Research article

# Microbially-Induced-Calcite-Precipitation (MICP): A biotechnological approach to enhance the durability of concrete using *Bacillus pasteurii* and *Bacillus sphaericus*

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## HIGHLIGHTS

- Microbial calcite-precipitation is used as a smart and eco-friendly approach to produce bio-based durable concrete.
- SEM, EDAX & DTA verified that the addition of *B. pasteurii* & *B. sphaericus* into mortar induced calcite bio-precipitation.
- Bacterial mortar had high restoration for load deflection & stiffer with less deformation than control, confirming its healing.
- Bacterial mortar exhibited improved physico-mechanical performance & durability with great potential for application.
- Microbial self-healing is an innovative bio-approach for repairing micro-cracks in concrete thus can reduce maintenance costs.

## ARTICLE INFO

## ABSTRACT

Developing bio-based self-healing concrete aims to minimize durability problems related to cracking. In this study, MICP was used as a smart and eco-friendly approach to produce bio-based durable materials. *Bacillus pasteurii* (BP) and *Bacillus sphaericus* (BS) were added into mortar mixtures with 0.25% and 0.5% cement weight. All treated samples exhibited a significant decline in water uptake, capillary permeability, and volume of permeable voids, as compared to control with no bacteria. All treated samples showed significant increase in compressive strength by 28–50%, after 28 days of curing. At the age of 120 days, the flexural strength of all treated samples was significantly increased by 19.29–65.94%. SEM imaging and EDAX confirmed that treated samples were denser with less voids due to MICP. DTA verified that the calcite amount and the crystallinity degree were improved in treated samples. Load deflection of bacterial Reinforced-Laminates had less deformation than control. Reloaded bacterial Reinforced-Laminates exhibited excellent restoration of physico-mechanical properties and performance, after 28, 90, and 120 days, confirming the healing process. Microbial self-healing is an innovative approach for continuous repair of micro-cracks in concrete, improving its durability, thus can reduce the maintenance costs.

## 1. Introduction

Concrete is the most widely used construction material, with over 6 million m³ produced worldwide every year, because of its high compressive strength, casting, and relatively low cost [1, 2]. It does, however, has limited tensile strength, and diminutive resistance to cracking. Cracks influence concrete durability, it arises easily due to external load, volumetric change induced by temperature variation or shrinkage [3, 4]. The produced cracks facilitate the entry of water, gases, salts, acids, and other agents into the concrete's matrix, thus, accelerate its degradation, corrosion, and shorten the service life of the structure. Remarkably, the maintenance of concrete structures significantly impacts the community budgets and environment [4, 5]. Also, traditional methods of repairing have some disadvantages, such as, operation restrictions during reconstruction, different thermal expansion coefficient of matrix base and added material, as well as environmental hazards [6].

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Developing bio-based self-healing concrete aims to reduce cracking-related durability issues. Therefore, researchers are trying to exploit self-healing approaches [2, 4, 5, 7, 8, 9, 10, 11]. Novel bio-materials, based on the enhancement of calcite-precipitation have been developed for self-healing of cracks [5, 7, 8, 12]. Microbial-induced-calcite-precipitation (MICP) is a bioprocess by which selected microorganisms can precipitate calcite through different pathways [5, 10, 13]. The addition of selected microorganisms into cementitious materials is considered cost-effective and eco-friendly method for micro-cracks repair. Microbial self-healing is a very promising technique, because it is a continuous process for healing of micro-cracks, leading to substantial improvement in the physico-mechanical properties of bio-Concrete [2, 4, 5, 12, 14]. Therefore, microbial self-healing can increase the service life of concrete structures without using costly and/or time consuming interventions [15, 16].

Bio-precipitation of calcite results from the metabolic activities of selected microorganisms in concrete which improves its overall behavior, and thus it is regarded as an autogenous self-healing process [2, 10]. Microbial self-healing of bioConcrete normally uses two metabolic pathways, either through urea hydrolysis via ureolytic bacteria and/or respiration by non-ureolytic one [5, 9, 11]. In this regard, ureolytic bacteria such as, *B. sphaericus* and *B. pasteurii* have gained a lot of interest in bio-cementation approaches [17, 18]. Both strains are common in the soil and aquatic habitat, they are Gram positive, aerobic, rod-shaped, non-pathogenic bacteria. They are urease positive, and are tolerant to high alkalinity [17, 18, 19]. Such bacteria, can biotransform urea into carbonate and ammonium [20]. The produced ammonia increases the pH, leading to calcite-precipitation inside the micro-cracks, thus sealing it [17]. Using sustainable eco-friendly biomaterials in building structures, can be alternative to traditional chemicals without likely causing any environmental or health hazards [5, 21]. Previous work in our lab succeeded to enhance the durability of bioConcrete through the addition of Egyptian *B. subtilis* and *B. megaterium* strains [2]. To complete such target, the present work aims to develop another sustainable bioConcrete using ureolytic bacteria. The new physico-mechanical properties were evaluated at different ages. The new bioConcrete was characterized, the healing activity, load deflection of bacterial Reinforced-Laminates, restoration of mechanical properties of the produced bioConcrete, and its durability were also evaluated.

## 2. Materials and methods

### 2.1. Materials

Glucose, beef extract, yeast extract, malt extract, peptone, agar, sodium bicarbonate (NaHCO$_3$), sodium carbonate (Na$_2$CO$_3$), and calcium lactate were obtained from Oxford chemicals (India). Hydrochloric acid (HCl), urea CO(NH$_2$)$_2$, potassium phosphate (K$_2$HPO$_4$), and sodium hydroxide (NaOH), sodium phosphate (Na$_2$HPO$_4$), sodium chloride (NaCl), and manganese sulfate (MnSO$_4$·2H$_2$O) were purchased from SAS-Chemicals CO (Mumbai, India).

### 2.2. Cultivation of bacteria

*Bacillus pasteurii* (DSM 33) and *Bacillus sphaericus* (DSM 396), were purchased from MIRCEN, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Bacterial colonies were sub-cultured on Luria-Bertani (LB) medium (10 g/L yeast extract, 5 g/L sodium chloride, 10 g/L peptone), with 20 g/L filter-sterilized urea. Bacterial strains were cultivated in broth medium, supplemented with 0.01 g/L of MnSO$_4$·2H$_2$O to enhance sporulation [2]. Cultures were incubated in shaking incubator (New Brunswick, CA) at 30 °C and 150 rpm, for 7 days. Bacterial growth was checked regularly and quantified using pour-plate counting and microscopic analysis.

### 2.3. Cement, sand, fine aggregates, water, and reinforcement-laminates

Medium well-graded sand of 2.2 fineness modulus was used for mortar mixture, that complies the Egyptian Standard Specifications (ESS) requirements [22]. Portland Cement (CEM-I) grade 42.5 N with ESS and fresh tap water with water/cement ratio 0.45 were used. Reinforced-Laminates with expanded wire mesh were locally produced, the strips were 0.7 kg/m$^2$, short and long way pitches were 1.0 cm and 2.0 cm, respectively. Strand thickness was 0.55 mm and its width was 0.6 mm.

### 2.4. Bacterial preparation and count

Bacterial cell pellets from 7 days-old bacterial culture were resuspended in sterile saline solution (0.9% w/v) before their addition. Bacterial suspensions (2× 10$^9$ CFU/mL) were used with two concentrations 0.25% and 0.50% of cement weight. Cement mortar mixtures was prepared, solidified, where treated samples and control were collected during the time course of 7–90 days. Small cubes from solidified mortar were homogenized, and 10 g were suspended in sterile saline solution (0.9 %v/v). Bacterial count was done using 10-folds serially diluted samples, where 1 mL of dilution was distributed on LB solid medium. Finally, the inoculated plates were incubated for 5 days at 30 °C, and the CFU/g was counted for both strains [2].

### 2.5. Mortar mixes and setting time

Dry mortar mixtures were weighed, mixed well using mechanical mixer, water was added and mixed well for 10 min according to Ferrocement Model Code [23]. Sand/Cement ratio (1:3 w/w) and Water/-Cement ratio (0.45 v/w) were prepared. Treated mortar samples were prepared with the addition of *B. sphaericus* or *B. pasteurii* (0.25 and 0.50%) of cement weight. Calcium lactate (0.25% and 0.125%) of cement weight was added (Table 1A). Test samples were remolded after 24 h, kept in wet cloth, and sprayed daily with tap water to keep the moisture. Initial and final setting time tests for the mortar paste were determined using Vicat-apparatus [22] and standard Water/Cement ratio was tested only prior to setting time examination [24].

## 3. Physical properties of mortar

### 3.1. Rate of water absorption, capillary permeability coefficient, and volume of permeable voids

Samples were dried in a drying oven for 3 days at 50 °C, then cooled at 3, 7, 28, 90, and 120 days after curing. Samples were coated with epoxy-resin to allow water to flow in only one direction. Using attached plastic sheets and elastic bands, mortar samples were tightly packed. Initial mass was determined after 2 h from submerging in water. Samples were removed and weighed after blotting-off excess water [25]. The gain in mass ($\Delta$m, kg/s) at time t (s), density of water (d), and exposed area of the sample (a, m$^2$) were used to calculate water absorption rate (I, m/s$^{0.5}$) based on Eq. (1).

$$I = \Delta m (a * d) \tag{1}$$

For capillary water absorption tests, samples were taken from the water curing pan at the age of 3, 7, 28, 90, and 120 days, then kept in the drying oven for 24 h at 50 °C. Samples were cooled down and immersed in water at 0.5 cm depth. Other surfaces were covered with impermeable adhesive tape to prevent water penetration. After 24 h, samples were taken out, dried with cloth, weighed, and finally the capillary permeability coefficient was calculated using Eq. (2) [9].

$$K = Q^2 / (A^2 * t) \tag{2}$$

**Table 1.** Experimental Mortar Mixture Proportions (A). The relationship between flexural strength and compressive strength of control and bacterial mortar samples (B).

**A. Mixture**

| Mixture | Samples | Sand/Cement | Water/Cement | Bacteria/Cement | Calcium Lactate/Cement |
|---|---|---|---|---|---|
| 1. | Control | 3:1 | 0.45 | 0.0 | 0.0 |
| 2. | B. sphaericus (BS50) | | | 0.5 % | 0.25 % |
| 3. | B. sphaericus (BS25) | | | 0.25 % | 0.125 % |
| 4. | B. pasteurii (BP50) | | | 0.5 % | 0.25 % |
| 5. | B. pasteurii (BP25) | | | 0.25 % | 0.125 % |

**B. Mixture**

Compressive and Flexural Strength (N/mm²)

Age (days)

| | 28 days | | | | 90 days | | | | 120 days | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Compressive Strength | Experimental Flexural | Calculated Flexural | Exp./Cal. Flexural | Compressive Strength | Experimental Flexural | Calculated Flexural | Exp./Cal. Flexural | Compressive Strength | Experimental Flexural | Calculated Flexural | Exp./Cal. Flexural |
| Control | 34.7 | 6.13 | 5.71 | 107% | 43.0 | 6.56 | 6.36 | 107% | 50.0 | 7.31 | 6.86 | 107% |
| BS50 | 49.1 | 7.88 | 5.74 | 137% | 54.1 | 8.75 | 6.51 | 134% | 53.4 | 11.81 | 7.26 | 163% |
| BS25 | 44.4 | 7.00 | 5.74 | 122% | 54.7 | 7.63 | 6.36 | 120% | 52.1 | 12.13 | 6.99 | 174% |
| BP50 | 51.1 | 7.38 | 5.82 | 127% | 51.1 | 7.50 | 6.43 | 117% | 56.1 | 8.72 | 6.99 | 125% |
| BP25 | 52.1 | 7.25 | 6.13 | 118% | 53.1 | 7.88 | 6.43 | 123% | 53.4 | 9.00 | 7.06 | 127% |

where, Q: Water absorbed (cm$^3$), K: Capillary permeability coefficient (cm$^2$/s), A: Sample's area in contact with water (cm$^2$), and t: Time elapsed (s).

Permeable void's volume was measured in accordance with the ASTM C642-06 [26]. Samples were dried at 110 °C for 24 h, then cooled after 7, 28, 90, and 120 days of curing. Sample's initial mass was taken, final mass was taken after 24 h from first contact with water, samples were removed and excess water was blotted-off using paper towel, weighed, and permeable void's volume was calculated using Eq. (3).

$$\textbf{Volume of permeable voids} = (\textbf{B} - \textbf{A}) \, / \, (\textbf{B} - (\textbf{B} - \textbf{V}) * 100 \quad (3)$$

where: A: dry weight, B: weight after immersion, and V: volume of sample.

### 3.2. Mechanical strength tests

The compression test was performed on prepared mortar samples based on Gandhimathi et al. [27] method. Test samples with 7*7*7 cm dimensions were cast then cured using wet cloth. After the specified time course (28, 90, and 120 days), all samples were tested for its maximum load using the compression machine, where cubes were tested until failure on 2000 kN hydraulic machine [28]. While, testing flexural strength were performed on prisms 40 × 40 × 160 mm in size using 10-tons capacity flexure machine. Samples were exposed to three-points loading and the flexural strength was determined using Eq. (4).

$$\textbf{Flexural strength} = 3PL/2d_1 d_2{}^2 \quad (4)$$

where, P is the maximum applied load to the sample (N), $d_1$ is the width of the sample (mm); $d_2$ is the height of sample (mm). L is the Span of the prism. Reinforced-Laminates with the dimensions 15 × 3 × 35 cm were cast using mortar mixture. Each lamina has expanded wire mesh 35*15 cm in mid height. Reinforced-Laminates were loaded until failure at mid-point and the deflection was determined using dial-gage in the Material Lab, Civil Engineering Dept, Faculty of Engineering, Menoufia University, Egypt [2]. The relationship between compressive and flexural strength was determined using Eq. (5) [29]:

$$\textbf{FSM} = 0.97\sqrt{\textbf{f\'cm}} \quad (5)$$

where, FSM: mortar's flexural strength, N/mm$^2$, and f'cm: mortar's compressive strength, N/mm$^2$.

### 3.3. Characterization using SEM, EDAX, and DTA

Mortar samples were analyzed using SEM [30] equipped with Energy Dispersive X-ray Spectrometer (EDAX) at the National-Research-Center (NRC), Cairo, Egypt. Magnifications of 1500X, 6000X, and 7000X were selected for imaging. Characterization of samples was done by Differential Thermal DT-50 Analyzer (Shimadzu Co., Kyoto, Japan) based on Ramachandran et al. [31] method. 20 mg samples were heated to 1000 °C, maintaining 20 °C/ min using α-Al$_2$O$_3$ as reference material and two thermocouples platinum-platinum-radium (Pt/Pt-13% Rh) [31].

## 4. Durability of treated cement mortar

### 4.1. Restoration of compressive strength

The control with no bacteria was loaded with ultimate load until complete failure, then half of this load was used as half failure load. Samples were loaded after 7, 28, 90 days from casting date with half of failure load, and cured by wet cloth during the tested period. Samples were reloaded and tested to determine the restoration after 28, 90, and 120 days [2].

### 4.2. Testing flexural strength of Ferrocement-Laminates

Reinforced-Laminates of 35 × 15 × 3 cm dimensions were cast; each lamina was reinforced with 15 × 35 cm expanded wire mesh in mid-height. Short and long way pitch were 1.0 cm and 2.0 cm, respectively. Strand thickness was 0.55 mm and the width was 0.6 mm. Reinforced-Laminates were loaded and tested until failure after 28, 90, 120 days of curing. Deflection of mid-point for each Reinforced-Lamina and its maximum load were measured [2].

Reinforced-Laminates were loaded with half failure load after 28 and 90 days from casting to assess flexural strength restoration. During the testing time, all samples were maintained wet, after that, samples were reloaded and examined for flexural strength restoration after 90 and 120 days, respectively.

### 4.3. Statistical analysis

Results were expressed as values means ± their standard deviation. One-way ANOVA using SPSS software (Version 17), and Tukey tests at *P-value ($\leq 0.05$)* were used to find significant differences.

## 5. Results and discussion

### 5.1. Bacterial count and setting times

Self-healing of cementitious materials is induced by some bacterial spp. through direct bioprecipitation of $CaCO_3$ and/or decomposition of calcium-containing compounds [2, 9, 10, 30, 32]. *Bacillus* spp. are alkaliphilic and their spores can be dormant for many years in the environment without nutrients. In this respect, many *Bacilli* spp. were able to induce $CaCO_3$-biomineralization, when applied to decomposed stones [2, 9]. Results (Figure 1) revealed no significant difference in *B. pasteurii* or *B. sphaericus* count during the whole time course. However, the count was reduced with time, probably due to nutrients depletion [2]. Both species were adapted with same rate and followed similar growth-pattern.

### 5.2. Setting time, water absorption rate, capillary permeability, and permeable voids

Initial setting time (Figure 2A) was reduced for all treated samples as compared with control (no bacteria). Final setting time was increased for all treated samples, probably because of bacterial or nutrients addition [2]. The initial and final setting time for all treated samples were ˃60 min and ˂10 h, respectively. Results suggest that addition of bacteria and calcium lactate into mortar speeded up its initial setting time with increasing its final setting time than the control. Results also suggest that the depletion of nutrients with time can delay the initial setting time of bioConcrete [2, 33].

The effect of bacterial addition was investigated during the age of 3–120 days. Data (Figure 2B) showed that water absorption rate was high at the beginning (2 h) which was gradually decreased. With bacterial addition, the water absorption was significantly decreased than the control (Figure 2B). It is suggested that MICP fills up the mortar pores with calcite, and hence, the water absorption was decreased. The over-time reduction in water absorption was also reported [2, 10] as a result of bacterial addition into mortar.

Results (Figure 2C) revealed that, at all tested ages, the permeable void's volume for all treated samples was reduced as compared with control. As porosity decreases, the permeability decreases, and the compressive strength increases. The permeable void's volume in mortar can be used to specify the mortar's durability. In compliance with previous results (Figures 2A, B, C), the capillary-permeability coefficient was decreased for all treated samples as compared with control (Figure 2D). After 120 days, the capillary of both *B. pasteurii* (BP 0.25% and BP 0.5%) and *B. sphaericus* (BS 0.25% and BS 0.5%) treated samples were decreased to 10.6%, 17.9%, 28.1%, and 10.6%, respectively, where BP 0.25% and BS 0.5% had the least value.

Healing in wet-dry environments is suggested to stimulate the bacterial growth and $CaCO_3$ bioprecipitation [34, 35]. Wet-dry environments are beneficial, where in such environments, the carbonation gets faster [36]. In the present work, all treated samples were kept moist for 120 days based on healing period reported by Wiktor and Jonkers [10] and Ahmed et al. [2]. Similarly, Xu and Wang [17] added *B. pasteurii* (ATCC 11859) into concrete and achieved self-healing after 28 days of curing, where all samples had significant decrease in water absorption. Results confirm that the metabolic activities of added bacteria probably had led to the bioprecipitation of $CaCO_3$, which not only seals the micro-cracks, but also closes the pores within the mortar matrix, thus reduce the transport of fluids, decrease capillary-permeability, water absorption rate, and permeable void's volume [2, 17, 37].
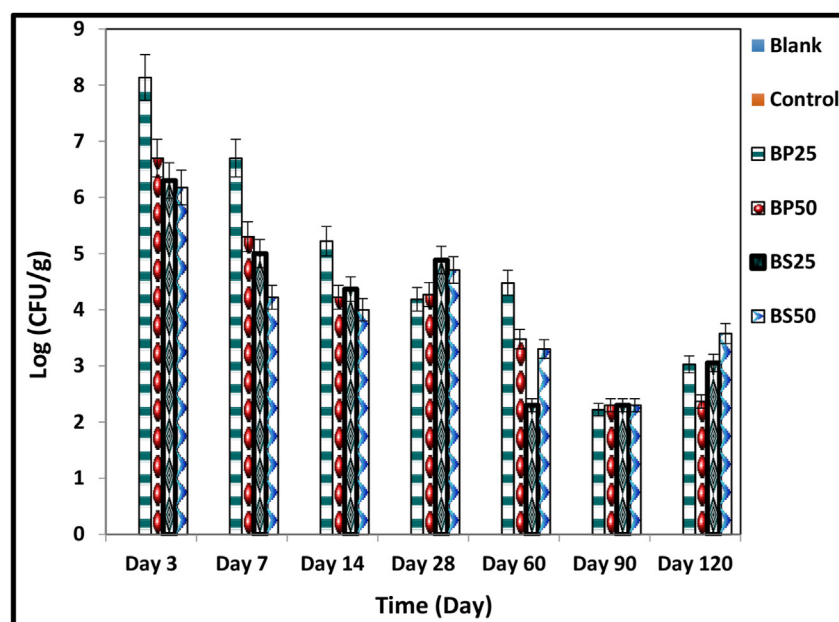


**Figure 1.** Log of bacterial count (CFU/g) of each bacterium (BP = *Bacillus pasteurii*, BS = *Bacillus sphaericus*) at 0.25 and 0.5% of cement weight, after 3, 7, 14, 28, 60, 90, and 120 days (Blank = LB Medium without bacteria, Control = Untreated Sample).
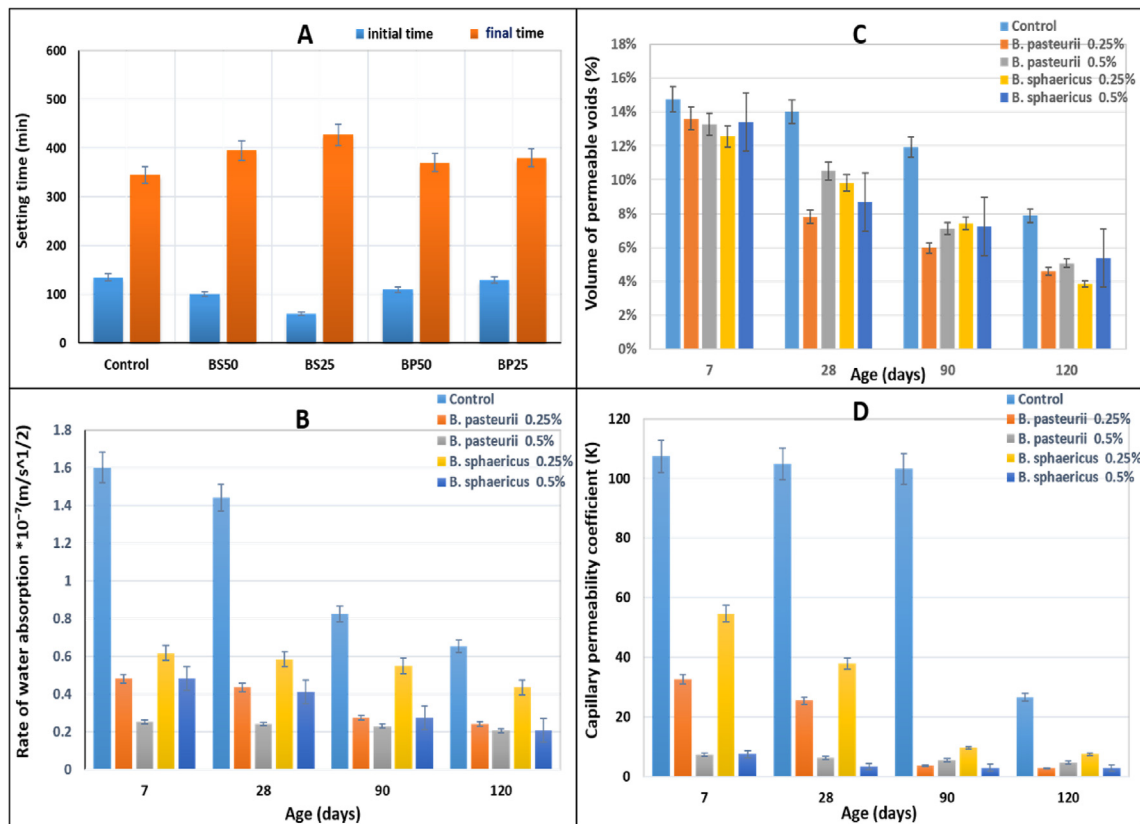
**Figure 2.** Initial and final setting times (**A**); Rate of water absorption (**B**); Volume of permeable voids (%) (**C**); Capillary permeability (**D**) for bacterial and control mortar samples (BS = *B. sphaericus,* BP = *B. pasteurii)* using 0.25% and 0.50% concentration of cement weight.

## 5.3. Mechanical strengths

A significant improvement in compressive strength for all treated samples was detected (Figure 3A). The highest compressive strength was for BP 0.25% and BS 0.25% as compared to control at same age. The compressive strength of BS 0.5%, BS 0.25%, BP 0.5%, and BP 0.25% were increased by 41%, 28%, 47%, and 50%, respectively, after 28 days. The compressive strength of BS 0.5%, BS 0.25%, BP 0.5% and BP 0.25% were increased by 26%, 27%, 20%, and 23%, respectively, after 90 days. The mechanical strength of treated samples was developed earlier than control, probably due to bacterial bio-precipitation filling the matrix pores (Figure 3A). This improvement reached its highest values after 28 days, which is the key point for design criteria, and thus proved the bioactivity of added bacteria. Similarly, Xu and Wang [17] reported remarkable increase in water tightness and compressive strength by 50% and 30%, respectively, after 28 days of curing, when *B. pasteurii* (ATCC 11859) was included as microbial-based self-healing system. Likewise, *B. subtilis* (AP91) addition enhanced the compressive strength by 31%, after 28 days [38]. Treated samples had an overtime improvement in its compressive strength until 120 days, which verify the continuous bioactivity of bacteria (Figure 3A). Also, Chahal et al. [37] reported that *B. pasteurii* induced calcite-precipitation in treated samples, which reduced its porosity; and improved its compressive strength by 22%. Authors described nano-deposition of $CaCO_3$ through MICP, which probably blocks the micro-cracks and tiny pores in cementitious materials, thus improves its mechanical properties [37, 38, 39, 40].

Similarly, all treated samples (Figure 3B) exhibited higher flexural strength at all tested ages. The highest flexural strength was that of BS 0.5% followed by BP 0.25%, after 28 and 90 days (Figure 3B). However, after 120 days, the flexural strength of BS 0.5%, BS 0.25%, BP 0.5%, and BP 0.25% were increased significantly by 61.59%, 65.94%, 19.29%, and 23.12%, respectively. MICP by *B. sphaericus* and *B. pasteurii,* improved

the physico-mechanical properties of treated mortar [14]. Previous work in our lab. by Ahmed et al. [2] reported enhanced physico-mechanical properties of treated samples, due to calcite-deposition by Egyptian *B. subtilis* and *B. megaterium* strains, where the compressive strength was improved by 21.4%, after 28 days.

## 5.4. SEM, EDAX, and DTA analyses

SEM micrographs revealed that treated samples were denser with less voids as compared to sample with no bacteria (Figure 4A, B, C, D, E, A′-C′). Bacterial addition can improve the micro-structure of bioConcrete by calcite-precipitation; which has been verified by SEM and EDAX analyses [2]. Likewise, Siddique et al. [16] reported that it was clearly seen by SEM that the concrete voids was filled in the treated samples. Calcite crystals were needle-like and rhombohedral-shaped (Figure 4D, E). MICP fills up pores within the mortar matrix, increasing its impermeability and enhancing micro-cracks sealing [2, 39].

EDAX (Figure 4F) specified that the precipitated compound was $Ca^{++}$, oxygen, and carbon of $CaCO_3$. The bacteria represent the nucleating sites for calcite-precipitation which is the main crystalline product involved in the healing process [40, 41]. Calcite in the form of $CaCO_3$ was verified by EDAX analysis, which increases the bioConcrete durability [2, 42]. Similarly, calcite crystals growth was observed by Wang et al. [43], who studied self-healing of *B. sphaericus* when added to cementitious paste. Overall, SEM images and EDAX confirmed $CaCO_3$ deposition within the micro-cracks of bioConcrete (Figure 4). SEM analysis agree with the reduction in water absorption and enhancement of physico-mechanical properties of produced bioConcrete [2, 10, 15, 44].

DTA Thermograms showed some endothermic peaks (Figure 5). The endothermic peaks at 61–68 °C were related to moisture; the 146–155 °C endothermic peaks were due to micro crystalline (C–S–H), 460–470 °C endothermic peaks were due to $Ca(OH)_2$ decomposition, and the
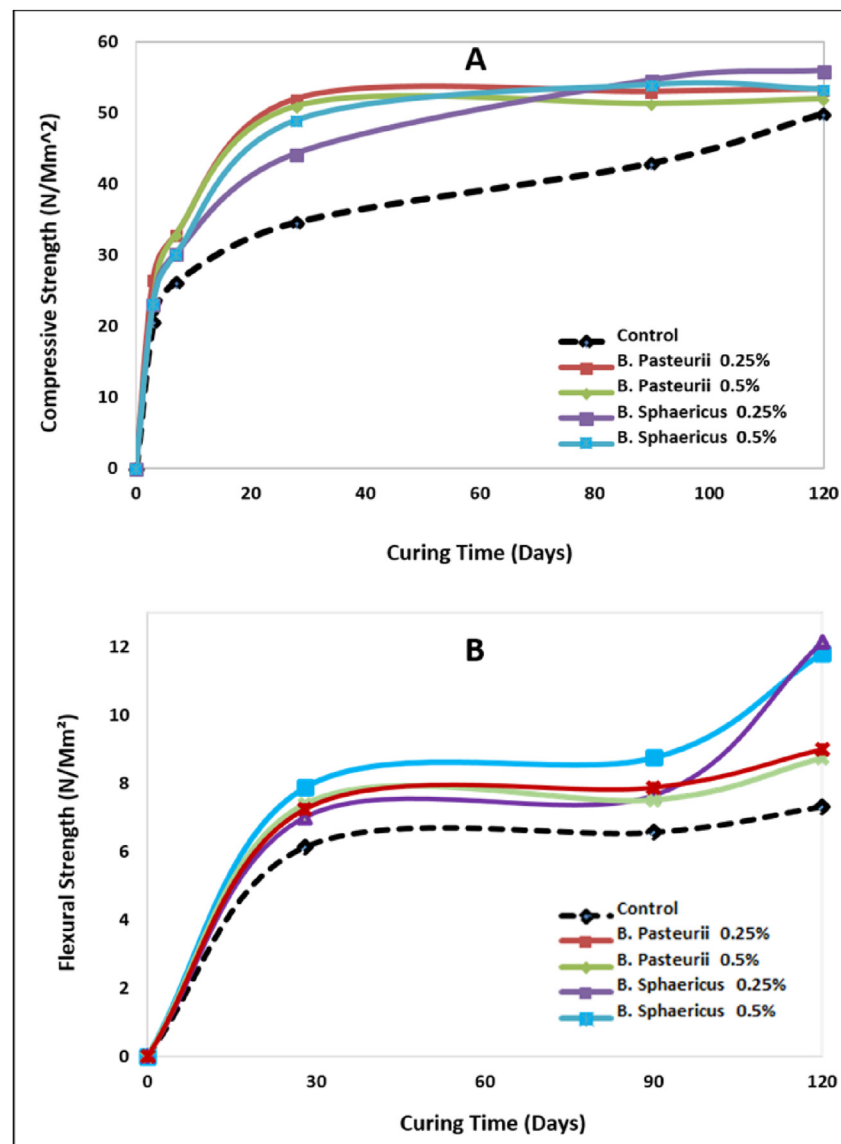
**Figure 3.** Compressive strength (**A**), flexural strength (**B**), for bacterial and control mortar samples using *B. sphaericus,* and *B. pasteurii* at 0.25% and 0.50% concentration of cement weight.

576–579 °C endothermic peaks were due to α, β quartz transformation [2, 31]. The endothermic peaks at 679–79 1°C were attributed to the breakdown of crystalline and amorphous parts of $CaCO_3$. The decomposition of $CaCO_3$ was shifted to higher temperature in the treated samples (686.87 °C, 686.88 °C, 690.86 °C, and 684.26 °C) than control (679.07 °C). DTA (Figure 5) indicates that the degree of $CaCO_3$ and its crystallinity were enhanced in treated samples than control. Self-healing by selected integrated bacteria could result in better healing as compared to autogenous healing of non-amended pastes [2, 9, 45]. Overall, the improvement in crystallinity degree with its stability enhanced the physico-mechanical properties of the produced bioConcrete.

### 5.5. Durability and restoration of mechanical strength

The main objective for developing bacterial self-healing is to achieve good healing performance when cracks occur [2]. For this objective, and to confirm the bacterial healing activity, treated samples were loaded with half failure load after 7, 28, and 90 days, then reloaded after 28, 90, and 120 days until failure, simultaneously. Results revealed significant increase in compressive strength for treated samples when compared with their

originals, at all tested ages (Figure 6A, B). After 28 days, the restoration of BS 0.5%, BS 0.25%, BP 0.5%, and BP 0.25% were 59.2%, 76.7%, 78.4%, and 66.3%, respectively, as compared with original ones. After 90 days, the restored compressive strengths in BS 0.5%, BS 0.25%, BP 0.5%, and BP 0.25% were significantly increased to 95.1%, 90.2%, 107.8, and 95.6%, respectively. While, after 120 days, the restored compressive strengths of BS 0.5%, BS 0.25%, BP 0.5%, and BP 0.25% were further increased to 103.1%, 95.2%, 103.8%, and 95.0%, respectively. The restoration for all treated samples, confirms the continuous bacterial self-healing (Figure 6). The compressive strength improvement in treated samples along with its decrease for the control assure the occurrence of self-healing. Both bacterial strains were capable to restore the mechanical properties of mortar to their original state confirming self-healing activity and improved durability [2]. After 120 days, BS and BP (0.5%) have the highest restoration than its original ones before cracking. Our results agree with those reported for mechanical strength restoration after the addition of *B. megaterium* or *B. subtilis* into mortar matrix [2]. Likewise, reloading have induced new micro-cracks in columns as reported by Al-Tabbaa et al. [46] without reopening formerly healed ones, probably due to full recovery of mechanical strength after self-healing activity.
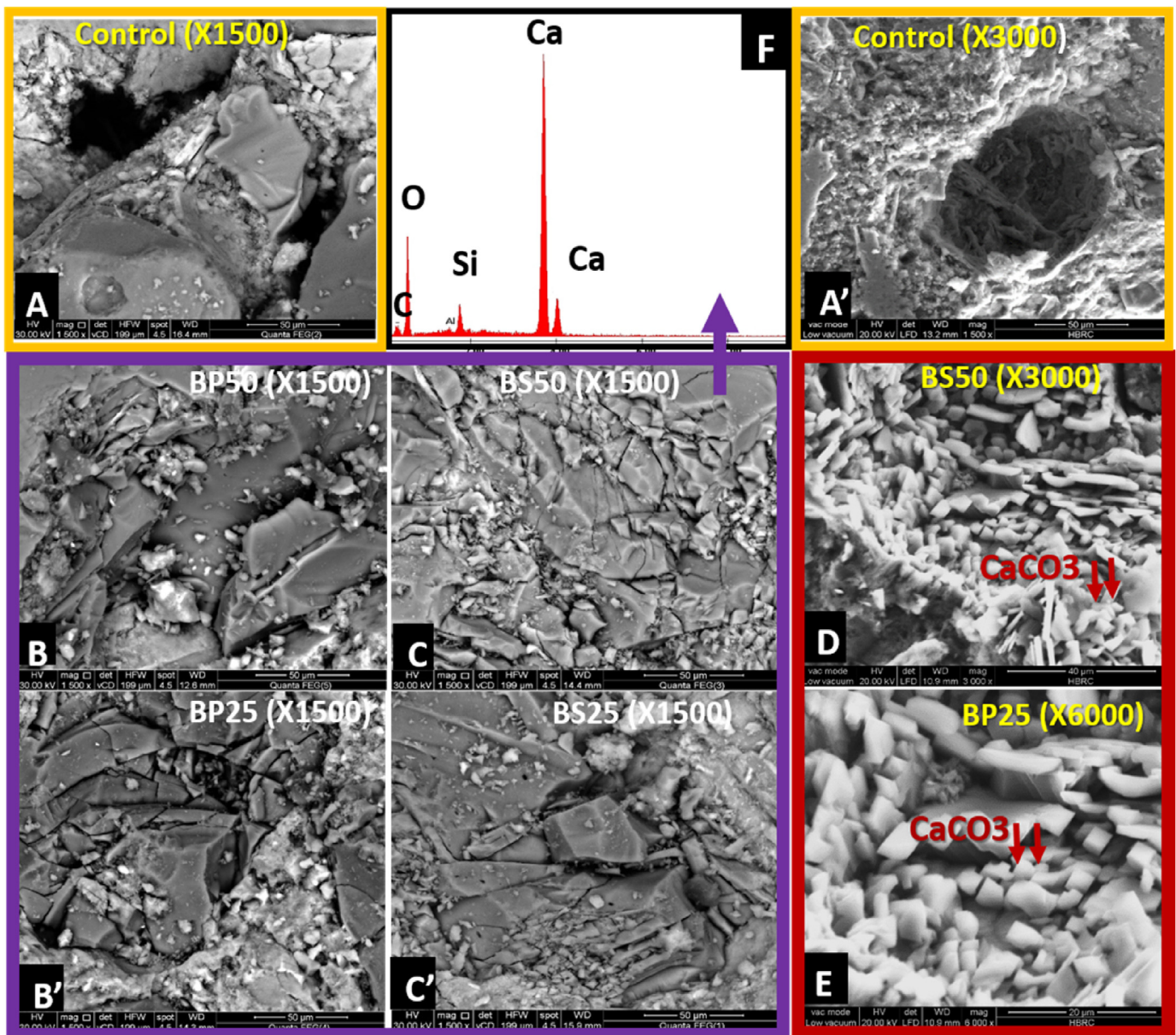
**Figure 4.** SEM photographs of control (**A, A′**) and bacterial mortar samples (**B, B′, C, C′, D, E**) at 1500X, 3000X and 6000X, BP25 and BP50 = *B. pasteurii*, BS25 and BS50 = *B. sphaericus* at 0.25% and 0.50% of cement weight, EDAX photographs (**F**) of bacterial mortar sample (*B. sphaericus*) showing elements of CaCO₃ after 28 days of curing.

## 5.6. Durability and restoration of Reinforced-Laminates under load deflection

After 28, 90, 120 days, the deflection of bacterial samples was less than control (Figure 7). Under the same load, all treated samples exhibited less deformation as compared with control. The bioactivity of bacteria induced stiffer behavior, under the same load. Damage development in cementitious materials result from stiffness decay, which is a function of crack opening [47]. Results (Figure 7A, B, C) suggested that the bioactivities of the added bacteria (*B. sphaericus* and *B. pasteurii*) have induced the same self-healing behavior in treated samples. Loaded samples exhibited similar behavior until half failure load (Figures 7A, B, C), and higher resistance to complete failure than control. Likewise, Ahmed et al. [2] reported the same behavior as a result of *B. megaterium* and *B. subtilis* inclusion into mortar, confirming durability enhancement. Results in Figure (7D) agree with the flexural strength enhancement of bacterial samples over control. After 28 days, flexural strength of BS 0.5%, BS 0.25%, BP 0.5%, and BP 0.25% were improved significantly by

58.33%, 50.00%, 41.67%, and 66.67%, respectively. Figure (7D) showed significant improvement of flexural strength for all treated samples than control. Generally, bacterial laminates exhibited improved flexural strength and more ductility (Figure 7A, B, C). Similarly, treated samples with 0.5% *B. subtilis* or *B. megaterium* showed ductile deflection with flexural enhancement than control [2]. Results (Table 1B) indicated that the values of experimental flexural strength of treated samples exceeded its calculated values. The experimental mechanical strength for treated samples were improved when compared to the calculated values, which gives a strong indicator for MICP enhancement of physico-mechanical properties and durability.

## 5.7. Restoration of Ferrocement-Laminates

The flexural strength (Figure 7D, E, F) was improved for all treated mortar samples as compared with original samples. All treated samples restored more flexural strength than the control. All treated samples at 90 and 120 days have higher flexural strength than their original before
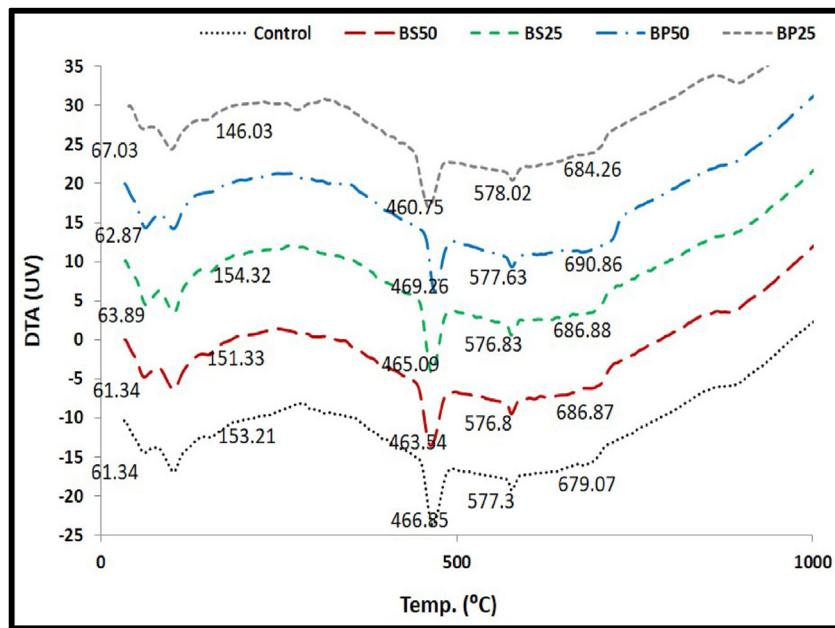
**Figure 5.** DTA Thermograms for control and bacterial mortar samples after 28 days of curing (C = control, BP25 and BP50 = *B. pasteurii*, BS25 and BS50 = *B. sphaericus* at 0.25% and 0.50% of cement weight.
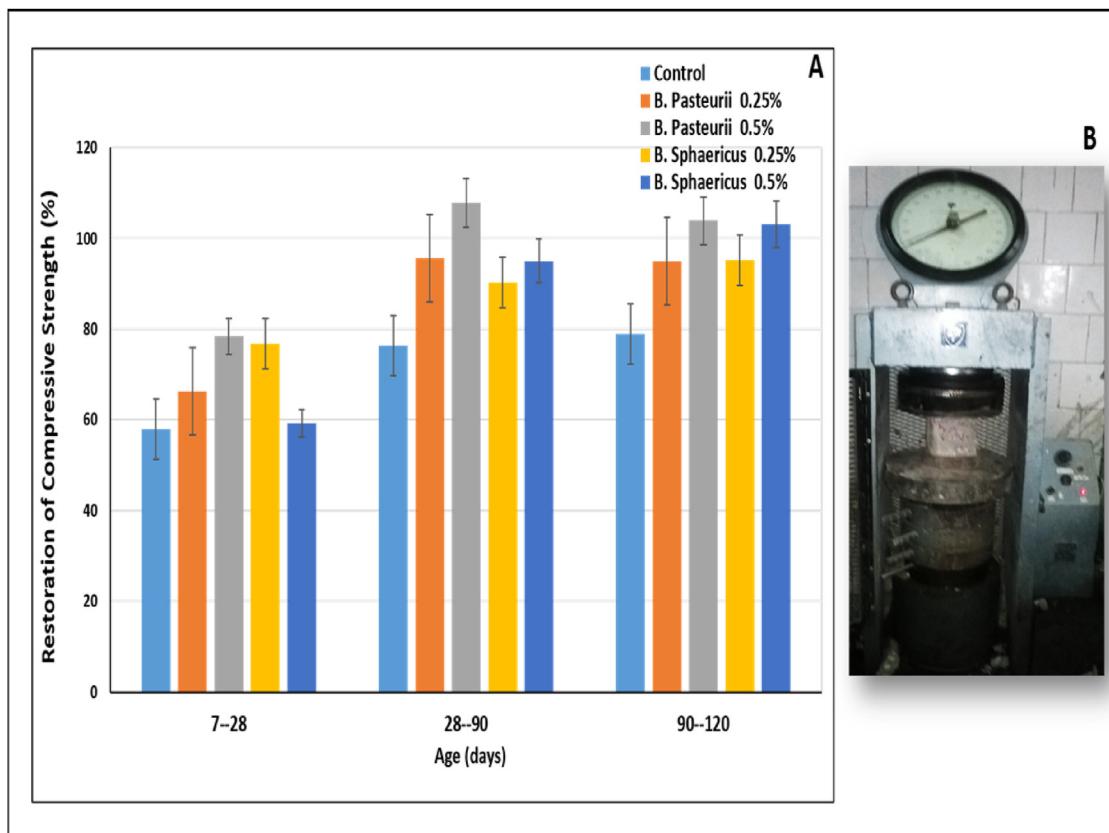


**Figure 6.** Restoration of compressive strength **(A)** for bacterial mortar samples loaded with half load failure, using *B. pasteurii* and *B. sphaericus* at 0.25% and 0.50% of cement weight, after 28, 90, and 120 days of curing, compressive strength measurement **(B).**

cracking. After 90 days, the restored flexural strengths of BS 0.5%, BS 0.25%, BP 0.5%, and BP 0.25% were 87.0%, 113.0%, 130.4%, and 103.8%, respectively, when compared with unloaded samples. After 120 days, BS 0.5%, and BS 0.25% had the highest restored flexural strength that were increased by 34.8% and 33.3%, respectively. The improvement

in flexural strength for treated mortar along with its decrease for control assure the occurrence of self-healing [2, 48, 49, 50]. Therefore, it could be concluded that *B. sphaericus* and *B. pasteurii* (BP and BS) were able to restore the mortar's mechanical performance to its original state, which is probably due their calcite-precipitation that was verified by SEM,
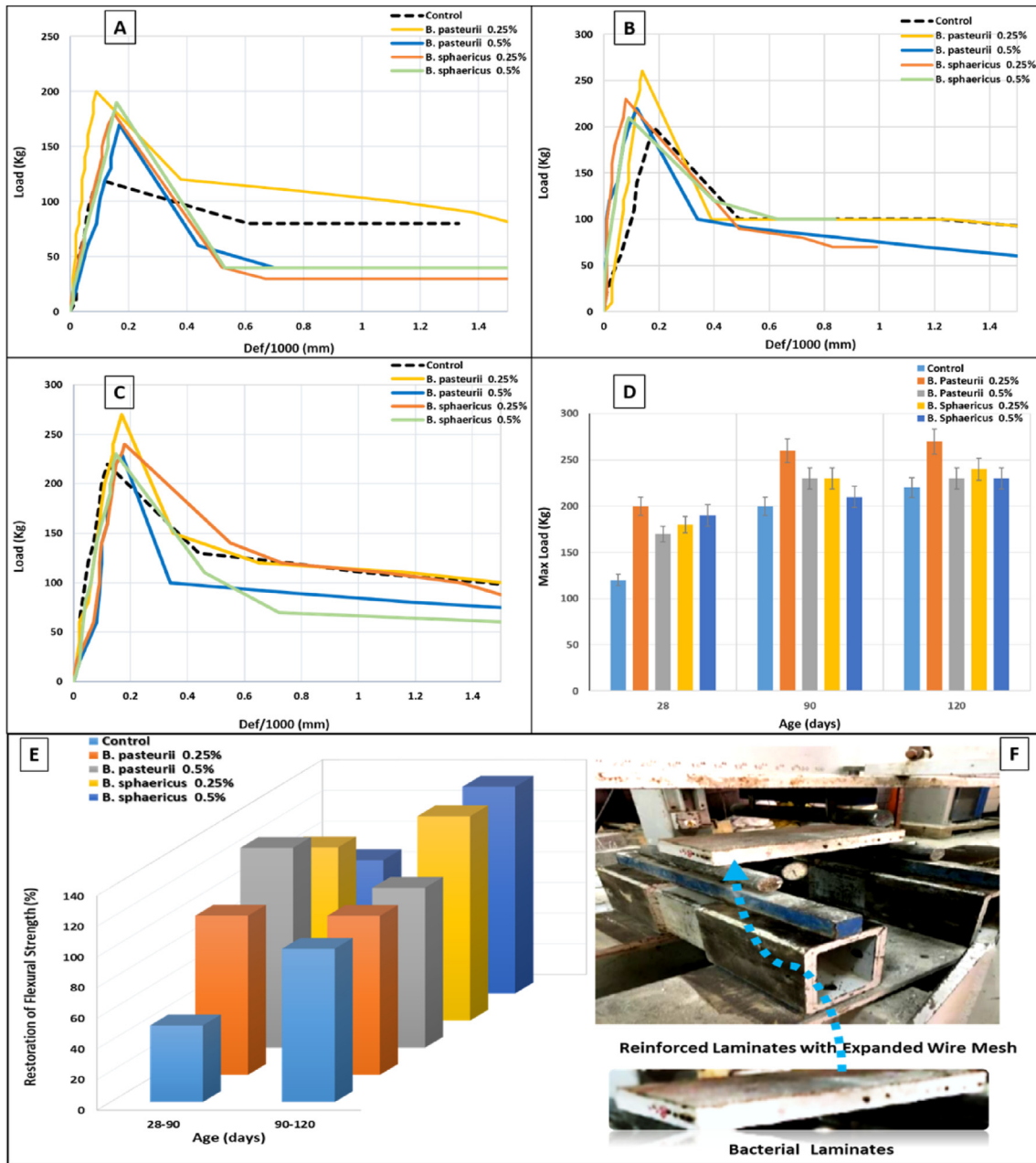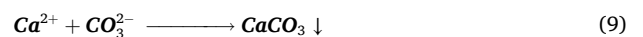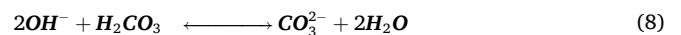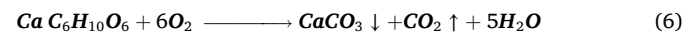
**Figure 7.** Load-Deflection of Reinforced-Laminates for bacterial and control mortar samples (with no bacteria) after 28 (**A**), 90 (**B**), and 120 days (**C**) of curing, Max. load (Kg) of Reinforced-Laminates (**D**) using *B. pasteurii* and *B. sphaericus* at 0.25% and 0.50% of cement weight. Restoration of flexural strength (**E**) of bacterial Reinforced-Laminates loaded with half load failure. Reinforced-laminates with expanded wire mesh (**F**).

EDAX, and DTA analyses. Thus, enhanced the overall durability of treated Ferrocement-Laminates.

Reloaded bacterial samples had high restoration performance, which is very promising for healing, these results agree with the enhancement of flexural strength for treated samples previously discussed. Results (Figure 7) suggest that *B. pasteurii* and *B. sphaericus* can heal internal micro-cracks caused by reloading, thus improve the mechanical performance for treated samples [2, 14, 48]. Overall results confirm the continuous self-healing of micro-cracks caused by reloading of half failure load, which is probably induced by *B. pasteurii* or *B. sphaericus* [2, 14, 17, 49, 50, 51]. Nano-sized calcite-precipitation can seal the micro-cracks in bioConcrete matrix, and thus enhance its overall durability and performance [2, 15, 30, 32, 48]. Results in Figure (8A, B, C) suggested that the incorporated bacteria can heal the internal micro-cracks through the bio-precipitation of CaCO₃. Generally*, both species in this study,* are involved in two main reactions, these are: 1. Bioconversion of calcium

lactate ($CaC_6H_{10}O_6$) and/or 2. Urea hydrolysis to induce $CaCO_3$ deposition [51, 52].

$CaC_6H_{10}O_6$ is converted into $CaCO_3$ as presented in Eqs. (6), (7), (8), and (9):

$$Ca\,C_6H_{10}O_6 + 6O_2 \longrightarrow CaCO_3 \downarrow + CO_2 \uparrow + 5H_2O \tag{6}$$

$$CO_2 + H_2O \longrightarrow H_2CO_3 \tag{7}$$

$$2OH^- + H_2CO_3 \longleftrightarrow CO_3^{2-} + 2H_2O \tag{8}$$

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3 \downarrow \tag{9}$$

Extra $CO_2$ is released during lactate decomposition, and higher pH shifts the equilibrium towards $CO_3^{2+}$ formation and $CaCO_3$ precipitates. This active precipitation results from exchange of ions through the
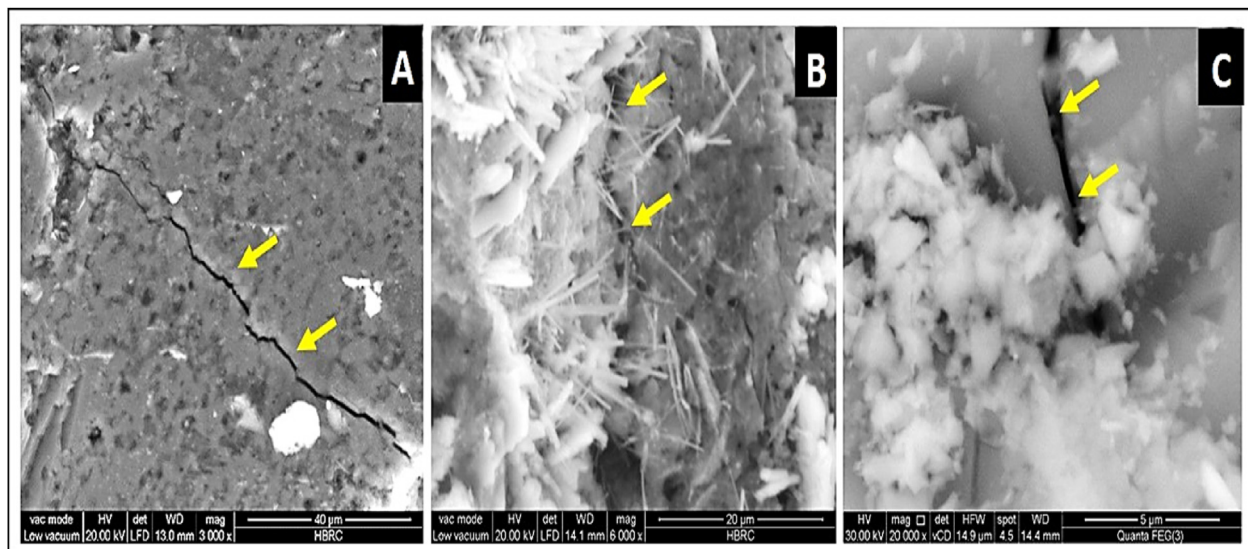
**Figure 8.** SEM photographs of control (**A**) showing micro-cracks after half-failure loading and bacterial mortar samples of *B. pasteurii* 0.5% (**B**) and *B. sphaericus* 0.25% of cement weight (**C**) during the healing process showing the initiation of CaCO$_3$ crystals precipitation. Arrows refer to the micro-cracks.

bacterial membrane and activation of Ca$^{2+}$ and/or Mg$^{2+}$ ion pumps, combined with CO$_3^{2+}$ production [52, 53]. The negatively charged bacterial cell surface attracts Ca$^{2+}$ for CaCO$_3$ precipitation, thus enhance self-healing as shown in Eqs. (10) and (11) [53, 54, 55].

$$Ca^{2+} + Cell \rightarrow Cell - Ca^{2+} \tag{10}$$

$$Cell - Ca^{2+} + CO_3^{2-} \rightarrow Cell - CaCO_3 \tag{11}$$
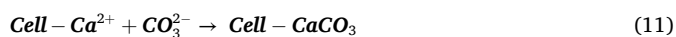
## 6. Conclusions

Bacterial self-healing technique has attracted great attention, since it is considered as sustainable and eco-friendly technique for continuous micro-cracks repair. In this study, *B. pasteurii* and *B. sphaericus* were capable to produce calcite crystals to block the micro-cracks in mortar matrix. *B. sphaericus* and *B. pasteurii* improved the physico-mechanical properties with high restoration for load-deflection. SEM, EDAX, and DTA analyses verified that both strains induced bio-precipitation of calcite, which filled up the matrix pores, decreased water absorption, capillary permeability, and volume of permeable voids, thus enhanced the physico-mechanical properties of bioConcrete. The bacterial bioactivity induced stiffer behavior, where under the same load; treated samples had less deformation as compared with control. Both bacterial strains are promising for bio-application, treated samples revealed better physical properties, mechanical performance, and self-healing of bio-Concrete. The work presented succeeded to implement two bacteria [*B. pasteurii* and *B. sphaericus*] to increase the durability of Reinforced-Laminates, optimum conditions were 0.25% of *B. sphaericus* or 0.50% of *B. pasteurii* with 0.125% of calcium lactate, which showed flexural strength restoration up to 2.3 and 2.6 folds, respectively. Microbial-based self-healing is considered one of the most promising techniques, since it is a continuous process for micro-cracks healing. The overall results are sufficiently positive to promote exploration towards enhancing durability via bioactive materials, thus, reducing the maintenance of cementitious structures.

## Declarations

### Author contribution statement

Amal A. Nasser, Noha M. Sorour, Rateb N. Abbas, Mohamed A Safan: Conceived and designed the experiments; Performed the experiments;

Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

### Data availability statement

Data will be made available on request.

### Declaration of interests statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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