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Bio self-healing concrete using MICP by an indigenous *Bacillus cereus* strain isolated from Qatari soil

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ABSTRACT

In this study, the self-healing process in concrete through microbial-induced calcium carbonate precipitation (MICP) performed by an adapted indigenous strain of *Bacillus cereus* isolated from soils in Qatar was investigated. This strain has advantage of withstanding and performing MICP in environments of 45–50 °C temperature and 80–100% relative humidity. Hence, it is considered a suitable candidate for self-healing in concrete. The performance of this new isolate was compared to that of *Sporosarcina pasteurii*, a well-studied strain for MICP in concrete. The strains were encapsulated in sodium alginate beads, which were then incorporated in the cement-sand mortar. It was observed that the selected local strain was able to fill cracks with widths ranging from 162 μm to 670 μm, while the *Sporosarcina pasteurii* strain was able to fill cracks with widths ranging from 200 μm to 4700 μm. Scanning electron microscopy (SEM) images provided evidence for the survival of the bacterial cells in the beads during the mixing of mortar and casting of the samples. The X-ray diffraction (XRD) spectra and SEM images confirmed the formation of calcium carbonates in the cracks. The local *Bacillus cereus* strain showed high urease activity and could be a viable and economical solution for the bio self-healing concrete through MICP where hot and humid climatic conditions are encountered.

1. Introduction

Reinforced concrete (RC), as a quasi-brittle material, is susceptible to cracking and deterioration. Cracks occur in RC structures, usually at an early age, due to several factors, including temperature, creep and shrinkage, and tensile loading. Chloride and carbon dioxide ingress through cracked concrete initiate the corrosion of the reinforcing steel. The expansive corrosion products apply tensile stresses on concrete, leading to further cracking and spalling of concrete and accelerating the aging process. The degradation is more severe in high temperature and relative humidity (RH) conditions, such as those encountered in the Middle East and the Arabian Gulf [1]. Hence, the durability of RC structures has become a significant concern in these regions and often leads to repetitive repairs and rehabilitation.

Self-healing concrete through microbial-induced calcium carbonate precipitation (MICP) is a potential approach to build a more resilient and sustainable RC infrastructure [2–4]. Self-healing concrete can heal cracks autonomously without interference while the structure is under service [5–7]. Most commonly, bio self-healing concrete is manufactured by adding MICP performing bacteria in the concrete. However, the environment inside the concrete matrix is hostile towards microorganisms due to the high pH of the concrete pore solution. These organisms do not survive the harsh mixing and pouring of concrete. Furthermore, cement hydration generates heat, making the bacterial cells inefficient if added directly into the concrete matrix [8]. For these reasons, the bacterial cells are encapsulated in natural biopolymers such as sodium

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alginates, carrageenan, and chitosan to ensure their viability inside a concrete matrix [9,10]. These polymeric beads are 10 μm to 1 mm in size. They encapsulate either the living cells or the spores of the MICP performing bacteria alongside the nutrients. In some cases, the nutrients are separately added to the concrete matrix, while the beads contain only the bacterial cells or spores. Polymeric beads should be strong enough to withstand the mixing and placing of concrete while being weak enough to break during crack formation [10,11].

Several bacterial species in the natural environment of sea, lake waters, and soils can precipitate calcium carbonates under favorable conditions [6,12,13]. The calcite precipitation by microorganisms depends on the pH of the environment, concentrations of calcium ions and dissolved inorganic carbon, and the availability of nucleation sites [5,14]. The most common pathway studied for engineering applications is the decomposition of urea with the help of bacterial urease enzymes [15,16]. Through the metabolic process, the living bacterial cells produce urease that works as a catalyst in the urea hydrolysis into carbonate and ammonium, both of which increase the pH and carbonate concentration around the bacterial cells. Urease, further, hydrolyzes to ammonia (NH_4^+), and carbonic acid (CO_3^{2-}). Since the walls of the bacterial cells are negatively charged, they attract the cations (Ca^{2+}) from the surroundings and react with carbonate ions that lead to the formation of calcium carbonate [12].

Researchers have tested different bacterial species for MICP to seal cracks in concrete [17–20]. Since most bacterial species are not viable at a basic pH of around 9, only those that are resistant to high pH have been successful. The most commonly used bacterium is *Sporosarcina pasteurii* [9,21–23]. However, there is a continuing search for other bacterial strains to increase the reliability of crack healing in concrete. For example, Jonkers et al. [8] employed *Bacillus pseudfirm* (DSM 8715) and *Bacillus cohnii* (DSM 6307) directly in concrete and observed that both *Bacillus* species were able to produce calcium carbonates in the presence of calcium lactate. Hassan et al. [24] used *Bacillus pseudofirmus* and *Diaphorobacter nitroreducens* strains in a mortar matrix. They observed that crack widths up to 372 μm were at least partially filled with carbonate precipitates by both strains.

Several techniques have been employed to immobilize the bacterial cells or spores and encapsulate the nutrients to protect the bio self-healing agents from the alkaline concrete environment. Some examples of encapsulation materials are silica sol-gel [25], hydrogels [26], lightweight clay particles [27], glass granules made from recycled glass [28], and ethylene oxide and propylene oxide [26], among others. Alazhari et al. [29] incorporated expanded perlite (EP) (0/4 mm) in the mortar. The spores of *Bacillus pseudofirmus* were immobilized in separate EP particles, while nutrients such as calcium acetate, yeast extract, and dextrose were encapsulated in separate EP particles. Once impregnated with spores and nutrients, the expanded perlite particles were coated with sodium silicate solution and dry cement to block the leakage of encapsulated materials. The self-healing process was more pronounced with 20% EP as aggregate replacement. It was observed that the MICP is dependent on the number of bacterial cells and the amount of calcium nitrates in the mortar samples. Wiktor and Jonkers [11] impregnated lightweight clay aggregates with bacterial spores and nutrients of calcium lactate and yeast extract. The impregnated clay particles contained 6% calcium lactate and 1.7×10^5 bacterial spores per gram. Cracks of up to 460 μm wide were filled with the carbonate minerals generated by the bacterial action upon breaking of clay particles. Zamani et al. [30] encapsulated *Bacillus pseudofirmus* bacterial spores and calcium lactate in polyurea capsules having sizes between 0.2 mm and 1.3 mm. It was observed through thermogravimetric analysis (TGA) that spores and calcium lactate were viable and could produce MICP after breaking of capsules that encountered the formed cracks. Zhang et al. [28] used expanded glass (EG) granules as spores and nutrient carriers in the cementitious materials. The EG has a porosity of up to 82% to 84%, hence can accommodate a higher amount of spores and nutrients. The size of EP particles varied from 0.5 mm to 2.5 mm. The EGs are made from recycled glass, and hence are economical and sustainable. Cracks up to a width of 111 μm were completely filled with calcium carbonate formed by *Sporosarcina pasteurii* immobilized alongside calcium lactate in the EG granules. The naturally occurring biopolymers such as sodium alginates, carrageenan, and chitosan are the other suitable candidates for encapsulation of spores and nutrients [4,10,31].

An extensive collection of indigenous Qatari MICP-performing bacterial strains was created in an effort to achieve sustainable biological soil stabilization processes to minimize soil erosion from wind loading [32–34]. Several strains of *Bacillus cereus* were shown to be capable of MICP in the harsh Qatari conditions and the calcareous soil characterized with a high pH. This unique and intrinsic characteristic of the local strains is a result of an adaptation route to tolerate the local weather and soil conditions [33]. These physical and chemical attributes could be useful in conditions encountered in concrete. Since the sensitivity of bacteria to high pH and dry and hot environment limits its ability for bio self-healing through MICP and results in low urease activity. That is why the highly adapted bacterial strains from Qatar represent a potential advancement for bio self-healing at weathered conditions.

Bacillus cereus is ubiquitous in the environment, such as in the soil, food, and vegetation [35]. Their spores are present in soil from 10^2 CFU g^{-1} to more than 10^5 CFU g^{-1}; it is thus a frequent soil contaminant. A minority of illnesses (2–5%) are caused by *Bacillus cereus* [36]. The ability of *Bacillus cereus* to cause illness is also related to the pathogenicity of the strain and particularly to the density of spores or cells in food [37]. However, *Bacillus cereus* and the closely related species, particularly *Bacillus subtilis,* are considered mainly in biotechnology applications after confirming their Generally Recognized As Safe (GRAS) status. For microbiological procedures, common L-1 culturing safety procedures are employed. For MICP at laboratory or field scale, reintroducing the strain in the soil or in concrete only requires the commonly used biosafety measures.

In this study, an indigenous bacterial strain, *Bacillus cereus* selected from the Qatari MICP-performing bacteria collection, was investigated for MICP in the concrete matrix. The strain has already been proven efficient in soil stabilization through MICP [34]. Therefore, this strain is expected to be a promising candidate for bio self-healing in concrete. Its performance is also compared to the commonly used bacterium *Sporosarcina pasteurii* for the application in concrete, obtained from the American Type Culture Collection (ATCC® 11857™). The urease activity and minerals production, the viability in sodium alginate beads, and the bio self-healing ability in the concrete are compared for the two strains. The findings are important for further applications of bio self-healing materials with harsh internal conditions.

2. Experimental program

2.1. Urease producing bacterial strains

In this study, a local bacterial strain *Bacillus cereus* (Q3.3) (Accession No. CP011151.1) [33], was used. *Bacillus cereus* strain Q3.3 was isolated from the soil in Qatar alongside six other strains, which were tested for the MICP capabilities [33]. The selected strain was observed to be the most promising strain based on the urease activity and calcite mineral formation at temperatures exceeding 40 °C in the field [33]. The capability of calcium carbonate precipitation, sustained growth, and the viability of these strains have been demonstrated elsewhere [33,34]. The strains were preserved in the Qatari Mineral Precipitating Strains Bank at –80 °C in 30% glycerol. They were recovered by striking them on the solid Luria-Bertani (LB) medium to obtain viable, fresh, and pure cells. The second strain used in this study is *Sporosarcina pasteurii* obtained from ATCC® with the reference code ATCC® 11857™.

2.2. Growth media

Two types of culture media, Luria Bertani (LB) Medium and Urea

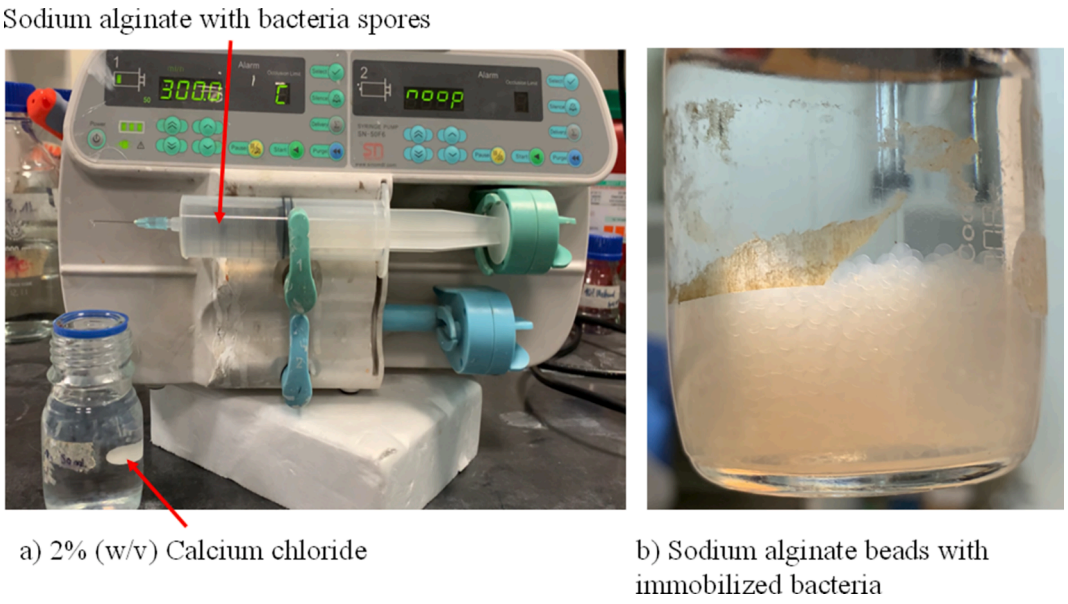

Fig. 1. Beading process; a) syringe pump is used for dripping sodium alginates in 2% CaCl2 solution, b) beads in the solution.

Table 1
Mortar mixture designs for self-healing through bacterial strains.

No.	Mixture type	Strain	Cement (g)	Sand (g)	Water (g)	w/c*	SP* (g)	Beads (g)	Urea (g)	YE* (g)	CN* (g)
1	Control mix (C)	–	666	1665	333	0.5	10	–	–	–	–
2	Control + beads (CB)	–	666	1665	333	0.5	10	20	–	–	–
3	Control + nutrients (CN)	–	666	1665	333	0.5	10	–	27	6	33
4	Control + nutrients + beads with S. *pasteurii* (SP-B-N)	*S. pasteurii*	666	1665	333	0.5	10	16	27	6	33
5	Control + nutrients + beads with *B. cereus* (BC-B-N)	*Bacillus cereus*	666	1665	333	0.5	10	16	27	6	33

*w/c = water-cement-ratio, SP = superplasticizers (Epsilon PC 485, polycarboxylate ether-based), YE = yeast extracts, CN = Calcium nitrate.

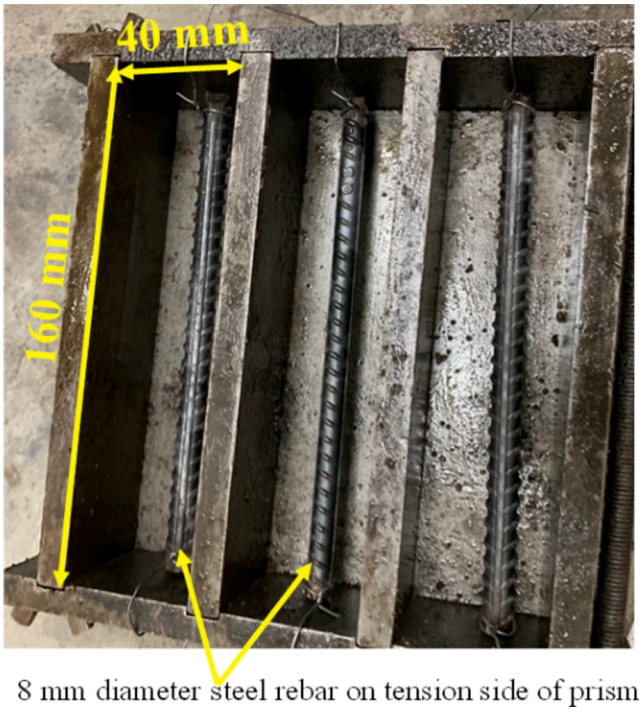

Fig. 2. The molds used to make the prism samples. A single rebar was embedded on the tension face for controlled crack generation.

Medium (UM) were used to grow the strains as inoculum with an initial OD_{600} of 0.15. The LB is composed of 5 g/l NaCl, 10 g/l tryptone, and 5 g/l of yeast extract. The UM was composed of 5 g/l NaCl, 2 g/l Peptone, 1 g/l glucose, 2 g/l KH_2PO_4, and 20 g/l of Urea 20 (filter sterilized). The solid medium was obtained by adding 18 g/l agar to each liquid medium. Then, the entire medium was sterilized by autoclaving at 121 °C for 20 min. The recovery of the strains was first made on solid media, and then they were inoculated in the liquid media to be incorporated in sodium alginate beads.

2.3. Determination of urease activity

Urease activity was measured for the two studied strains by determining the amount of ammonia in the solution using the hypochlorite-phenol assay with modifications [26,27]. Briefly, the crude enzyme (100 µl) was added to 900 µl of urease buffer (pH 7.5) containing 1 mM EDTA, 50 mM HEPES and 20 g/l urea. The mixtures were incubated at 25 °C for 10 min and then cooled in an ice-water bath. The enzyme-buffer solution (100 µl) was rapidly added to 200 µl of a phenol-nitroprusside solution composed of phenol 70 g/l and nitroprusside 0.34 g/l dissolved in Milli-Q water and stored at 4 °C in a dark bottle. 200 µl of freshly prepared buffered sodium hypochlorite solution was used to stop the reaction. The sodium hypochlorite solution (pH 10.2) was stored in a dark bottle at room temperature and contained the following quantities per liter 1.75 g NaOH, 5.9 g Na_2HPO_4, and 20 ml of 5% bleach. After 30 min of incubation at room temperature, the absorption measurements were obtained using a spectrophotometer set at 640 nm. The standard curve was performed using serial dilutions of a

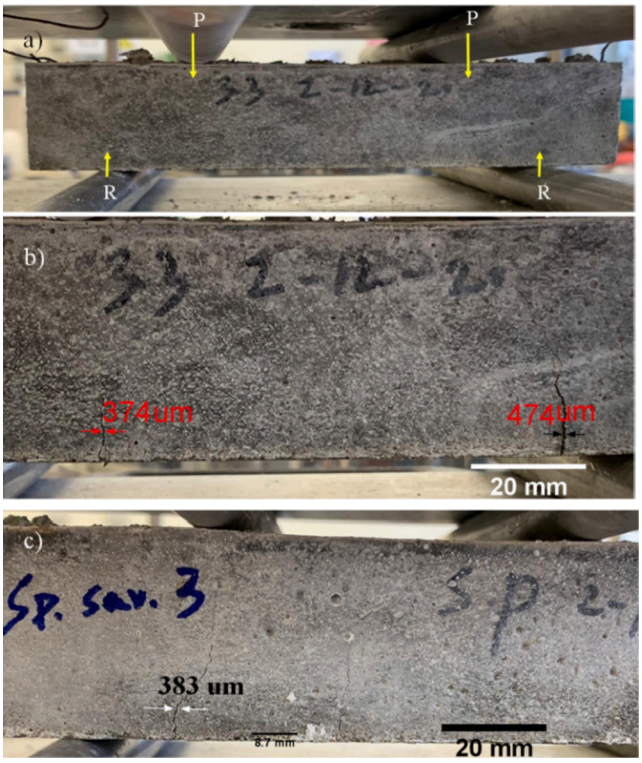

Fig. 3. Crack generation in prism samples. a) four-point bending loading set-up; b) cracks generated in the *Bacillus cereus* sample; c) cracks generated in the *Sporosarcina pasteurii* sample.

2×10^{-4}M of ammonium chloride stock solution. The standards were run at the same time the assays were done. The cultures and the urease activity were carried out in triplicate for each strain.

2.4. Immobilization of spores by encapsulation

2.4.1. Preparation of bacterial spores

Cultures were prepared in 50 ml sterile falcon tubes containing 25 ml of UM. The first inoculum was adjusted to obtain $OD_{600} = 0.15$. Next, the bacterial cultures were incubated for 7 days at 30 °C in a shaker set at 150 rpm. At the end of the incubation period, the falcon tubes were placed in a water bath set at 80 °C for 10 min and then were immediately placed in an ice bath for 5 min to induce spores. Subsequently, the germination of the spores was induced by culturing in UM medium for two days at 30 °C with agitation at 150 rpm to ensure that more than 90% of the cells are spores. The spores were then harvested by centrifugation at 5000 g for 15 min at 4 °C. The supernatant was discarded and the remaining suspension containing spores were washed twice with 0.1% (w/v) NaCl solution. The sporulation was confirmed using the malachite green staining method.

2.4.2. Manufacturing of beads

The beading process is illustrated in Fig. 1. Ten grams (10 g) of sodium alginate was mixed in 1 L of autoclaved distilled water making it a 1% w/v sodium alginate solution. The mixing was carried out for 120 min using a magnetic stirrer to achieve a homogeneous solution. Then the sodium alginate solution was sterilized by exposing it to UV for 12 h. Once the cells are converted into the spores in the media, from the previous step, the mixture was added to the sodium alginates solution to obtain a final concentration of 10^7 CFU/ml. The mixture was stirred for another 20 min at 42 °C to maintain the gel soluble. The mixture was sucked in a 60 ml syringe and it was introduced into a 2% calcium chloride solution, drop by drop, as shown in Fig. 1 (a). A syringe pump was used for this purpose. For larger quantities and rapid beads production, peristaltic pumps could be used. However, the sterilization of the pipes and pumps would be required. Fig. 1 (b) shows the beads in the $CaCl_2$ solution. Although the needle diameter was 0.23 mm, the droplets were up to 2 mm in diameter when formed in the $CaCl_2$ solution.

2.4.3. Recovery of the bacterial spores after the encapsulation process

The encapsulated bacterial spores were enumerated using the standard plate count method as colony-forming units per milliliter (CFU/ml). Alginate beads (0.1 g) were re-suspended in 9.9 ml of 10% sodium citrate solution. The solution containing beads was vortexed for 5 min at room temperature. The number of cells was determined by plate count on LB agar, serial dilutions of the dissolved beads were plated in triplicate and incubated at 30 °C for 24 h [38]. The evaluation of the spores' viability before and after encapsulation was carried out in triplicates. The encapsulation yield (EY) was calculated according to Eq. (1) [10],

$$EY\ (\%) = N/N_0 \times 100\% \quad (1)$$

where N is the number of viable encapsulated spores and N_0 is the number of initial spores before the encapsulation process.

2.4.4. Nutrients

The nutrients for the bacterial strains such as calcium nitrate, yeast extracts, and urea, were added as dry ingredients in the mortar matrix. They were not encapsulated in beads alongside the spores to avoid any growth before concrete cracking. The selected set of nutrients has been used by several researchers previously [10,13,24,26].

2.5. Self-healing in cementitious matrix

Table 1 presents the mixture proportions of the mortar. Five mixtures were prepared: the control mix, a mix with the beads only, a mix with the nutrients only, and two mixes with beads containing bacteria and the nutrients in the mortar matrix. The control mix contained cement, sand,

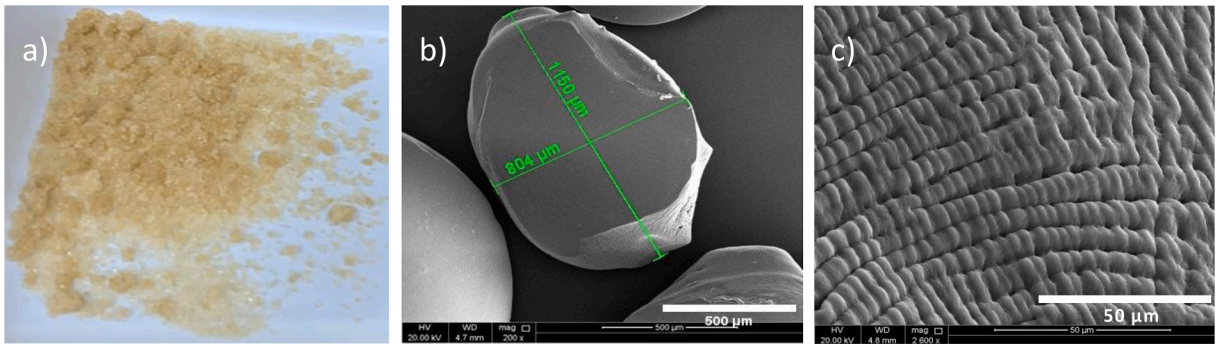

Fig. 4. a) Beads after drying at 45 °C for three days; b) an SEM image of a bead showing the shape and dimensions, and c) SEM close-up showing the porous surface of the beads.

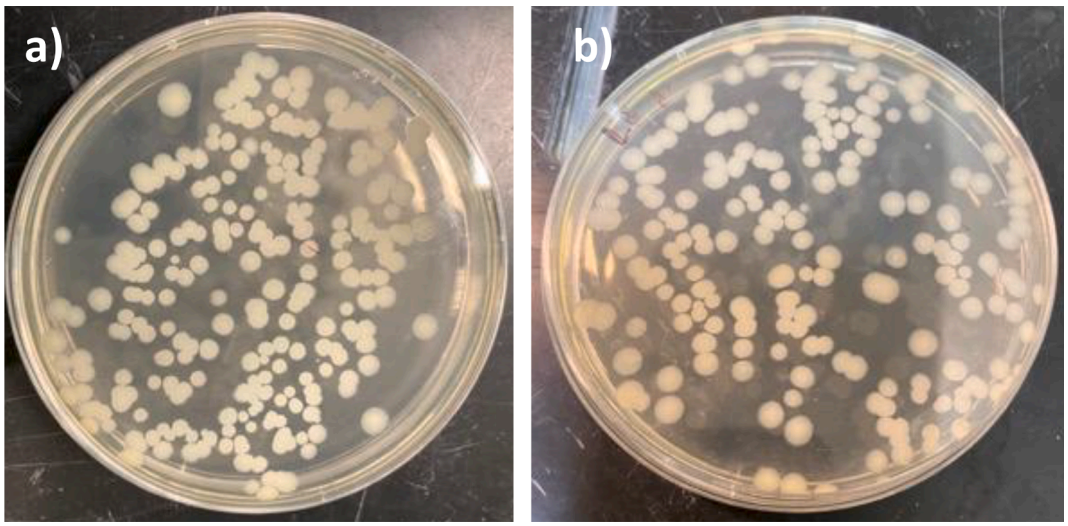

Fig. 5. Example of CFU (10^6/ml) for strain Q3.3, a) before encapsulation; b) after encapsulation.

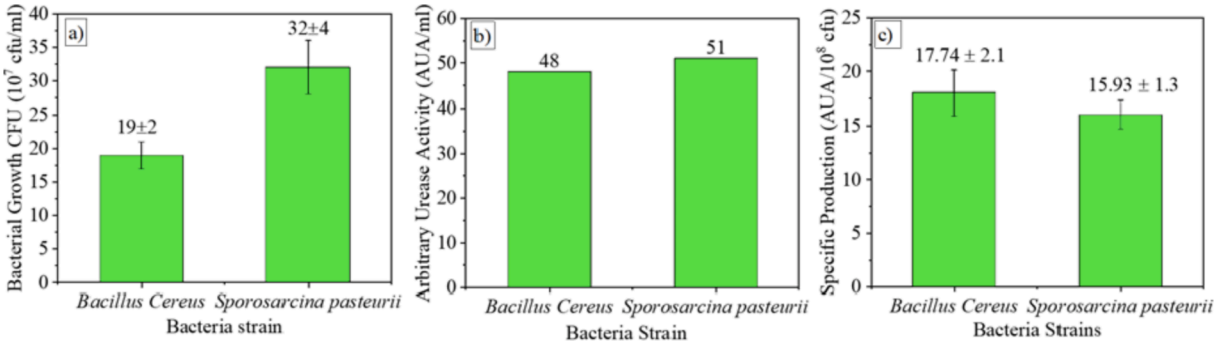

Fig. 6. Comparison of local strain *Bacillus cereus* and commonly used *Sporosarcina pasteurii*, a) growth in control media; b) arbitrary urease activity (AUA); and c) specific production.

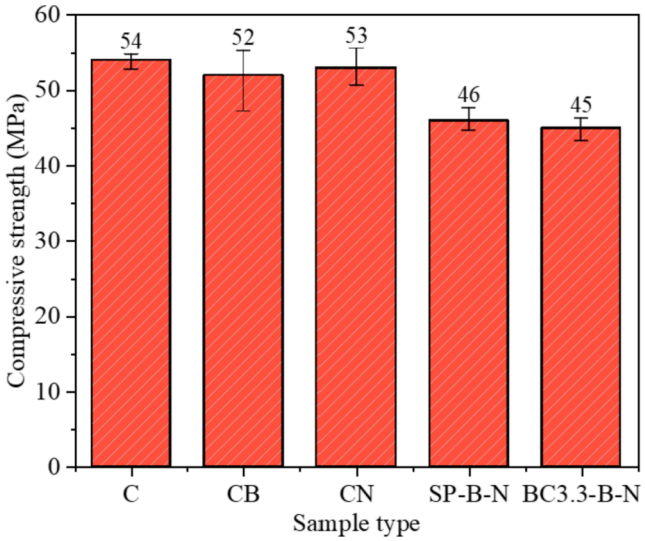

Fig. 7. Effects of beads and nutrients on compressive strength of mortar. C = control, CB = Control with Beads, CN = Control with nutrients, SP and BS are *Sporosarcina pasteurii* and Bacillus cereus, respectively.

and water. The cement to sand ratio was 1:2.5, whereas the water-to-cement ratio was 0.5. The second mix was prepared to observe the effects of sodium alginate beads on the compressive strength of the mortar. The added amount of beads was 3% by weight of cement while keeping the other ingredients the same as the control mix. Since urea, yeast extract, and calcium nitrate could also affect the concrete properties, a mortar mixture was prepared with these nutrients while the other ingredients were the same as the control mix. Two other mixes were prepared by adding sodium alginates beads containing either *Sporosarcina pasteurii* (ATCC® 11857™) or *Bacillus cereus* spores, and the nutrients were mixed in the mortar matrix. The beads were dried for 3 days at 45 °C before adding them into the mortar. The urea was added by 4% of the cement weight (27 g), yeast extract was 0.85% of the cement weight (4 g), and the calcium nitrate was 5% of cement weight (33 g). The decision to add 3% beads by weight of cement was made based on the amount of mortar required per mix to cast three prisms and three cube samples and the number of beads that could be obtained from 1 L of sodium alginate solution, which was approximately 16 g. This makes about 3% of cement weight used per mix. In addition, the mixture resembles the work done on self-healing with sodium alginate beads by others [10,11,24]. The selected amounts of nutrients (by weight of cement) were sufficient for bacteria to get enough nutrients to grow as will be demonstrated later.

2.6. Samples for compressive strength and crack generation

The effect of sodium alginate beads and the nutrients on the compressive strength of mortar was evaluated on 50 × 50 × 50 mm cubes prepared according to ASTMC109/C109M-20 [39]. To investigate the self-healing process in the cementitious matrix through the mineral

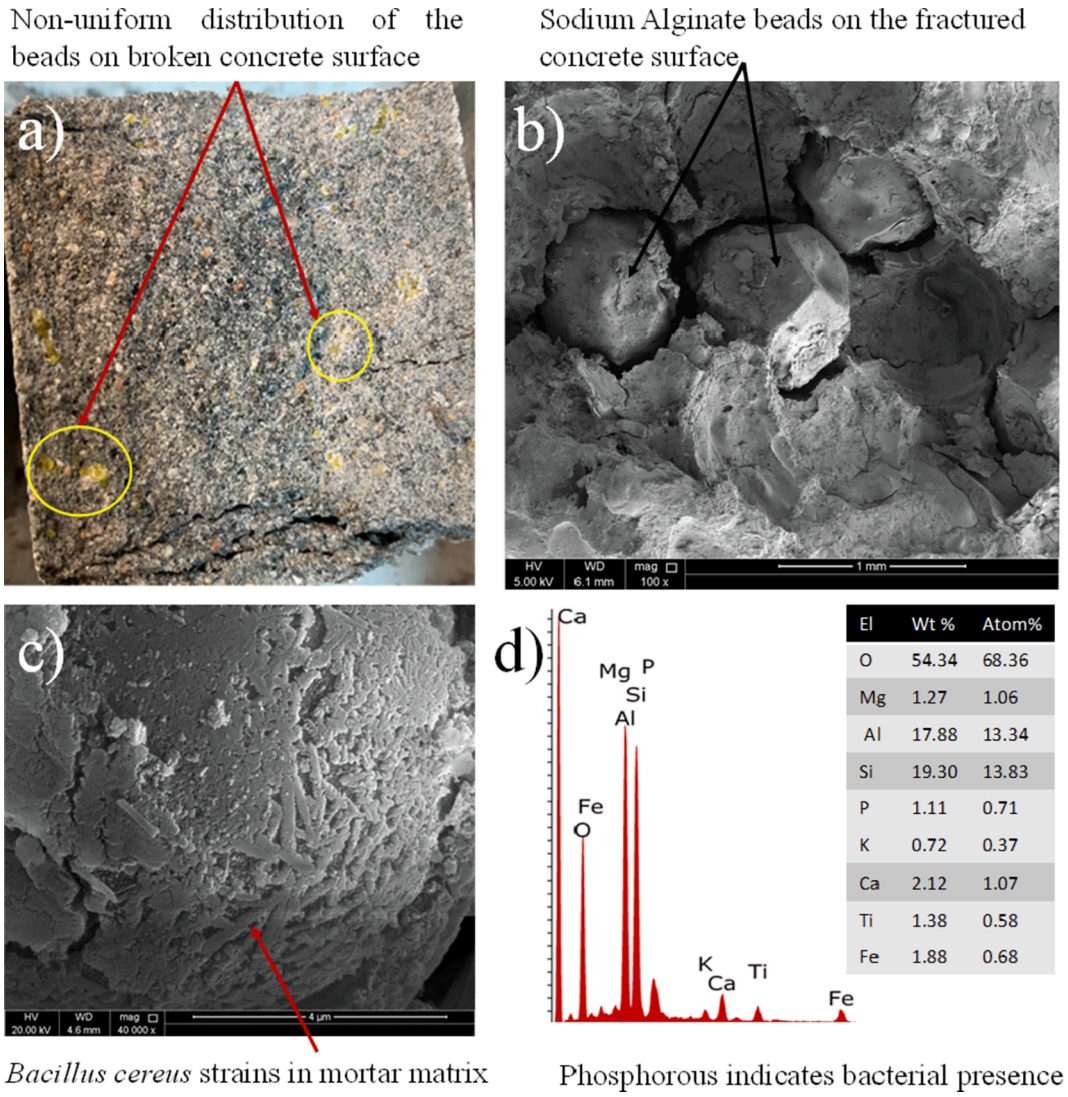

Fig. 8. a) A broken surface of a cube sample shows the spatial distribution of the beads inside the mortar; b) beads inside the mortar matrix; c) Bacillus cereus strains in the mortar matrix, showing their viability in the alkaline mortar matrix, and d) presence of phosphorous in EDX, which is an indicator of bacterial presence.

precipitation, singly reinforced prism mortar samples with dimensions of 40 × 40 × 160 mm were cast, as shown in Fig. 2. Having a steel rebar on the tensile face of the prisms facilitates generating well-distributed cracks in the prisms. The embedded steel rebar was 8 mm in diameter and 155 mm to 160 mm in length. Cubes and prisms were cured under 20 ± 3 temperature and 95% ±5 RH for 28 days.

Three cubes from each mix were tested under compressive load using a hydraulic universal testing machine. Whereas three prism samples were loaded under four-point bending using a manual hydraulic machine. Circular stainless steel bars of 16 mm diameter were used as reaction supports at the bottom and the top-loading points of the prism samples as shown in Fig. 3. The load was applied manually until three or four cracks appeared in the samples. The width of the cracks was measured through digital image analysis using ImageJ software [40]. The images were taken with a high resolution digital camera. The formed cracks had widths ranigng from 100 μm to 800 μm. Since there was no shear reinforcement, the cracks appeared in the shear zone on both sides of the prism. The location of the loading and support points could be changed to form more cracks. One prism sample with *Sporosarcina pasteurii* was broken was tied up with steel wires. This sample could provide insights into whether the broken portions of concrete can also be healed and held together by the calcium carbonate minerals.

It is expected that the beads were broken or distorted in the formed cracks and the bacterial spores were exposed to the moisture and nutrients in the mortar matrix. The cracked prism samples were immersed in distilled water to increase the urease activity of the bacteria and generate mineral precipitation.

2.7. SEM and XRD analysis of precipitation

The microstructure and morphology of calcite precipitation were observed with the help of SEM images of the crack surfaces. An FE SEM NOVA® instrument was used for SEM images. The mineralogy of calcites was investigated with EDX and XRD analysis. A PAN Analytical® EMPYREAN instrument with Cu-Kα radiations was used for the XRD analysis. The scan was carried out for 2θ values between 10° and 90° with a step size of 0.01°.

3. Results and discussion

3.1. Manufacturing of beads

Fig. 4 (a) shows the dried beads. One liter of sodium alginate yielded about 16 g of dried beads. Fig. 4 (b) presents the size and shape geometry of the beads through the SEM images. The dry beads were up to 1 mm in width and breadth. Hence, the shape and geometry of dried sodium

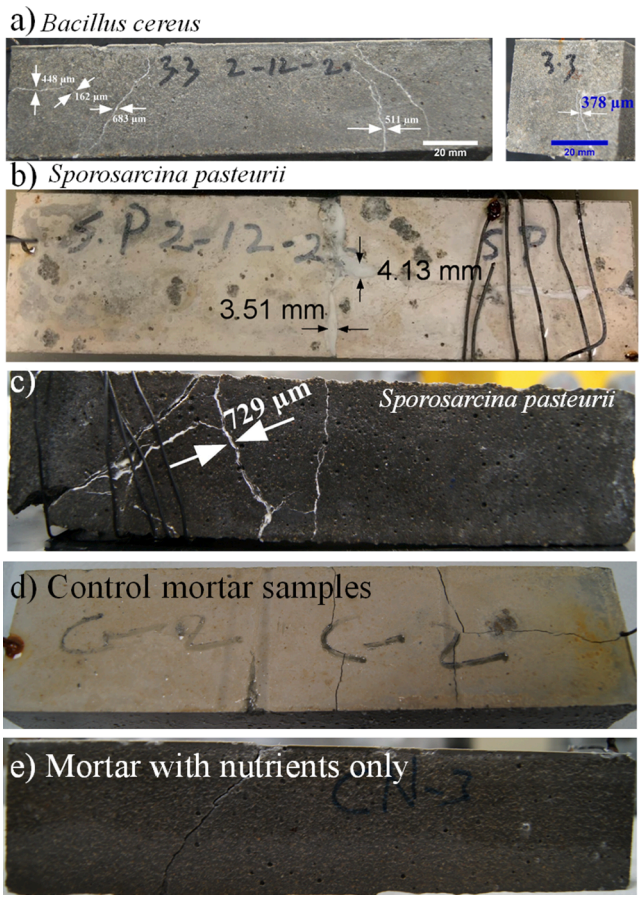

Fig. 9. Self-healing process: cracks filled with calcium precipitate minerals formed by bacterial strains. a) Cracks filled by *Bacillus cereus*; b) and c) mineral precipitation by *Sporosarcina pasteurii* filled up to 4.32 mm wide cracks; d) and e) no precipitation in the control mortar samples and mortar with nutrients only, respectively.

Fig. 10. A broken prism sample with separated pieces bonded together with calcium carbonate formed by bacteria.

alginate beads resembled the sand particles in concrete. The walls of the beads were porous and could absorb water and swell (Fig. 4 (c)). When in wet conditions, beads had a jelly-like structure and they were flexible, whereas they became hard and brittle in dry conditions. Therefore, they could resist the mixing as they become wet and flexible during concrete processing and break in dry conditions when the cracks form.

3.2. Recovery of the bacterial spores after the encapsulation process

The encapsulation yield (EY) was evaluated using the standard plate count method with the assumption that each colony originates from a single cell (or spore), counted as colony forming units (CFU/ml). Fig. 5 shows the (CFU/ml) through serial dilution of *Bacillus cereus* before and after encapsulation. The dried beads were introduced in the UM and left for three days. Then, serial dilutions were performed. The results of EY for encapsulated *Bacillus cereus* (Q3.3) and *Sporosarcina pasteurii* were (%) 92.25 ± 3.4 and 91.76 ± 2.1, respectively. Thus, there was no significant difference between the two strains in terms of encapsulation recovery.

3.3. Measurement of urease activity

The bacterial growth in terms of CFU and urease activity was determined in the culture using 20 g/l urea after 3 days of incubation. The relative growth in the control media, arbitrary urease activity (AUA), and specific productions of both strains are compared in Fig. 6 (a), (b), and (c), respectively. The growth of local *Bacillus cereus* was 19 $\pm 2 \times 10^7$ CFU/ml, whereas for *Sporosarcina pasteurii* the same was $32 \pm 4 \times 10^7$ CFU/ml. The growth in the selected time was almost double for *Sporosarcina pasteurii* compared to the *Bacillus cereus* in the urea media. The arbitrary urease activity was 48 AUA/ml and 51 AUA/ml for *Bacillus cereus* and *Sporosarcina pasteurii*, respectively, which was almost the same for both strains. On the other hand, Bacillus cereus and *Sporosarcina pasteurii* specific urease activities were 17.74 ± 2.1 AUA/10^8CFU and 15.93 ± 2.1 AUA/10^8CFU, respectively. The *Bacillus cereus* strain showed a higher potential for mineral production compared to the *Sporosarcina pasteurii*. ANOVA analysis at 95% confidence level revealed that the significant variance *p value* is less than 0.05 for the specific production of urease (AUA) between the local strain (*Bacillus cereus*) and the *Sporosarcina pasteurii*.

3.4. Compressive strength with beads and nutrients

Although sodium alginate beads are strong enough to resist the mixing process, they are porous and have jelly-like structures, which could affect the strength of the concrete. In addition, the urea and sugary ingredients of yeast extract could negatively affect the concrete's mechanical properties. The parametric study on the effects of beads and nutrients was not carried out as the focus of the study was to investigate whether the local *Bacillus cereus* strain encapsulated in sodium alginate beads could perform MICP and seal a concrete crack. Therefore, only the impact of the employed beads' quantity on the concrete strength was studied. Fig. 7 shows the effects of beads and nutrients added, alone and in combination, on the mortar's compressive strength. It was observed that the beads up to 2.5% had no significant impact on the compressive strength of the concrete. Control samples had an average strength of 54

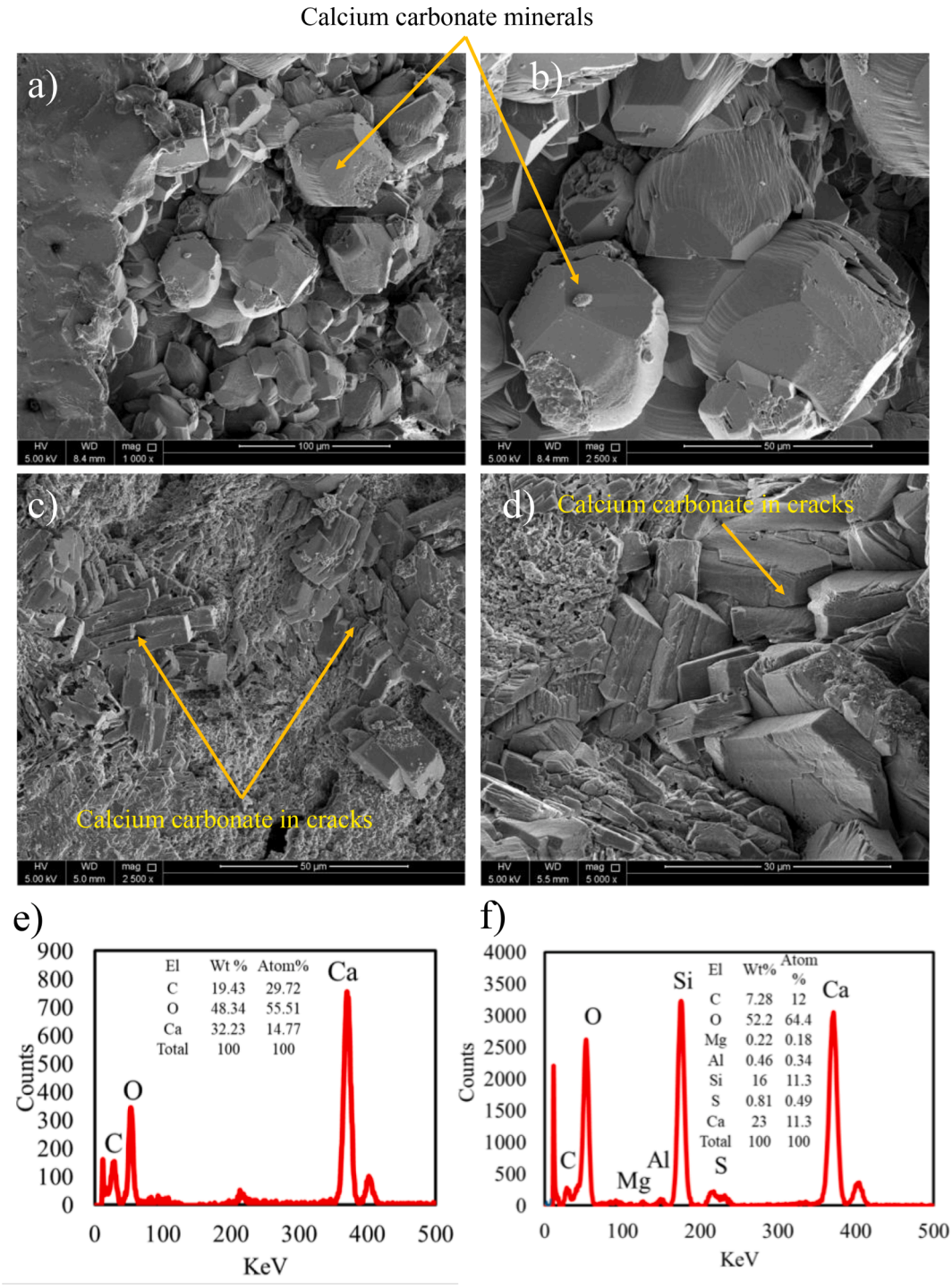

Fig. 11. SEM images of white precipitation inside the cracks, a) and b) *Bacillus cereus* samples; and c) and d) *Sporosarcina pasteurii* samples; f) EDX on the white precipitation, and e) EDX results for mortar away from the crack zone.

MPa with minimum and maximum values of 53 MPa and 55 MPa, respectively. At the same time, the samples with the beads showed an average compressive strength of 52 MPa with minimum and maximum values of 48 MPa and 54 MPa, respectively. The samples with nutrients had an average compressive strength of 53 MPa with the minimum and maximum values of 50 MPa and 55 MPa, respectively. The difference in strength falls within the expected variability of mortar samples with no modifications.

On the other hand, the strength was reduced when the nutrients and the beads are used together. The average strength of the mixes containing *Sporosarcina pasteurii* and *Bacillus cereus* bacteria spores in the beads and nutrients in the mortar matrix were 46 MPa and 45 MPa, respectively. The loss in strength was 15% and 17%, respectively, for the samples of two bacterial strains. A similar range of strength loss in the mortar matrix is observed with 3% sodium alginate beads by Hassan et al. [24]. The relatively small reduction in the strength can be easily compensated by adjusting the w/c ratio of the mixes.

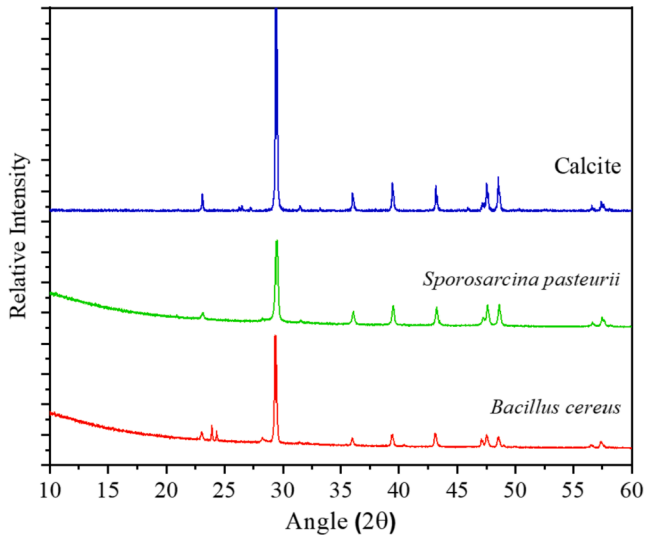

Fig. 12. XRD analysis of crack filling material in the samples with Sporosarcina pasteurii and Bacillus cereus. XRD peaks confirm the presence of calcium carbonates minerals with both types of strains.

3.5. Viability of beads and bacterial strains

Fig. 8 (a) and (b) show the distribution of the beads in the mortar matrix of a cube sample and the SEM image of a broken surface, respectively. The number of beads added was 2.5% (16 g) by the weight of cement. This small amount reduces the possibility of uniform distribution of the beads in the mortar matrix. In addition, after drying, the beads tend to stick together, which further impairs their uniform distribution. It is observed in Fig. 8 that the beads were locally concentrated in the mortar matrix at different locations. This reduces the possibility of cracks passing through the beads. In this study, the beads were separated with rodding and vibration; however, it is recommended to coat the beads with silica to prevent them from agglomerating. Fig. 8 (c) shows the viability of the strains in the cementitious matrix after crack formation. Elongated thin cells of *Bacillus cereus* of length about 1 μm were visible. Fig. 8 (d) presents EDX spectra at the location where bacterial cells were seen. The phosphorous peaks were observed, which is an indicator of bacterial presence.

3.6. MICP in cementitious matrix

As mentioned earlier, after the cracks were formed, the prism samples were immersed in distilled water to facilitate bacterial growth and urease activity. The samples containing the encapsulated spores of *Bacillus cereus* and *Sporosarcina pasteurii* are shown in Fig. 9 after 4 days of immersion underwater. The formed cracks were filled with white precipitates of calcium carbonate minerals. Those cracks, which passed through the beads, were completely sealed. Cracks with widths ranging from 162 μm to 683 μm were filled with white precipitates by the local strains of *Bacillus cereus*. Whereas, with *Sporosarcina pasteurii*, cracks up to 4.13 mm wide were filled with carbonate precipitates. It is noteworthy that the crack widths were uncontrollable during the loading, and hence were different in each sample. Each strain was only able to fill the available widths. That is why the size of the filled cracks was different for the two strains. Excess calcium precipitates were observed at the surface of prism samples for both strains, indicating that higher crack widths might also be filled.

One of the prism samples was broken during the cracking process, and the pieces were held together with steel wires wrapped around it. It was observed that the separated pieces were glued together with the calcium carbonate formed by *Sporosarcina pasteurii*, as shown in Fig. 9 (b),(c), and Fig. 10.

The control mortar, nutrients, and samples with beads were also immersed underwater after cracking. Fig. 9 (d) and (e) show the control and the samples with nutrients only, respectively. These samples did not have calcite precipitation, and empty cracks were visible after 14 days of immersion underwater.

Fig. 11 presents the SEM images of the cracked portions in prism samples. Fig. 11 (a) shows the white precipitate from the *Bacillus cereus* sample. Whereas Fig. 11 (b) shows the shape of these calcite particles at a 2500x magnification. Fig. 11 (c) and (d) show magnified images from the samples having *Sporosarcina pasteurii* embedded in the beads at 2500x and 5000x magnifications, respectively. The rhombohedral and cubic-shaped calcium carbonates minerals were observed on the cracks in both types of samples. Fig. 11 (e) shows the element composition through EDX spectra of white precipitates in the crack zone. The EDX spectra for the mortar composition away from the crack is shown in Fig. 11 (f). The white precipitates only yielded the elements of calcium carbonates, that is, calcium, carbon, and oxygen. Whereas the mortar's EDX showed silica, aluminum, magnesium, calcium, oxygen, and carbon. The weight percentage of each element also confirms that the precipitates are of calcium carbonate.

The XRD analysis of white precipitates filling the cracks was also performed to observe the mineralogy. Fig. 12 shows the XRD spectra of the material filling produced by *Sporosarcina pasteurii* and *Bacillus cereus*. Also, a spectrum of calcite is shown for reference purposes. Both materials were similar in mineralogy: calcium carbonate minerals (calcite). The minor XRD peaks at an angle from 22.5 to 25 may be due to impurities from the cementitious matrix.

4. Conclusions and recommendations

The self-healing process in concrete through microbial-induced calcium carbonate precipitation (MICP) produced by two bacterial strains is investigated. One of the strains, *Bacillus cereus*, was isolated from local Qatari soil. This strain was selected based on its growth, urease activity, and mineral production capabilities compared to the other strains found in the Middle Eastern soils. The second strain was *Sporosarcina pasteurii* (ATCC® 11857™), a commonly studied bacteria species in the laboratory for MICP in concrete.

- It was observed that local *Bacillus cereus* are capable of producing mineral precipitation in the same quantities as *Sporosarcina pasteurii* in the urea growth media.
- The experiments demonstrated that the *Bacillus cereus* strain could withstand the harsh environment of concrete and it is able to produce calcium carbonate minerals.
- The cracks of up to 683 μm were filled with the produced minerals. In addition, the MICP generated by *Sporosarcina pasteurii* bonded together separated concrete pieces and filled cracks up to 4 mm.
- The sodium alginate beads were up to 2.5% by weight of cement, reducing the concrete compressive strength by up to 18%. Whereas the added quantity of the nutrients such as yeast extract (0.85% by weight of cement), urea (4% by weight of cement), and calcium nitrate (5% by weight of cement) had negligible affects on the compressive strength of concrete.
- The results presented here are from a preliminary study on the local strains. An extensive study on the durability characteristics of self-healing concrete with these strains is required, where permeability, sorptivity, and corrosion resistance are investigated. For future research, it is recommended to investigate the MICP of these bacteria at high temperatures up to 50 °C and humidity up to 100%, which are commonly encountered in many parts of the world. In addition, parametric studies to determine the optimum quantities of beads and nutrients in concrete are needed.

CRediT authorship contribution statement

Muazzam Ghous Sohail: Formal analysis, Data curation, Writing – original draft, Visualization, Writing – review & editing. **Zulfa Al Disi:** Methodology, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Nabil Zouari:** Conceptualization, Methodology, Validation, Writing – review & editing, Resources, Funding acquisition, Supervision, Project administration, Investigation. **Nasser Al Nuaimi:** Conceptualization, Methodology, Supervision, Writing – review & editing, Project administration, Investigation. **Ramazan Kahraman:** Resources, Funding acquisition, Investigation, Formal analysis, Writing – original draft, Supervision, Project administration. **Bora Gencturk:** Resources, Funding acquisition, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Validation. **Debora F. Rodrigues:** Resources, Funding acquisition, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Yucel Yildirim:** Conceptualization, Methodology, Resources, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

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