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Investigation of Utilizing Bacteria to obtain Self-Healing of Invisible Cracks in Mortar

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ABSTRACT
Bio mortar is used to deal with cracks by providing a special type of bacteria along with nutrients to the ingredients of the mortar during the mixing. Once the cracks appear, bacteria in the mortar are activated by oxygen and water and begin calcite precipitation, which automatically fills the cracks inside the mortar without any exterior interference. This research investigated the effects of varying proportions of (type of bacteria and nutrients; concentration of bacteria; bacteria and nutrient percentage) on the properties of self-healing mortar. Investigate the steps of preparation of cell suspensions of bacteria. Investigate obtaining self-healing of invisible cracks in mortar. Forty mortar mixtures were designed and tested. Three types of bacteria were utilized: Bacillus pasteurii DSM 33, Bacillus sphaericus DSM 396, and Bacillus subtilis H50620/9. Two concentrations (2×109 - 2×1010) Colony Forming Units per milliliter from DSM 33 and DSM 396 were used. Calcium lactate, calcium acetate, and urea were added as a nutrient to bacteria. Nutrients is added by in proportions ranging from 0.25, 0.50, and 1% of cement weight. Bacterial suspension is added by (0.5, 1, 4, and 10% of cement weight). Bacillus subtilis encapsulated in calcium alginate beads added by (0.25, 0.5, 1, and 4% of cement weight). The specimens were cracked by two different methods, which are visible artificial cracks and invisible natural cracks. Tests were performed for compressive strength and flexural strength. The microstructure analysis of the bacteria mortar has been done utilizing SEM to ensure that calcium carbonate (calcite) has indeed filled the cracks. Thermogravimetric analysis was performed to determine the degree of hydration for bacteria paste. The ANOVA test was conducted on the compressive strength test results by the linear model. The results show all the independent variables affect the dependent variable (the compressive strength). The best results in the increase of compressive strength reached 69.8% and 66.4% at 7 days and 120 days, respectively, in mortar mix which contains 0.5% bacteria DSM 396 with 0.25% calcium lactate. The expected decrease in compressive strength as a result of loading did not occur significantly, that shows the bacteria are working, precipitating calcite at the onset of invisible cracks formation, which assures that self-healing in that case has occurred.

Keywords: *Bacteria, Self-healing mortar, Invisible cracks, Thermo gravimetric analysis, Calcite precipitation.*

1. Introduction

A major problem with the mortar is that it is vulnerable to cracking, as it's a brittle composite cementitious material that cracks easily. Cracks can appear at any time during the service life of a building due to many different factors, such as overloads, shoddy construction procedures, shrinkage, and temperature variations. There are the traditional methods of repair and the coefficients of thermal expansion of materials used differ, these materials are incompatible for mortar repair. So, the inspection and maintenance process are not always an easy thing to do and is costly. To solve the problems of traditional mortar repair procedures, an effective healing approach must be created. Finding a sustainable approach to not only limit fracture propagation but also repair them without the need for any interference is the best solution to this problem. Self-healing mortar is considered a relatively "smart" material that presumably heals itself upon cracking, thereby saving time, costs involved in maintenance, and reaching places of repair that are impossible to reach. Self-healing in mortar is the ability of the mortar to detect damage and recover damaged properties through the usage of available resources in the matrix of mortar [1-4].

In general, there are two classifications of self-healing: autogenous self-healing and autonomous self-healing. Traditional mortar does intrinsically possess some naturally self-healing capacity and can heal limited-width cracks due to excess non-hydrated or unreacted cement particles in the mortar, referred to as autogenous healing of mortar. The essential autogenous crack healing efficiency is time-dependent, with the process leading to significant concrete expansion via the crack wings, further hydration of cementitious particles, and crystallization of CaCO3. In most of the traditional mortar mixtures, almost 20-30% of the cement is left dehydrated. Reducing crack width is an autogenous process. Autogenous healing can be possible in the following ways CaCO3 or Ca $(OH)_2$ formation to block cracks, hydration of dehydrated particles of cement, blocking of cracks by the presence of impurities in water, and expansion/swelling of calcium-silicate hydrate (CSH) gel. Autonomous self-healing involves designed additives to be mixed in the cementitious matrix for healing purposes and also uses particular techniques to transport such additives. Autonomic healing is the mechanical process of repairing damaged structures. A lot of autonomous self-healing approaches have been proposed, and they mainly include vascular, microcapsule, electrodeposition, bacteria, embedding shape memory alloys, and induction energy [5-7].

Bio mortar can be used to treat cracks by adding a special type of bacteria along with a calcium-based nutrient to the ingredients of the mortar at the time of the mixing process that can produce and precipitate calcium limestone (calcium carbonate). Once moisture enters through newly formed cracks, latent but viable bacterial spores immobilized in the mortar matrix become metabolically active. These cracks will then be repaired by microbial calcite precipitation. Microbiologically Induced Calcite Precipitation is a technique that falls under the biomineralization field of science. Mechanism of self-healing: Bio-healing materials are based on the bacteriology field of study, a branch of microbiology. Biotechnological healing technologies are categorized under the genus bacillus group of bacteria which are highly resistant to alkaline environments. An approached pathway for bacterial healing consists of providing a source of nutrients for bacteria and obtaining precipitated CaCO3 as a result of the metabolic process. It is hypothesized that bacteria can form CaCO3 precipitations and biologically induce chemical precipitations in which an organism creates an optimal extracellular micro-environment of mineral phases, known as bio-mineralization. Hence the development of one of the methods of bio-healing mortar. Bacteria are the most effective microorganisms because of their ability to precipitate certain helpful chemicals to be employed in self-healing mortar. Certain characteristics must be present for the bacteria to mineralize. Firstly, the bacteria should be capable of surviving in an extremely alkaline environment. Second, the bacteria should be able to create enormous quantities of minerals that will seal the newly formed cracks. Because concrete constructions are built to last at least 50 to 100 years, bacteria should be able to survive for that long. As a result, for biological induced mineralization, a specific group of alkaliphilic spore-forming bacteria was chosen. Several species from the genus bacillus have been used in mortar. Third, because the mortar matrix contains oxygen due to diffusion through matrix capillaries, the bacteria that are integrated into mortar must also be oxygen brilliant. Fourth, the bacteria should be able to withstand high calcium ion concentrations and create more CaCO3. Fifthly, it should be able to sustain high-pressure conditions as if it were implanted into the matrix. Also, the bacteria selected should be thermophilic, because during the hydration process of cement large amount of heat is developed [8-11].

Bacillus is the only group of bacteria that can survive this high alkaline environment. Finally, the bacteria used in mortar must be non-pathogenic. Different Bacillus strains of spore-forming bacteria have been used by researchers in their studies for self-healing of mortar, such as Bacillus Pasteurii, Bacillus sphaericus, and Bacillus subtilis [12,13]. Bacillus pasteurii is also known as sporosarcina pasteurrii. It has an ability to form into

precipitate when calcium lactate comes into contact with it in the presence of water droplets. Xu et al. used Sporosarcina pasteurii and calcium nitrate as the self-healing agent to study the self-healing of mortar cracks with crack width in the range of 20–450 μm [14]. Sporosarcina pasteurii, when used under favorable conditions in concrete can continuously precipitate calcite that has a coarse crystalline structure that readily adheres to concrete because it is highly insoluble in water. Besides this, a durability study on concrete beams treated with bacteria, exposed to alkaline, sulfate and freeze thaw environments were also studied. The durability performance increased with increase in the concentration of bacteria. Also, Sporosarcina pasteurii, urea, and yeast extract were used as a carbonation agent for internal carbonation of reactive magnesia cement pastes and Sporosarcina pasteurii was used with concentration 1.5 ×108 CFU [15]. Vijeth N Kashyap, Radhakrishna, crack healing and improvement of physical properties of cement paste, mortar and concrete are studied. It is done by the addition of bacterial strains Bacillus Sphaericus and Sporosarcina Pastuerii. It is found that these bacteria when added concentrations of 106 cells/ml of water to cement composites increased by about 39.8% and 33.07% in paste. The strength increase is found to be 18.3% and 12.2% for Bacillus Sphaericus and Sporosarcina Pastuerii respectively for concrete [16]. Bacteria from Bacillus Pasteurii type were used. Calcium Lactate was added as a nutrient for bacteria. The study showed that bacterial mortars have higher compressive and flexural strength and less water absorption than the control mortar mix. The results showed a 32% decrease in water absorption for 0.25% of cement at 120 days and 150% increase in the 28-day compressive strength of control mortar was achieved with the addition of 0.25% bacterial cement. The flexural strength of 0.25% bacterial addition was improved by 145% of the control mortar. SEM images revealed the presence of CaCO3 produced [17].

Bacillus sphaericus is strictly aerobic gram-positive rod-shaped bacterium. Bacillus sphaericus, a common soil bacterium that induces the precipitation of calcite exhibits its positive potential in selectively consolidating simulated fractures in the consolidation of sand. Bacillus Sphaericus are pore forming bacterium, dormant for several years and would be able to withstand extreme temperature. De Muynck et al. used the bacillus sphaericus for creating a CaCO3 surface treatment and investigated the changes in water absorption and gas permeability of the resulting specimens. The results indicated the bio deposition of a CaCO3 layer on the surface of mortar specimens leading to a decrease in the permeation properties. Achal et al. investigated the effects of this same Bacillus sp. bacteria on the durability and crack repair in cement structures to find that over 50% of the porosity in their mortar specimens reduced and that chloride ion permeability [18]. Achal et al. utilized Bacillus Sphaericus CT-5 with ratio of 0.47 to cement weight added directly to the mortar during mixing. After 28 days, increase in compressive strength of 36% and water absorption 6 times less than reference specimen was obtained [19]. Various studies examining the effects of employing bacteria as a self-healing agent for mortar crack restoration have recently been undertaken. However, some points are still worthy of consideration: such as the fact that there is no standard for microbial self-healing agent and bacterial concentration. The steps of bacteria culturing and preparation of cell suspension of bacteria with different concentrations are unknown in the field of civil engineering in Egypt. Mechanical test approaches are mainly based on compressive strength to prove the effectiveness of healing processes seems to indicate that it is not enough and that it needs other tests to confirm that self-healing occurs. All of these elements have an impact on self-healing potential and should be researched further.

2. EXPERIMENTAL PROGRAM

2.1. Description of materials

Three types of bacteria were utilized in this research, namely Bacillus pasteurii DSM 33, Bacillus sphaericus DSM 396 and Bacillus subtilis H50620/9. The bacterial liquid from DSM 33 and DSM 396 was directly added to the mortar, but the third type Bacillus subtilis was encapsulated in calcium alginate beads. Bacillus is urease-positive, a gram-positive bacterium and can precipitate calcium carbonate via the process of mineralization when given a calcium source [20]. DSM 33 and DSM 396 strains were purchased from the Microbiological Resources Centre in the Faculty of Agriculture, Ain Shams University. Bacillus subtilis encapsulated in calcium alginate beads was purchased from a supplier in France. Fig. (1) show the used bacteria strains in their stock form. Calcium acetate and calcium lactate was selected as a source of calcium and a nutrient for bacteria. Also, Urea is used as a nutrition for the bacteria.

Ordinary cement CEM I 42.5 N was utilized in this research and it matches E.S.S 4756-1/2013[21]. The physical and mechanical properties are measured and presented in “Table I”. Natural siliceous sand with fineness modulus 2.91, specific gravity 2.59 and unit weight 1.7 t/m3 was used as fine aggregate according to the E.S.S 1109:2008 [22] and it was free from impurities. The grading curves for sieve analysis of used sand is shown in

Fig. (2). Distilled water from a water distillation system type DESA 0040 was used to bacteria preparation. Potable water was used for both mixing and curing.

(a)

(b)

(c)

Fig. 1 The used bacteria (a) DSM 33, (b) DSM 396, and (c) Bacillus subtilis encapsulated in calcium alginate beads

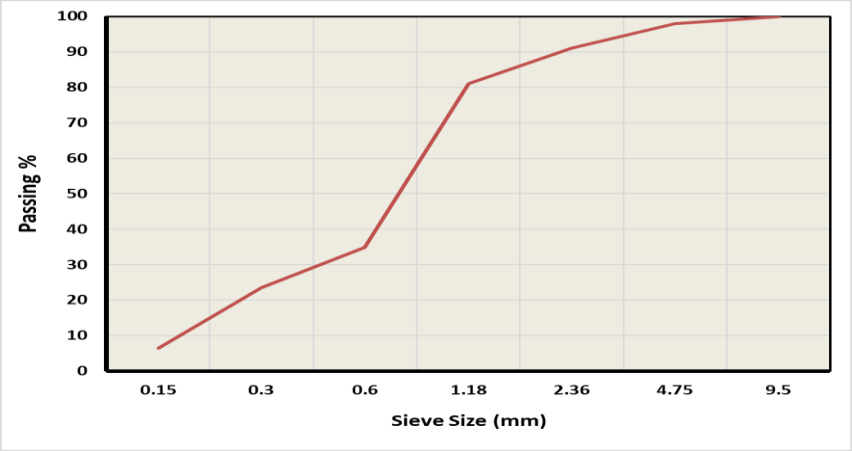


Fig. 2 the grading curves of used sand

TABLE I. PROPERTIES OF THE USED CEMENT.

Property	Test results
Specific Gravity	3.15
Fineness – Blaine measurement (cm2/gm)	3550
Initial setting time (min)	150
Final setting time (min)	250
7 days mortar compressive strength (MPa)	34
28 days mortar compressive strength (MPa)	55

2.2 Bacteria culturing and preparation of cell suspension of bacteria with different concentrations

This section discusses the method for growth and increasing the number of bacteria strains. the bacteria stock was stored in a fridge before being opened under sterile conditions. According to the supplier's recommendation, the culture media prepared by dissolving 13 grams of nutrient broth in one distilled water liter as shown in Fig. (3). The pH level of the culture media was adjusted to 7.2 by pH meter. The culture media in the container was sterilized by autoclaving at 121°C and a pressure of 1.5 bar for 20 minutes using autoclave device type SX-700 as shown in Fig. (4). Then, at room temperature, the media was then allowed to cool. Bacteria were extracted using a loop then suspend in the flasks tube containing the culture media as shown in Fig. (5). This process called inoculation of bacteria; takes place in a device of a laminar flow cabinet type AURA HZ48 that can maintain a working area devoid of contaminants. Now a cell suspension consisting of culture media and bacteria has become ready. The cell suspension inside flasks was left in a rotary shaking incubator as shown in Fig. (6) with 30°C and a shaking rate of 150 rpm for 3 days to incubate bacteria and initiate bacteria growth.

Fig. 3 preparation of the culture media

Fig. 4 Autoclave device

Fig. 5 Inoculation of bacteria

Fig. 6 Incubator shaker

Fig. 7 to obtain the cell suspension at the concentration of (2×109 - 2×1010) CFU/mL

A sample of the media with the bacteria was taken to check the rate of the growth and sporulation yield using light microscopic analysis. The culture was streaked and stored at room temperature on nutrient agar plates. CFU counting was done using serial dilutions and the spread plate technique. After the incubation period was extracted a certain quantity of culture and its bacterial cells were isolated, then these isolated cells were diluted to the required volume with distilled water and thence were serially diluted. By using solid medium (nutrient agar medium containing 13 grams of nutrient broth disperse in l liter of distilled water and 20 grams of powder Agar) colonies of bacterial cells were counted. The total count of colony-forming units of bacteria DSM 33 equals 4×1011 cfu/ml and bacteria DSM 396 equals 1×1012 cfu/ml. The bacteria culture was put inside 4℃ fridges until use. To obtain the cell suspension at the concentration of (2×109 - 2×1010) cfu/ml for each type can be diluted cell suspension with culture media as shown Fig. (7) keeping in mind that the amount of nutrient broth per bacterium should be the same in all the concentrations [23]. The above procedure was repeated for any quantity of cell suspension. All microbiological assays have been done at Seed and Tissue Pathology Lab, Faculty of Agriculture, Mansoura University, El-Mansoura, Egypt.

Calcium Alginate was used as a carrier (microcapsules) for bacterial spores Bacillus subtilis H50620/9 and nutrients. Palin et al. utilized calcium alginate in a similar method [24] to encapsulate the bacteria and nutrition.

According to the supplier's recommendation in France and from the manufacture data sheet on the product, we present steps of preparation of bacterial agent and encapsulation process that done in France. The preparation of the bacterial-based self-healing agent began with the cultivation of bacterial strains. The bacterial strains were grown in basal media 121 and its derivatives121A and 121B to produce the spores. Bacteria growth consists of 8 g of nutrient broth, 1 g of potassium chloride, and 0.25 g of magnesium sulfate per litter adjusted to pH 7.0–7.2. The culture was incubated in a shaker for 72 h at 120 rpm and a temperature of 36 °C until spores appeared. The gram and spore stain procedures were used to confirm spore production under a microscope. The spores were harvested using a centrifuge machine to reduce the presence of vegetative cells. Heavy particles are pushed away from the axis of rotation by centrifugal force, as a result, spores depose at the bottom of the test tube, creating a pellet. The spores were then encapsulated in calcium alginate as follows. The capsules were made by mixing a solution of 7.5 g of sodium alginate, 0.5 g of yeast extract, 7.8 g of hydrochloride, and the bacterial spores (6.1×106 cfu/ ml). A magnetic stirrer was used to homogeneously mix the solution, resulting in a 1.5 % bacterial sodium alginate solution. To form the calcium alginate beads, the solution was manually dropped via syringe into a coagulate solution containing 8 g of calcium chloride and 4 g of calcium lactate combined in 400 ml of sterilized water. After 20 min, the formed beads were extracted from the calcium chloride solution, washed twice in sterilized water, and dried at 37℃ for 24 h. The capsules formed have a particle size of about 150 μm. These steps are almost identical to this research [25,26].

2.3. Mix proportions, Casting, and curing

Forty bacterial mortar mixtures were designed and tested the different effects of the varying proportions of five different factors (type of bacteria, concentration of bacteria, bacteria/cement ratio, types of nutrients, and nutrients /cement ratio). There is a description of all mortar mixtures in "Table II". All the mortar mixtures were made with (1 Cement: 2 Sand: 0.4 Water). Three different percentages of calcium lactate and calcium acetate were 0.25%, 0.50%, and 1% of cement weight. Urea added percentage of 0.25%, 0.5%, 2%, and 2.5% of cement weight. Bacteria added with ratios of (0.50%, 1%, 4%, and 10% of cement weight. A portion of the mixing water was replaced by bacterial suspension. Bacillus subtilis H50620/9 encapsulated in calcium alginate beads added to mortar with ratios (0.25%, 0.5%, 1%, and 4% of cement weight). The measured amounts of sand and cement were carefully mixed for two minutes without water in a mechanical fixed horizontal pan mixer. Add nutrition to the mixes and mix for two minutes at a low speed. Water and bacteria liquid were poured over the mixture. Then mix for approximately five minutes until the mixture is uniformly distributed. Finally, three layers of fresh mortar are being poured into molds and each layer is compacted using the vibrating table for 30 seconds. The mortar specimens were cubes with dimensions of 70 mm x 70 mm x 70 mm and beams with dimensions of 40 x 40 x 160 mm.

TABLE II. THE DETAILED DESCRIPTION OF ALL MORTAR MIXTURES.

Mix code	Types of bacteria	Bacteria concentrations (cfu/ml)	Bacteria /cement	Types of Nutrients	Nutrient /Cement	Cement Kg/m^3	Bacteria Kg/m^3	Water Kg/m^3	Sand Kg/m^3	Nutrient Kg/m^3
M 43	-	-	-	-	-	679.27	-	271.71	1358.54	-
M 44	DSM 33	2×10^9	0.5%	C. L	0.25%	678.57	3.39	268.04	1357.15	1.696
M 45	DSM 33	2×10^9	1%	C. L	0.5%	677.80	6.78	264.34	1355.61	3.389
M 46	DSM 33	2×10^9	1%	C. L	0.25%	678.57	6.79	264.64	1357.15	1.696
M 47	DSM 33	2×10^9	4%	C. L	1%	676.26	27.05	243.46	1352.53	6.763
M 48	DSM 33	2×10^9	10%	C. L	1%	676.26	67.63	202.88	1352.53	6.763
M 49	DSM 33	2×10^{10}	0.5%	C. L	0.25%	678.57	3.39	268.04	1357.15	1.696
M 50	DSM 33	2×10^{10}	1%	C. L	0.5%	677.80	6.78	264.34	1355.61	3.389
M 51	DSM 33	2×10^{10}	1%	C. L	0.25%	678.57	6.79	264.64	1357.15	1.696
M 52	DSM 33	2×10^{10}	4%	C. L	1%	676.26	27.05	243.45	1352.53	6.763
M 53	DSM 33	2×10^{10}	10%	C. L	1%	676.26	67.63	202.88	1352.53	6.763
M 54	DSM 396	2×10^9	0.5%	C. L	0.25%	678.57	3.39	268.04	1357.15	1.696
M 55	DSM 396	2×10^9	1%	C. L	0.5%	677.80	6.78	264.34	1355.61	3.389
M 56	DSM 396	2×10^9	1%	C. L	0.25%	678.57	6.79	264.64	1357.15	1.696
M 57	DSM 396	2×10^9	4%	C. L	1%	676.26	27.05	243.45	1352.53	6.763
M 58	DSM 396	2×10^9	10%	C. L	1%	676.26	67.63	202.88	1352.53	6.763
M 59	DSM 396	2×10^{10}	0.5%	C. L	0.25%	678.57	3.39	268.04	1357.15	1.696
M 60	DSM 396	2×10^{10}	1%	C. L	0.5%	677.80	6.78	264.34	1355.61	3.389
M 61	DSM 396	2×10^{10}	1%	C. L	0.25%	678.57	6.79	264.64	1357.15	1.696
M 62	DSM 396	2×10^{10}	4%	C. L	1%	676.26	27.05	243.45	1352.53	6.763
M 63	DSM 396	2×10^{10}	10%	C. L	1%	676.26	67.62	202.88	1352.53	6.763

M 64	DSM 33	2×10^9	1%	C. A	0.5%	677.91	6.78	264.38	1355.82	3.390
M 65	DSM 33	2×10^9	10%	C. A	1%	676.48	67.65	202.94	1352.95	6.765
M 66	DSM 33	2×10^{10}	1%	C. A	0.5%	677.91	6.78	264.38	1355.82	3.390
M 67	DSM 33	2×10^{10}	10%	C. A	1%	676.48	67.65	202.94	1352.95	6.765
M 68	DSM 396	2×10^9	1%	C. A	0.5%	677.91	6.78	264.38	1355.82	3.390
M 69	DSM 396	2×10^9	10%	C. A	1%	676.48	67.65	202.94	1352.95	6.765
M 70	DSM 396	2×10^{10}	1%	C. A	0.5%	677.91	6.78	264.38	1355.82	3.390
M 71	DSM 396	2×10^{10}	10%	C. A	1%	676.48	67.65	202.94	1352.95	6.765
M 72	DSM 33	2×10^9	1%	Urea	2%	673.21	6.74	262.55	1346.42	13.464
M 73	DSM 33	2×10^9	3%	Urea	2%	673.21	20.12	249.09	1346.42	13.464
M 74	DSM 33	2×10^9	1%	Urea	0.5%	677.80	6.78	264.34	1355.61	3.389
M 75	DSM 33	2×10^9	5%	Urea	2.5%	671.69	33.58	235.09	1343.38	16.792
M 76	DSM 33	2×10^9	0.5%	-	0	679.35	3.4	268.34	1358.7	0
M 77	DSM 33	2×10^9	10%	-	0	679.35	67.94	203.80	1358.7	0
M 78	-	-	0	C. L	0.25%	678.57	0	271.43	1357.15	1.696
M 79	B.S	-	0.25%	-	-	679.27	1.7	-	1358.54	-
M 80	B.S	-	0.5%	-	-	679.27	3.40	-	1358.54	-
M 81	B.S	-	1%	-	-	679.27	6.8	-	1358.54	-
M 82	B.S	-	4%	-	-	679.27	27.17	-	1358.54	-

Calcium Acetate = C. A, Calcium Lactate = C. L, bacillus pasteurii DSM 33 = DSM 33, Bacillus sphaericus DSM 396 = DSM 396, Bacillus subtilis H50620/9 encapsulated in calcium alginate beads = B.S.

2.4. Creation of cracks

Two different methods were used to investigate the ability of bacteria to self-heal in the event of a crack. To monitor the healing of the cracks, visible artificial cracks were created in the fresh mortar by introducing a thin aluminum plate by 0.5 mm thickness into the wet mortar at the time of casting. The plates were removed before the final setting of mortar, leaving a 0.5 mm wide crack on the upper surface of the specimens. The specimens were visible in the crack as shown in Fig. (8). Then, the specimens were removed from the molds after 24 h and cured in water. The specimens were removed from water after 14 days and 28 days, and the crack widths were measured with a Stereo-Microscope with Camera System OLYMPUS SZ61 that was found in the Seed and Tissue Pathology Lab in the Faculty of Agriculture, Mansoura University. Microscopic observations were taken to see the formation of a white precipitate produced by bacterial activity on the specimens. The experimental devices and crack measurements are illustrated in Fig. (8). The invisible natural cracks were created at an early age. To measure the ultimate load in all mixes, at the age of 7 days from casting, the cubes were removed from the curing water and loaded until failure. Then, conjugate sets of cubes from mortar were applied with a load equal to 30 - 40% of the max compressive load of the cubes. In some loaded samples, slight cracks appear after loading, which can be seen by visual inspection. Now loaded specimens and other unloaded specimens from each mix were ready. These loaded cubes go back to curing to give the bacteria a chance to start calcite precipitation and heal cracks. After 28, 56, and 120 days, both loaded and unloaded specimens from each mix tested until failure to measure the compressive strength after precipitation calcite [27].

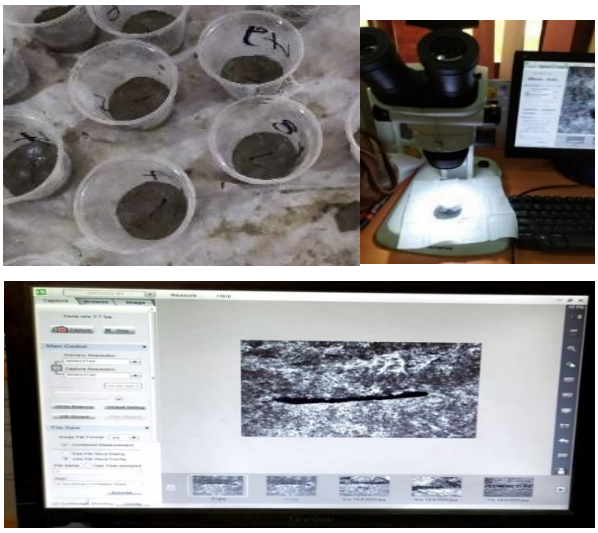


Fig. 8 the experimental devices for visible crack measurements

2.5. Testing procedure

- Compressive strength

The compressive strength of mortar was determined after 7, 28, 56, and 120 days from the casting. The loaded specimens were tested to determine the compressive strength until failure at 28, 56, and 120 days. Three cubes were tested at each age, and the averages of three samples for each age were taken and tabulated. The strength of the mixes was measured using a hydraulic testing machine type ELE, which had a capacity of 2000 KN in Materials Testing Laboratory, Faculty of engineering, Delta University for Science and Technology as shown in Fig. (9). the compression test was carried out according to BS EN 12390-3:2019 [28].

Fig. 9 Compressive strength test

- Flexural strength

The flexural specimens of mortar were subjected to a three-point loading test after 28 and 120 days from the casting. An average of three tested specimens for each age was taken. The flexural testing of the mixes was measured using the hydraulic compression-flexural test with a total capacity of up to 300 kN, according to BS EN 12350-5 [29].

- Scanning Electron Microscope (SEM)

SEM is a powerful instrument that permits the characterization of heterogeneous surfaces, gives a high-quality and clear stereoscopic image, and enables detailed information about the sample microstructure. The effect of biological calcium carbonate precipitation was investigated using SEM on the specimen's mortar. Some damaged parts of specimens were obtained after a compressive strength test at 120 days to be observed using an electronic microscope type JEOL JSM-651OLV with a magnification capacity of 300,000 times in the Faculty of Agriculture located at Mansoura University.

- Thermal Gravimetric Analysis (TGA)

TGA was performed to determine the chemically bound water of bacteria blended cement pastes and assess the degree of hydration. Six cement pastes in total were prepared by varying the three types of bacteria used and their concentration. The blended cement paste nomenclatures by weight are shown in "Table III". The water to cement (W/C) ratio was selected at 0.35. The percentages for calcium lactate were 0.50% of cement weight, the percentages for bacteria were 1% of cement weight. The dynamic heating ramp varied between 25 ^{o}C and 1000 ^{o}C with a heating rate of 5 ^{o}C/min under a nitrogen atmosphere at 28, 56, and 90 day). TGA was run at HBRC as shown in Fig. (10). Cement hydrates can be classified into three basic stages in general. The evaporable water and the hydrates' decomposition are represented in the first stage, which occurs from 100 ^{o}C and 400 ^{o}C represents the dehydration reaction (Ldh). The dehydroxylation of Portlandite (Ldx) are represented in the second stage, which occurs between 400 and 600 ^{o}C. The decarbonation of $CaCO_3$ (Ldc) are represented in the last stage, which occurs between 600 and 1000 ^{o}C [٣٠]. According to Bhatty [٣١], the hydration degree of cement paste can be calculated using the following equations Eq (6) and Eq (7):

$$W_B = Ldh + Ldx + 0.41\,(Ldc) \qquad \textbf{Eq (1)}$$

$$\alpha = \frac{W_B}{0.24} \qquad \textbf{Eq (2)}$$

TABLE III. PASTE MIX DETAILS FOR TGA TEST

Mix	Cement (gram)	Water (gram)	Water - Bacteria	Bacteria (gram)	Calcium Lactate (gram)
Control	400	140	140	-	-
DSM 33- 2×10^{9}	400	140	136	4	2
DSM 33- 2×10^{10}	400	140	136	4	2
DSM 396- 2×10^{9}	400	140	136	4	2
DSM 396- 2×10^{10}	400	140	136	4	2
Bacillus subtilis encapsulated in calcium alginate beads	400	140	140	4	-

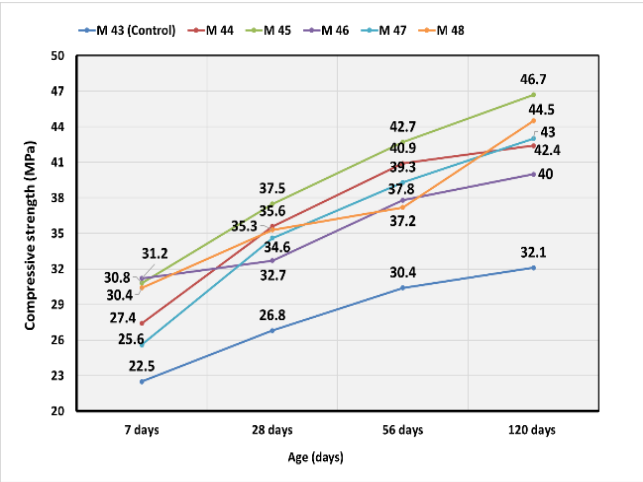
Fig. 10 Preparation cement pastes specimens for TGA test.

3. RESULTS AND DISCUSSION

3.1. Compressive strength

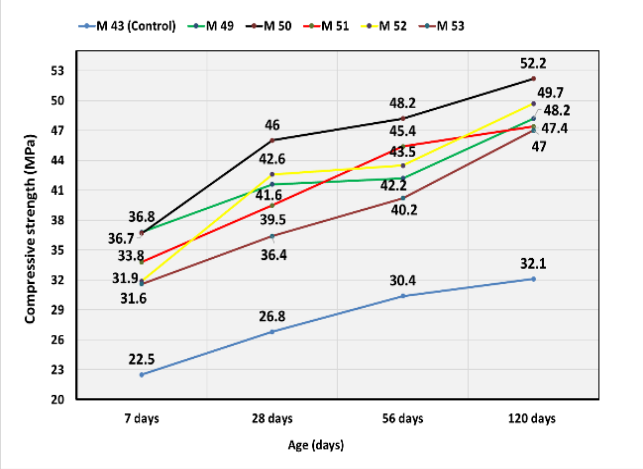
The impact of bacteria on the compressive strength for forty mortar mixtures with different conditions are shown in "Table IV". Results in "Table IV" reveal that mixes containing bacteria of all ages have a higher compressive strength than their control mix 43 of the same age. The only parameters that changed were adding the healing agents. Thus, this leads to the conclusion that the increase in compressive strength is due to use of bacteria in mix design. By mortar mixes prepared with different percentages of bacteria and nutrients, results revealed that containing a higher percentage of bacteria and nutrients was not a requirement to give the best result in compressive strength. When compared to the control mix, it was found that the best results in the increase of compressive strength reached 69.8% and 66.4% at 7 days and 120 days, respectively, in mix 54, which contains 0.5% bacteria DSM 396 with 0.25% calcium lactate. When type of bacteria (DSM 33), nutrient (calcium lactate) and concentration of bacteria (2×109 cfu/ml) were fixed, it was found that the percentage of bacteria/cement and the percentage of nutrient/cement that gave the best result was 1% bacteria/cement with 0.5% calcium lactate. This is verified in Mix 45 as shown in Fig. (11). When the type of bacteria (DSM 396), nutrient (calcium lactate) and concentration of bacteria (2× 109 cfu/ml) were fixed, it was found that the percentage of bacteria/cement and percentage of nutrient /cement which gave the best result was 0.5% bacteria /cement with 0.25% calcium lactate. This is verified in mix 54 as shown in Fig. (12 a). The same result was found when the concentration of bacteria increased to 2× 1010 cfu/ml. It was found that the percentage of bacteria/cement and the percentage of nutrient /cement which gave the best result was 0.5% bacteria/cement with 0.25% nutrient. This is verified in mix 59 as shown in Fig. (12 b). On the other hand, when changed the percentage of bacillus subtilis H50620/9 encapsulated

in calcium alginate beads between (0.25, 0.5, 1, and 4) % of weight of cement, it was found that the best result in compressive strength was 1% bacteria/cement. This is verified in mix 81 as shown in Fig. (13).



M 43: Control **M 46: 1% DSM 33 + 0.25% C. L**
M 44: 0.5% DSM 33 + 0.25% C. L **M 47: 4% DSM 33 + 1% C. L**
M 45: 1% DSM 33 + 0.5% C. L **M 48: 10% DSM 33 + 1% C. L**

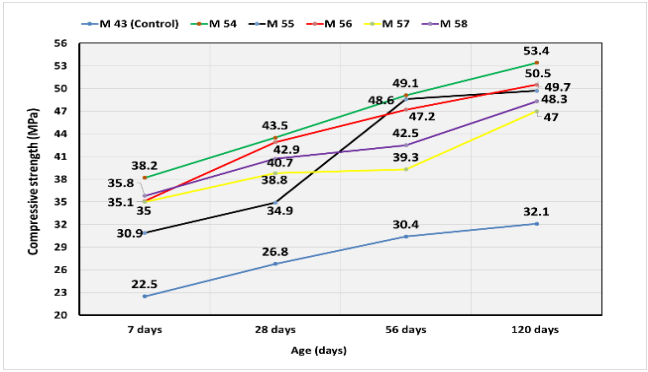
(a)



M 43: Control **M 51: 1% DSM 33 + 0.25% C. L**
M 49: 0.5% DSM 33 + 0.25% C. L **M 52: 4% DSM 33 + 1% C. L**
M 50: 1% DSM 33 + 0.5% C. L **M 53: 10% DSM 33 + 1% C. L**

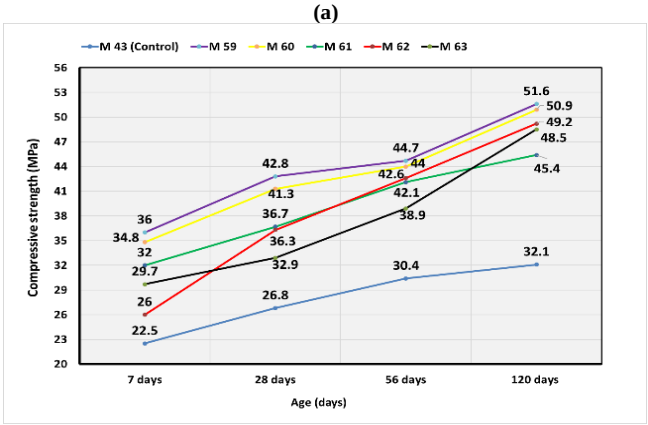
(b)

Fig. 11 Effect of percentage of bacteria and nutrient on compressive strength at different ages with DSM 33 bacteria, (a) 2× 10^9 cfu/ml (b) 2× 10^{10} cfu/ml concentration, and calcium lactate nutrient.



M 43: Control **M 56: 1% DSM 396 + 0.25% C. L**
M 54: 0.5% DSM 396 + 0.25% C. L **M 57: 4% DSM 396 + 1% C. L**
M 55: 1% DSM 396 + 0.5% C. L **M 58: 10% DSM 396 + 1% C. L**

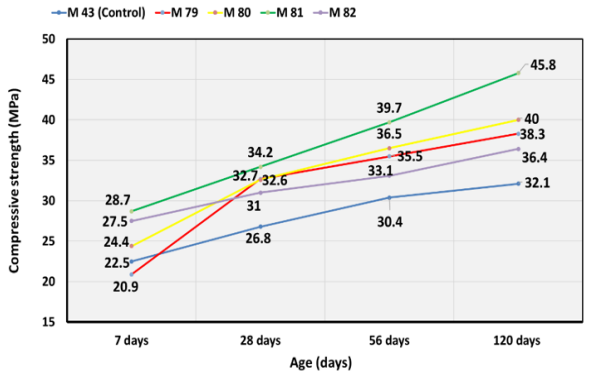
(a)



M 43: Control **M 61: 1% DSM 396 + 0.25% C. L**
M 59: 0.5% DSM 396 + 0.25% C. L **M 62: 4% DSM 396 + 1% C. L**
M 60: 1% DSM 396 + 0.5% C. L **M 63: 10% DSM 396 + 1% C. L**

(b)

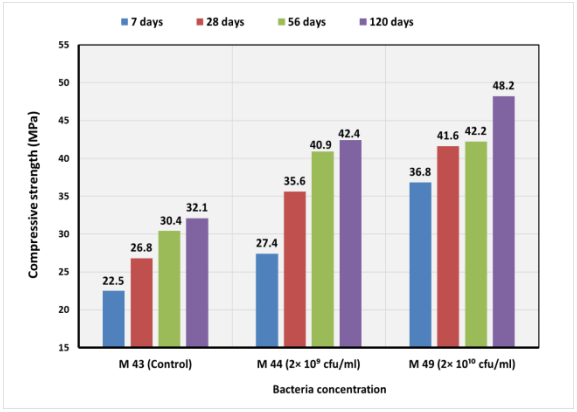
Fig. 12 Effect of bacteria/cement percentage as well as nutrient/cement percentage on compressive strength at different ages with DSM 396 bacteria, (a) 2× 10^9 cfu/ml (b) 2× 10^{10} cfu/ml concentration, and calcium lactate nutrient.



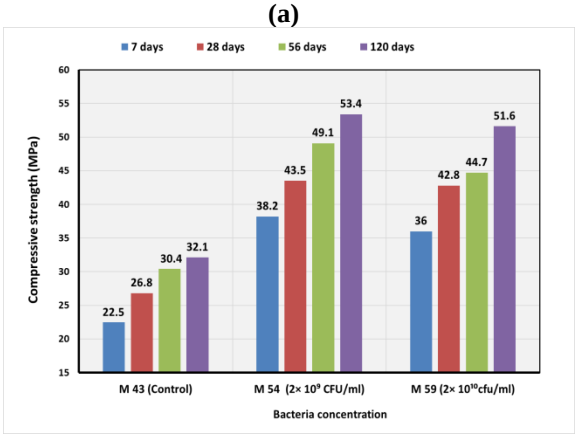
M 79: 0.25% B. S **M 81: 1% B. S**
M 80: 0.5% B. S **M 82: 4% B. S**

Fig. 13 Effect of bacillus subtilis encapsulated in calciumalginate beads percentage on compressive strength at different ages.

The purpose of this research was also to see how variations in bacterial concentration affected the compressive strength of mortar. The difference in the compressive strength results is larger when the bacteria concentration increases to 2×1010 cfu/ml. For instance, the gap between mix 44 and mix 49 is 6 MPa at 28 days and 5.8 MPa at 120 days, as shown in Fig. (14 a). On the other hand, the gap between mix 54 and mix 59 is 4.4 MPa at 56 days, as shown in Fig. (14 b). As the bacteria concentration is increased, the effect of bacteria on compressive strength becomes apparent with the type of bacteria DSM 33 and, on the contrary, with the type of bacteria DSM 396. If compare three types of bacteria used in this study, it was found that in terms of compressive strength, the Bacillus sphaericus DSM 396 type generally surpasses the other two types, Sporosarcina Pasteurii DSM 33 and Bacillus subtilis H50620/9 encapsulated in calcium alginate beads, as shown in Fig. (15). When compare three types of nutrients used in this study, it was found that in terms of compressive strength, the calcium lactate type generally surpasses the other two types. For instance, when the type of bacteria Sporosarcina Pasteurii DSM 33, concentrations of bacteria 2×109 cfu/ml, percentage of bacteria/cement 1%, and percentage of nutrient/cement 0.5% were fixed, it was found that the best results in compressive strength were mixed with nutrient type calcium lactate, as shown in Fig. (16). Also, the effect of the addition of nutrients alone or bacteria alone on compressive strength was studied. Also, there are mixes without nutrients, such as mix 76 and mix 77, with varying percentages of bacteria. It can be seen that the addition of nutrient alone in mix 78 slightly reduces the compressive strength of mortar mixes compared to those of control mixes at the start, and then a slight increase in compressive strength occurs at ages of 56 days and 120 days. As for the mixes 76 and 77, which have bacteria and there is no nutrient, when compared to the control mix, it was found that there was an increase in compressive strength and this increase was caused by the percentage of bacteria in mix 76 reaching 10% and 25.9% at the ages of 28 days and 56 days, respectively.



(a)



(b)

Fig. 14 Effect of bacteria concentration on compressive strength at different ages with bacteria (a) DSM 33, (b) DSM 396, and concentration (2× 10^9 and 2× $10^{\prime\prime}$ cfu/ml).

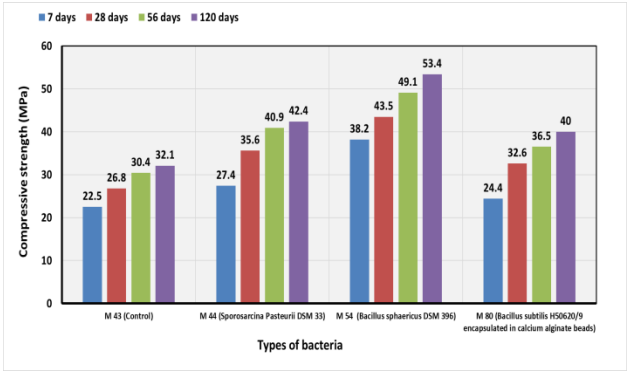


Fig. 15 Effect of bacteria type on compressive strength at different ages for mortar mixes.

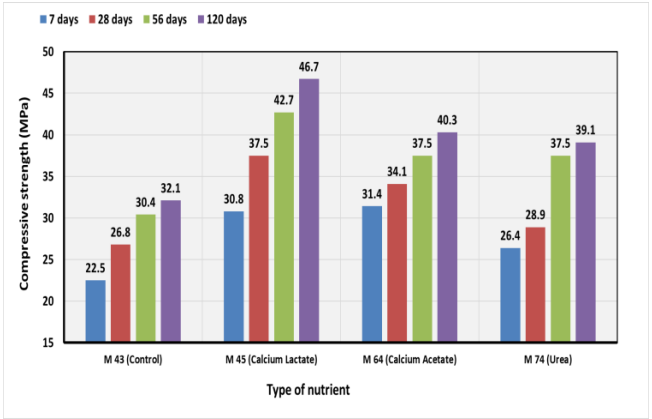


Fig. 16 Effect of nutrient type on compressive strength at different ages for mortar mixes with bacteria DSM 33 and concentration 2× 10^9 cfu/ml.

Specimens loaded were tested to determine the compressive strength until failure at 28, 56, and 120 days. The purpose of this experiment was to see if the mortar specimens could regain their strength after being cracked.

Compressive strength values of control specimens decreased by 17% at 28 days and 19% at 120 days when loaded specimens were compared to reloaded specimens. The results of the compressive strength test revealed that there was an increase in strength for most specimens when compared to control specimens at the ages of 28, 56, and 120 days as illustrated in "Table IV" and shown in Fig. (17). This means that bacteria can heal internal micro-cracks caused by loading and the ability to restore the mechanical properties of mortar to their original state.

When calcium lactate as a nutrient in mixes from mix 43 to mix 63 was fixed and changed between the two types of bacteria used and their concentration, it was found that results of the compressive strength show the effect of the percentage of bacteria and nutrient on the recovery of specimens reloaded of compressive strength cracked. The percentage of bacteria and nutrient which gave the best results was 10% bacteria/cement with 1% calcium lactate. As the percentage of bacteria and nutrients increases, so does the recovery percentage of compressive strength of the reloaded cracked samples compared to the original specimens of the same mixture. For instance, at the age of 28 days, mix 63 has a compressive strength of 35.1 MPa for reloading specimens while the loaded specimen original has 32.9 MPa, as shown in Fig. (18). At the age of 120 days, mix 54 has a compressive strength of 55.6 MPa for reloading specimens, while the loaded specimen original has 53.4 MPa, as shown in Fig. (18). By way of explanation, at the age of 28 days, mix 71 has a compressive strength of 37.4 MPa for reloading specimens, while the loaded specimen original has 37.8 MPa. No severe decrease in compressive strength values of bacterial mortar mixes and a decrease in compressive strength values of control mix specimens assure that self-healing has occurred. This suggests that internal microcracks created by loads have the potential to repair and restore the mechanical properties of mortar by using bacteria.

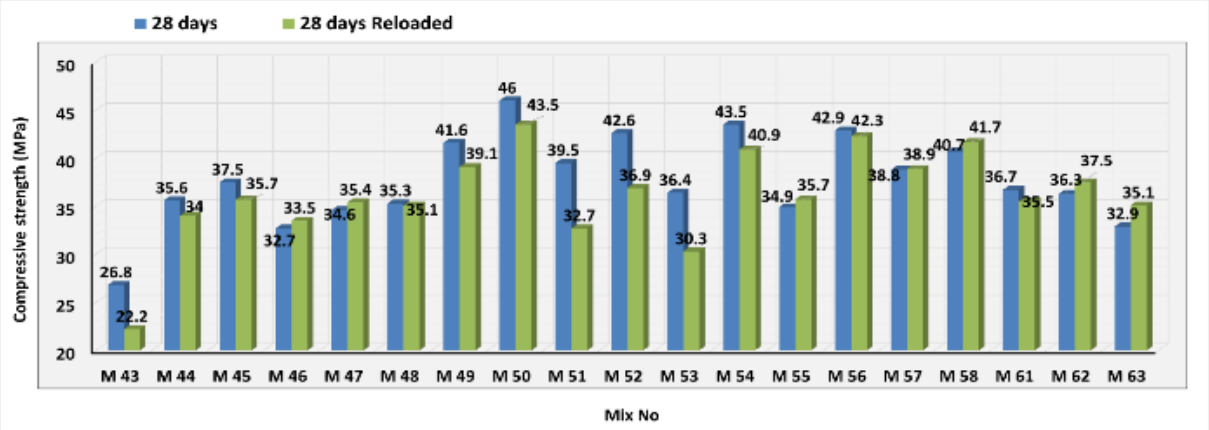


Fig. 17 Effect of bacteria on the recovery of compressive strength of reloaded cracked specimens at 28 days.

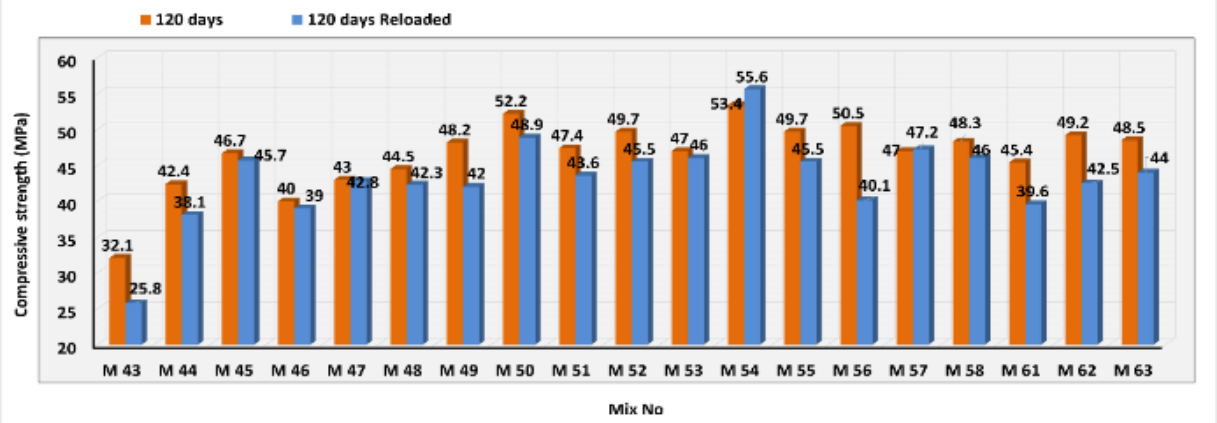


Fig. 18 Effect of bacteria on the recovery of compressive strength of reloaded cracked specimens at 120 days.

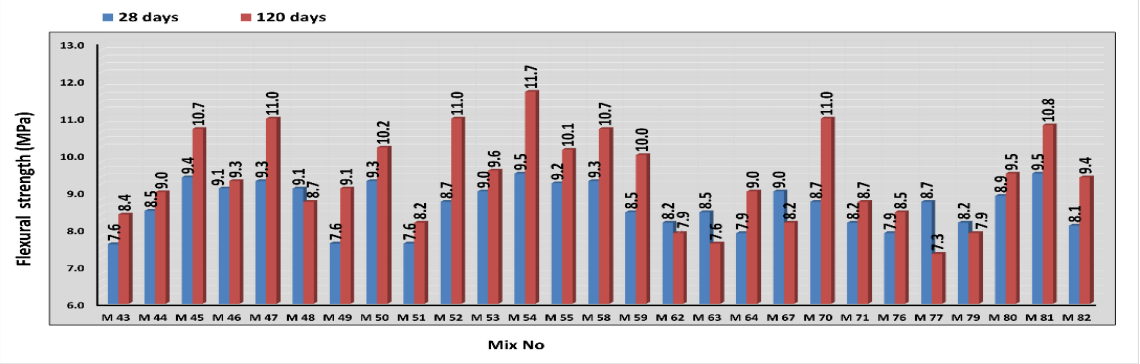


Fig. 19 Flexural strength results at 28 and 120 days.

TABLE IV. RESULT OF COMPRESSIVE STRENGTH AND FLEXURAL STRENGTH TEST.

	Compressive strength (MPa)				Compressive strength (MPa) of reloading samples			Flexural strength (MPa)	
Mix code.	7 days	28 days	56 days	120 days	28 days	56 days	120 days	28 days	120 days
M 43	22.5	26.8	30.4	32.1	22.2	24.9	25.8	7.6	8.4
M 44	27.4	35.6	40.9	42.4	34	37.4	38.1	8.5	9.0
M 45	30.8	37.5	42.7	46.7	35.7	43.5	45.7	9.4	10.7
M 46	31.2	32.7	37.8	40	33.5	38.1	39	9.1	9.3
M 47	25.6	34.6	39.3	43	35.4	41.1	42.8	9.3	11.0
M 48	30.4	35.3	37.2	44.5	35.1	35.2	42.3	9.1	8.7
M 49	36.8	41.6	42.2	48.2	39.1	38.3	42	7.6	9.1
M 50	36.7	46	48.2	52.2	43.5	45.6	48.9	9.3	10.2
M 51	33.8	39.5	45.4	47.4	32.7	42.6	43.6	7.6	8.2
M 52	31.9	42.6	43.5	49.7	36.9	40.8	45.5	8.7	11.0
M 53	31.6	36.4	40.2	47	30.3	41.3	46	9.0	9.6
M 54	38.2	43.5	49.1	53.4	40.9	48.1	55.6	9.5	11.7
M 55	30.9	34.9	48.6	49.7	35.7	40.8	45.5	9.2	10.1
M 56	35.1	42.9	47.2	50.5	42.3	43	40.1	—	—
M 57	35	38.8	39.3	47	38.9	43.9	47.2	—	—
M 58	35.8	40.7	42.5	48.3	41.7	45.8	46	9.3	10.7
M 59	36	42.8	44.7	51.6	40.2	42.2	—	8.5	10.0
M 60	34.8	41.3	44	50.9	39.8	42.8	—	—	—
M 61	32	36.7	42.1	45.4	35.5	36.4	39.6	—	—
M 62	26	36.3	42.6	49.2	37.5	40.2	42.5	8.2	7.9
M 63	29.7	32.9	38.9	48.5	35.1	40.7	44	8.5	7.6
M 64	31.4	34.1	37.5	40.3	34.5	31.8	28.7	7.9	9.0
M 65	32.4	33.6	42.6	46.9	31	39.5	40.5	—	—
M 66	25.5	31	35.2	39.8	27.5	37.5	39	—	—
M 67	21.2	29	30.3	40.2	29.9	32.4	33.9	9.0	8.2
M 68	30.5	33.5	40.1	43	—	—	—	—	—
M 69	29.4	31.5	39.4	41.2	—	—	—	—	—
M 70	32.7	34.5	40.9	48.9	32.5	36.4	47.8	8.7	11.0
M 71	34.5	37.8	43.5	49.3	37.4	42	49	8.2	8.7
M 72	19.6	21.5	29.7	34.1	15.2	16.8	14.8	—	—
M 73	19.3	26.5	34.8	37.2	16.4	17.9	15.6	—	—
M 74	26.4	28.9	37.5	39.1	19.8	22.3	20	—	—
M 75	24.8	27.1	35	38.9	18.1	19.5	13.9	—	—
M 76	21.9	29.5	38.3	42.5	27.1	31.5	30	7.9	8.5
M 77	21.4	28.7	30	35.7	22.9	25.3	27.9	8.7	7.3
M 78	29.2	32	35.4	37.6	—	—	—	—	—
M 79	20.9	32.7	35.5	38.3	29	30.7	33.4	8.2	7.9
M 80	24.4	32.6	36.5	40	27.5	31	34.5	8.9	9.5
M 81	28.7	34.2	39.7	45.8	30.5	36.4	40.9	9.5	10.8
M 82	27.5	31	33.1	36.4	25	26.8	30	8.1	9.4

3.2. Flexural strength

The results of the flexural strength test revealed that there is a significant increase in the strength of the bacterial mortar in all mixes compared to the control mortar, which indicates a positive effect of such bio additive on the flexural strength of mortar, as illustrated in "Table IV". For example, the percentage of bacteria/cement and the percentage of nutrient/cement which gave the best results in most of the mixes, with fixing other variables, was 0.5% bacteria/cement and 0.25% nutrient. A significant increase of mix 54 was 25% and 39.3% of the flexural strength of control mortar at the ages of 28 and 120 days, respectively, mix 54, which contains 0.5% bacteria DSM 396 with 0.25% calcium lactate. When the type of bacteria (DSM 33), nutrient (calcium lactate), and concentration of bacteria (2×109 cfu/ml) were fixed, it was found that the percentage of bacteria/cement and the percentage of nutrient/cement which gave the best result was 4% bacteria /cement with 1% calcium lactate. This is verified in mix 47 as shown in Fig. (19). The same result was found when the concentration of bacteria was increased to 2×1010 cfu/ml. It was found that the percentage of bacteria/cement and the percentage of nutrient/cement which gave the best result was 4% bacteria /cement with 1% nutrient. This is verified in mix 52. When the type of bacteria (DSM 396), nutrient (calcium lactate), and concentration of bacteria (2× 109 cfu/ml) were fixed, the percentage of bacteria/cement and percentage of nutrient/cement that produced the best results was 0.5% bacteria/cement with 0.25% calcium lactate, as shown in mix 54. When the concentration of bacteria increased, it was found that the same percentage was retained. This is verified in mix 59. When type nutrient (calcium acetate) was fixed and exchanged in types of bacteria (DSM 33 and DSM 396) with two concentrations (2× 109 and 2× 1010 cfu/ml), it was found that the best result in flexural strength was 1% bacteria /cement with 0.5% calcium acetate. This is verified in mix 70. On the other hand, when exchanged the percentage of bacillus subtilis H50620/9 encapsulated in calcium alginate beads between (0.25, 0.5, 1, and 4) % of weight of cement, it was found that the best result in flexural strength was 1% bacteria/cement. This is verified in mix 81. This study was also aimed at investigating the effects of different types of bacteria on the flexural strength of mortar mixes. If compare the three types of bacteria used in this study, it was found that in terms of flexural strength, the Bacillus sphaericus DSM 396 type generally surpasses the other two types, as shown in Fig. (20).

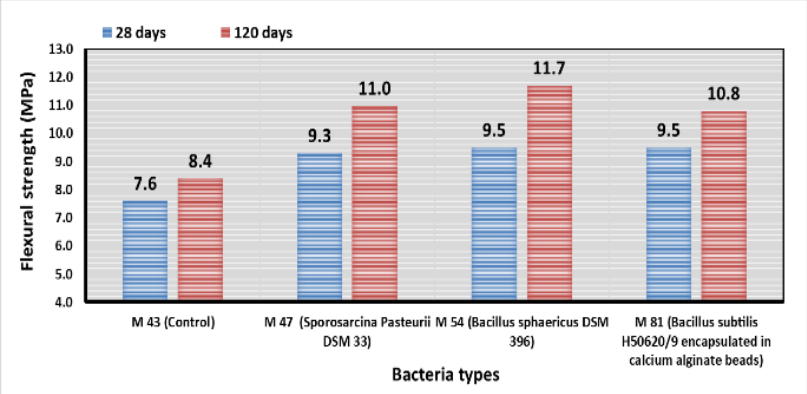


Fig. 20 Effect of bacteria types on flexural strength at 28 and 120 days.

3.3. SEM analysis

SEM tests were used to search for evidence of microbial calcium carbonate precipitation s shown in Fig. (21). SEM tests were used to examine some specimens with the highest compressive strength. The SEM micrographs were carried out after 120 days of casting with different the magnifications ranging from 2000X to 6000X. From SEM analysis, calcium carbonate as calcite crystals could be clearly distinguished within the pores of bacterial mortar in the form of a layer of white precipitates all over the of the specimen; knowing, in control specimens that were made without any addition of bacterial cells not done observed. It is shown that calcite crystals are precipitated by bacterial cells, leading to filling pores, this gives rise to more strength and durability for the mortar. The presence of crystalline calcite associated with bacteria indicated that bacterial cells act as nucleating sites for precipitation of calcium carbonate during the mineralization process. Also, as the concentration of bacterial cells increases, the precipitated calcium carbonate content rises, this is checked in mixes mix 50 and mix 59 as shown in Fig. (21). Varied shapes and sizes of calcium carbonate crystals were evident in SEM micrographs of bacterium specimens made with different concentrations of bacterial cells. The mixes with bacteria DSM 33 with a concentration of 2×10^{10} cfu/ml compared to others gave the best results. These results support evidence of microbial calcite precipitation by types of bacteria used in this work.

Deposition of ettringite crystals with long needle shape and in bacterial mortar specimen in mix 54 were observed as shown in Fig. (21).

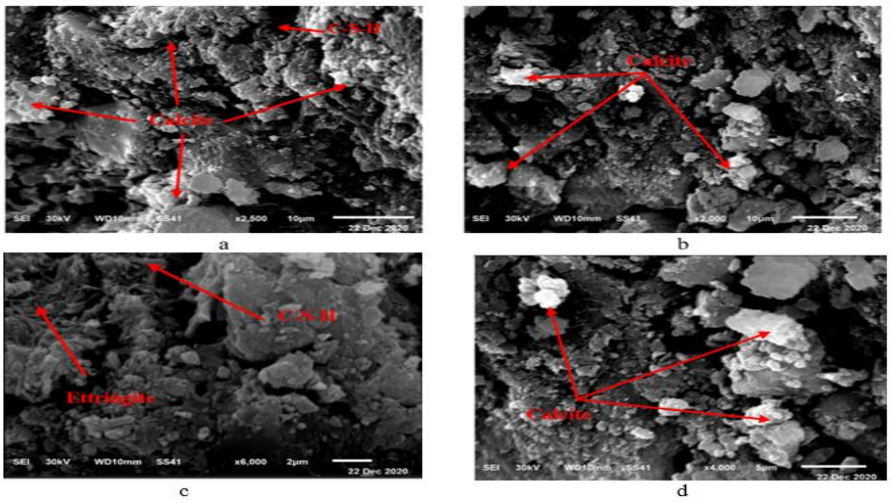


Fig. 21 SEM images at 120 days (a) Mix 54 (b) Mix 59, (c) Mix 43 control and (d) Mix 50

3.4. Thermogravimetric analysis (TGA)

To estimate the hydration degree of paste bacteria from TGA results, the chemically bound water content should be known according to method of Bhatty [٣١]. The chemically bound water is dependent on several factors such as the particle size distribution, the water/ cement percentage, the type of additive, and its percentage. In this thesis, the chemically bound water content **(W_B)** was estimated by the mass loss between 100 and 1000 ºC according to the following equation [W_B = Ldh + Ldx + 0.41 (Ldc)]. The degree of hydration **(α)** blended cement pastes was calculated according to the following equation [$\alpha = \frac{W_B}{0.24}$]. Where Ldh, Ldx, and Ldc are the relative mass loss on TGA curves during dehydration of C-S-H between 100 and 400 ºC, dehydroxylation of Ca $(OH)_2$ of portlandite recorded between 400 and 600 ºC, decarbonation of $CaCO_3$ recorded between 600 and 1000 ºC. All results of TGA values are presented from "Table V". The mass loss values has been obtained from TGA performed on blended cement pastes. These values are presented in "Table VI". The degree of hydration of cement pastes blended by bacteria is presented in Fig. (22). Some of TGA curves of samples extracted from mixes are presented in Fig. (23).

The comparison of the degree of hydration between the control mix and cement pastes containing bacteria at 28, 56, and 90 days shows a significant difference. Also, it can be noticed that the short-term hydration degree of the control mix was rapidly increasing. After 56 days, however, the evolution of the hydration degree slowed down. The degree of hydration for control mix was 55.03, 59.87, and 61.28% at 28, 56, and 90 days respectively. In bacteria mixed pastes, the inclusion of bacteria greatly enhanced the hydration kinetics, notably at 28 days, according to the results. For example, in mix DSM 396 2×10^9 the degree of hydration increased 19.4 and 12% for 28 and 90 days compared to the control mixture. Similarly, in the mix DSM 396- 2×10^{10}, the degree of hydration increased by 16.2, 11, and 10.5 % for 28, 56, and 90 days respectively compared to the control mixture. Meanwhile, in the mix Bacillus subtilis H50620/9 encapsulated in calcium alginate beads, the degree of hydration exhibited an enhancement by 13.2, 7.5, and 6 % for 28, 56, and 90 days respectively compared to the control mixture. These results lead to an important fact, which is that the degree of hydration is increased with the use of bacteria and the degree of hydration is increased with an increase in calcite.

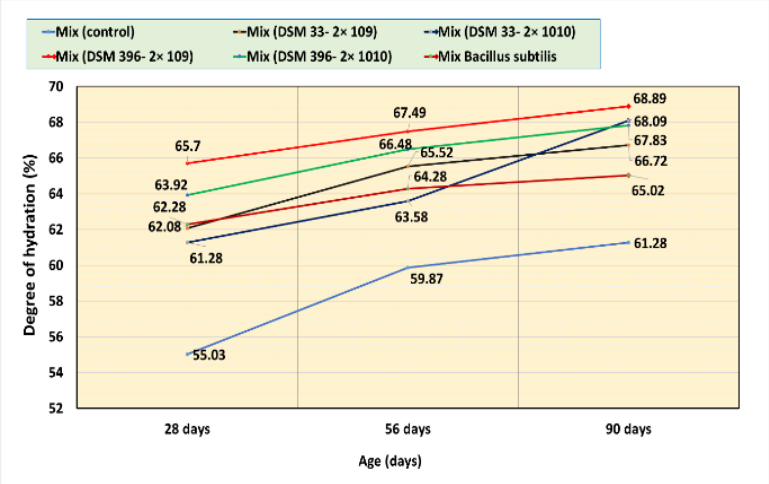
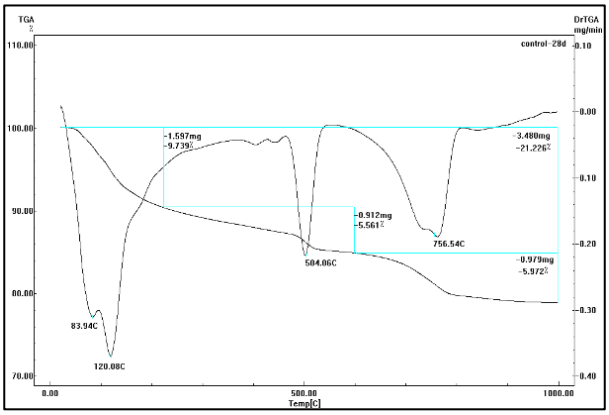


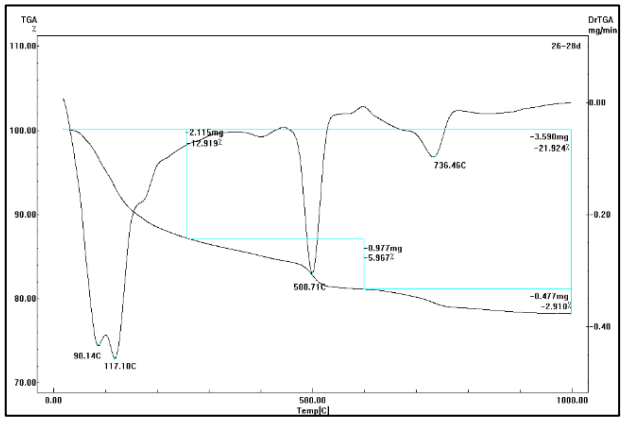
Fig. 22 Degree of hydration of cement pastes blended by bacteria

TABLE V. THE CALCULATIONS FOR THE CHEMICALLY BOUND WATER CONTENT (W_B) AND THE DEGREE OF HYDRATION (α)

Mix No	Age (days)	M_{sample} (mg)	Mass loss values during TGA test				Ldh	Ldx	Ldc	W_B (mg)	W_B (%)	α (%)
			$M_{105\,^oC}$ (mg)	$M_{400\,^oC}$ (mg)	$M_{600\,^oC}$ (mg)	$M_{1000\,^oC}$ (mg)						
Mix (control)	28	14.19	13.54	12.45	11.92	11.51	1.09	0.53	0.41	1.79	13.21	55.03
	56	16.39	15.77	14.43	13.91	12.92	1.34	0.52	0.99	2.27	14.37	59.87
	90	16.22	15.54	14.17	13.64	12.7	1.37	0.53	0.94	2.29	14.71	61.28
Mix (DSM 33 - 2× 10^9)	28	16.34	15.64	14.19	13.56	12.95	1.45	0.63	0.61	2.33	14.90	62.08
	56	16.32	15.56	13.98	13.31	12.83	1.58	0.67	0.48	2.45	15.72	65.52
	90	16.38	15.45	13.67	13.14	12.74	1.78	0.53	0.4	2.47	16.01	66.72
Mix (DSM 33 - 2× 10^{10})	28	15.26	14.61	13.25	12.65	12.19	1.36	0.6	0.46	2.15	14.71	61.28
	56	15.76	15.04	13.57	12.95	12.45	1.47	0.62	0.5	2.30	15.26	63.58
	90	15.14	15.57	13.84	13.37	12.53	1.73	0.47	0.84	2.54	16.34	68.09
Mix (DSM 396 - 2× 10^9)	28	16.29	15.54	13.97	13.27	12.83	1.57	0.7	0.44	2.45	15.77	65.70
	56	16.37	15.6	13.89	13.27	12.79	1.71	0.62	0.48	2.53	16.20	67.49
	90	16.25	15.87	14.52	13.48	12.91	1.35	1.04	0.57	2.62	16.53	68.89
Mix (DSM 396- 2× 10^{10})	28	14.03	13.39	12.06	11.5	11.1	1.33	0.56	0.4	2.05	15.34	63.92
	56	14.25	13.6	12.2	11.59	11.2	1.4	0.61	0.39	2.17	15.96	66.48
	90	14.45	14.11	12.4	12.1	11.4	1.71	0.3	0.7	2.30	16.28	67.83
Mix Bacillus subtilis	28	15.85	15.2	13.8	13.17	12.58	1.4	0.63	0.59	2.27	14.95	62.28
	56	17	16.18	14.57	13.86	13.43	1.61	0.71	0.43	2.50	15.43	64.28
	90	16.65	15.91	14.24	13.62	13.15	1.67	0.62	0.47	2.48	15.60	65.02



Mix (control) - 56 days



Mix (DSM 396) – 56 days

Fig. 23 Sample of TGA curves of cement paste of mix control and mix (DSM 396- 2×10^9 cfu/ml).

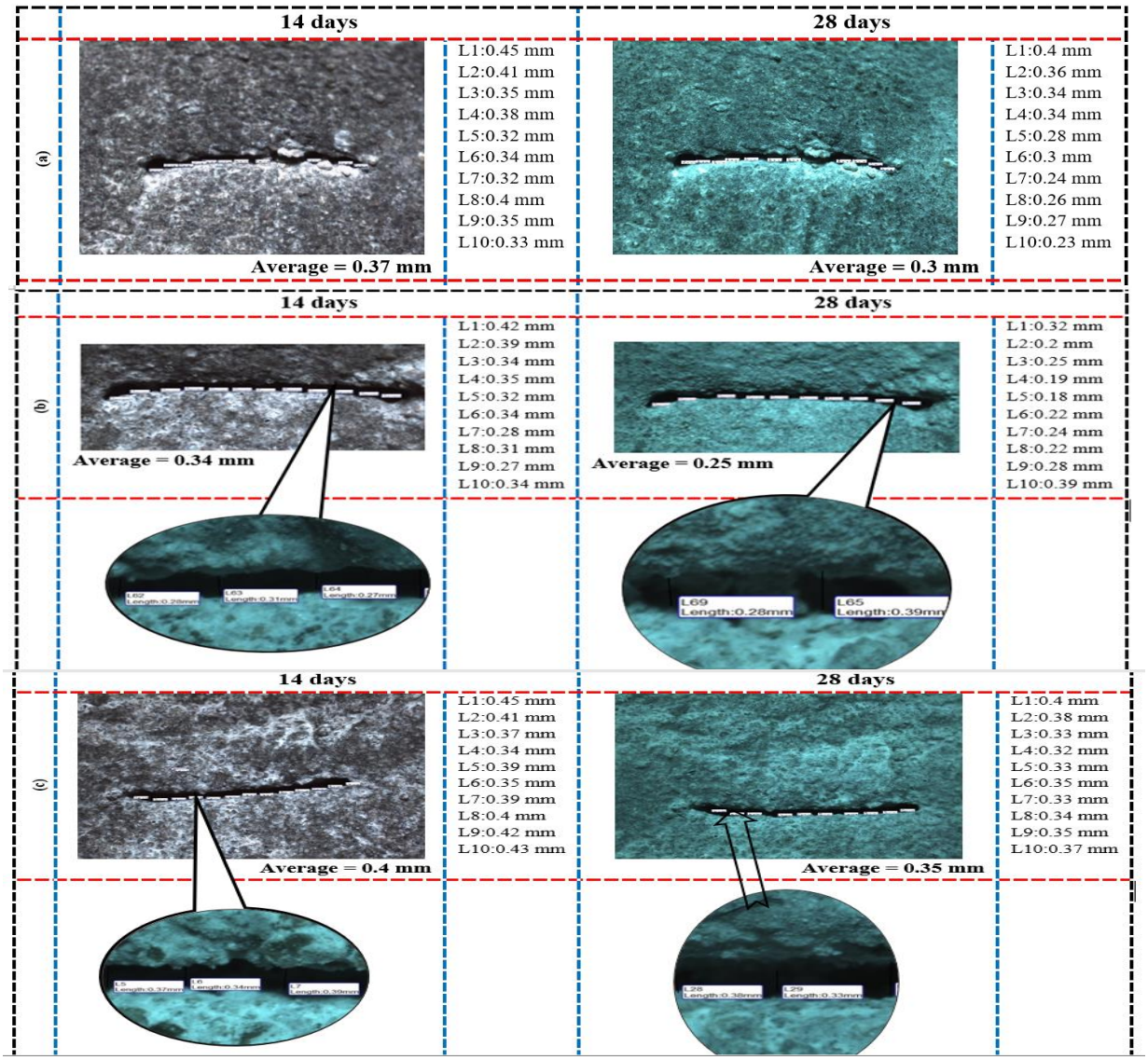


Fig. 24 Stereomicroscopic images showing the evolution of artificial crack after 14 days and 28 days of water curing(a) Mix 50 (1% DSM 33 - 2×10^{10} + 0.5% C. L), (b) Mix 54 (0.5% DSM 396 - 2×10^9 + 0.25% C. L), and (c) mix 44 (0.5% DSM 33 - 2×10^9 + 0.25% C. L)

3.5. Results of visible crack-healing

The mortar specimens were removed from the water after 14 days and 28 days, and the crack widths were measured with a Stereo-Microscope with Camera System OLYMPUS SZ61 to visualize self-healing. Fig. (24) shows images of microscopic crack healing of mortar specimens with bacteria after 14 days and 28 days of curing in water. There was no significant change in the width of the crack of the control specimen, so this artificial crack healing was slow and limited. Crack healing in normal mortar is probably due to autogenous healing, which is an inherent property of cementitious materials. The mortar specimens incorporating microbial (bacteria + nutrients) showed self-healing as shown in Fig. (24). At the same time, white products were noticed around the cracks and it can be seen that some of the cracks are partially filled with this white precipitate. It was identified as the precipitation of calcite through microstructural investigations. For example, the crack surfaces of mix 50 were closed approximately 26% at 14 days and 40% at 28 days, in another meaning at 14 days, 0.13 mm from out of 0.5 mm (the initial width of the crack) crack was filled by microbial carbonate precipitations. Also, at 28 days, 0.20 mm from out of 0.5 mm (the initial width of the crack) crack was filled by microbial carbonate precipitations. Thus, it appears that adding the suspension of bacteria has had a major healing impact on specimens. In mortar mix 54, the initial crack width was around 0.5 mm, at 14 days the average crack width was obtained 0.34 mm and at 28 days the average crack width was of around 0.25 mm, it can be observed that the crack was filled by calcite crystals as shown in Fig. (24). The crack widths varied around 0.40 mm in mix 44 mortar specimen (0.5% DSM 33 - 2×10^9 + 0.25% C. L) at 14 days. While at 28 days the crack widths varied around 0.35 mm as shown in Fig. (24). After comparing the results of the two types of cracks, it was concluded that natural cracks seem to heal better than artificial cracks.

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3.6. Descriptive statistics analysis evaluations of the tests results

Statistical analysis was used to analyze the data obtained from experimental results of some mixes to see if the main variables in the experimental program had a statistically significant effect on the measured values or not. Using the Analysis of Variance (ANOVA) statistical method, this section examined the effects of type of bacteria, concentration of bacteria, bacteria/cement percentage, types of nutrients, and nutrients/cement percentage on the compressive strength of self-healing mortar. ANOVA is the most frequent statistical procedure for determining the percent contribution of each component and factor interactions from the results of an experiment. The most important results of ANOVA are the p-values; which is the level of significance, when the p-value of the factor is equal to or less than 0.05, the factor is significant and vice versa [٣٢]. By applying ANOVA, compressive strength was assigned as the dependent variable while the experimental test parameters (age of test, percentage of bacteria and nutrients, and type and concentration of bacteria were selected as the independent factors (independent variables (inputs)) in mixes from mix 43 to mix 63. The analysis has been performed at 0.05 significance level to identify the statistical significance of experimental parameters which are age of test (7 days - 28 days- 56 days - 120 days), percentage of bacteria and nutrients (0% B + 0 % C.L - 0.5% B

+ 0.25 % C.L – 1 % B + 0.5 % C.L - 1 % B + 0.25 % C.L - 4 % B + 1 % C.L – 10 % B + 1 % C.L) , and type and concentration of bacteria (control, DSM 33-2×10^9, DSM 33-2× 10^{10}, DSM 396-2×10^9, DSM 396-2×10^{10}) on the compressive strength of mortar. The stepwise regression analysis was utilized to determine the type of relationship between the independent variables to determine the percentage of difference that can be explained in the dependent variable (compressive strength) by the independent variables and to determine the R^2 to know the effect percentage of each independent variable on the dependent variable. Results of ANOVA statistical analyses have been displayed in "Table VI". From the ANOVA test, the addition of bacteria and nutrients had a significant effect on the compressive strength results.

TABLE VI. SAMPLE OF RESULTS OF TGA TEST FOR MIX (DSM 396- 2× 10^9 CFU/ML).

28 days, M_{sample} = 16.29 [mg]	
"Temp"	**"TGA"**
"C"	"mg"
26.07	16.29
43.33	16.28
85.23	15.86
105.0	15.54
125.7	15.17
166.9	14.73
187.7	14.59
208.1	14.48
249.5	14.33
291.1	14.21
311.7	14.16
332.5	14.11
394.8	13.97
415.7	13.92
436.4	13.87
457.3	13.83
478.1	13.79
498.9	13.60
519.6	13.37
540.5	13.33
581.9	13.29
602.7	13.27
623.5	13.25
644.3	13.23
686.0	13.16
706.7	13.12
727.5	13.06
748.3	12.98
769.1	12.95
789.9	12.93
810.8	12.92
831.5	12.91
852.3	12.89
873.1	12.87
893.9	12.86
935.5	12.84
956.3	12.84
977.2	12.83
998.0	12.83

56 days, M_{sample} = 16.37 [mg]	
"Temp"	**"TGA"**
"C"	"mg"
19.01	16.37
37.71	16.36
79.92	15.96
99.51	15.60
119.8	15.24
161.3	14.73
181.9	14.58
202.6	14.47
243.9	14.31
285.5	14.18
306.2	14.13
327.0	14.08
389.3	13.94
410.1	13.89
431.1	13.85
451.7	13.81
472.4	13.76
493.3	13.62
514.1	13.39
534.7	13.32
576.2	13.28
597.0	13.27
617.9	13.26
638.8	13.23
659.5	13.20
680.2	13.16
701.0	13.11
721.9	13.05
763.5	12.92
825.7	12.88
846.6	12.86
867.6	12.84
929.8	12.80
950.6	12.80
971.6	12.79
992.4	12.79

Table VI. Results of ANOVA statistical analyses for mortar mixes.

ANOVA [a]						
Model		Sum of Squares	df	Mean Square	F	**Sig.**
1	Regression	2545.117	1	2545.11	128.58	**.004**
	Residual	1623.033	82	19.793		
	Total	4168.150	83			
2	Regression	2973.484	2	1486.74	100.80	**.016**
	Residual	1194.666	81	14.74		
	Total	4168.150	83			

As shown in Fig. (25), the mean is nearly equal to zero and the standard deviation is 0.988, Also, the normality of the independent variable (compressive strength) is clarified by the comparison between the reality and what was expected. As shown in Fig. (26), the comparison between reality and what was expected was very close to the actual match.

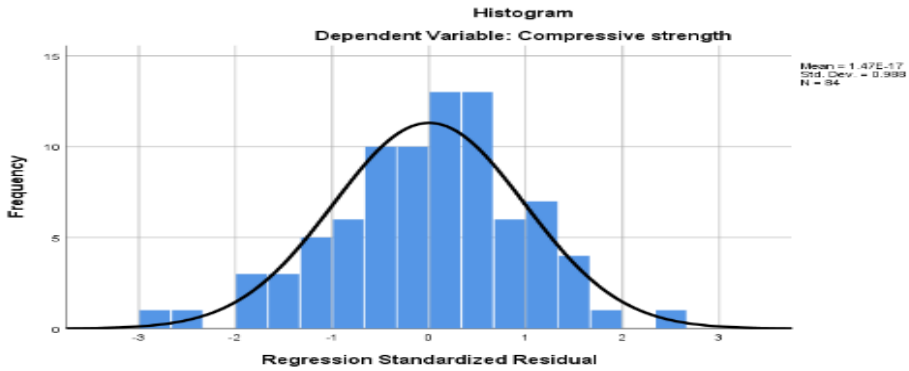

Fig. 25 Regression standardized residual of the dependent variable compressive strength

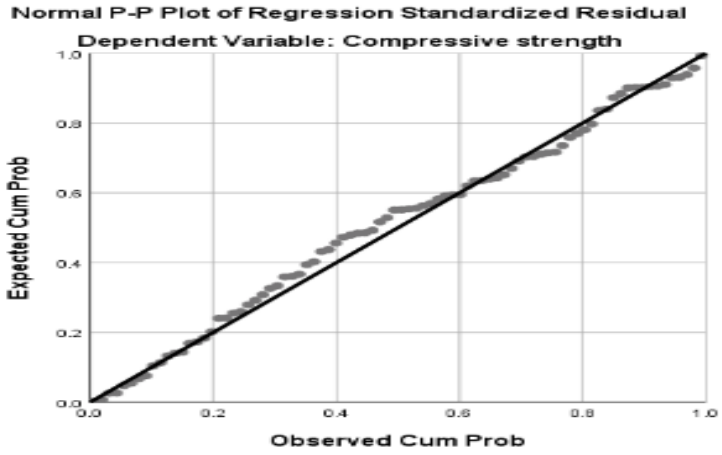

Fig. 26 The histogram of the most affecting the compressive strength

4. Conclusions

The consequences of this research provide contribution towards the understanding of the effects of bacteria on the performance of mortar mix and it can serve as a guidance for other researchers dealing with optimization of bio-based self-healing mortar mixtures. Several conclusions could be derived based on the results obtained in this research as follows:

- Most mixes containing bacteria and nutrients in all ages have a higher compressive strength than control mixes (without bacteria) of the same age.
- The best results in the increase of compressive strength reached 69.8% and 66.4% at 7 days and 120 days, respectively, in mix with 0.5% bacteria DSM 396 with 0.25% calcium lactate.
- As the bacteria concentration is increased, the effect of bacteria on compressive strength becomes apparent with the bacteria DSM 33.
- The increase in compressive strength was owing to calcium carbonate precipitation on the bacteria cell surfaces within the pores, which was confirmed by SEM.
- DSM 396 type generally surpasses the other two types and calcium lactate generally surpasses the other two types of nutrients.
- The addition of nutrients alone reduced the compressive strength compared to control mix. The presence of nutrients with bacteria is very important in increasing the compressive strength.
- The expected decrease in compressive strength as a result of loading did not occur significantly, and the decreasing in compressive strength values of control mix specimens mostly shows the bacteria are

working, precipitating calcite at the onset of crack formation, which assures that self-healing in that case has occurred.

- The increase in the flexural strength of the bacterial mortar shows a positive effect of bacteria on the flexural strength.
- SEM analysis proved the presence of calcite crystals could be clearly distinguished within the pores of bacterial mortar in the form of a layer of white precipitates all over the specimen.
- Invisible cracks seem to heal better than visible artificial cracks.
- The degree of hydration is increased with use of bacteria. For example, mix DSM 396- 2×10^9 the degree of hydration increased by 19.4 and 12% for 28 and 90 days compared to the control mix. This was confirmed by the TGA test
- The results of ANOVA test show all the independent variables affect the dependent variable (the compressive strength). The effects of all variables on compressive strength results are determined to be statistically significant since the p-values of parameters are less than 0.05.

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