compared to compressive strength of control mortar.

Also, at the age of 56 days all bacterial mortar increased such as SpM1, PaM1 and SuM1 was 136%, 135% and 113% respectively when compared to compressive strength of control mortar.

Moreover, at the age of 90 days all bacterial mortar increased such as SpM1, PaM1 and SuM1 was 170%, 162% and 122% respectively when compared to compressive strength of control mortar.

This means that the compressive strength for bacterial and control mortar specimens decreased at all ages in the case of exposure to acids, but there are improvements in compressive strength with time for all bacterial mortar specimens compared to compressive strength of control mortar.

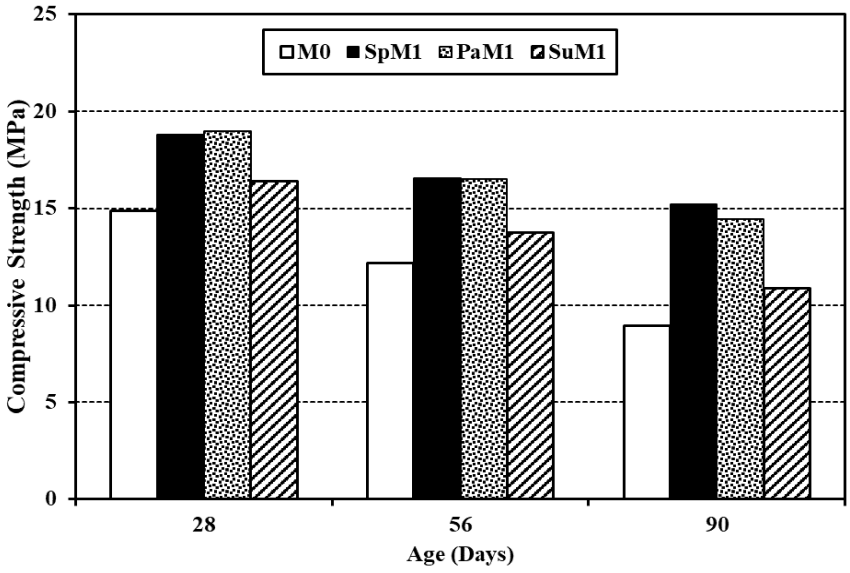


Fig. 11. Compressive strength test results for different mortar mixes after exposure to 1.5%sulfuric acid at different ages.

i. FERROCEMENT LAMINATES

ii. FLEXURAL STRENGTH OF FERROCEMENT LAMINATES

Reinforced laminates were tested under flexure after 28, 56, 90 days of curing until failure. The load-deflection of relationships of reinforced laminates with bacterial mortar and control mortar were concluded at the same age.The improvement of the flexural strength of bacterial samples over control sample. At the age of 28 days, the maximumflexural loadingof laminates value of SpM1, PaM1 and SuM1 were 134%, 115% and 113% compared to control mortar, respectively. At the age of 56 days, the maximum flexural loading of laminates value of SpM1, PaM1 and SuM1 were 116%, 105% and 107 % compared to control mortar, respectively. At the age of 90 days the maximum flexural loading of laminates value of SpM1, PaM1 and SuM1 were 131%, 115% and 113% compared to control mortar, respectively. The findings of the current study are consistent with those reported by [**3**] , where two bacteria, bacillus pasteurii and bacillus sphaericus, were successfully employed to enhance the durability of reinforced-laminates. The test results are given in **Table 3**. These findings further support the considerable potential of these bacterial strains and calcium lactate in augmenting the mechanical properties and durability of Reinforced-Laminates. **Fig. 12-13-14** shows the load deflection curve of the bacterial mortar over the control mortar. Generally, bacterial laminate proved to have a higher flexural strengthdue to the specimen have the same dimension.

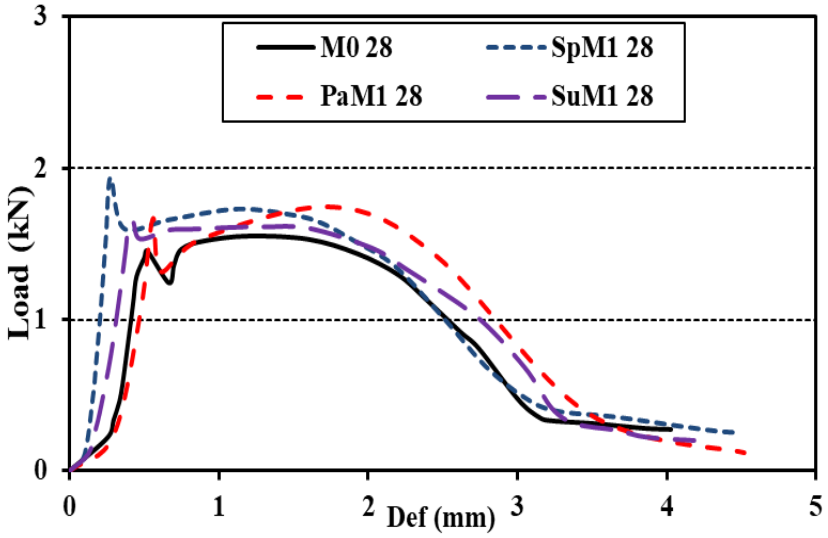


Fig. 12. Load-deflection of reinforced laminates after 28 days of curing.

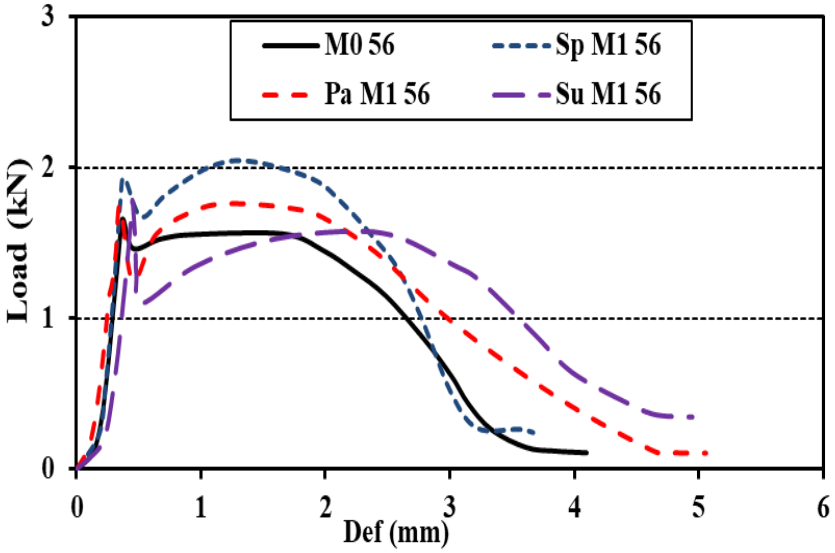


Fig. 13. Load-deflection of reinforced laminates after 56 days of curing.

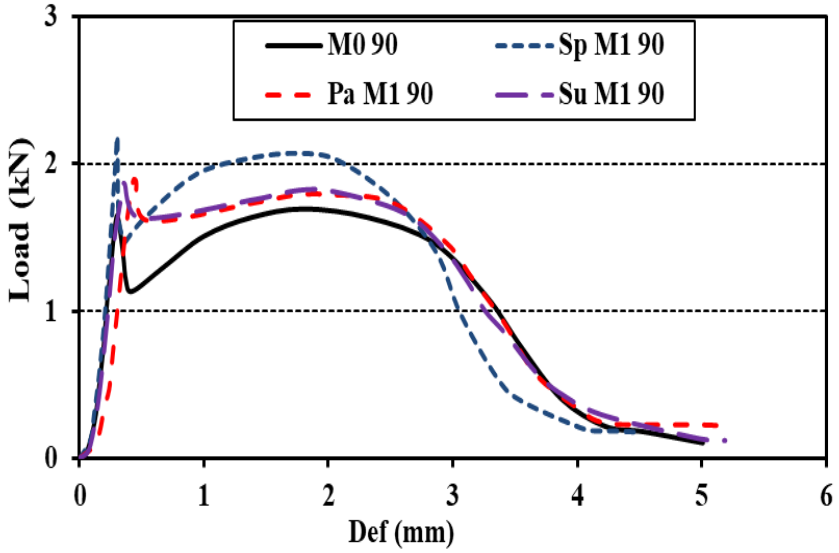


Fig. 14. Load-deflection of reinforced laminates after 90 days of curing.

Table (3): Test results of ferrocement laminates at different ages

Specimens ID	Original behavior (without preloading)														
	28 days					56 days					90 days				
	Load (kN)	%	Max Deflection (mm)	Toughness (kN.mm)	%	Load (kN)	%	Max Deflection (mm)	Toughness (kN.mm)	%	Load (kN)	%	Max Deflection (mm)	Toughness (kN.mm)	%
F0	1.45	100	4.09	3.79	100	1.66	100	4.1	4.05	100	1.65	100	5.01	5.31	100
FSp	1.94	134	4.494	4.55	120	1.93	116	3.67	4.89	121	2.17	131	4.5	5.77	109
FPa	1.67	115	4.52	4.44	117	1.75	105	5.06	5.21	128	1.89	115	5.22	5.76	109
FSu	1.64	113	4.184	4.29	113	1.78	107	4.95	5.36	132	1.87	113	5.19	5.82	110

Behavior after preloading										
Specimens ID	28-56 days					56-90 days				
	Load (kN)	%	Max Deflection (mm)	Toughness (kN.mm)	%	Load (kN)	%	Max Deflection (mm)	Toughness (kN.mm)	%
F0	1.75	100	3.375	3.57	100	1.80	100	4.772	4.88	100
FSp	2.07	118	3.89	4.69	131	2.25	125	4.029	6.06	124
FPa	1.81	103	5.55	5.03	141	2.03	113	4.61	6.31	129
FSu	1.83	105	5.301	5.17	145	1.85	103	5.02	5.87	120

IV. FLEXURAL TOUGHNESS OF FERROCEMENT LAMINATES

As given in **Table 3,**the toughness of Ferro-cement laminates was calculated by using the experimental load deflection curves as shown in **Fig.15.**

In this study toughness indices on Ferro-cement laminates were calculated by using experimental load deflection curves.

At the age of 28 days, the toughness of SpM1,PaM1 and SuM1 were 120%, 117% and 113%of flexural strength of control mortar, respectively.

At the age of 56 days, the flexural strength value of SpM1, PaM1 and SuM1 were 121%, 128% and 132% of flexural strength of control mortar, respectively.

At the age of 90 days the toughness value of SpM1, PaM1 and SuM1 were 109%, 109%and 110% comparing with thetoughness of control mortar, respectively. It can be observed that inclusion of bacteria increased the toughness values as shown in **Fig.15**. It can be observed that inclusion of bacteria increased the toughness values for all bacteria to control sample.

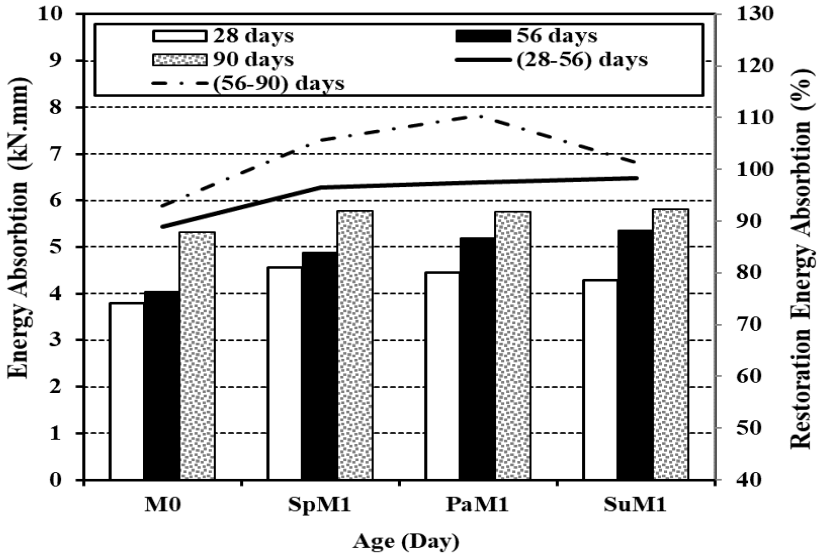


Fig. 15. Toughness results of ferrocement laminates at different ages.

V. RESTORATION OF FERRO-CEMENT LAMINATES

Samples were loaded after 28 and 56 days from casting date with half of failure load at 28 and 56 days then reloaded at 56 and 90 days until failure simultaneously. Results of max load revealed that there is an increase in strength for all bacterial mortar when compared to the original samples at the age of 56 and 90 days as shownin**Fig. 16,Fig. 17**and **Table3** the Restored samples were compared to original samples.

At the age of 56 days, the restored load of SpM1, PaM1 and SuM1 were 118%, 103% and 105% compared to max load of control samples. At the age of 90 days, the restored load of SpM1, PaM1 and SuM1 were 125%, 113% and 103% compared to max load of control samples. Increasing in max load value of bacterial mortar specimens such as SpM1, PaM1 and SuM1 and decreasing the max load values of control mortar specimen assure that self-healing of bacterial mortar occurred.

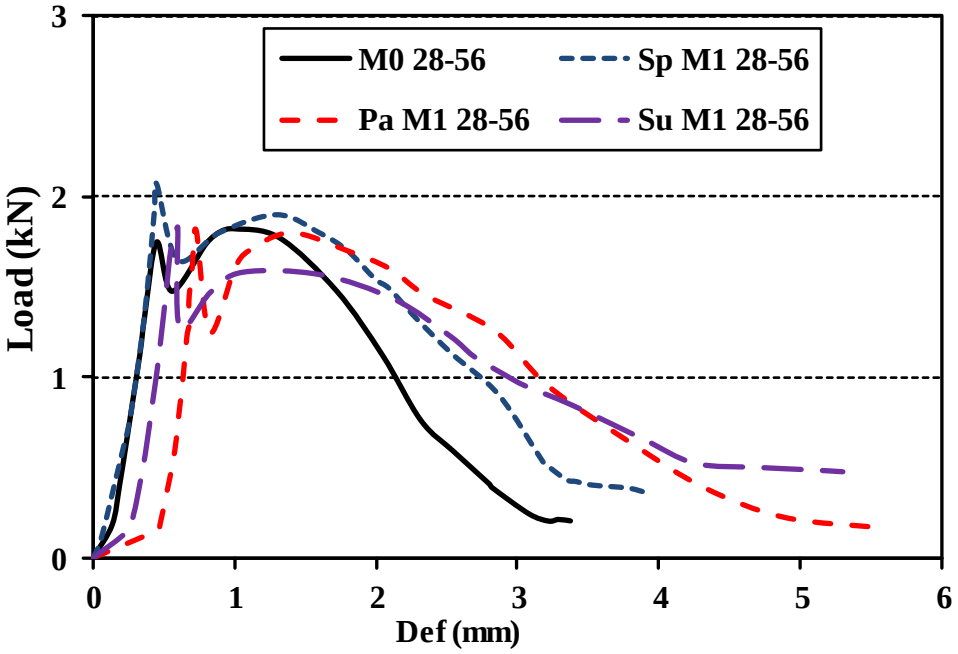


Fig. 16. Load-deflection of preloadingferrocementlaminatesat28-65 days of curing.

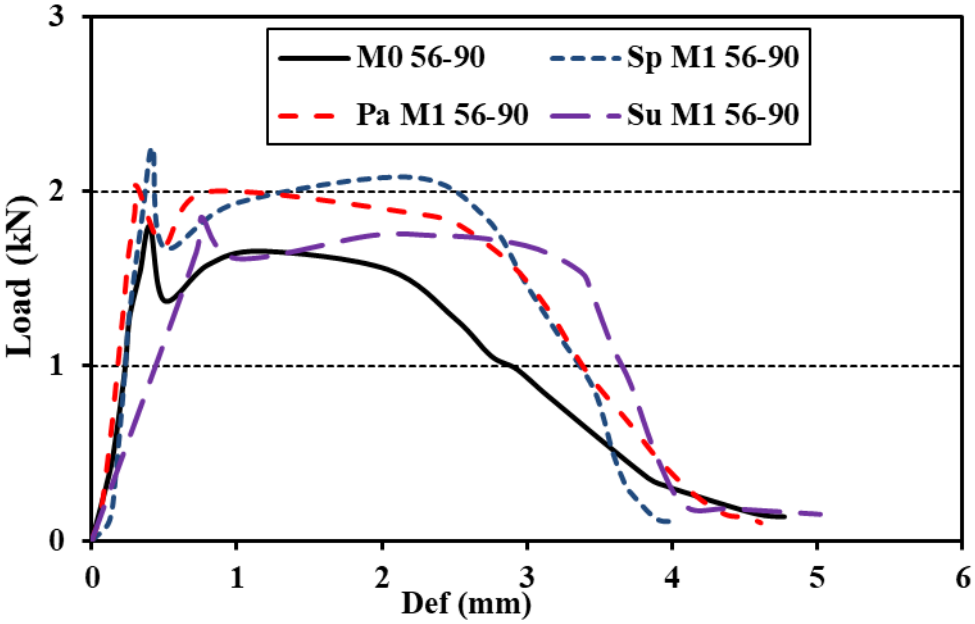
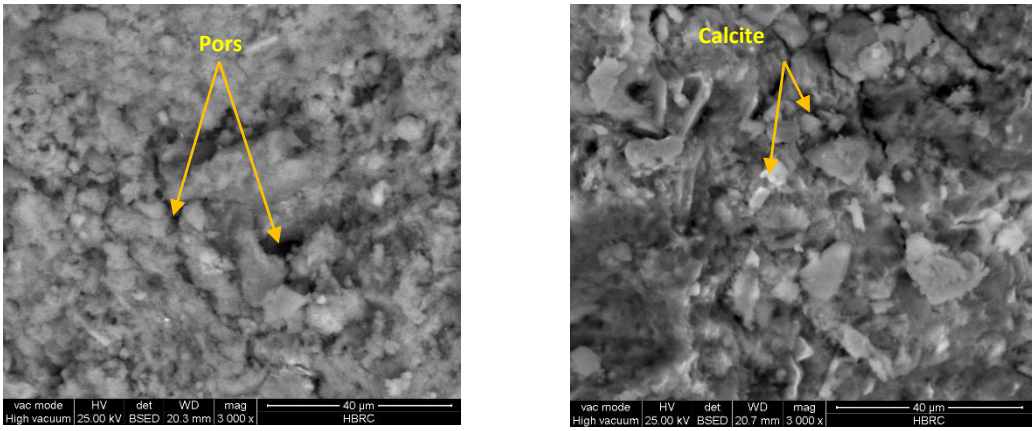


Fig. 17. Load-deflection of preloading ferrocement laminates56-90 days of curing.

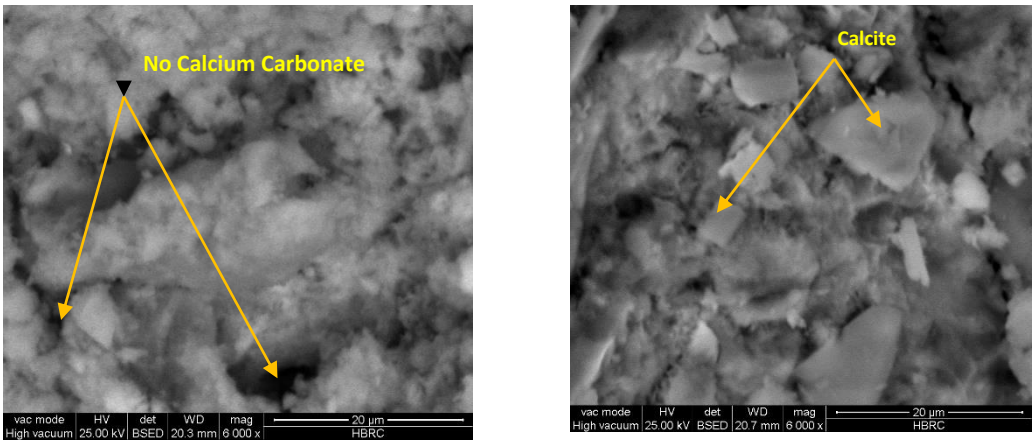
SCANNING ELECTRON MICROSCOPY (SEM)

The SEM micrographs were carried out for specimens at 28 days. **Fig.18.** showsSEM pictures for Control (without bacteria) and Bacterial mortar specimens, it 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 mortar specimens were compared using SEM pictures at the same magnifying. magnifying X3000 showed control mortar specimens had many voids compared with bacterial mortar specimens. **Fig.19** at magnifying X6000 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 [**37**].



(A) **(B)**

Fig.16. SEM images (3000X) after 90 days of curing for mixes (A) M0 and (B) PaM1.



(A) **(B)**

Fig.17.SEM images (6000X) after 90 days of curing for mixes (A) M0 and (B) PaM1.

VI. CONCLUSION

The bacterial self-healing technique has garnered significant attention due to its reputation as a sustainable and eco-friendly method for repairing continuous micro-cracks. This study bacillus sphaericus, bacillus pasteurii and bacillus subtilis can produce calcite crystals, which can effectively block micro-cracks in the mortar matrix.The main conclusions of the experimental work of this study are summarized and highlighted in the following:

- The different types of bacteria improvement influence on the properties of mortar. The flowability of mortar gradually decreased by using different types of bacteria Moreover, the setting time reduced.
- Bacillus sphaericus, Bacillus pasteurii and Bacillus subtilis improved the physical-mechanical properties with high restoration for load-deflection.Reduction rate of water absorption about (30-70) % compare with control cement mortar.
- The addition of 0.5% bacterial Bacillus subtilis (SuM1) of cement and 0.25% calcium lactate results in a significant increase of 138% in compressive strength after 90 days. Bacterial samples achieve compressive strength earlier than the control samples due to the filling of pores with bacterial precipitation.
- Precipitated calcium carbonate in pores enhances the bonds of the concrete microstructure, leading to increased flexural strength. The maximum increase in flexural strength occurs at 90 days, becoming 149% of the flexural strength for the addition of 0.5% bacterial (SpM1) Bacillus sphaericus of cement and 0.25% calcium lactate of the control mortar.
- The ability of the bacteria to provide recovery of mechanical properties also was assessed. The flexural and compressive strength recovery ranged about (28% and 13%) at early ages and (9% and 49%) at later ages, respectively, which would be sufficient for many structural applications.
- The studies investigatethe compressive strength for bacterial and control mortar through acid resistance test with 1.5%H_2SO_4(sulfuric acid) revealed that the strengthof bacterial mortarspecimens decreased at all ages, but there are improvements in compressive strength by 70% compared to control after 90 days.Additionally, further research is needed to examine the bonding between the calcium carbonate precipitate and the mortar substrate, and to determine ways to tailor the MICCP process to promote increased bonding.
- Addingbacteriato the mortar enhancedthe flexural stiffness of preloading ferrocement laminates at the age of 56-90 dayscompared with control.
- SEM micrographs reveal that the bacterial mortar exhibits a significantly reduced number of voids compared to the control mortar. One significant enhancement is the reduced water absorption, which indicates a decreased propensity for water to infiltrate the material, capillary permeability thus enhanced the physical mechanical properties of bio-Concrete. Additionally, Future research on microbial concrete must consider selection of bacteria from a minimum-nutrient environment point of view, which can optimize the production cost of microbial concrete.

REFERENCES

[1]. S. O. Ahmed, A. A. Nasser, R. N. Abbas, M. M. Kamal, M. A. Zahran, and N. M. Sorour, "Production of bio concrete with improved durability properties using alkaliphilic Egyptian bacteria," 3 Biotech, vol. 11, pp. 1–15, 2021.

[2]. T. H. Nguyen, E. Ghorbel, and H. Fares, "Bacterial self-healing of concrete and durability assessment," Cement and Concrete Composites, vol. 104, p. p 33-40, 2019.

[3]. A. A. Nasser, N. M. Sorour, M. A. Saafan, and R. N. Abbas, "Microbially-induced-calcite-precipitation (MICP): a biotechnological approach to enhance the durability of concrete using bacillus pasteurii and bacillus sphaericus," Heliyon, vol. 8,p. p 79-98, 2022.

[4]. H. G. Kwak, S. J. Ha, and J. K. Kim, "Non-structural cracking in RC walls: Part I. finite element formulation," Cement and Concrete Research, vol. 36, pp. 749–760, 2006.

[5]. H. Wahid, S. Ahmad, M. A. M. Nor, and M. A. Rashid, "Prestasi kecekapan pengurusan kewangan dan agihan zakat: perbandingan antara majlis agama islam negeri di malaysia," Journal Ekonomi Malaysia, vol. 51, no. 2, pp. 39–54, 2017.

[6]. P. Ryparova, Z. Prosek, H. Schreiberova, P. Bily, and P. Tesarek, "The role of bacterially induced calcite precipitation in self-healing of cement paste," Journal of Building Engineering, vol. 39, pp. 223-244, 2021.

[7]. N. K. Dhami, M. S. Reddy, and A. Mukherjee, "Improvement in strength properties of ash bricks by bacterial calcite," Ecological Engineering, vol. 39, pp. 31–35, 2012.

[8]. A. U. Charpe, M. V. Latkar, and T. Chakrabarti, "Microbially assisted cement – a biotechnological approach to improve mechanical properties of cement," Construction and Building Materials, vol. 135, pp. 472–476, 2017.

[9]. V. Wiktor and H. M. Jonkers, "Quantification of crack-healing in novel bacteria-based self-healing concrete," Cement and Concrete Composites, vol. 33, pp. 763–770, 2011.

[10]. V. Achal, A. Mukherjee, and M. S. Reddy, "Microbial concrete: way to enhance the durability of building structures," Journal of Materials in Civil Engineering, vol. 23, pp. 730–734, 2011.

[11]. S. Gupta, H. W. Kua, and S. D. Pang, "Healing cement mortar by immobilization of bacteria in biochar: an integrated approach of self-healing and carbon sequestration," Cement and Concrete Composites, vol. 86, pp. 238–254, 2018.

[12]. L. S. Wong, A. F. M. Oweida, S. Y. Kong, D. M. Iqbal, and P. Regunathan, "The surface coating mechanism of polluted concrete by Candida ethanolic induced calcium carbonate mineralization," Construction and Building Materials, vol. 257, p. 82-94, 2020.

[13]. W. De Muynck, K. Cox, N. De Belie, and W. Verstraete, "Bacterial carbonate precipitation as an alternative surface treatment for concrete," Construction and Building Materials, vol. 22, pp. 875–885, 2011.

[14]. S. A. Abo-El-Enein, A. H. Ali, F. N. Talkhan, and H. A. Abdel-Gawwad, "Application of microbial biocementation to improve the physic-mechanical properties of cement mortar," HBRC Journal, vol. 9, no. 1, pp. 36–40, 2013.

[15]. V. Senthilkumar, T. Palanisamy, and V. N. Vijayakumar, "Comparative studies on strength characteristics of microbial cement mortars," International Journal of Chemtech Research, vol. 6, no. 1, pp. 578–590, 2014.

[16]. J. Xu and X. Wang, "Self-healing of concrete cracks by use of bacteria-containing low alkali cementitious material," Construction and Building Materials, vol. 167, pp. 1–14, 2018.

[17]. B. Madhu Sudana Reddy and D. Revathi, "An experimental study on effect of Bacillus sphaericus bacteria in crack filling and strength enhancement of concrete," Materials Today: Proceedings, vol. 19. pp. 803–809, 2019.

[18]. S. K. Ramachandran, V. Ramakrishnan, and S. S. Bang, "Remediation of concrete using micro-organisms," ACI Mater. J., vol. 98, pp. 3–9, 2001.

[19]. M. V. S. Rao, V. S. Reddy, M. Hafsa, P. Veena, and P. Anusha, "Bioengineered concrete - A sustainable self-healing construction material," Research Journal of Engineering Sciences, vol. 2, pp. 45–51, 2013.

[20]. EN 196-16, "Method of testing cement—part 1: Determination of strength," CEN: Brussels, Belgium, 2016.

[21]. Egyptian Standard Specifications, "Composition, specifications and conformity criteria for common cements," vol. ESS 4756-1, 2013.

[22]. E.S.S. No. 1109, (2008) "Aggregate", Egyptian Standard Specification, Ministry of Industry, Cairo, Egypt.

[23]. L. Dong, L.-B. Lv, and R. Lai, "Molecular cloning of Tupaia belangeri chinensis neuropeptide Y and homology comparison with other analogues from primates," National Center for Biotechnology Information, vol. 33, no. 1, pp. 75–78, 2012.

[24]. M. Luo and C. Qian, "Influences of bacteria-based self-healing agents on cementitious materials hydration kinetics and compressive strength," Construction and Building Materials, vol. 121, pp. 659–663, 2016.

[25]. ASTM 403-92, "Standard Test Method for Time of Setting of Concrete Mixtures by Penetration Resistance," Annu. B. ASTM Stand., 1999.

[26]. ASTM C 1437, "Standard test method for flow of hydraulic cement mortar," Annu. B. ASTM Stand., vol. 04, p. 02, 2020.

[27]. ASTM C 1585, Standard test method for measurement of rate of absorption of water by hydraulic cement concretes, United States, 2013.

[28]. A. Naji Givi, S. Abdul Rashid, F. N. A. Aziz, and M. A. M. Salleh, "The effects of lime solution on the properties of SiO2 nanoparticles binary blended concrete," Composites Part B: Engineering, vol. 42, no. 3, pp. 562–569, 2011.

[29]. M. Oulu and R. Şahin, "Pore structure analysis of hardened cement mortars containing silica fume and different nano-powders," Construction and Building Materials, vol. 53, pp. 658–664, 2014.

[30]. ASTM C642. Standard test method for density, absorption, and voids in hardened concrete. Philadelphia (PA): ASTM; 2001.

[31]. EN 196-1, "Method of testing cement—part 1," Determ. Strength; CEN Brussels, Belgium, 2016.

[32]. ACI 544, 1988, "Design considerations for steel Fiber Reinforced concrete." International Concrete Abstracts Portal. Vol.85, No.5, PP.1–15.

[33]. H. N. Marsh et al., "Measurement of Properties of Fiber Reinforced Concrete," American Concrete Institute, vol. 15, pp. 433–439,1988.

[34]. M. F. Ghazy, A. A. Maaty, and G.R.Mohamed, "The effect of self-healing by bio-precipitation by bacteria on the properties cement based materials-Areview".American Journal of Engineering Research, Vol. 11,pp. 182-201,2022

[35]. H. Chen, C. Qian, and H. Huang, "Self-healing cementitious materials based on bacteria and nutrients immobilized respectively," Construction and Building Materials, vol. 126, pp. 297–303, 2016.

[36]. M. Wu, X. Hu, Q. Zhang, D. Xue, and Y. Zhao, "Growth environment optimization for inducing bacterial mineralization and its application in concrete healing," Construction and Building Materials, vol. 209, pp. 631–643, 2019.

[37]. N. Chahal, R. Siddique, and A. Rajo, "Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of concrete incorporating silica fume," Construction and Building Materials, vol. 37, pp. 645–651, 2012.