



Evaluation of Portland and Pozzolanic cement on the self-healing of mortars with calcium lactate and bacteria

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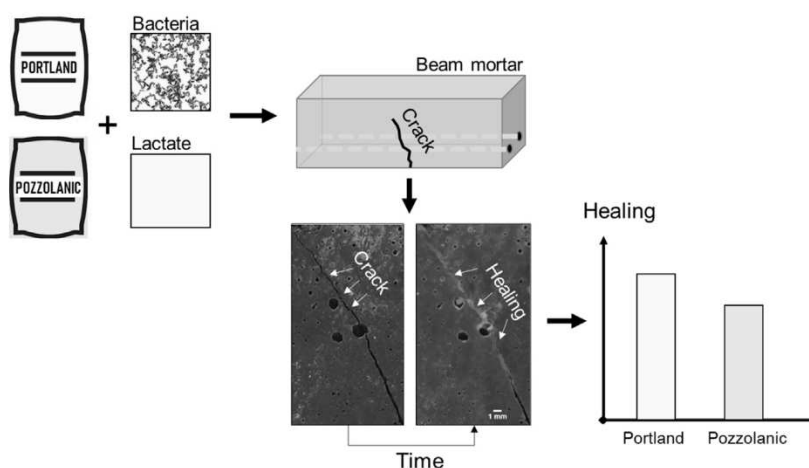
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HIGHLIGHTS

- Self-healing of Portland and Pozzolanic cement mortars with bacteria was studied.
- Self-healing in Portland cement mortar is higher than Pozzolanic cement mortar.
- Self-healing on mortars with lactate only was observed.
- Crack filling from self-healing shows presence of calcite.

GRAPHICAL ABSTRACT



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ABSTRACT

This article studies the effect of Portland and Pozzolanic cement on the crack-healing of mortars prepared with *Bacillus pseudofirmus* bacteria and calcium lactate as a nutrient. Mixtures were prepared with the same content of cement, water, sand, and Light Weight Aggregate. Results showed an inverse relationship between crack width and self-healed area, with most of the healing occurring between 0 and 21 days of sample conditioning. The healing on samples with calcium lactate only and calcium lactate + bacteria was significantly higher than in control samples. Results indicate that Portland cement better promotes the self-healing of cracks compared to Pozzolanic cement.

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1. Introduction

Concrete is one of the most used construction materials, explained by its good workability, high compressive strength and

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durability, and low cost-effectiveness ratio. The concrete industry is one of the largest in the world, with more than 6 million m³ produced each year [1], and also one of the most contaminating industries, contributing to 7% of CO₂ emissions, the main gas responsible for global warming and climate change [2]. In addition, large amounts of CO₂ are produced in cement plants by the combustion of raw materials used for clinker production, one of the materials necessary for cement production. This combustion needs to increase the temperature up to 1500 °C, therefore a considerable energy quantity is also required [3,4]. Concrete is a brittle material that fractures under tensile and compressive loading, although compressive strength is higher than tensile strength. For this reason, steel reinforcement is installed in order to carry the tensile forces after cracking [5]. This cracking is not necessarily the failure of reinforced concrete, unless the prevailing crack width exceeds a threshold. However, cracks increase concrete permeability and the pass of undesirable fluids, exposing concrete reinforcing steel to oxygen and moisture, and external agents such as chloride and sulfate ions among others that cause steel corrosion, concrete degradation, and potential structural failure [6]. In addition, cracks cause leakage in concrete structures, such as water reservoirs, roofs and water pipes. These leaks negatively affect functionality [5]. Hence, controlling width and sealing cracks increase durability and service life of concrete structures, reduce concrete consumption, use of raw materials, and CO₂ emissions, and reduce the effects of global warming and climate change.

The mitigation of cracking in concrete structures could be carried out by manmade repair, which in some cases is not an efficient solution because this demands high direct and indirect costs, may result in low repair durability, and frequently encounters difficult access to the damaged concrete, such as cracks in underground concrete structures [5]. In comparison, self-healing cracks in concrete is beneficial and avoids most of the problems mentioned above [7]. For example, self-healing cracks blocks the crack pathway, preventing water leakage, and inhibiting ingress of aggressive ions, water and oxygen, making corrosion of steel reinforcement more difficult. In other words, self-healing concrete could represent an attractive solution to mitigate cracking, increasing durability, and hence contribute to the lessening the environmental problems related to concrete production and repair.

Researchers use different techniques for increasing the self-healing capacity of concrete: mineral admixtures, adhesive agents, and bacteria [5,8–10]. Recently, self-healing concrete using bacteria agents has gained popularity. The basis of this method is the incorporation of mineral-precipitating bacterial spores with nutrients in the concrete mixture. The spores, or dormant bacteria, become active upon contact with water and start metabolizing and precipitating calcium carbonate. This formation of the healing material is the result of bacterial synthesis of available nutrients into calcium carbonate (CaCO₃), which is a bacterial byproduct that can fill cracks to a certain degree and thereby reseal the concrete matrix. Biomineralization is the name of the mechanism performed by the bacteria, and this process leads to the formation of mineral materials produced by living organisms [8]. Other examples of biomineralization found in the environment and human body are shells, bones, teeth, and kidney stones. These organic-inorganic structures are produced by a selective extraction and absorption of ions from the environment, reaching a high degree of organization [11].

Jonkers and Schlangen [10] proposed a self-healing mechanism with bacteria based on the metabolic conversion of suitable organic compounds to calcite. Although researchers have proven the effectiveness of certain bacteria in the crack-healing in concrete [8,12–17], it is not yet a method applicable in practice because there are high costs involved and there is a

lack of performance data. For example, the influence of cement type on the performance of bacteria self-healing has not been reported. This could be an important effect, since cements have different chemical composition that generates different products after hydration, such as calcium hydroxide [18–22]. Another important factor that affects bacterial activity and biomineralization is temperature [23–27]. Since concrete structures could be exposed to very different temperatures depending on location, altitude, and specific climatic conditions, it is important to understand the interaction of environmental variables with self-healing concrete.

In this research, the authors used two types of commercially available cement produced in Chile, i.e., a Portland cement and a pozzolanic cement, in order to evaluate the feasibility of this technique for concrete applications. The study of different types of cement is particularly important in countries like Chile, Italy, China, the USA and other countries where, for geological reasons, limestone is not readily available and hence it is more expensive than pozzolans [28–30]. In addition, the study analyzes the effect of temperature on the self-healing of concrete with bacteria to evaluate the possibility of using this method in areas with vastly different climates and temperature variation. This research was limited to the comparative study of self-healing mortars prepared with Portland and pozzolanic cement at one conditioning temperature of 23 °C. In addition, the research was limited to the study of the conditioning temperature effect on the self-healing of mortars prepared with Portland cement only.

2. Materials and test methods

2.1. Cultivation of bacteria

The *Bacillus pseudofirmus*, a spore-forming bacterium that is facultative alkaliphilic, was selected for this study. Researchers investigated the potential for spore-formation and calcite production of this strain [12,31,32], confirming that is suitable for the crack healing of concrete. *B. pseudofirmus*, imported from the Belgian Co-ordinated Collections of Micro-Organisms (BCCM) code LMG 17944, grow in a basic medium (pH range between 7.5 and 11.4) and tolerates surges in external pH [23]. The lyophilized bacteria stocks were stored at –80 °C in glycerol before cultivation at the microbiology laboratory of the faculty of medicine, Universidad del Desarrollo. The liquid medium for bacteria culture, prepared following the supplier recommendations, consisted of 5 g peptone, 1 g meat extract, 20 g Agar, 2 g yeast extract, and 5 g NaCl dissolved in 1 L distilled water. For the pH adjustment, a solution consisting of 4.2 g NaHCO₃ and 5.3 g Na₂CO₃ dissolved in 100 ml distilled water was added to the liquid medium. Once the liquid medium achieves a pH of 9.7, and was sterilized by filtration (cellulose acetate, pore size 0.22 µm), it was left for 24 h in the incubator to verify the inexistence of microorganism growth. The lyophilized bacteria was inoculated into the liquid medium to create an initial bacteria culture, which was used to inoculate a second culture to enhance spore formation according to Jonkers et al. [15]. Bacteria cultures were aerobically incubated for 24 h in Erlenmeyer flasks on a shaker table at 190 rpm and 37 °C to increase sporulation. The spectrophotometer available at the microbiology laboratory monitored bacteria growth by optical density at 620 nm (PHOmO, Autobio). Bacteria culture were centrifuged for 20 min at 6000 rpm and re-suspended twice in sterile distilled water. The final concentration of *B. pseudofirmus* was 1.5 × 10⁹ cells/mL, measured using the most probable number method cultivation-dilution technique. *B. pseudofirmus* bacteria were stored in sterile distilled water at 4 °C for the preparation of the self-healing agent explained below.

2.2. Production and impregnation of the healing agent into the lightweight aggregate

The healing agent is produced by blending the bacteria agent, calcium lactate (200 g/L), and yeast extract (5 g/L) with a spatula in a sterile glass. The yeast extract content was determined by previous researchers [33]. The healing agent was impregnated into Lightweight Aggregate (LWA), with the objective of protecting bacteria against a non-favorable environment during the mechanical mixing of concrete. LWA is an expanded clay, i.e., a light and porous aggregate suitable for the impregnation of the healing agent. LWA maximum particle size was 4.75 mm, with a fraction of 5.8% smaller than 0.15 mm (Table 1). The water absorption of LWA was measured using ASTM C1761-13b [34] laboratory standard for light aggregates. The water absorption after 24 h is 12.01% (Table 2). For the impregnation, oven dried LWA was soaked for approximately 12 h in the healing agent. The healing agent used was 12% of the LWA weight. At this stage, the bacteria changes from being contained in a liquid environment to a solid one; hence, this conversion is considered a critical point in the study. The soaked LWA was sealed in plastic bags at 4 °C to reduce the risk of bacteria activation before the mixing process.

2.3. Viability of bacteria

To verify *B. pseudofirmus* viability, the bacteria population was counted using the most probable number method at different stages of the project. This technique quantifies the number of bacteria in different samples, and measures bacteria concentration in both liquid and solid media.

The population of the bacteria diluted in distilled water (Section 2.1) was measured by obtaining a sample of 1 ml. Then, the sample was diluted serially in a tube with 9 ml of distilled water 0.85% NaCl. The procedure was repeated in series until reaching a 7:10 dilution. From each dilution, 5 drops of 5 µl were inoculated in an area of a Petri dish divided into 5 zones according to the dilution factor. The drops with bacteria were left at 25 °C to grow. The value obtained according to the number of inoculum grown by dilution is extracted from the population estimation table used in the most probable number method. The concentration of *B. pseudofirmus* in the culture or bacteria agent was 1.5×10^9 cell/ml, which is a suitable density according to other researchers [15]. At this stage of the experimental procedure, it is important to have a high bacteria concentration because impregnation and concrete mixing are harsh conditions for microorganisms. Hence, the expected trend indicated that the bacteria density would decrease in the next stages of the experimental work. The second count was performed on the self-healing agent impregnated in the LWA because the bacteria goes from being contained in a liquid environment to a solid one. Following the same procedure described above, a 1 ml sample was obtained from the LWA 24 h after the impregnation of the self-healing agent for most probable number analysis, obtaining a concentration of 1.5×10^7 cel/g. Researchers typically have not reported bacteria count on the LWA before mixing [35]. The third count was performed on a hardened mortar. In

Table 1 Particle size distribution of LWA.

Sieve Number	Size [mm]	Passing [%]
3/8"	9.5	100
4	4.75	65.7
8	2.36	23.7
30	0.6	15.8
50	0.3	11.1
100	0.15	5.8
200	0.075	0.2

Table 2 Water absorption percentage of LWA.

Time [h]	Absorption [%]
4	7.74
24	12.01
48	12.21
72	16.99

this case, the sampling methodology is slightly different to that applied for liquid samples. Instead of 1 ml, 1 gr of mortar with bacteria was obtained from a sample of fresh mortar and from a sample of hardened mortar after 28 days of preparation. The samples of mortar were ground and diluted in a test tube with 9 ml of saline solution and then mix by pulse-vortexing for 30 s. The observed density was 3.2×10^6 cel/gr and 2.6×10^6 cel/gr for the fresh mortar and hardened mortar after 28 days, respectively. As expected, the bacteria concentration decreased from the culture to the hardened mortar.

2.4. Mortar mixture design

For each cement type, three mortar mixture were prepared: Control mixture (C), Lactate only (L), and Bacteria mixtures (B) (Table 3). Mixtures with Lactate only are included because good self-healing results were observed by Stuckrath et al. [31] in this type of mortars. All the mixtures were prepared with the same amount of cement, free water, sand and LWA. In the control mixture (C) LWA was pre-soaked in tap water. In the lactate mixture (L) LWA was pre-soaked in calcium lactate (without the healing agent). In the bacteria mixtures (B) LWA was pre-soaked with the healing agent, containing bacteria, lactate and yeast, as previously described.

Water to cement ratio was fixed at 0.50 in all mortars in order to increase cement hydration, reducing the potential autogenous self-healing of concrete (by late hydration of unreacted cement particles), and isolating the self-healing effect of bacteria.

Two representative Chilean cement types were used to study the self-healing behavior: ordinary Portland cement (PT) and common Pozzolan cement (PZ). The tests used for characterization of the cement types were the specific weight, according to NCh 154 of 69 [36]. The average specific gravity for PT and PZ was 3.19 g/ml (SD = 0.0089 g/ml) and 3.15 g/ml (SD = 0.0115), respectively. Table 4 shows the chemistry composition of cements.

2.5. Specimen preparation, curing and experimental matrix

Flexural beams (40 mm × 40 mm × 160 mm) and cubical specimens (50 mm) were prepared for the visual self-healing quantification and compressive strength, respectively. Each specimen was made in triplicate for statistical purpose. All specimens were demolded 24 h after mixing and then, specimens were cured in tap water at room temperature (15–20 °C) for 28 days.

Flexural beams were cracked in the three point bending frame applying a monotonic load. To avoid a brittle failure, the flexural beams were reinforced using two steel wires (diameter = 2 mm, length = 160 mm). The wires (Fig. 1) were placed parallel to the 160 mm axis at 10 mm from the bottom of the flexural beam with a separation of 20 mm. Each flexural beam sample was placed in the loading machine together with the three point bending frame. Each sample was loaded until approximately the same load to achieve a similar cracking level.

Once the specimens were cracked, they were separated in three groups. Each group of flexural specimens were left in tap water at 4, 23, and 40 °C to study the effect of temperature on the self-

Table 3
Mixing proportions of mortar specimens.

Mixture ID	Description and Cement type	Cement [kg/m ³]	Water [kg/m ³]	Sand [kg/m ³]	LWA [kg/m ³]	W/C
C – PT	Control – Portland	530	265	690	300	0.5
C – PZ	Control – Pozzolanic	530	265	690	300	0.5
L – PT	Lactate only – Portland	530	265	690	300	0.5
L – PZ	Lactate only – Pozzolanic	530	265	690	300	0.5
B – PT	Lactate + Bacteria – Portland	530	265	690	300	0.5
B – PZ	Lactate + Bacteria – Pozzolanic	530	265	690	300	0.5

Table 4
Chemical composition of Portland and Pozzolanic cements.

Compound	Type of cement	
	Portland (%)	Pozzolanic (%)
SiO ₂	20.0	23.0
Al ₂ O ₃	5.4	5.8
Fe ₂ O ₃	3.3	2.8
CaO	64.5	61.5
MgO	1.6	1.9
SO ₃	2.7	2.5

healing of cracks. The cracks were periodically monitored to quantify the self-healing degree of mortars, as explained in more detail below. The experimental matrix (Table 5) shows a summary of the conducted test. The cracking and observation of the self-healing process was applied to all the mortars fabricated with Portland cement, and only to the mortars fabricated with Pozzolanic cement and cured at 23 °C.

Cubic specimens were fabricated with Portland cement only to measure compressive strength. These tests were used to study the effect of lactate and bacteria on compressive strength at 3, 7, and 28 days from casting [37]. The compressive strength test was conducted only on mortars prepared with Portland cement and cured at 23 °C (Table 5).

2.6. Cracking and self-healing process of flexural specimens

After 28 days of curing, flexural specimens were loaded to induce flexural cracking mainly at the bottom of the specimen. The load was applied by a universal testing machine configured for three-point bending tests. For each test, the peak load and deformation was recorded by the machine control system. The cracked specimens were soaked in plastic containers with tap water. Each container with groups of cracked flexural specimens was conditioned for 63 days at constant temperatures of 4, 23, and 40 °C. Each specimen was removed from water after 7, 14, 21, 35, and 63 days of cracking for crack-healing quantification by image analysis.

2.7. Image analysis of the self-healing

The assessment of the self-healing was conducted by image analysis. The beam specimens were removed from water to be photographed each 7 days to observe the changes of crack width

for cracks in all specimens. Twelve-megapixel images were obtained from a constant distance using a digital camera, Fujifilm ×10 and lens combination giving a resolution of 22 pixels per mm (45.5 μm/pixel). The combination of the camera and lens was more appropriate than the optical microscope available, because the resolution of the images allowed the detection of cracks, and the image size covered the entire crack. In addition, the handling of the beam specimens in the optical microscope was difficult because its design is for the analysis of relatively flat specimens. Images of the same regions of interest at different times were aligned to quantify changes in crack width over time. The segment length was set constant at 1.6 mm. For any given crack, the number of crack segments was chosen as required. The healing at time t can be calculated as follows:

$$w(t)\% = \frac{w_i - w_t}{w_i} \times 100 \quad (1)$$

where w_i is the initial crack width, and w_t the width at time t .

On the other hand, it was calculated that the healing of crack area for specimens before and after healing could be accomplished by setting the crack area threshold gray level. Then the area repair was calculated according to the Eq. (2)

$$a(t)\% = \frac{a_i - a_t}{a_i} \times 100 \quad (2)$$

where a_i is the initial crack area, and a_t the area at time t .

2.8. SEM and XRD

After 63 days of immersion, a sample of healing material was removed with a scalpel from inside the cracks. The healing material was analyzed using scanning electron microscopy (SEM) and XRD to study the morphology of the material. For the SEM, the JEOL microscope model JSMT300LV was used and with a voltage of 15 kV. For the analysis, the sample was dried super critically and coated with gold to make it conductive. Through the SEM, it was also possible to perform a compositional analysis X-ray energy dispersion spectrometry (EDX), which consists of identifying the elements present and establishing their concentration by measuring the energy of the X-rays that are generated in a sample subjected to electronic bombardment. The microscope AZtec Oxford EDX detector was used for this procedure. XRD enabled identifying the minerals present in a sample by its crystalline structure [38].

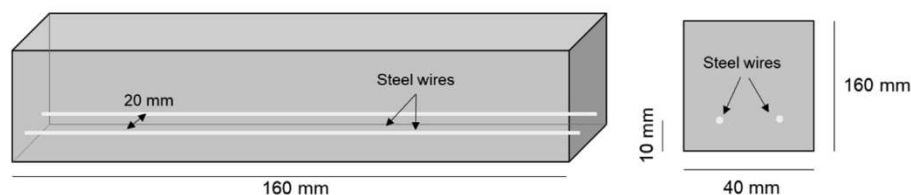


Fig. 1. Diagram of the mortar flexural beam and location of the reinforcement wires.

Table 5
Experimental matrix.

		Type of cement					
		Portland (PT)			Pozzolanic (PZ)		
		Control (C-PT)	Calcium Lactate (L-PT)	Calcium Lactate + Bacteria (B-PT)	Control (C-PZ)	Calcium Lactate (L-PZ)	Calcium Lactate + Bacteria (B-PZ)
Temp. (°C)	4	Healing	Healing	Healing			
	23	Healing	Healing	Healing	Healing	Healing	Healing
	40	Strength	Strength	Strength			

2.9. Chromatography

Chromatography consists of a separation method in which two immiscible phases are brought into contact, one phase is stationary and the other is mobile. This study used the ion exchange chromatography (IEC), in which the ionic components of the sample are separated by selective exchange with counterions of the phase. The negatively charged particles (positively) join the stationary phase positively charged (negatively) and are retained, while the positively charged particles (negatively) are rejected by the matrix and eluted. The elution of negatively charged particles (positively) is achieved by changing the pH of the solvent until it equals its isoelectric point or until its net charge is reversed [38]. With use of IEC, a relationship was sought between the water used for curing before the self-healing process and after two months of containing the self-healed samples. This examination aimed to detect whether elements of the water can affect the curing process. Chromatography analysis was conducted with the IC 930 Compact Flex Metrohm chromatograph controlled with the MagIC Net software. The software allows the automation of the equipment, controlling all the peripheral devices for handling liquids and recording results.

3. Analysis and discussion of results

3.1. Compressive strength on cubic specimens prepared with Portland cement

The objective of this test was to study the effect of lactate only and bacteria on the compressive strength of cubic samples prepared with Portland cement. The results showed the same trend for the three mixtures (Fig. 2) with a rapid increase in strength after 7 days of curing and reduced strength gain rate after the 7 days. After 28 days of curing, the compressive strength was relatively similar. The total compressive strength achieved after 28 days of all cubic samples was within the same range of 42–43 MPa and the difference between mixtures was too small to be statistically different ($p = 0.6908$, calculated using ANOVA test and 95% confidence level). However, the strength of samples prepared with calcium lactate (L) and with calcium lactate plus bacteria (B) have the highest strength after three days of curing, with 14.9% ($p = 0.0044$) and 11.8% ($p = 0.0070$) more than the average strength of control samples. After seven days of curing the strength of L and B samples is 10.6% ($p = 0.1118$) and 9.3% ($p = 0.0909$) greater than control samples, respectively. Therefore, results show that the addition of calcium lactate accelerates the compressive strength gain of the samples. However, after 28 days of curing the healing agent does not modify the compressive strength of the mixture.

3.2. Crack width distribution and relationship between crack width and self-healed area

The objective of the three-point bending test was to induce cracks in hardened mortar samples for the self-healing study.

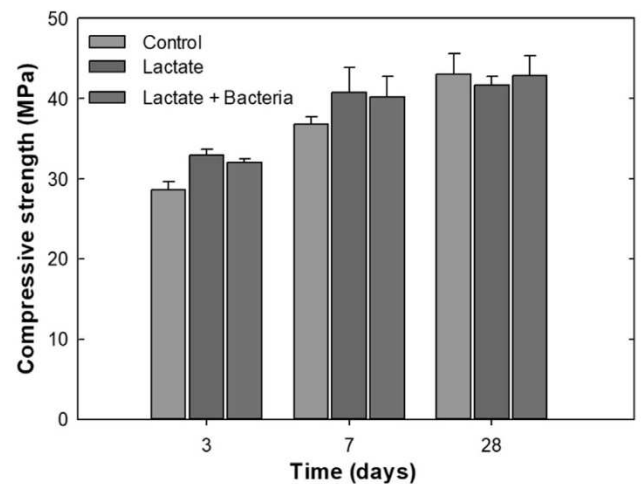


Fig. 2. Compressive strength of cubic specimens prepared with Portland cement after 3, 7, and 28 days of curing.

The method is effective to induce cracks; however, crack dimensions are uncontrolled during the test and every sample cracks differently. This difference is relevant for a self-healing concrete study because researchers have reported that cracks wider than 0.4 mm do not self-heal [17]. In the current study, the authors analyzed the cracks propagated in the three-point bending tests by statistical analysis, which considered all the flexural specimens fabricated in this research. A Beta probability distribution described well the average crack width measured in 139 cracks from all of the hardened mortar samples ($\alpha = 2.86$, $\beta = 12.76$, mean = 1.83). Fig. 3a indicates that crack width is within 0.125 mm and 0.225 mm for more than 60% of the cracks, and the maximum crack width was 0.475 mm.

During the self-healing study, described in detail below, the authors observed an inverse relationship between crack width and self-healed area. In other words, the wider the crack, the lower self-healing observed in the crack. Fig. 3b shows the crack width versus the self-healed area 63 days after sample cracking. The two-parameter, exponential decay curve fits the average measurements relatively well. In addition, Fig. 3b shows that all the cracks with a width <0.075 mm self-healed. These are important factors to consider in the analysis presented below, since, as previously mentioned, the crack width was different among different samples. For clarity, only one bar showing one standard deviation was included in Fig. 3b and other figures.

3.3. Qualitative analysis of images on concrete cracks

Fig. 4a shows an example of the self-healing observed in a specimen of hardened mortar prepared with Portland cement and bacteria, cured at 23 °C. Fig. 4a shows the crack immediately after

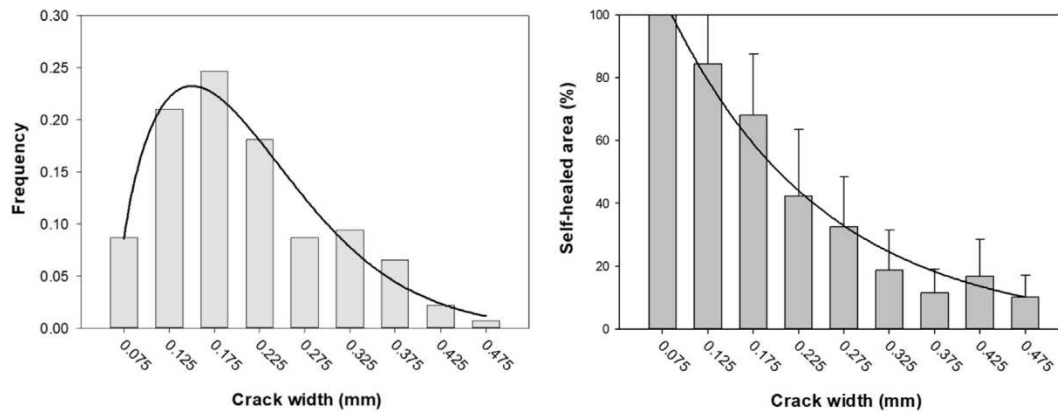


Fig. 3. (a) Crack width frequency measured on all hardened mortar samples (b) Relationship between crack width and self-healed area after 63 days for all samples.

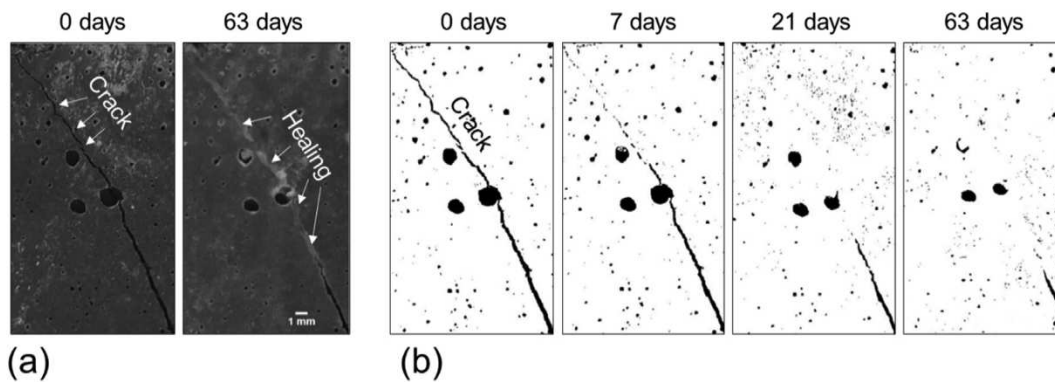


Fig. 4. (a) Image of self-healing observed in a Portland cement + bacteria samples immediately after cracking (0 days) and after 63 days (b) Processed images of self-healing evolution with time.

flexural loading (0 days) and at the end of the 63 days curing period. In addition to 0 and 63 days, the researchers obtained images after 7, 14, 21, and 35 days to follow the healing evolution of cracks. For a better visualization of the cracks and measurement of the self-healed area, the contrast, sharpness, and other image properties of the images were adjusted using ImageJ[®] software. Fig. 4b shows the filtered images after 0, 7, 21, and 63 days of curing, images after 14 and 35 days were omitted due to space limitations. The images after 0, 7, 21, and 63 days were selected because they show an important change in the crack healing. The healing gradually starts after 7 days with material precipitation in the narrow part of the crack. After 21 days, approximately two thirds of the crack is completely covered and the narrowing effect is observed in one part of the wider section of the crack. The crack healing between 21 days and 63 days is similar, indicating that most of the healing occurs between 0 and 21 days; in other words, only a small fraction of the crack healed between day 21 and 63 days.

3.4. Effect of lactate and bacteria on the self-healing of samples with Portland cement

Fig. 5a shows the self-healed area measured in samples prepared with Portland cement and conditioned for 63 days after curing at 23 °C and for different initial crack widths. Fig. 5a shows that 100% of cracks smaller than 0.1 mm width self-heal for the different mixture types studied (control, lactate only, and lactate + bacteria). However, results for crack width larger than 0.1 mm show a difference for different mixture types. Control mixtures have an

average self-healed area of 65%, 14%, and 12% for crack widths between 0.1 and 0.2 mm, 0.2–0.3 mm, and 0.3–0.4 mm, respectively. This average self-healed area is smaller than the self-healed area of 86%, 100%, and 27% achieved by the mixtures with lactate only, and the self-healed area of 82%, 44%, and 36% achieved in mixtures with bacteria, both for crack widths between 0.1 and 0.2 mm, 0.2–0.3 mm, and 0.3–0.4 mm, respectively. Fig. 5b shows the percentage of cracks with a self-healed area larger than 75%. The authors propose this indicator because it represents the number of cracks that achieved a relatively high healing over the total number of cracks observed. The 75% is an arbitrary percentage selected by the authors; however, this percentage yields a significant number of self-healed cracks over the total number of cracks. Results in Fig. 5b show that only 28% of the cracks observed in control samples have a self-healed area larger than 75%. Conversely, 73% and 53% of the cracks from lactate and bacteria samples, respectively, have a self-healed area larger than 75%. The results indicate that lactate and lactate + bacteria mixtures have a higher capability of self-healing cracks than control samples. In addition, results show autogenous self-healing of control mixtures for small cracks, and that cracks wider than 0.4 mm do not self-heal for all samples (Fig. 4a). It is important to recognize that specimens with lactate only achieved a higher self-healed area and a higher number of cracks with a self-healed area >75% than specimens with lactate + bacteria.

These results are consistent with Stuckrath et al. [31] who compared the healing of a mortar prepared with lactate only, with lactate + bacteria, and a control mixture. After 100 days conditioning, the healing median for the mortars with lactate only was 94%,

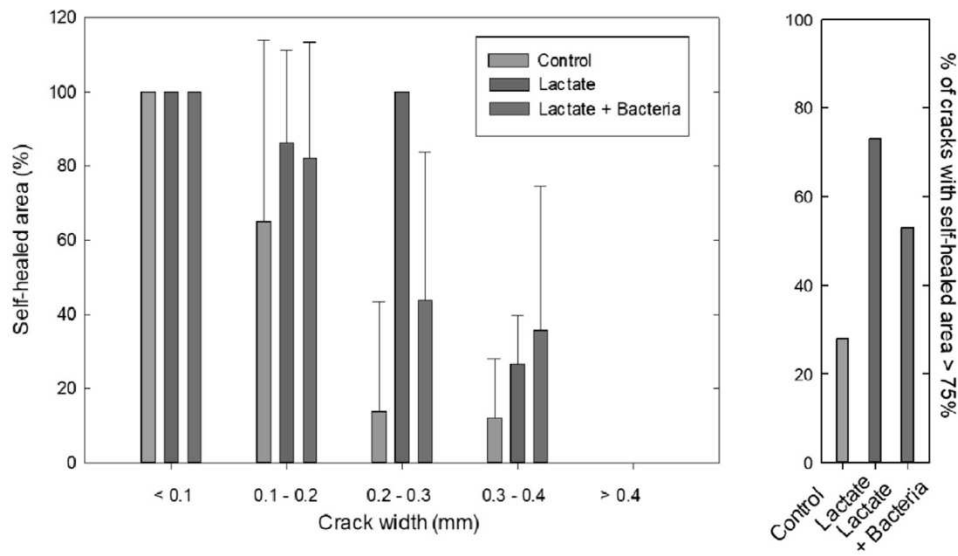


Fig. 5. (a) Self-healed area measured in Portland cement samples cured at 23 °C for 63 days for different crack width (b) Percentage of cracks with a self-healed area larger than 75%

while for the lactate + bacteria was 77%, and for the control mixture was 30%, confirming that adding lactate only increases the self-healing capabilities of concrete mortars.

3.5. Effect of lactate and bacteria on the self-healing of samples with Pozzolan cement

Fig. 6a shows the self-healed area measured in samples prepared with Pozzolan cement and conditioned for 63 days at 23 °C and for different initial crack widths. Fig. 6a shows that in control samples, 80% of the cracks with width smaller than 0.1 mm self-healed. However, 100% of cracks smaller than 0.1 mm width self-healed in samples with lactate only and lactate + bacteria. The difference between control samples and samples with lactate and lactate + bacteria is more evident. Control mixtures have an average self-healed area of 30% and only 2% for crack widths between 0.1 and 0.2 mm and 0.2–0.3 mm, respectively. This average self-healed area is smaller than the self-healed area of 85% and 56%, achieved by the mixtures with lactate only, and the self-healed area of 82% and 44%, both for crack widths

between 0.1 and 0.2 mm and 0.2–0.3 mm, respectively. Self-healing for cracks wider than 0.3 mm was not observed in Pozzolan samples with T = 23 °C curing temperature. Fig. 6b shows the percentage of cracks with a self-healed area larger than 75%. Results were similar to those obtained for Portland mixtures. Only 29% of the cracks observed in control samples have a self-healed area larger than 75%, while 62% and 68% of the cracks from lactate and bacteria samples, respectively, have a self-healed area larger than 75%. Again, the results indicate that lactate and bacteria mixtures contribute to the self-healing of cracks. Results from control mixtures show less autogenous self-healing than mixtures prepared with Portland cement, and mixtures with lactate + bacteria have the highest percentage of cracks with a self-healed area larger than 75%.

3.6. Effect of cement type: Pozzolan vs. Portland healing ratio

The ratio between the self-healing of Pozzolan vs. Portland samples is suitable for evaluating the effect of cement type. A ratio lower than 1.0 indicates a better healing performance of Portland

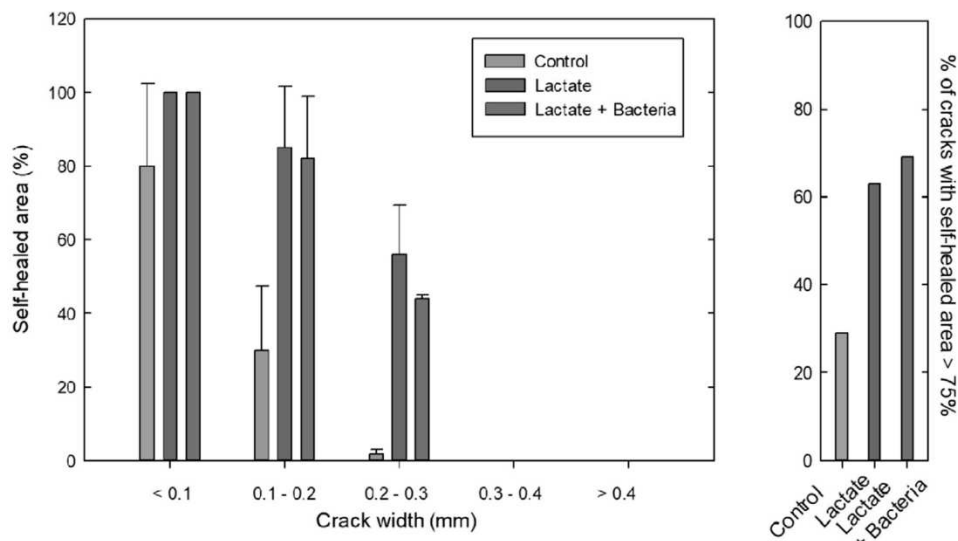


Fig. 6. (a) Self-healed area measured in Pozzolan cement samples cured at 23 °C for 63 days for different crack width (b) Percentage of cracks with a self-healed area larger than 75%

cement, and a ratio higher 1.0 indicates a better healing performance of Pozzolanic cement. Fig. 7 indicates that Portland cement favors the self-healing of control mixtures, since the ratio achieved was 0.80, 0.46, and 0.13 for crack width <0.1 mm, 0.1–0.2 mm, and 0.2–0.3 mm, respectively. The positive effect of Portland cement on the self-healing for lactate mixtures is less evident than for control mixtures, with ratios of 1.0, 1.0, and 0.56 for crack width less <0.1 mm, 0.1–0.2 mm, and 0.2–0.3 mm, respectively. For mixtures with lactate + bacteria, all the ratios were very close to 1.0, indicating a small effect on the self-healing of cracks for the testing conditions. Crack widths of 0.3–0.4 and >0.4 mm were not available for either type of cement; hence, it was not possible to calculate the ratio. However, they were included in Fig. 7 for consistency with previous graphs.

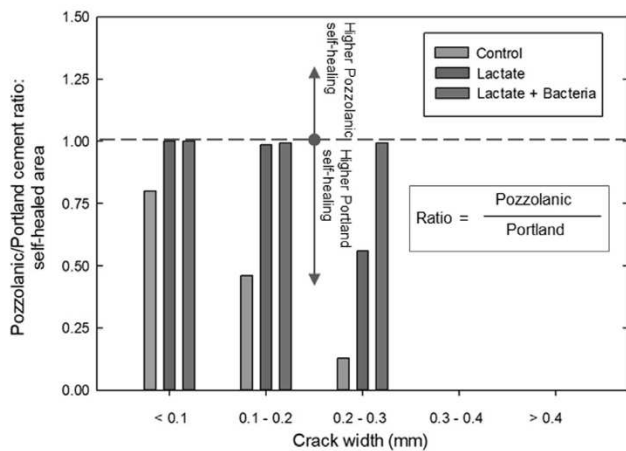


Fig. 7. Ratio of self-healed area measured in Pozzolanic cement samples over Portland cement samples cured at 23 °C for 63 days for different crack width.

3.7. Effect of temperature on the self-healing

Fig. 8 shows the self-healing results on control samples and lactate + bacteria samples prepared with Portland cement and conditioned for 63 days at different temperatures. The analysis of cracks on samples conditioned at 4 °C showed no healing after 63 days, confirming that low temperatures do not produce self-healing in control mortars and lactate + bacteria mortars. Nevertheless, curing temperatures of 23 °C and 40 °C produce different self-healing of mortars for different crack widths.

In control mortars (Fig. 8a), narrow cracks <0.1 mm self-healed 100% for 23 °C and 83% for 40 °C, respectively. Crack widths 0.1–0.2 mm show a similar trend for control mortars with 65% and 35% for 23 °C and 40 °C, respectively. Crack width between 0.2 and 0.3 mm, however, show a different trend with higher self-healing measured on samples conditioned at 40 °C, while cracks width 0.3–0.4 mm showed a self-healed area of 12% for mixtures conditioned at 23 °C while and 0% healing was observed at 40 °C.

Results with lactate + bacteria (Fig. 8b) confirm that 23 °C is a temperature that increases the self-healed area for all crack widths. This finding is consistent with the characteristic of *Bacillus pseudofirmus*, the mesophile spore used in this study, as that bacteria grows best in moderate temperature, typically between 20 and 40 °C. For narrow cracks <0.1 mm the self-healed area was 100% for both temperatures of 23 °C and 40 °C. Crack widths 0.1–0.2 mm show a similar trend with 82% and 49% for 23 °C and 40 °C, respectively. Crack width between 0.2 and 0.3 mm, was 44% and 10%, while cracks width 0.3–0.4 mm showed a self-healed area of 36% for mixtures conditioned at 23 °C.

3.8. Crack-repair versus time in one crack from each sample

The self-healing measurement at 0 and 63 days considered the crack width. The crack evolution, or crack-repair versus time consisted in the measurement of the total self-healing area without considering the initial width because the intention was to follow

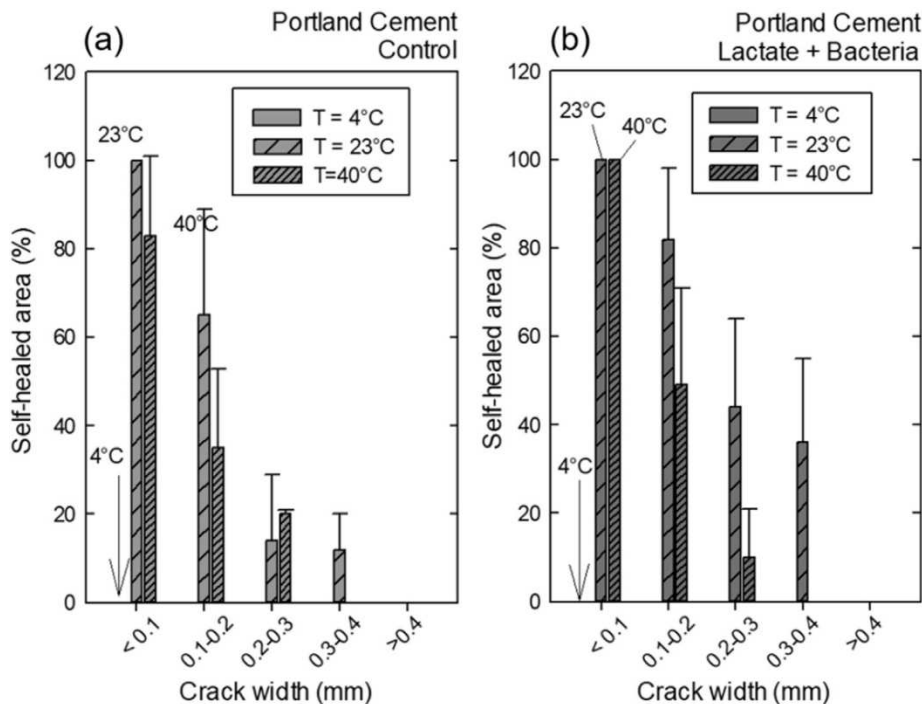


Fig. 8. Effect of temperature on Portland cement (a) control samples (b) Lactate and Bacteria samples.

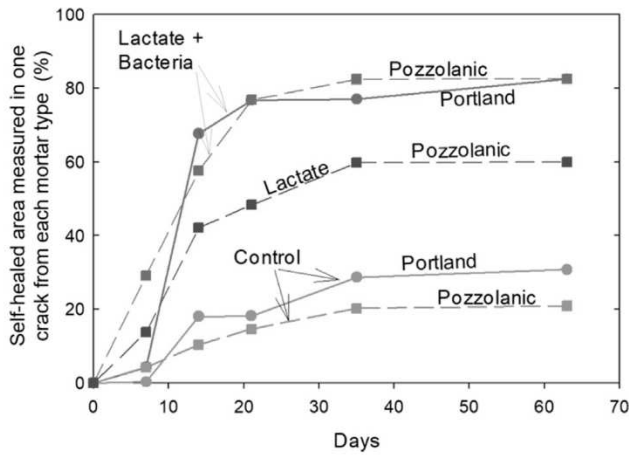


Fig. 9. Effect of time on the self-healed area measured in one from each mortar type, without considering crack width, samples cured at 23 °C for 63 days.

the crack-healing evolution. In addition to 0 and 63 days, crack-healing measurements were conducted in one crack only after 7, 14, 21, and 35 days. Fig. 9 shows the total self-healed area for one crack sample between 0 and 63 days. Since only one crack is analyzed for crack evolution, Fig. 9 does not totally follow the trends presented above, which considers all the cracks of the sample. Pozzolanic control specimen increased the total self-healed area gradually until day 35 after initial cracking. Portland control specimens show a similar trend; however, the total self-healed

Table 6
XRD characterization of crack-filling precipitates after healing.

Components	Formula
Calcite, syn	CaCO ₃
Vaterite	CaCO ₃
Quartz low	alpha-SiO ₂
Albite, calcian	(Na,Ca)(Si,Al) ₄ O ₈
Portlandite	Ca(OH) ₂

Table 7
XRD characterization of light weight aggregate (LWA).

Components	Formula	Content (%wt)
Kaolinite	Al ₂ Si ₂ O ₅ (OH) ₄	56.95
Quartz low	Alpha-SiO ₂	41.76
Lime	CaO	1.29

area is larger than in Pozzolanic control specimens. The Pozzolanic sample with Lactate only shows a higher total self-healed area that increased rapidly until day 14 with continued healing until day 35. Unfortunately, measurements of Portland samples with Lactate only were not available for this analysis at 7, 14, 21, and 35 days. Lactate + Bacteria samples show a similar behavior with a rapid increase in the total self-healed area until day 21. After that, the healing plateaus and remains relatively constant. The Lactate + Bacteria curve in Fig. 9 is consistent with the images shown in Fig. 4, since after 21 days the sample images show a small increase in total self-healed area. Results also indicate that 7 days is the minimum expected time to observe self-healing and after 14 days of curing crack-healing is approximately two thirds of the final total self-healed area.

3.9. XRD and SEM

The XRD analysis was conducted for characterization of the precipitated material obtained from self-healed cracks. However, the quantity of precipitated material obtained with a bistoury from each mixture type was very small and made XRD quantitative analysis unfeasible. Nevertheless, the amount of precipitated material obtained from Portland cement with lactate + bacteria samples (in the order of 0.4 g) made the XRD qualitative (instead of quantitative) analysis viable only for that mixture. The XRD analysis conducted indicated that precipitated material (Table 6) contains Calcite, Vaterite, Low Quartz, Albite, and Portlandite. Hydrated particles of cement adjacent to, or in the precipitated material obtained from cracks explain the existence of Portlandite. Calcite and Vaterite crystals, the most abundant material found in the crack-precipitated material, are polymorphisms of calcium carbonate and have the same chemical formula; however, they differ in their crystallographic properties. The presence of Low Quartz was initially difficult to explain; however, an additional quantitative XRD (Table 7) conducted on the LWA used in the study gives an important presence of Low Quartz and explains Table 6 results.

SEM images were also obtained from crack-precipitated material (Fig. 10a). Figures showed rhombohedral formations similar to CaCO₃ crystals; however, an important quantity of amorphous material normally has lower hardness than crystals and therefore is not necessarily a good precipitated material to protect cracks in concrete. The EDS spectrum (Fig. 10b) shows, in weight, high amounts of Carbon (13.51%), Oxygen (54.76%), and Calcium (25.00%), which confirms the appearance of CaCO₃ crystals obtained from XRD analysis. The presence of silicon shows a relationship to the Albite found in the XRD. This crystal has a hardness of 6 to 6.5 on the Mohs scale, which makes it a suitable crystal for

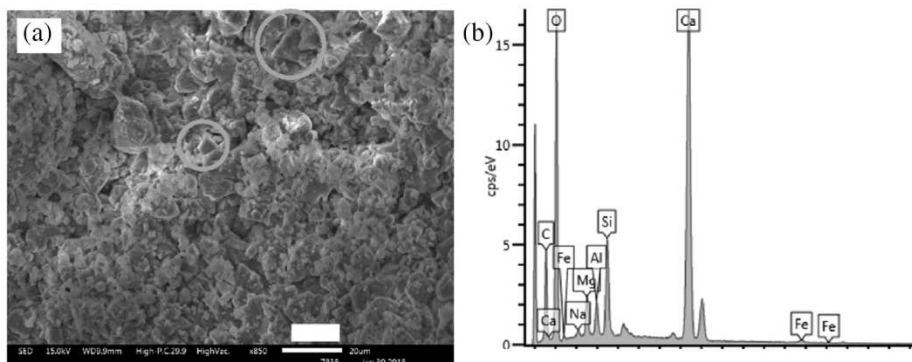


Fig. 10. (a) SEM images of crack-filling precipitates after crack-healing from a sample fabricated with Portland + bacteria, (b) EDS spectrum.

Table 8

Results of the chromatography, Sample 1 corresponds to the water before submerging the concrete specimens. Sample 2 corresponds to water after 63 days of submersion.

Cations	Sample 1 (mg/L)	Sample 2 (mg/L)
Sodium	40.73	263.34
Potassium	24.92	277.94
Calcium	49.85	4.28
Magnesium	7.006	0.49

sealing, although is not abundant. The Magnesium and Iron found in the spectrum were not detected by the XRD, and could not form crystals.

3.10. Chromatography

The chromatography was conducted on water used for soaking the flexural specimens. The first water sample was collected at the beginning of the experiment; before soaking the samples for the 63 day conditioning. Hence, the first water sample did not have contact with concrete specimens. After the 63 days of keeping the samples submerged, a second water sample was analyzed. The results (Table 8) show a decrease in calcium and magnesium cations, and an increase in sodium and potassium. The decrease in calcium ions is consistent with the hypothesis that the reaction to seal the cracks takes calcium from some external source, so that part of the calcium cations found in the water would be responsible for the formation of calcium carbonate in the cracks. A logical explanation for the increase of sodium and potassium cations is the transfer of concrete components (for example, chemicals in sand, LWA) to the water [39].

4. Conclusions

This study evaluated crack-healing mortars produced with Portland and Pozzolanic cements prepared with self-healing agents based on lactate and bacteria. In addition to conventional sand, lightweight aggregates were used to impregnate the self-healing agents. The results obtained from the study lead to the following conclusions:

- After 28 days of curing, the healing agent does not modify the compressive strength of the mortars prepared with Portland cement. Control, lactate, and lactate + bacteria mortar specimens showed similar strength after 28 days curing.
- A Beta probability distribution described well the average crack width from cracked flexural beam samples. An inverse relationship between crack width and self-healed area was observed.
- Crack healing starts after 7 days with material precipitation in the narrow part of the crack. Results show that most of the healing occurs between 0 and 21 days, and only a small fraction of the crack heals after that.
- The crack healing of Portland and Pozzolanic cement mortar samples prepared with lactate only, or lactate + bacteria, is significantly higher than in control samples. In addition, results show that Portland cement better promotes the self-healing of cracks compared to Pozzolanic cement.
- Samples prepared with Portland cement showed the highest crack self-healing at 23 °C in comparison with 40 °C and 4 °C. Self-healing was not observed in samples conditioned at 4 °C.
- XRD analysis indicated that precipitated material obtained from sealed cracks contains calcite, vaterite, Low Quartz, Albite and Portlandite. SEM analysis showed rhombohedral formations similar to CaCO₃ crystals, which was consistent with EDS spectrum analysis.

- Overall, results show that Portland and Pozzolanic cement mortar samples prepared with lactate or lactate + bacteria, promote the self-healing of cracks.

CRediT authorship contribution statement

Álvaro González: Project administration, Supervision, Conceptualization, Writing - original draft, writing - review & editing. **Araceli Parraguez:** Methodology, Formal analysis, Investigation. **Liliana Corvalán:** Methodology, Resources. **Néstor Correa:** Methodology, Investigation. **Javier Castro:** Resources, Writing - review & editing. **Claudia Stuckrath:** Conceptualization, Methodology, Writing - review & editing. **Marcelo González:** Writing - review & editing, Data curation.

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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