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ORIGINAL ARTICLE

Production of bioconcrete with improved durability properties using Alkaliphilic Egyptian bacteria

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Abstract

Microbial-based self-healing of concrete represents innovative technology for improving micro-crack sealing. Microbial bioactivity can induce calcite-precipitation in concrete, which seals micro-cracks. In this respect, two Egyptian bacterial isolates were selected and identified, as *Bacillus subtilis* (Bs) and *Bacillus megaterium* (Bm) using MALDI-TOF/MS-Biotyper®. Peak patterns of the bacterial ribosomal proteins showed a high match between samples and standards, which verified species consistency. Bs and Bm were added to the mortar mixture in two concentrations (0.5%, 1%) of cement weight, then the mechanical and physical properties were tested throughout a 180-day time course. The compressive strength of Bm0.5 bacterial mortar samples was increased by 21.4% after 28 days, as compared to control. The rate of water absorption of Bm samples was decreased by 12.4% after 180 days. Bacterial mortar samples showed significant restoration of compressive strength than the original samples by 44%, 21%, and 52.6% for Bs1, Bm0.5, and Bs0.5, respectively. SEM and EDAX analyses confirmed that bacterial samples were denser with fewer voids than the control, as a result of microbial nanosized calcite-precipitation. DTA verified that the amount of $CaCO_3$ and its degree of crystallinity were increased in the bacterial mortar samples. Load–deflection of reinforced-laminates for bacterial mortar samples showed ductile behavior and less deformation as compared to control. In this work, novel concrete with improved mechanical and physical properties has been developed using selected Egyptian microorganisms, it can promote self-healing of micro-cracks with improved durability of the concrete. The application of self-healing bioconcrete can reduce the inspection and maintenance costs.

Keywords Concrete · Micro-cracks · Self-healing · *Bacillus subtilis* · *Bacillus megaterium*

Introduction

Microorganisms act as a renewable resource for many novel products that can be widely used in medicine, agriculture, and industrial applications. Therefore, it is essential to utilize their important bio-products to tackle the ever-growing challenges in life. In this regard, scientists inspired by nature have fabricated self-healing concrete that relies on the metabolic bioactivity of certain bacteria (Dick et al. 2006; Ryparová et al. 2021). The benefits of microbial bioactivity were investigated in the protection of limestone monuments, specifically their ability to induce calcite-precipitation under suitable environmental and nutritional conditions (Rodriguez-Navarro et al. 2003).

On one hand, cement is the main component of concrete, which is the most widely used material in construction, due to its relatively low cost and its high compressive strength (Schlangen and Joseph 2009). The cement industry in Egypt represents one of the oldest and important industries

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affecting the Egyptian economy. Egypt produces ~ 60 million tons per year and occupies the 12th position in the world, Egypt's cement exports can reach 13 million tons with an annual turnover of ~ one billion dollars (https://www.statista.com/statistics/507174/egypt-production-of-cement/retrived April 1, 2019). However, crack formation is a common phenomenon in concrete structures as a result of its limited tensile strength (Schlangen and Joseph 2009; Ryparová et al. 2021). The cracks threaten the durability of concrete structures, where liquids and gases can penetrate its matrix causing damage. Cracks can lead to leakage problems or entrance of harmful materials, causing deterioration of the matrix and/or corrosion of embedded steel-reinforcement. Furthermore, cracks may develop and get wider, thus, the reinforcement can be vulnerable to other severe conditions, and once the reinforcement starts to corrode, the whole structure can collapse. These cracks are the main reason for a decreased service life of concrete; therefore, it is advisable and economical to restrict the development of early-aged small cracks (Achal et al. 2011; Xu et al. 2020).

On the other hand, self-healing approach represents a promising way, where the pre-addition of certain healing agents to the concrete can heal the early-aged cracks (Dhami et al. 2012). Research on crack healing focused on using, either chemical healing agents (Li and Yang 2007) or biological methods, where the integrated bacteria together with a suitable calcium source were added during the mixing of concrete (Wiktor and Jonkers 2011; Ryparová et al. 2021). However, conventional synthetic healing polymers used for cracks repair are considered expensive and not eco-friendly, therefore, biological repair technique is highly encouraged (Li 2009). Interestingly, bacteria inducing calcite-precipitation has the potential for self-healing of concrete. It is more compatible with the concrete matrix, and it is considered eco-friendly (Wang et al. 2012). A bacterial-based self-healing concrete has been studied to extend the concrete service life (Wiktor and Jonkers 2011; Ryparová et al. 2021). The concept has been enhanced by developing a liquid bacterial-based repair system that can be injected into the porous concrete network (Sangadji et al. 2013). The added bacteria can produce nanosized calcite-based materials, a process that results in sealing and water tightening of micro-cracks. Selected bacteria can precipitate $CaCO_3$ and/or other inorganic minerals in their microenvironment, and the process is strongly dependent on environmental conditions. Therefore, bacterial-based self-healing bioconcrete can self-repair damage resulting in decreased maintenance and increased service life of concrete (Wiktor and Jonkers 2011). These new materials used in constructions can control damage rather than preventing it by featuring healing mechanism, thus could be economical than the traditional techniques (Jonkers 2013; Xu et al. 2020; Ryparová et al. 2021). Due to the aforementioned reasons, the main objective of this work was to develop sustainable self-healing bioconcrete using selected calcite-producing bacteria isolated from Egyptian soil. The isolated bacteria were selected and identified using MALDI-TOF/MS Biotyper®. The mechanical and physical properties of the new bioconcrete were characterized. Calcite-precipitation was verified using SEM, EDAX and DTA analyses. Also, the healing activity, load–deflection of reinforced-laminates, and the durability for the new bioconcrete were evaluated, for the first time after 1 year.

Materials and methods

Chemicals

Sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), glucose, malt extract, beef extract, yeast extract, calcium lactate, and peptone were obtained from Oxford laboratory chemicals (India). Hydrochloric acid (HCl), sodium hydroxide (NaOH), and potassium phosphate (K_2HPO_4) were of analytical grade. Sodium chloride (NaCl), sodium phosphate (Na_2HPO_4), manganese sulfate ($MnSO_4{\cdot}2H_2O$), citric acid, and agar were purchased from SAS Chemicals CO (MUMBAI). All solutions were prepared using double distilled water.

Selection and cultivation of calcite-producing bacteria

Ten grams' alkaline soil sample from Wadi El-Nitron, Behera governorate, Egypt, was mixed with 90 mL of 0.9% NaCl solution, tenfolds serially diluted and spread onto the selected media, then incubated at 30 °C for 48–96 h. The medium composition (g/L), 5 g peptone, 3 g beef extract, 0.42 g $NaHCO_3$, and 0.53 g Na_2CO_3, the pH was adjusted to 9.5 using 1 M NaOH. The selection was followed by picking the colonies and sub-culturing to get pure colonies of the isolates on Luria–Bertani (LB) agar medium containing; g/L (10 g yeast extract, 10 g peptone, and 5 g sodium chloride). The pH of the medium was adjusted to 9.5. The high pH medium was used to grow and select alkaliphilic bacilli. *Bacilli* spp. was chosen for this study because of their ability to form spores and survive in an alkaline environment (Jonker et al. 2010; Khaliq and Ehsan 2016). Selected bacterial isolates were cultured in LB broth medium, supplemented with manganese ($MnSO_4{\cdot}2H_2O$; 0.01 g/L) to enhance the bacterial sporulation. Cultures were aerobically incubated in 2 L Erlenmeyer flasks using a rotary shaking incubator (New Brunswick, CA) at 150 rpm for 7 days at 30 °C. The growth and sporulation yield of bacteria were regularly checked and quantified using microscopic analysis and pour-plate count method.

Identification of selected bacteria using MALDI-TOF/MS biotyper®

Twenty-four hours grown microbial isolates were identified using matrix-assisted laser desorption/ionization time of flight–mass spectrophotometry Biotyper (MALDI-TOF/MS, Bruker). To identify a microorganism, the sample was mixed with 1 µL of matrix solution (cinnamic acid or benzoic acid derivate), placed on the target plate to dry. Samples were exposed to short laser pulses, which vaporize the microorganism together with the matrix, leading to ionization of the ribosomal proteins. The time of flight (TOF) of the analyte to reach the mass detector was precisely measured (Seng et al. 2010; Wieser and Schubert 2016). The degree of ionization, as well as the mass of the proteins determines their individual TOF. Based on this TOF information, a specific spectrum pattern was detected as particular sample fingerprint, which is unique for each species, and the sample can be processed in 5–7 min (Wieser and Schubert 2016). Based on the ionization of co-crystallized samples, the ions were accelerated and their TOF is measured in a vacuum flight tube. The computer software automatically compares the collected spectra with a reference databank containing a wide variety of relevant isolates, and generates a numerical value called *score value* based on the similarities between the observed and stored database, this *score value* provides information about the validity of the identification method. A *score value* above 2.0 is generally considered to be a valid species, while values between 2.0 and 1.7 represent reliable genus identifications (Sauer et al. 2008; Wieser and Schubert 2016).

Sand, fine aggregates, cement, water and reinforcement

Medium well-graded sand of fineness modulus 2.2 used for mortar complies an Egyptian Standard Specifications (ESS) requirement and Ordinary Portland (CEM-I) cement with grade 42.5 N confirmed Egyptian Standard Specifications (ESS) requirements (ES, 4756-1/2007). Fresh tap water was used with water/cement ratio of 0.45. Expanded Wire Mesh reinforced-laminates were locally produced and available commercially in the Egyptian market were employed, and the strips were weighed 0.7 kg/m^2.

Bacterial suspension preparation and bacterial count

Identified bacterial cultures were incubated for seven days to ensure sporulation in a shaking incubator with 150 rpm at 30 °C, washed by repeated centrifugation at 10,000 rpm for 10 min. Finally, cell pellets were resuspended in sterile saline solution (0.9% NaCl), before their addition to the cement mortar mixture. The optical density of the bacterial cultures and the plate count method were used to prepare culture cell suspensions with a final cell density of 2×10^9 CFU/mL; in two concentrations (0.5% and 1%) of the cement weight. After cement mortar preparation and solidification, both control and treated cement mortar samples were collected for bacterial counts time course after 3, 7, 28, 60, and 90 days. For the bacterial count time course, 10 g of each sample was added to 90 mL of sterile 0.9% NaCl solution and left overnight to release the bacterial cells and spores from the mortar samples, then ten folds' serial dilutions of each sample were prepared, and 1 mL of the final dilution was dispensed on LB solid medium in triplicates for each dilution. Finally, the plates were incubated at 30 °C for 6 days and the CFU/mL in each plate was counted during the time courses for *B. subtilis* (Bs) and *B. megaterium* (Bm). All microbiological assays have been done at the Microbial Biotechnology Lab., Microbial Biotechnology Department, (GEBRI), University of Sadat City, Egypt.

Mortar mixes and setting time

The dry mortar mixture was weighed and mixed using mechanical mixer for five minutes, water was poured and the mixing process lasted for 10 min. Mortars proportions were in accordance with Ferrocement Model Code (Ferrocement Model Code 2001). Sand/cement Ratio was 1:3 (w/w). Water/cement ratio was 0.45 (v/w). Samples were prepared for the mortar mixture with the addition of *B. subtilis* or *B. megaterium* (0.5% and 1%) of cement weight and compared to the control mortar mixture. Calcium lactate was added (Table 1. Supplementary material) and the mortar was cast in molds for different tests. Test samples were remolded after 24 h and kept in a wet cloth. The samples were kept in moisture by spraying with water every day till the testing day. Initial and final setting time tests were carried out using Vicat apparatus for cement paste (E.S.S. 4756/2007). The cement was used without any addition. Standard water/cement ratio was experimented on cement only before the setting time tests. Another four cement pastes were mixed using the same water ratio with the addition of bacteria and calcium lactate to investigate the effect of bacterial addition on the setting time (Erşan et al. 2015).

The rate of water absorption

Mortar samples were dried in an oven for three days at 50 °C, and then cooled at different ages of 3, 7, 28, 120, and 180 days of moist curing. The sides of the mortar samples were covered with epoxy resin to allow the flow of water in one direction. The samples were sealed tightly with attached plastic sheets and protected by an elastic band in

its position. Samples were kept partly and their initial mass was determined after their immersion in water to a depth of 5 mm. The readings started with the sample initial mass for a period of 2 h from initial water submerging. Then samples were removed and weighed after blotting off excess water (Givi et al. 2011). The gain in mass (Δm, kg/s) at time t (s), exposed area of the sample (a, m^2), and density of water (d), were used to obtain the rate of water absorption (I, $m/s^{0.5}$) according to the following equation:

$$I = \Delta m/(\text{a.d})$$

Compressive and flexural strength tests

The compression test was conducted on the prepared mortar based on Gandhimathi et al. (2015) method. Test samples with dimensions of 7 × 7 × 7 cm were cast and cured using a moisturized wet cloth. For each age, three samples were prepared. After the specified period for the time course (3, 7, 28, 120, and 180 days) all the samples were tested for their maximum load in the compression testing machine. The cubes were tested on a hydraulic machine 2000 kN capacity. They were tested up to failure (Ramadas et al. 2019). Flexural strength tests were carried out on mortar prisms of size 160 × 40 × 40 mm on flexure testing machine of capacity 10 tons. For each age, three samples were prepared, and the tests were carried out for the control and all bacterial mortar samples. The flexural samples were subjected to three-point loading test. The strength was analyzed at 28, 120, and 180 days. The flexural strength is calculated from the formula as given:

$$\text{Flexural strength} = 3PL/2d_1d_2^2$$

Where, P is the maximum applied load to the specimen (N), d_1 is the width of the specimen (mm); d_2 is the depth of specimen (mm). Reinforced-laminates with the dimensions 35 × 15 × 3 cm were cast using five mortar mixes and loaded on flexural testing machine (Fig. 3). Each lamina has an expanded wire mesh 35 × 15 cm in mid-height. Laminates were covered by wet cloth and loaded at mid-point until failure. Deflection of mid-point of reinforced lamina was measured using dial gauge at the age of 28 days. Load–Deflection of reinforced-laminates for mortar samples were measured in the Properties of Material Laboratory, Civil Engineering Department, Faculty of Engineering, El-Menufyia University, Egypt (Zahran et al. 2014).

Stopping the hydration

Hydration was stopped using alcohol–acetone method after 7 and 28 days of curing. The stopping solution was prepared as (1:1 v/v) of methyl alcohol and acetone. A 10 g sample was ground and stirred in 100 mL of stopping solution, filtered through sintered glass funnel (G4), washed with the same solution three times, and finally with ether. Each sample was dried at 80 °C for 24 h and kept in air-tight bottles inside desiccators until the time of analysis. The samples were analyzed using scanning electron microscopy (SEM) and differential thermal analyses (DTA).

Characterization using SEM, EDAX, and DTA

The morphology of the samples after 28 days of curing were analyzed using scanning electron microscope (SEM) equipped with an Energy Dispersive X-ray Spectrometer (EDAX), in National Research Center, Cairo, Egypt, based on Jonkers et al. (2013) method. Three magnifications of 100×, 6000×, and 7000× were selected for the imaging. Samples were analyzed using differential thermal analyses (DTA) as described by Ramachandran et al. (2002). The DT-50 thermal analyzer apparatus (Shimadzu Co., Kyoto, Japan) consists of a tubular furnace enclosing the sample holder and thermocouples connected to a recording instrument. The sample holder is made of standard alumina; it consists of massive support provided with a movable cover. The support holds crucibles, made of platinum. A total of 15–20 mg of the samples were taken in an alumina crucible and heated in nitrogen atmosphere up to 1000 °C, maintaining 20 °C/min heating rate and using α-Al_2O_3 as the reference material. Two thermocouples platinum–platinum radium (Pt/Pt-13% Rh) were used; one for the determination of the furnace temperature, and the other is the differential thermocouple. One junction is placed in the center of the standard material. The furnace and differential temperature were automatically recorded.

Statistical analysis

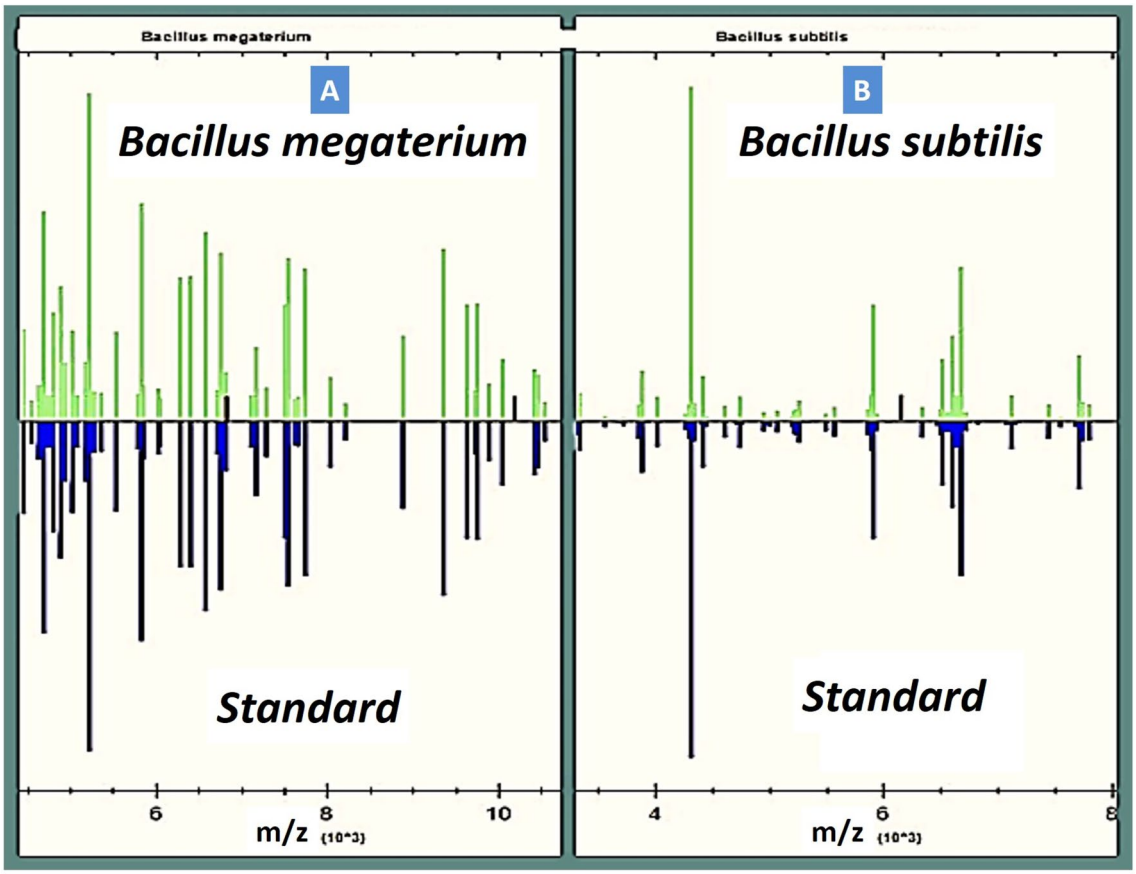
Experimental data are average of three replicates, results were expressed as the means ± standard deviation, significant differences analysis were determined using ANOVA and Tukey tests at P value (≤ 0.05). Analyses were carried out using SPSS software (Version 17).

Results and discussion

Bacterial identification

Self-healing of concrete has been reported by some bacterial spp. mainly of the genus *Bacillus* by the $CaCO_3$-precipitation through direct precipitation or ureolytic decomposition of calcium-containing compounds which can seal micro-cracks

(Jonkers et al. 2010; Xu et al. 2020; Ryparová et al. 2021). The genus *Bacillus* is alkaliphilic and its spores can stay dormant in alkaline environment without nutrients for many years. In this regard, two alkaliphilic bacilli isolates were selected and identified as *B. subtilis* and *B. megaterium,* using MALDI-TOF/MS Biotyper® with a high score for species consistency (Fig. 1). The high pattern match between the sample peaks and the standard peaks mass spectrum pattern of bacterial ribosomal proteins (Fig. 1) confirms the efficiency of bacterial identification using MALDI-TOF/MS Biotyper® (Seng et al. 2010). Recently, MALDI-TOF/MS is widely used for the analysis of biomolecules in many laboratories (Marvin et al. 2003; Wieser and Schubert 2016). Selected microbial isolates were identified as bacteria, family Bacillaceae candidates' *B. subtilis* and *B. megaterium*, these isolates were originally isolated from Wadi El-Nitron, Behera governorate, Egypt (Fig. 1). MALDI-TOF/MS Biotyper® is a fast, highly accurate identification method (Wieser and Schubert 2016). Many *bacilli* spp. have been isolated and were able to induce $CaCO_3$-biomineralization, when applied on decayed stone (Jroundi et al. 2010). Among the bacteria used in biomineralization, *B. subtilis* is the most prominent, and *B. cereus* was investigated for monument repair (Castanier et al. 2000). It was also evaluated to induced $CaCO_3$-biomineralization in buildings and statues in France (Anne et al. 2010). Other *Bacilli* spp. were *B. megaterium*, *B. pumilus, B. thuringiensis* and *B. alkalinitrilicus* (Cacchio et al. 2003; Wiktor and Jonkers 2011). The addition of bacteria, mainly of the genus *Bacillus*, were studied for cracks-filling and increasing the compressive strength



Analyte (Name)	Organism (Best Match)	Score (C-Value)	Organism (Second Best Match)
1 (+++)(B)	*Bacillus subtilis*	2.63	*Bacillus subtilis*
2 (+++)(A)	*Bacillus megaterium*	2.39	*Bacillus megaterium*

Fig. 1 Comparison matching patterns of *B. megaterium* Egyptian isolate with standard mass spectrum pattern of ribosomal proteins for *B. megaterium* DSM 2894 **a**; and matching patterns of *B. subtilis* Egyptian Isolates with standard mass spectrum pattern of ribosomal proteins of *B. subtilis* DSM 5552 **b**. Score 2.300–3.000 (+++) highly probable species identification, Score 2.000–2.299 (++) secure genus identification, probable species identification

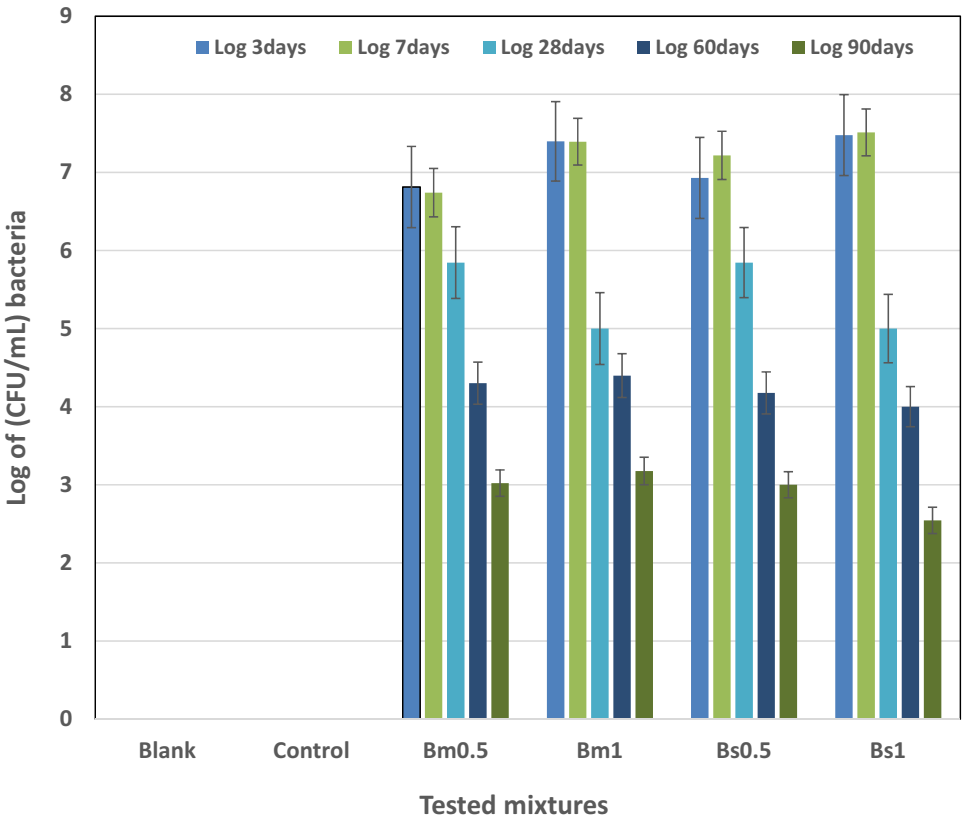


Fig. 2 Log of bacterial count (CFU/mL) of each bacterium, Bm (*B. megaterium*), Bs (*B. subtilis*) using 0.5 and 1% concentration of cement weight for 3, 7, 28, 60 and 90 days; (Blank: medium without bacteria, Control: untreated sample with no bacteria)

through $CaCO_3$-precipitation (Park et al. 2012). In this regard, bacteria capable of producing minerals were used to repair limestone monuments (Tiano et al. 1999; Dick et al. 2006) and fill the micro-cracks in cementitious materials (De Muynck et al. 2008; Xu et al. 2020; Ryparová et al. 2021).

Bacterial count and setting times

Bacterial-induced self-healing mechanism is based on the metabolic conversion of suitable organic compounds to calcite; which is a crystalline form of $CaCO_3$, and it is precipitated when certain bacteria grow in a medium supplemented with a suitable calcium source; e.g. the bioconversion of calcium formate with Portland cement present in the mortar paste according to the following reaction (Barabesi et al. 2007):

$$Ca\left(CHO_2\right)_2 + Ca(OH)_2 + O_2 \rightarrow 2CaCO_3 \downarrow\downarrow + 2H_2O$$

Results (Fig. 2) show that there was no significant difference in the bacterial count between *B. subtilis* and *B. megaterium* during the whole time course. However, the bacterial count was decreased during the time course which can be suggested as a result of nutrients depletion in the mortar mixture. Also, both species follow the same growth trend and were adapted at the same rate with the new environmental conditions in the mortar.

Setting time and rate of water absorption

The initial setting time was decreased for all bacterial cement samples, while the final setting time was significantly increased for Bs0.5 and Bm0.5 samples only, as compared to the control with no bacteria (Fig. 3a). In case of Bs1 and Bm1, the initial setting time was significantly decreased as compared to both Bs0.5 and Bm0.5 and the control. Generally, the addition of retarders (as nutrients) slows down the hardening of the cement mortar i.e. increases the initial setting time by stopping its rapid set, however, it does not change the hydration products' composition (Lea 1988; Abalaka 2011). Likewise, Xu and Wang (2018) investigated the influence of bacterial spores and nutrients (peptone and beef extract) on the setting time of the calcium sulphoaluminate-cement, bacterial spores' addition has a slight effect on the setting time, while both initial and final setting time were insignificantly increased at low nutrients concentration ($< 1.5\%$), and was significantly increased by the addition of $^{>}3\%$ nutrients.

The influence of bacterial addition on the absorption of water for the mortar samples was investigated (Fig. 3b),

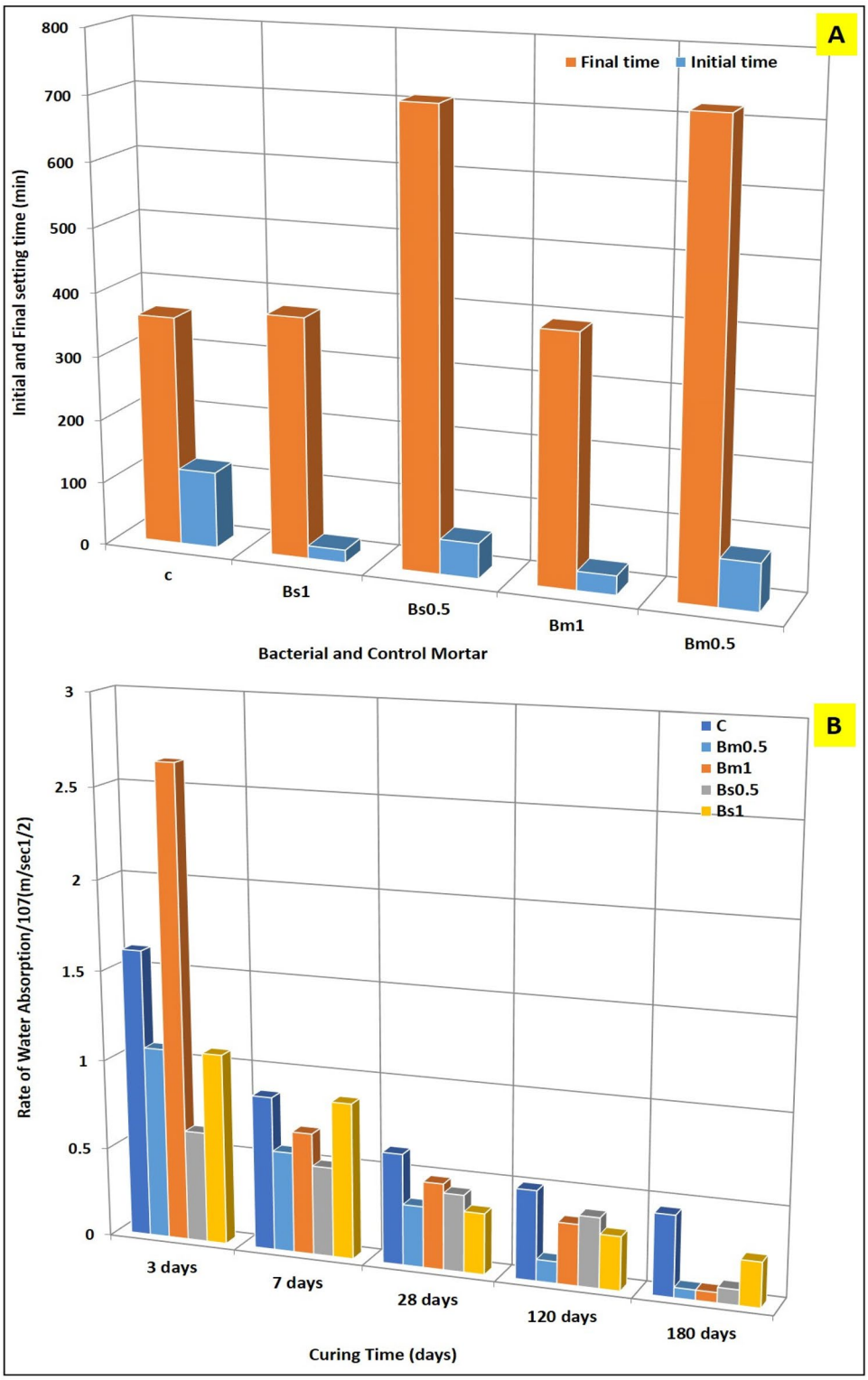


Fig. 3 Initial and final setting times **a**; Rate of water absorption **b** for bacterial and control mortar samples (*C* control, *Bm: B. megaterium, Bs: B. subtilis)* using 0.5 and 1% concentration of cement weight

during the age of 3–180 days. Results (Fig. 3b) showed that the water absorption of the bacterial mortar samples was reduced with the addition of selected bacteria. At the age of 3 days, the rate of water absorption for bacterial mortar samples was decreased except for Bm1 as compared to the control with no bacteria. At later ages, the water absorption rate was decreased in all bacterial mortar samples. After 7 days, more than 12-folds significant activity for the bacterial mortar sample Bm1 (0.68×10^{-7} m/sec$^{0.5}$) was obtained as compared to 0.056×10^{-7} m/sec$^{0.5}$, after

180 days. Minimum water absorption for all bacterial mortar samples was recorded after 180 days (Fig. 3b). Results obtained (Fig. 3b) for the overtime reduction of water absorption suggest that the added bacteria biochemically induced $CaCO_3$-precipitation that was responsible for filling up the pores in the cement mortar, which in turn decreased the absorption of water as reported by Wiktor and Jonkers (2011) and Chahal et al. (2012). Indeed, healing in wet–dry environments has been previously reported to enhance the bacterial growth and $CaCO_3$-precipitation when compared to wet conditions (Wang et al. 2014; Tziviloglou et al. 2016). It is expected that wet–dry environments are beneficial, as in such environments carbonation is faster (El-Turki et al. 2010). Additional $CaCO_3$ can be produced as a result of the reaction between the atmospheric and/or generated CO_2 and the $Ca(OH)_2$ present in the cement. In the present work, all bacterial mortar samples were placed in a moist environment for 180 days to generate a suitable amount of healing based on a similar healing period reported by Wiktor and Jonkers (2011). After incubation, all the samples showed a significant reduction in the water absorption (Fig. 3b), however, the degree of reduction could vary, while the tendency was in line with the crack-healing condition as verified by its new mechanical and physical properties. Similar results were reported by Xu and Wang (2018) who embedded *Sporosarcina pasteurii* (ATCC 11,859) in concrete as bacterial-based self-healing system and attained crack closure in 28 days, where all the samples showed a remarkable decrease in the water absorption, and 30% increase in the compressive strength, as compared to the control mortar. Calcite-precipitation not only seals the micro-cracks in the bacterial mortar samples, but also plugs the pores within its matrix, thus reduce the transport of fluids and decrease the rate of water absorption (Chahal et al. 2012; Ryparová et al. 2021).

Compressive and flexural strengths

Results obtained (Fig. 4a) show an increase in the compressive strength over time for the bacterial mortar samples (Bs and Bm) when compared with the control. After 28 days, the compressive strength of Bm0.5 bacterial samples was increased significantly by 21.4% as compared to the control. Likewise, Schwantes-Cezario et al. (2019) reported that the compressive strength was improved by 31% using *B. subtilis* strain AP91 in the mortar mixture. The compressive strength of Bm0.5 bacterial samples showed a significant increase to 121.4%, 119.1%, and 112.4%, at age of 28, 120, and 180 days, respectively, as compared to the control. The increase in the compressive strength for bacterial mortar samples over time, confirms the substantial activity of the added bacteria until the age of 180 days (Fig. 4a). Likewise, Chahal et al. (2012) reported that calcite-precipitation induced by the added bacteria is responsible for filling up the pores in the mortar samples, thus reducing its porosity; which in turn increases the compressive strength and decrease the water absorption of the mortar samples. Similarly, the addition of *Sporoscarcina pasteurii* in the mortar increased the compressive strength by 22% as compared to the control, as well as reduced the water absorption by four-folds (Chahal et al. 2012). Other authors described that the nano-deposition of $CaCO_3$ by the added bacteria probably closes the micro-cracks in cementitious materials, which increases the compressive strength (Achal et al. 2011; Ghosh et al. 2019; Ryparová et al. 2021).

Bacterial and control mortar samples were tested in flexure (Fig. 4b). It was observed that the flexural strength was increased for the bacterial mortar samples as compared to the control, except for Bm1 and Bm0.5 after 28 and 180 days, respectively (Fig. 4b). The flexural strength of Bs1 bacterial mortar samples was increased significantly to 114.3%, 111.7%, 121% as compared to the control mortar at the age of 28, 120, 180 days, respectively. It is suggested that the activity of the bacterial mortar, biochemically induced calcite-precipitation, which in turn increased the flexural strength (Gupta et al. 2008). A similar trend was obtained by Gupta et al. (2008) who compared the flexural strength of immobilized bacterial-cement mortar samples with mortar control and reported that the compressive and flexural strengths were improved.

Restoration of compressive and flexural strengths

The purpose of developing self-healing bioconcrete is to achieve good healing performance when cracks occur in the concrete. For this objective, to confirm the healing activity of the produced bioConcrete, the bacterial mortar samples after 28 days of curing were loaded with 60% of the ultimate load. Those samples were kept moisturized for 6 months, and then the compressive and flexural strengths were measured again after one year. All bacterial mortar samples showed a significant restoration of compressive strength (Fig. 5a) than the original samples by 12.5%, 44%, 21%, and 52.6% for Bm1, Bs1, Bm0.5 and Bs0.5, respectively. Furthermore, the bacterial mortar samples (Fig. 5b) showed higher restoration of flexural strength than the original ones, after 28 days of curing by 100%, 67%, 51%, and 127% for Bm1, Bs1, Bm0.5, and Bs0.5, respectively. Overall results obtained (Fig. 5) suggest that the added bacteria *B. subtilis* and *B. megaterium* have the ability to heal the internal micro-cracks caused by the loading, thus, improved the mechanical properties for all loaded bacterial mortar samples as compared to the original ones (Gupta et al. 2008; Du et al. 2019).

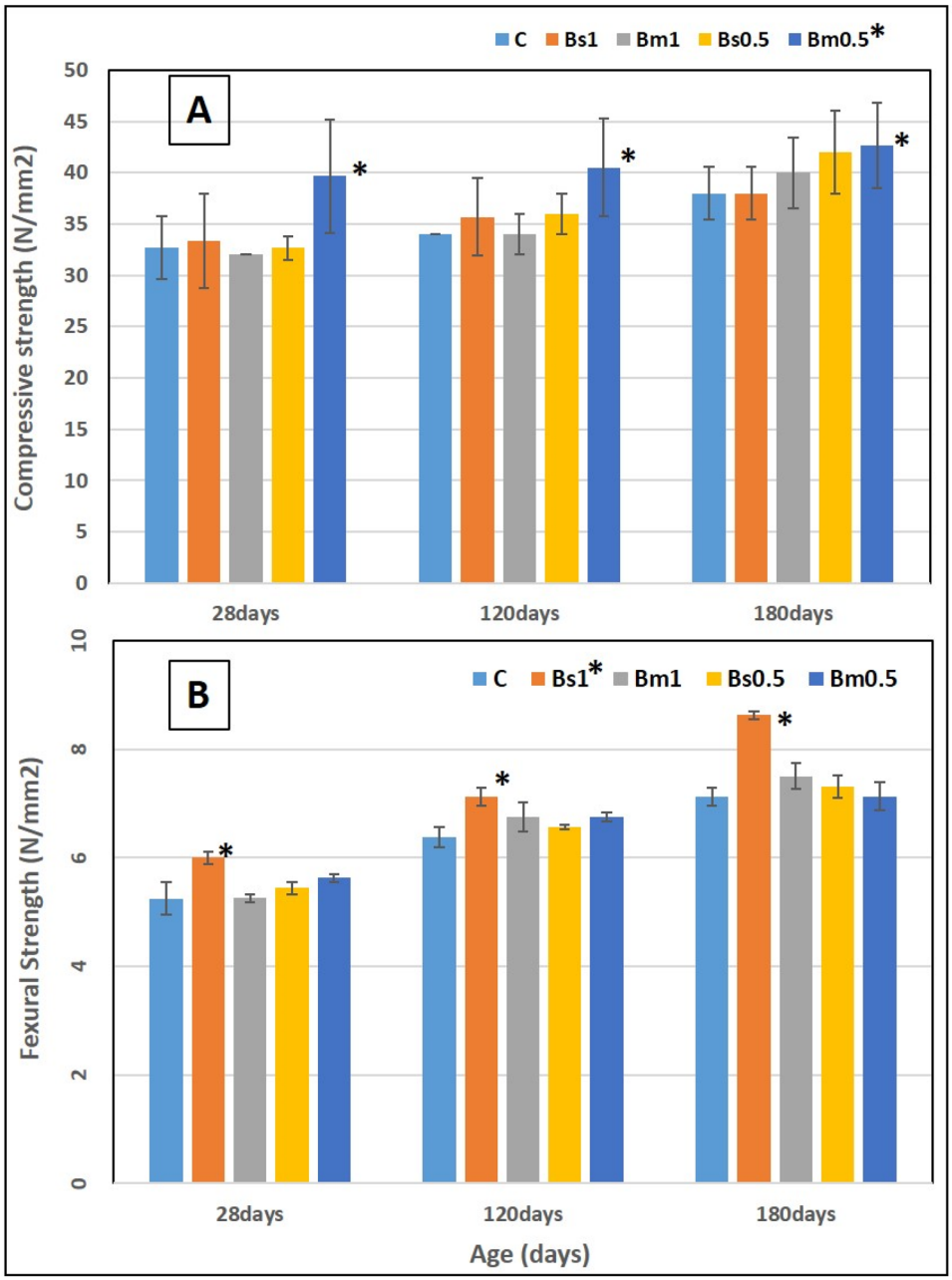


Fig. 4 Compressive strength **a** and flexural strength **b** for bacterial and control mortar samples (*C:* control, *Bm: B. megaterium*, *Bs: B. subtilis*), using 0.5 and 1% concentration of cement weight. *Significant *P* value < 0.05

Restoration of reinforced concrete laminates under load–deflection

Reinforced-laminates were tested under flexure after 28 days of curing until failure. All bacterial mortar samples showed ductile behavior and their deflection was better than the control mortar (Fig. 6). Ferrara et al. (2018) reported that the evolution of damage in cementitious materials results from stiffness decay, which is a function of crack opening that can be estimated. The obtained results (Fig. 6) suggest that the metabolic activities of the added bacteria have induced stiffer behavior in the mortar samples as compared to the control. Calcite is the primary crystalline product involved in the healing process, where the microbial metabolic activities induce calcite-precipitation in the presence of a suitable calcium source and nucleation sites provided by the bacteria. Under the same load, all bacterial mortar samples have less deformation as compared to the control mortar (Fig. 6). Likewise, Al-Tabbaa et al. (2019) reported that reloading of concrete columns induced new cracks without reopening of previously healed ones, which was probably due to the complete recovery of strength following self-healing, however,

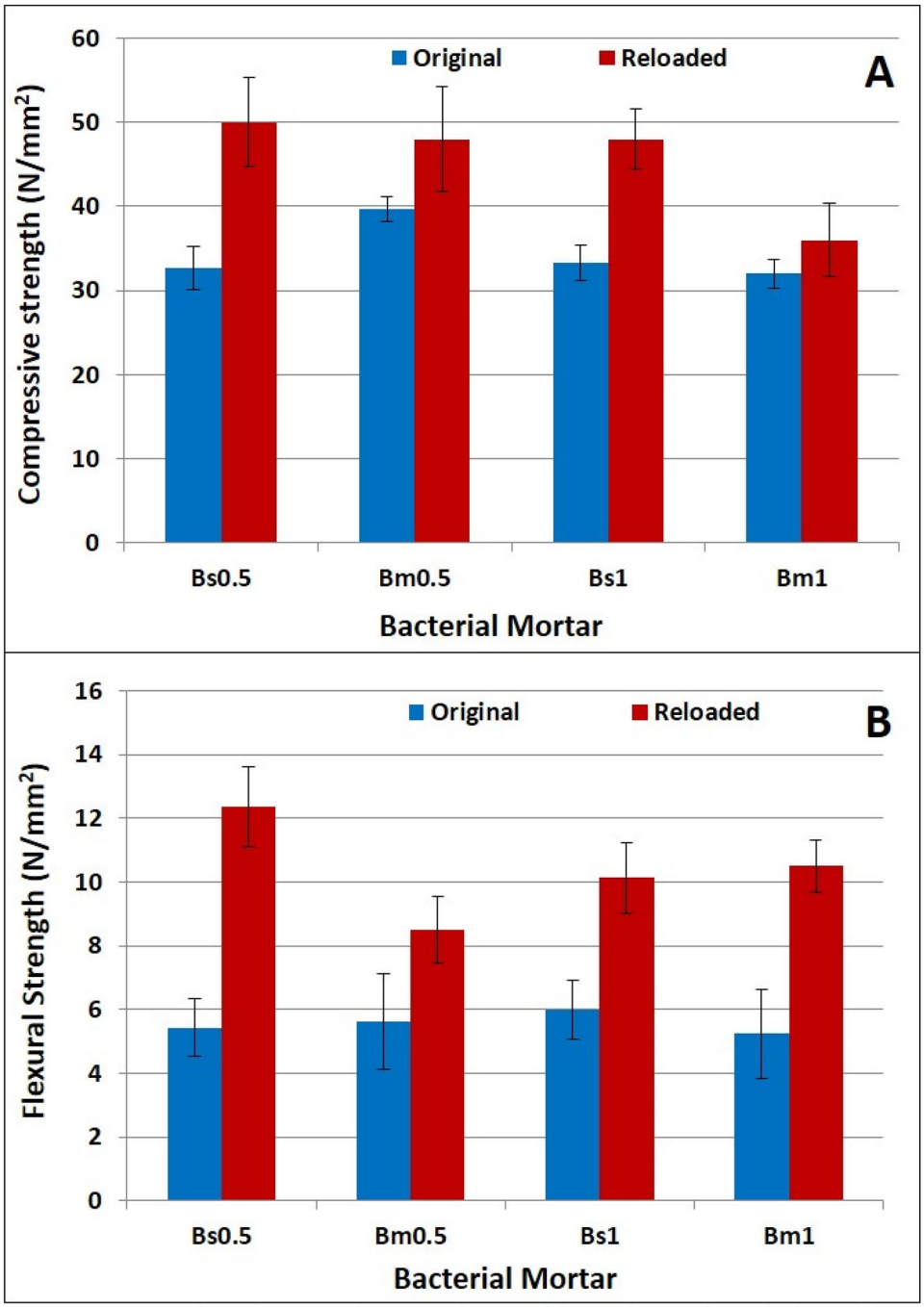


Fig. 5 Restoration of compressive strength **a** and flexural strength **b** for bacterial mortar samples loaded with 60% of ultimate load, *Bm*: *B. megaterium*, *Bs: B. subtilis*, using 0.5 and 1% concentration of cement weight

when these columns were exposed to impact-loading, the control exhibited a continuous loss in stiffness, while, the treated samples showed stiffness recovery up to ~99%. Furthermore, original reinforced-laminates bacterial samples were compared to the reloaded bacterial samples with 60% of ultimate flexure at the same age, after one year. Figure 7 shows a comparison between the original and reloaded state for load–deflection of *B. subtilis* and *B. megaterium* laminates. Reloaded samples showed similar behavior of loading until 60% of the ultimate load (Fig. 7a, b), and higher resistance to complete failure than the original ones of Bs1 and Bm1. The original bacterial samples were stiffer than the reloaded samples (Fig. 7a, b). However, reloaded bacterial samples Bs0.5 and Bm0.5 have high restoration and higher ultimate load than the original ones (Fig. 7c, d) as well as a wider area under the load–deflection curve. At the age of 120 days, different behavior of restoration for the bacterial samples was observed. The original Bs0.5 and Bs1

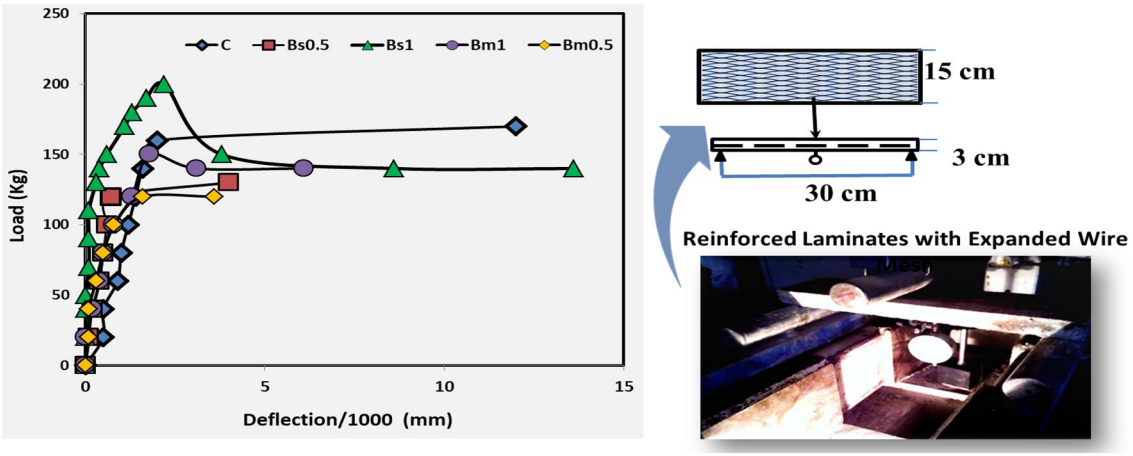


Fig. 6 Load–deflection of Reinforced-laminates for Bacterial and Control Mortar samples with no bacteria after 28 days of curing, (*C:* control, *Bm: B. megaterium*, *Bs: B. subtilis*) using 0.5 and 1% concentration of cement weight

bacterial samples have brittle behavior than reloaded bacterial samples (Fig. 7e, f), while the ultimate load was higher in reloaded samples of Bm0.5 and Bm1 than the original samples (Fig. 7g, h), as well as high restoration for reloaded bacterial samples and brittle behavior for original samples. The high restoration behavior in reloaded bacterial samples is very promising, and the results obtained agree with the improvement of flexural strength for bacterial mortar samples that were previously discussed. Results obtained confirm the self-healing of micro-cracks caused by loading 60% of ultimate load, which is probably through the bioactivity of the added bacteria, either *B. subtilis* or *B. megaterium* (Gupta et al. 2018; Du et al. 2019). The microbial activity induces nanosized calcite-precipitation which can seal the micro-cracks in cementitious materials, and thus can increase its durability (De Muynck et al. 2008; Du et al. 2019; Ryparová et al. 2021).

SEM and EDAX analyses

SEM and EDAX analyses (Fig. 8) verified that the bacterial addition into the concrete improved the microstructure of concrete by calcite-precipitation. SEM micrographs were carried out after 28 days of curing with the magnification of 100×, 6000×, and 7000×. SEM micrographs revealed that the bacterial mortar has fewer voids and denser as compared to the control mortar (Fig. 8). Calcite-precipitation in bacterial mortar samples was visualized by SEM, the precipitation acts as a barrier, increasing the impermeability of the concrete, thus decreasing the water absorption (Achal et al. 2011). Likewise, Vijay et al. (2017) reported that the biomineralization techniques give promising results in sealing the micro-cracks in concrete. Also, SEM imaging (Fig. 8) shows the presence of dense areas as a result of calcite-crystals precipitation in the bacterial mortar samples. It is clearly seen that the voids (Fig. 8b, c) were filled in the bacterial concrete samples (Siddique et al. 2016). It is suggested that calcite-crystals in the form of $CaCO_3$ were precipitated by the integrated bacterial cells which fill the pores in the mortar samples as confirmed by EDAX (Fig. 8d). Similarly, calcite crystal growth in bacterial mortar was reported by Dupraz et al. (2009), Wang et al. (2012), and Daskalakis et al. (2015). EDAX analysis during SEM imaging (Fig. 8d) showed that the precipitated compound was composed of calcium ion, oxygen, carbon of $CaCO_3$, and a silicon peak related to the sand particles. Selected microorganisms directly participated in calcite-precipitation by providing the nucleation sites, which is the primary crystalline product involved in the healing process (Barabesi et al. 2007). Overall, SEM imaging and EDAX analysis confirmed the deposition of $CaCO_3$ within the bacterial samples microcracks (Fig. 8). The obtained results agree with the decrease in the water absorption, improvement of mechanical properties for bacterial mortar samples as compared to the control (Wiktor and Jonkers, 2011; Hung and Su, 2016; Xu et al. 2020; Ryparová et al. 2021).

Differential thermal analysis (DTA)

Differential thermal analysis was observed after 28 days of curing (Fig. 9), the DTA thermograms of the control and the bacterial mortar samples, showed endothermic peaks at most peak temperatures. DTA analysis showed that added bacteria were able to precipitate $CaCO_3$ crystals in the micro-cracks (Fig. 9). The endothermic peaks at 59–64 °C were related to the moisture in the control and bacterial mortar samples;

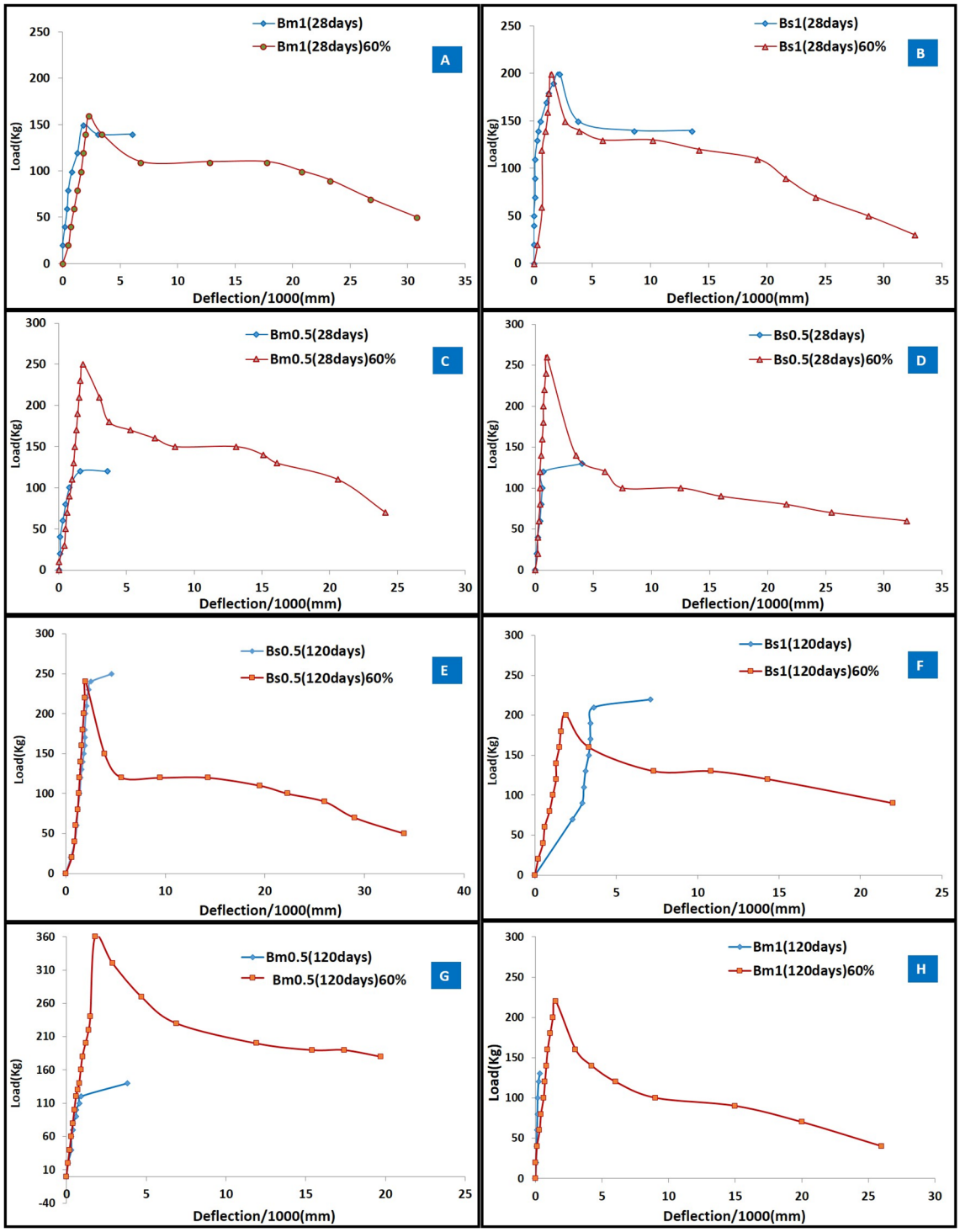


Fig. 7 Restoration of load–deflection for reinforced concrete laminates of original (Bm and Bs) and reloaded bacterial mortar samples (Bs and Bm 60%), after 28 and 120 days of curing, Bm: *B. megaterium,* Bs: *B. subtilis,* at 0.5 and 1% concentration of cement weight

the endothermic peaks at 148–152 °C were due to the microcrystalline (C–S–H), while, the endothermic peaks at 476–480 °C and 566–571 °C were due to the decomposition of Ca $(OH)_2$, and α, β quartz transformation, respectively (Ramachandran et al. 2002). However, the endothermic peaks at 657–717 °C can be attributed to the decomposition of the amorphous and crystalline parts of $CaCO_3$. The decomposition of $CaCO_3$ is shifted to the higher temperature

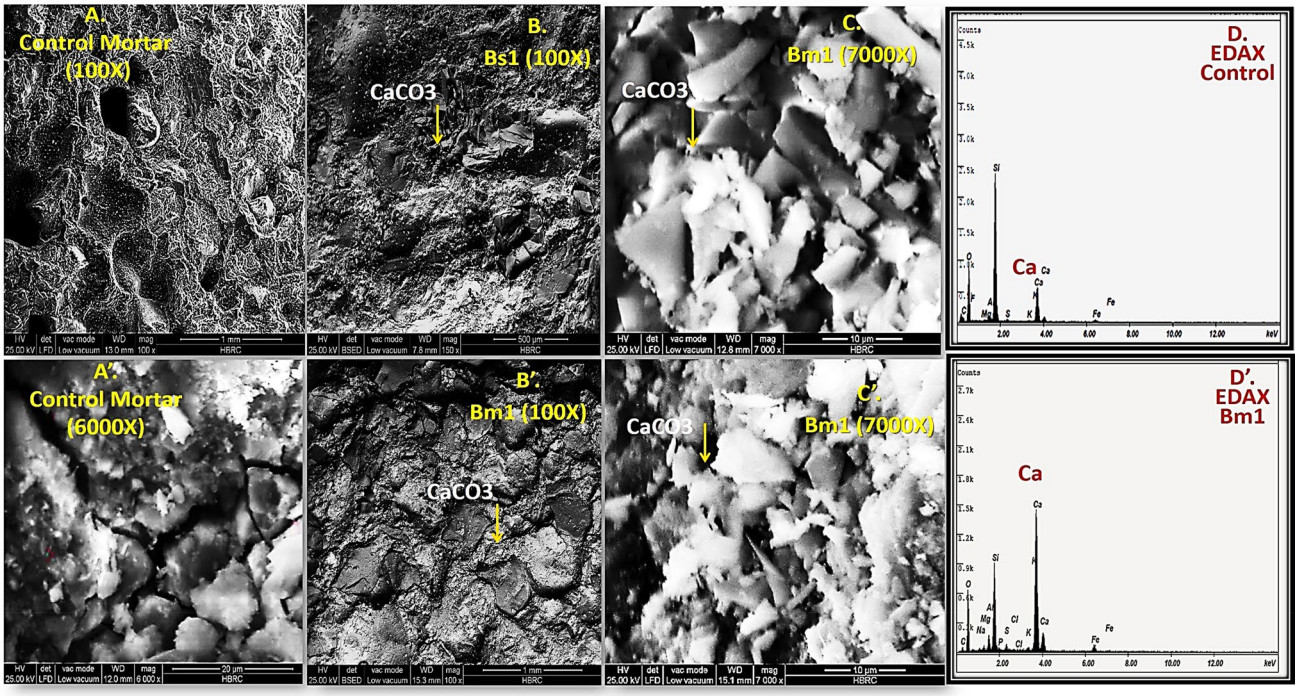


Fig. 8 SEM photographs of control **A**, **A′** and bacterial mortar samples **B**, **B′**, **C**, **C′** at 100×, 7000×, Bm1: *B. megaterium,* Bs1: *B. subtilis* at 1% concentration of cement weight, EDAX photographs of control and bacterial mortar sample (*B. megaterium*) showing elements of $CaCO_3$ after 28 days of curing **D, D′**

in bacterial mortar samples (690.5 °C, 688 °C, 716.2 °C, and 687.9 °C) than the control (657.4 °C); this indicates that both the amount and the degree of crystallinity of $CaCO_3$ was increased in bacterial mortar samples as compared to the control (Fig. 9). Overall results revealed that increasing the degree of crystallinity with its stability improved the bioconcrete physical and mechanical properties (Narayanan and Ramamurthy 2000; Ryparová et al. 2021).

Conclusions

Recently, the bio-based self-healing technique has attracted substantial attention as it is considered an eco-friendly and more sustainable model for micro-cracks repair in cementitious materials. Sometimes, manual repair of cracks is hard in case of limited access to the cracked zone; therefore, bio-based bacterial self-healing techniques is promising in sealing internal micro-cracks. *B. megaterium* and *B. subtilis* improved the mechanical properties and showed high restoration for load–deflection of reinforced-laminates. SEM and EDAX analysis verified that the addition of *B. subtilis* and *B. megaterium* in mortars induced the bio-precipitation of nanosized $CaCO_3$ crystals, which is responsible for filling up the pores and thus, decreasing the rate of water absorption. The metabolic activity of the bacteria induced ductile behavior in mortar samples, as compared to control, which confirms the durability of bacterial mortar samples. Both bacterial strains showed great potential for application in cementitious materials since their addition, exhibited better mechanical performance, physical properties, and durability. The addition of selected microorganisms for the repair of micro-cracks in cementitious materials should continue to be studied to prevent early deterioration. Research findings should stimulate more efforts to bridge the gap between the realistic field and the laboratory conditions for self-healing research. This research succeeded to implement selected alkaliphilic bacteria to improve the mechanical properties of bioconcrete, optimum conditions were 0.5% *B. subtilis* or *B. megaterium* with 0.25% calcium lactate as calcium precursor and curing for 28 days. The initial results are sufficiently positive for further investigation towards reducing the requirement for inspection and maintenance of concrete structures. However, further scale-up and implementation on a live construction site are still needed.

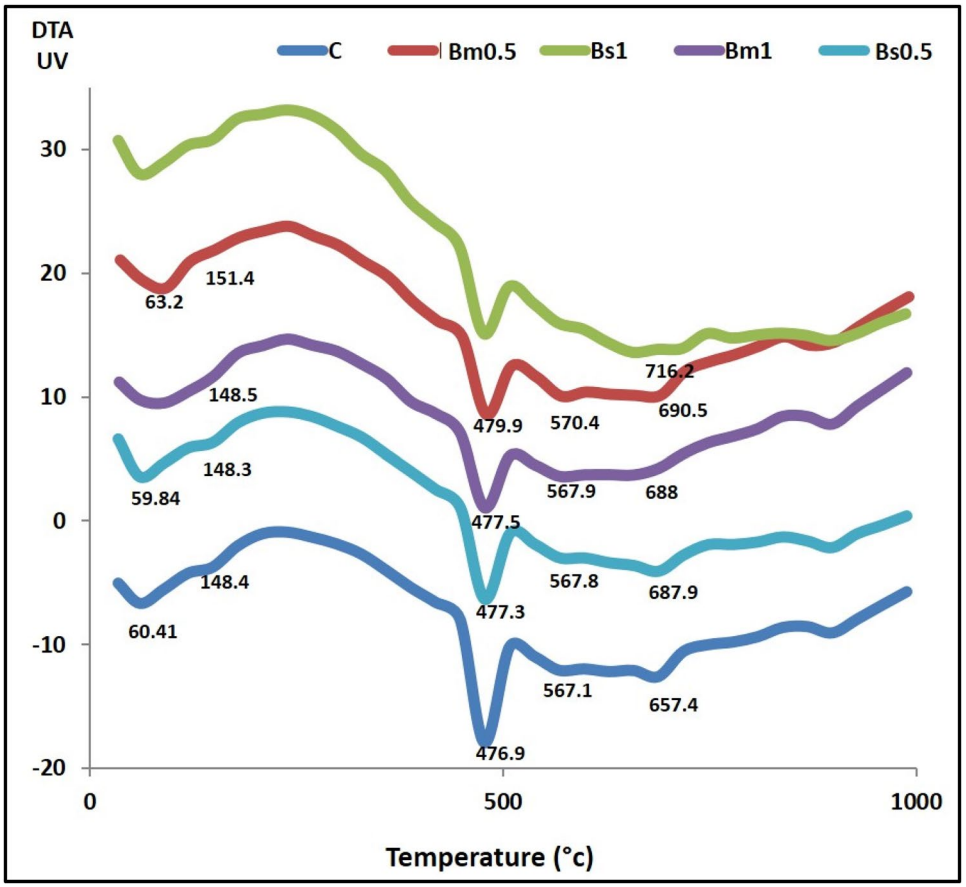


Fig. 9 DTA Thermograms for control and bacterial mortar samples after 28 days of curing, (*C:* control, *Bm: B. megaterium*, *Bs: B. subtilis*)

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13205-021-02781-0.

Declarations

Conflict of interest All authors declare that there are no financial/commercial conflicts.

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