

Biocementation technique for sealing cracks in concrete water storage tanks

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SUMMARY

Cracking in concrete structures is a very common pathology. For the particular case of water storage tanks, the cracks reduce the capacity of the structure to retain water, which compromises its utilization. The main objective of the current thesis is to study the technique of biocementation as an alternative for the treatment of cracks in water storage tanks in the context of ultimate limit state marked by several water loss due to failure to retain and storage water. This treatment has emerged as a "green", by reducing the gases emissions, and innovative technique for the treatment and rehabilitation of the cracks using non-pathogenic bacteria that precipitates calcium carbonate with the help of enzyme urease that performs the hydrolysis of urea providing ideal conditions for the development of the chemical process. This treatment was applied in artificial cracks made in concrete plates with 40 mm width and 200 mm length, with crack openings of 0.1, 1 and 10 mm. In the case of 10 mm opening samples, sand - chosen because it as a cheap material that can be easily compacted - was added to fill the voids with subsequent treatment by biocementation. The efficiency of the treatment was investigated by carrying out sealing tests through variable water head under 10 kPa pressure and the results were explained by images of thermographic camera and ultrasonic pulse velocity tests. The presence of biocement was confirmed by mineralogical analysis of the precipitate, microscopic images and 3D modelling, extracted from the cracks after breaking the plates through flexural bending tests. The results obtained were very satisfactory for all crack openings investigated, because it was possible to reduce 90% of the amount of water flowing through the crack considering the case before treatment as the reference case. This indicates that the amount of biocement precipitated was able to seal the cracks independently from their opening width.

Keywords: Biocementation, bacteria, repair, retrofit, water storage tanks; watertightness.

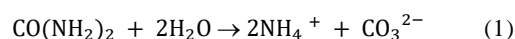
1. INTRODUCTION

Cracking in concrete structures is a very common pathology present in built elements. It is developed due to several factors, from design errors to execution problems and poor maintenance, deviating the components' performance mechanisms in use. For case of water storage tanks, the cracks reduce the capacity of these structures to retain water, which compromises their adequate utilization.

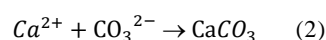
In developed countries of Europe and North America, almost all water supply systems were built a few decades ago and, currently, water service management entities are faced with the challenge of maintaining, operating, and managing them efficiently to guarantee the supply of the necessary quantities of water and with adequate quality to the populations. The aging of the water supply infrastructure and the respective equipment (from the abstraction to the consumer) is a natural and inevitable process and, as the different components of the system approach the end of their useful life, the volume of water losses tends to increase, the occurrence of ruptures and interruptions of supply becomes more frequent and the costs of maintenance of the systems get higher. The assets of these systems have significant civil construction components (e.g., pipelines, water storage tanks and pumping station buildings) whose degradation is reflected in the occurrence of holes and cracks that lead to water losses and poor performance.

It is fundamental to repair such structures to increase their watertightness, to avoid water losses and to increase their service life. Biocementation is emerging as an alternative repair technique to the traditional ones used for sealing cracks and rehabilitation of cracked elements.

Biocementation consists of a "green" and innovative technique that uses non-pathogenic bacteria or enzymes to produce biocement [1]. This biocement is the calcium carbonate (CaCO_3) precipitated after hydrolysis of urea ($\text{CO}(\text{NH}_2)_2$) that occurs with the help of the urease enzyme. This results in the carbonate ion (CO_3^{2-}), described by Equation 1.



The precipitation of calcium carbonate is supported by the release of ammonium (NH_4^+) which results in an increase in pH. The carbonate ions (CO_3^{2-}) react with the calcium ions (Ca^{2+}) supplied in the feeding solution and the precipitation of calcium carbonate occurs (Equation 2). The most common mineral forms of biocement are calcite or vaterite, being calcite the most stable one because it is insoluble.



There are several types of bacteria that produce urease enzymes, such as *Myxococcus xanthus* [2], *Bacillus sphaericus* [3], *Bacillus cereus* [4] and *Mytilus californianus* [5]. *Bacillus pasteurii* is the bacteria specie selected to be used in this work since it is the most used in biocementation of soils and other construction materials.

The current study aims at analyzing the technique of biocementation applied to water storage tanks, which are structures in contact with water under pressure. The specific objectives are: (i) to study how to apply this technique in water storage tanks, in particular when the cracks are vertical, like in the lateral tank walls, (ii) to evaluate the efficiency of the treatment concerning watertightness achievement, when applied to cracks with openings ranging from 0.1 to 10 mm, and (iii) to study how to evaluate the response of the treatment in substrates that will be in direct contact with water, such as in the interior surface of water storage tanks. Such topics are novel and have not been investigated so far.

Biocementation treatment was applied in artificial cracks made in concrete plates with 4 cm thickness and 20 cm length, with openings widths of 0.1, 1 and 10 mm. Sand was added to the large cracks with openings of 10 mm width before the treatment. Three plates were treated for each opening width. Some tests were carried out before, during and after the treatment of the cracks to evaluate the evolution of sealing capacity caused by increasing amounts of biocement precipitated during the different treatment rounds. The laboratory tests are distinguished in two types: concrete characterization tests and crack and sealing characterization tests. Included on this latter classification are the characterization tests of the sealing material (i.e., the mineralogical analysis and the optical microscopy), the verification tests of the sealing system (i.e., the watertightness test, the ultrasonic pulse velocity and thermography) and the test of suitability of the treatment for the recovery of structural capacity (i.e., the flexural tensile test).

2. MATERIALS AND METHODS

2.1. Concrete plates

Prefabricated unreinforced concrete slabs were used, with total porosity of 29.69 %, and an open porosity of 11.7 %. Two slabs were bought, and each was cut into three equal parts to obtain squares of 20×20×4 cm (Figure 1) to create 12 samples of equal dimensions. The cracks were artificially created in these squared pieces, as described in the subsequent sections.

2.2. Bacteria and feeding solution

The bacteria *Bacillus pasteurii* were grown in a culture medium (i.e., 20 g/L yeast extract, 10 g/L of ammonium sulphate and 0.13 M Tris buffer pH 9.0) at 30°C until reaching an optical density of 1 measured for 600 nm (OD_{600}) ($\sim 10^8$ cells/mL). The feeding solution was prepared with 0.5 M of calcium chloride (calcium source) and 0.5 M of urea, to which 1:10 diluted growth medium, 2.12 g/L of sodium bicarbonate and 10 g/L of ammonium chloride were added.

2.3. Sand

The cracks with 10 mm opening width were filled with a quartzite uniform-graded size river sand (ref. APAS 30), with particles with diameters between 0.06 mm and 0.25 mm and with a relative density $G_s = 2.66$. This sand was poured into the volume of the crack to reach a dry volumetric weight of 15 kN/m³.

2.4. Execution of the cracks

Cracks are typically characterized according to several parameters such as [6]: opening, extension, depth, orientation, and location, by diagnostic methods [7] like visual inspection, echo impact, thermography, ultrasound pulse velocity and techniques that uses equipments such as magnifying glasses, cameras, scales, tape measures, fissure meters, among others. Cracks can be classified according to their actual activity, that is with or without relative movement. The activity of the crack is quite important for the classification of the respective type and, consequently, the causes that originated it [8].

In the present study, the object of study is the repair of cracks at levels 0, 1, 2 and 3 of the [9] classification – with widths from 0,1 to 10 mm - that are considered passive fissures without activity. A greater focus has been devoted to the assessment of the effects of these cracks in constructions, especially in water storage tanks.

The cracks were artificially created in prefabricated concrete slabs (Figure 1a), after splitting them in the middle in controlled manner and re-joining the two halves with the desired opening, like those caused over the lifetime in concrete components [10]. A flexural traction press was used for splitting the plates (Figure 1d). This procedure was adopted to maintain the natural roughness of the surfaces inside the cracks, which is important for fixing the sealing material.

The crack opening was controlled with spacers (Figure 2b). Three different openings with 0.1, 1 and 10 mm were defined, with three samples assigned to each type. For this purpose, spacers of 1 and 10 mm were used, since the openings of 0.1 mm were achieved simply by placing the broken pieces together without anything between them. The two pieces were immobilized to prevent the relative movement between the two parts. One restriction was the need of a fixing component that could be removed later, so fastening clips (Figure 2c) that pressed both sides of the samples against each other were selected, allowing to obtain the final samples.

The samples were identified depending on the crack width: Samples 1, 2 and 3 were those with the crack opening 0.1 mm; Samples 4, 5 and 6, the ones with the crack opening of 1 mm; Samples 7, 8 and 9, those with 10 mm opening and finally the Samples 10, 11 and 12 were intact samples, used as reference for comparison.

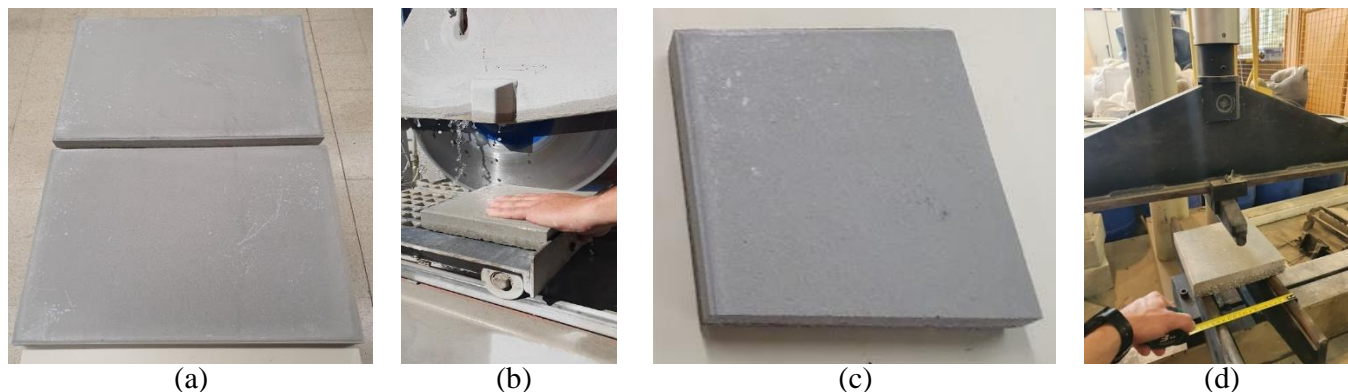


Figure 1. Concrete material: (a) prefabricated concrete slabs; (b) saw cutting the slabs; (c) parts obtained after the cut; (d) flexural traction press machine to break the parts in the middle.

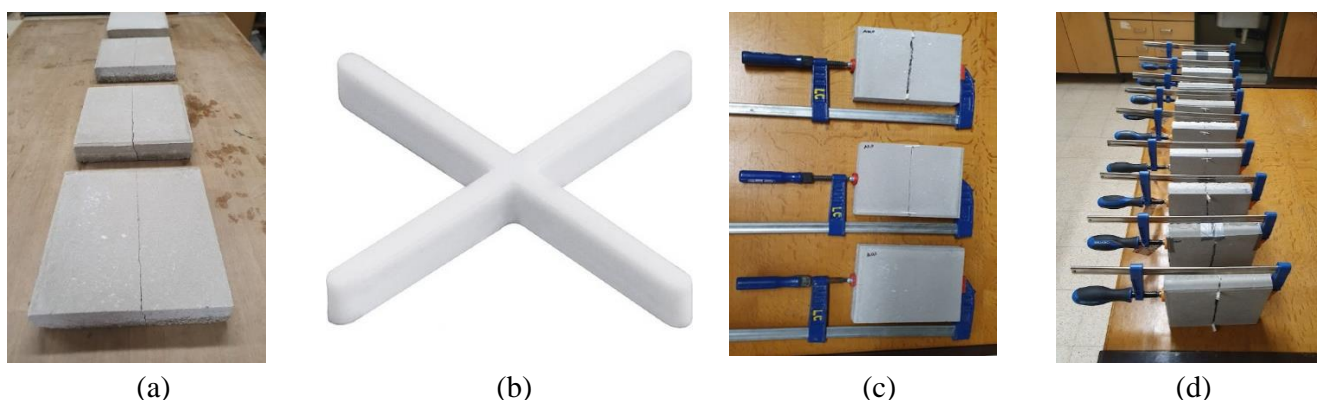


Figure 2. Execution of the cracks: (a) split parts; (b) spacer used to control the opening; (c) fastening clips; (d) samples 1 to 9 already fixed.

Exceptionally, the cracks with 10 mm opening – Samples 7, 8, 9 – were filled with APAS30 sand. The final porosities of the sand in each crack were roughly 71.2 % (Sample 7), 70.4 % (Sample 8) and 72.0 % (Sample 9).

A plastic film was placed on the side surfaces of the samples and on the bottom (Figure 3a), so that the piece was isolated from the outside by a protective layer which allows the application of the fluid with bacteria without leaking out of the sample. Paraffin was also applied (Figure 3b) to make the back of the sample and its lateral edges waterproof. Finally, a resistant adhesive tape was applied (Figure 3c) aligned with the centre of the crack, to isolate the internal part of the crack and create inside a small tank.

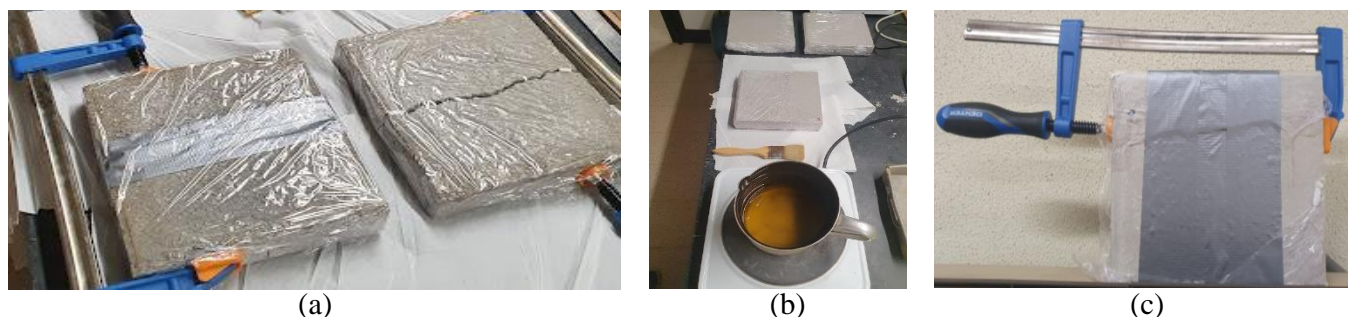


Figure 3. Isolation of the plate: (a) plastic film and resistant adhesive tape applied; (b) paraffin used to make the sample waterproof; (c) resistant adhesive tape applied to isolate the internal part of the crack.

2.5. Treatment by biocementation

The treatment was applied using a syringe to insert the fluids in the interior of the cracks. The needles allowed punching the fluid through the adhesive tape placed on the surface of the crack (Figure 4a). Filling was carried out from the inside to outside of the crack, as in an injection process. The treatment was divided in two or three rounds, each with the duration of 10 days. For each round, bacteria and feeding solution were added in the first five days, and only the feeding solution was added in the last five days. The quantities of bacteria and feeding solution were: 2, 7 and 10 mL, respectively to 0.1, 1 and 10 mm cracks, and it was defined prior to the beginning of the treatment according to the theoretical volume of the empty crack. Two rounds were done for Samples 1 to 3 (0.1 mm), while three complete rounds were necessary for the Samples 4 to 9 (1 and 10 mm), in order to attain watertightness. Figure 4b shows the 0.1 mm sample during the application of the first treatment round, while Figure 4d depicts the same sample during the second (and final) round.

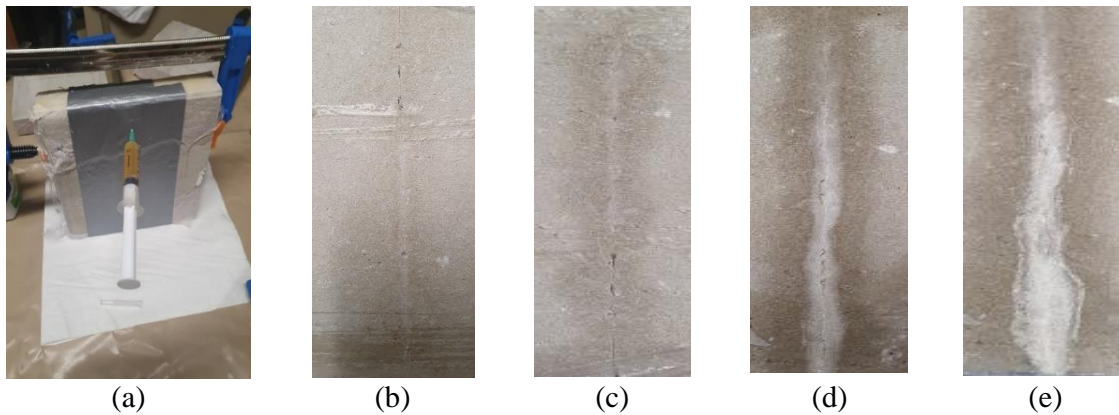


Figure 4. Application of the treatment: (a) needle used to the inside-out application; (b) sample after 2nd day injection of 1st round; (c) sample after 5th day injection of 1st round; (d) sample after 1st round completed; (e) sample after 2nd round completed.

2.6. Experimental tests

2.6.1. Watertightness tests

The watertightness tests consisted of variable water head tests, in which water flow through the cracks was measured along time. Acrylic pipes (with an outer diameter of 12 cm and a wall thickness of 0.5 cm) were installed on the top of the cracks (Figure 5a), with a metric scale to measure the evolution of water height and filled with 1 m of water in order to create the water columns. A cut on the paraffin and on the plastic film was also done (Figure 4b) so the water could get rid of any external obstacle to its natural flow. These tests were carried out after each treatment round and the water level was measured along time to assess the crack treatment tightness. This is fundamental to simulate the water pressure inside a water storage tank.

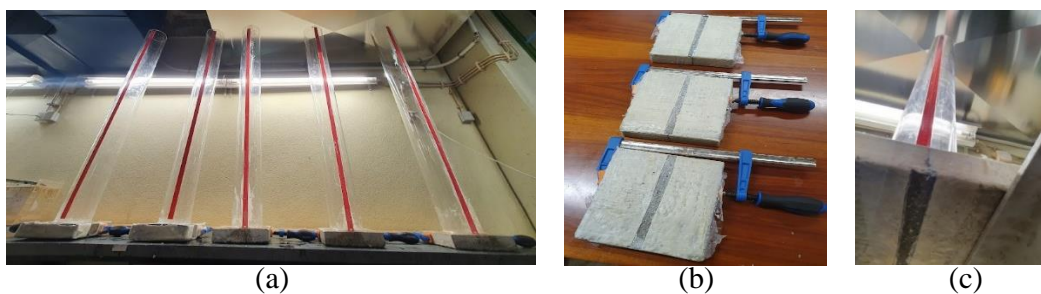


Figure 5. Watertightness test setup: (a) columns placed on the samples; (b) cut on the paraffin and plastic film surface to enable the water's flow; (c) bottom vision of the apparatus.

The initial water height was 1 m ($h = 1$ m). The time necessary for water level to drop 1 cm was measured with a chronometer and registered. The flow rate of the drained water was computed using Equations 3, 4 and 5.

$$D_i = D_e - 2 \times e = 11 \text{ cm} \quad (3)$$

$$A_i = \frac{\pi \times D_i^2}{4} = 95,03 \text{ cm}^2 \quad (4)$$

$$Q_j = \frac{\frac{h_{j-1} - h_j}{2} + \frac{h_j - h_{j+1}}{2}}{\frac{t_j - t_{j-1}}{2} + \frac{t_{j+1} - t_j}{2}} \times A_i \quad (5)$$

where D_i and D_e are the inner and outer acrylic pipe diameters, respectively (cm), e is the pipe wall thickness (cm), A_i is the inner cross-sectional area (cm²), j is time (s), Q_j is the outflow at time j (cm³/s) and h_j is the water height at time j (cm).

The initial flow rates at time $j=0$ were computed for each round of treatment i and compared to assess the relative improvement achieved with the treatment by using Equation 6.

$$\text{Relative improvement [\%]} = \left(1 - \frac{Q_i}{Q_{i-1}}\right) \times 100 \quad (6)$$

This indicator reflects the enhancement of the watertightness with the increasing amount of precipitated biocement in traduces in each treatment round, assessing the effectiveness of the treatment

2.6.2. Thermography

A *Flir IX Series* thermographic camera (Figure 6) was used to get the images from the samples by the temperatures difference between them, so it could evaluate empty regions with lack of sealing by biocement. The procedure consisted of pre-heating the samples in an oven at 105 °C for 24 hours, followed by adding distilled water at room temperature (colder than in the oven) inside the cracks to enable viewing the preferential flow paths of heat through their interior.

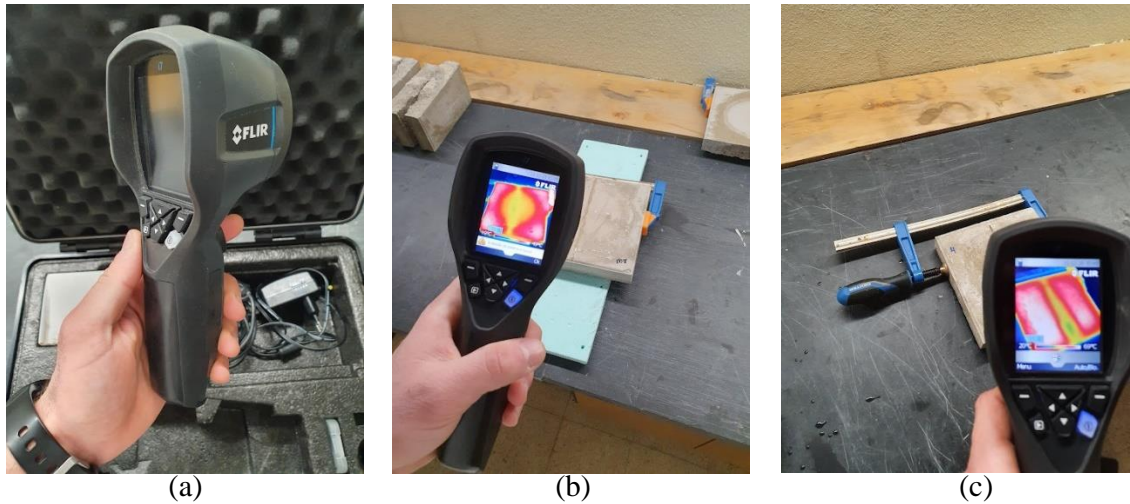


Figure 6. Thermography equipment: (a) camera *Flir IX Series*; (b) general thermographic image; (c) thermographic image indicating the crack region.

2.6.3. Ultrasonic pulse velocity

The ultrasonic velocity through the plate and sealed crack was measured using PUNDIT equipment. The ultrasonic wave generated had a frequency of 54 kHz and was applied using 35 mm diameter probes between the two opposite surfaces of the samples. This test was carried out on all untreated samples (reference case) and after each round of treatment. The results allowed to decide when to stop the treatment as the velocity was getting similar to the one of the previous rounds, or to that measured in the intact sample. This is because the velocity is higher in the absence of voids since waves propagate faster in solids than in fluids. Therefore, the velocity increases to values approaching those measured in intact plates with the increasing amount of biocement along the interior of the crack, filling it.

2.6.4. Other tests

Finally, new bending tests were carried out in the samples after the complete treatment to test the structural resistance of the treated crack. A load was applied to force splitting through the crack. This also had the advantage of having access to the mineral precipitated in the inner crack surfaces, which was sent for mineral analysis to confirm that it was calcium carbonate.

3. RESULTS AND DISCUSSION

3.1. Water flow measured in the watertightness tests

The results of the watertightness test at the end of each round are presented in Table 1. Flows are also referred as $Q1$, the initial flow ($j=0$) after the first treatment round, $Q2$, the initial flow ($j=0$) after the second treatment round, and $Q3$, the initial flow ($j=0$) after the third round and I , improvement (%). Table 1 also presents the comparison between the different rounds considering the improvement achieved through percentages over the subsequent rounds (Equation 6). The cells in the table are filled with a colour

scale according to the effectiveness of the treatment that is the attained level of satisfaction (green – satisfactory; yellow – adequate; orange – reasonable and red – unsatisfactory), with the legend indicated below the table.

The results presented in Table 1 confirm that the treated samples have improvements of, at least, 90% when compared to the reference situation (the untreated sample with a crack). This indicates that biocementation treatment is efficient to repair cracks when watertightness must be recovered.

Table 1. Results of the relative improvement to the watertightness aspect during the different rounds of treatment.

Evolution of the treatment										
Sample		Q reference [cm ³ /s]	Q1 [cm ³ /s]	I to the reference [%]	Q2 [cm ³ /s]	I to the 1 st round [%]	I to the reference [%]	Q3 [cm ³ /s]	I to the 2 nd round [%]	I to the reference [%]
0.1 mm	Sample 1	18	0.19	98.93%	0.00	100.00%	100.00%	-	-	-
	Sample 2	22	3.96	81.94%	1.53	61.26%	93.01%	-	-	-
	Sample 3	19	0.06	99.67%	0.00	100.00%	100.00%	-	-	-
1 mm	Sample 4	79	23.76	70.00%	2.09	91.21%	97.36%	0.73	65.27%	99.08%
	Sample 5	178	45.23	71.44%	38.01	15.96%	76.00%	7.36	80.65%	95.35%
	Sample 6	158	33.94	80.95%	21.12	37.78%	88.15%	17.82	15.63%	90.00%
10 mm	Sample 7	2923	-	-	-	-	-	19.01	-	99.35%
	Sample 8	2923	-	-	-	-	-	23.76	-	99.19%
	Sample 9	2923	-	-	-	-	-	15.84	-	99.46%

Note: ■ 100 % - 95 %; ■ 95 % - 80 %; ■ 80 % - 50 %; ■ 50 % - 0 %

Note: The flow which has been compared in all situations is the initial at instant $j=0$ since it is the highest one due to the water's pressure (one meter).

Furthermore, two treatment rounds were sufficient for the samples with 0.1 mm cracks (i.e., Samples 1, 2 and 3), while, for the others, the three rounds were necessary to reach the same level of effectiveness. It is also worth to note that the results were very similar for the samples with cracks of 10 mm opening with the addition of sand, which demonstrates that the combined treatment with sand is viable and promising, with levels of effectiveness close to 100 % (totally satisfactory). The curves of the evolution of the water level along time presented in Figure 7 are the best-case and worst-case situations. Samples 3, 4 and 9 have shown the best results with flat outflow curves (Figure 7a), which can also be seen in the Table 1 from the relative improvement of 100 %, 99.08 % and 99.46 % compared to the pre-treatment situation, respectively. On the other hand, Samples 2, 6 and 8 have shown the worst results with slightly sharper curves (Figure 7b), with relative initial outflow improvements about 93.01 %, 90.00 % and 99.19 %, respectively.

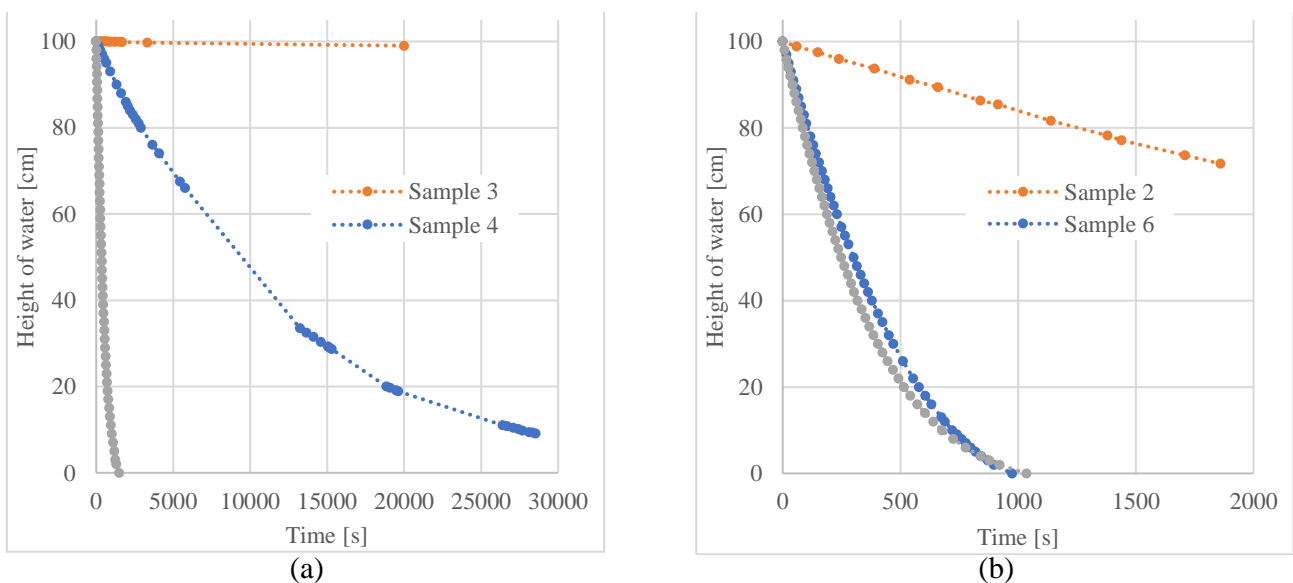


Figure 7. Best-case samples in watertightness tests (a); worst-case samples in watertightness tests (b).

3.2. Confirmation of watertightness

3.2.1. Thermography

The images obtained by the thermographic camera can be correlated to the results of the sealing tests. The greater the difference between the temperatures of the two media is, the higher the contrasts provided by the thermographic camera display are, as shown in Figure 8 (only the best and the worst results from the sealing tests are presented, both for the upper and bottom views of the samples). The colours correspond to the efficiency of the treatment: green are the empty spaces, while the red and yellow are the filled ones.

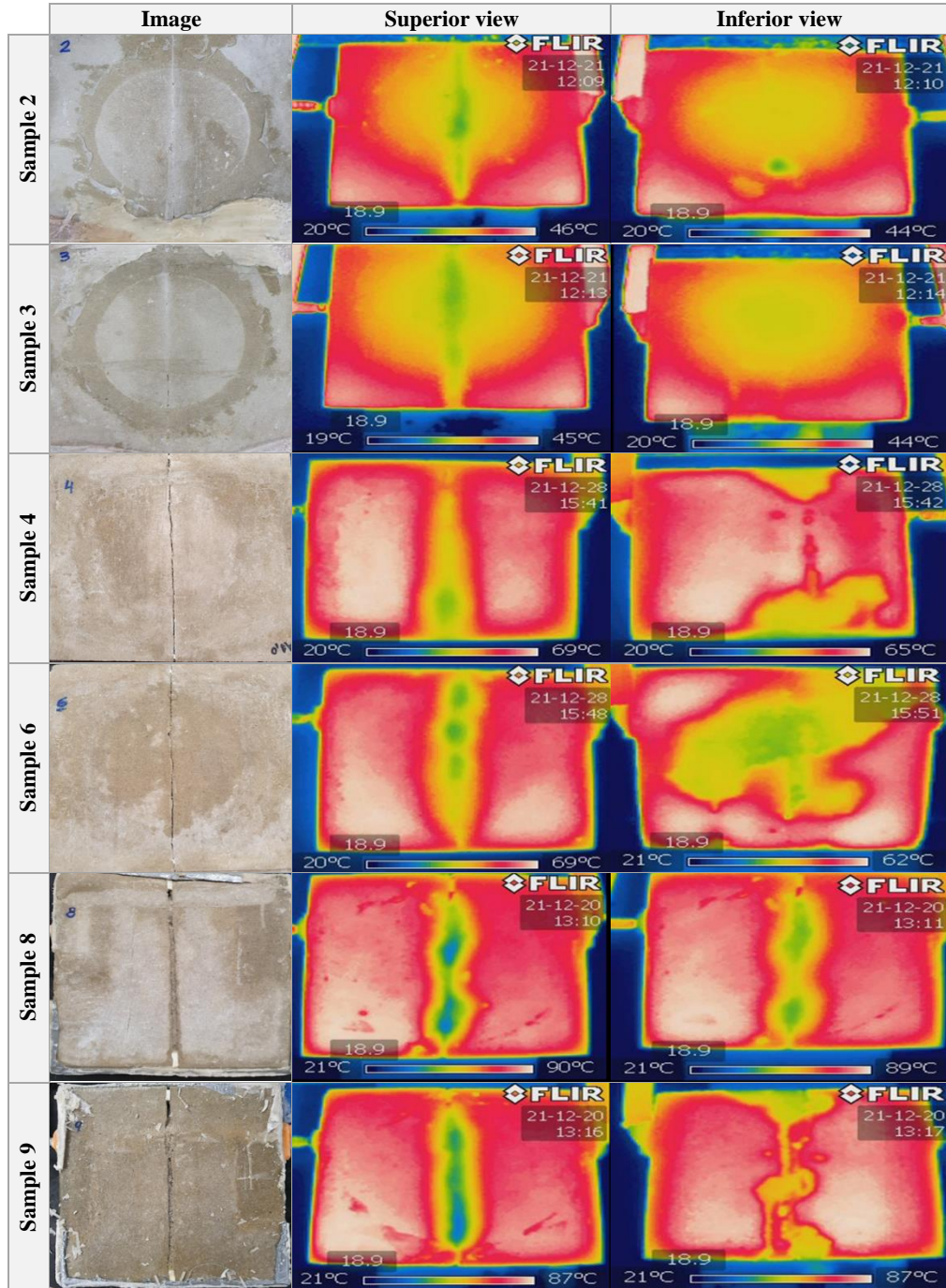


Figure 8. Results of thermographic tests after the conclusion of the treatment.

The comparison between the images from the upper and bottom views allow understanding if sealing was achieved for the entire thickness of the sample. From the differences observed between the two views, in particular in the bottom ones, it is possible to observe locations where the treatment was not well applied, requiring special attention and new rounds of treatment. This also reflects the same worst-case scenarios of the previous tests by showing a hole in the centre of Sample 2 with a preferential flow path for water. Some discontinuity was also observed for Samples 6 and 8 which have shown to be the worst in each category due

to more significant flow in green-yellow areas. Finally, the best-case were found for the Samples 2, 6 and 8 which showed more uniform colours in their surfaces accordingly to the watertightness test results.

3.2.2. Ultrasonic pulse velocity tests

The results of the ultrasonic velocity tests are presented in Table 2 in terms of wave the propagation time. This propagation time was measured and can be directly compared for all samples since the distance between the probes is the same. In this table, $M1_i$ is the value measured after the 1st round but before the watertightness test and $M1_f$ indicates the value measured after the 1st round and after the watertightness test, being the same for $M2$ and $M3$.

The comparison of the improvement between the treatment round are also presented in Table 2. The colours follow a variation such that the greens correspond to shorter and more satisfactory measurement times because the cracks are better sealed, while the red colours indicate the opposite. The yellow and orange colours are intermediate, and the legend is indicated below the table.

Table 2. Propagation times measured in the ultrasonic velocity tests.

Sample		Reference measure [μ s]	Reference measure with sand [μ s]	M1 _i [μ s]	M1 _r [μ s]	M2 _i [μ s]	M2 _r [μ s]	M3 _i [μ s]	M3 _r [μ s]
0.1 mm	1	53.80	-	49.73	49.70	49.30	48.17	-	-
	2	54.03	-	48.40	48.50	47.73	47.67	-	-
	3	55.43	-	48.83	49.23	47.50	47.47	-	-
1 mm	4	112.43	-	50.90	50.90	48.30	48.23	48.40	48.23
	5	71.40	-	50.47	50.53	48.77	49.53	48.90	48.90
	6	116.53	-	48.97	48.67	48.03	47.90	48.13	47.63
10 mm	7	0.00	131.93	54.40	-	49.73	-	48.47	48.90
	8	0.00	144.57	56.47	-	51.57	-	49.97	50.63
	9	0.00	310.90	51.30	-	49.60	-	47.97	48.73

Note: ■ 47 – 49 μ s; ■ 49 – 51 μ s; ■ 51 – 60 μ s; ■ 60 – 100 μ s or 0 μ s

Note: The reference measure with sand is only applicable to Samples 7, 8 and 9 with crack opening of 10 mm.

The samples with 0.1 mm cracks – Samples 1, 2 and 3 – showed very good results, with a significant improvement in the propagation time of the ultrasonic waves, which varied from approximately 54.50 μ s, in the pre-treatment, to average values of 47.70 μ s after the last round. These values are essentially close to the reference values of 47.40 μ s for samples with 0.1 mm openings, demonstrating the effective filling of the cracks.

Likewise, the 1 mm crack opening samples - samples 4, 5 and 6 - had their propagation times respectively 112.43, 71.40 and 116.53 μ s before treatment, that changed to 48.23, 48.90 and 47.63 μ s after the three treatment rounds. These values are also similar to the reference values for this category, 48.10, 48.00 and 47.60 μ s, indicative of biocement deposition.

Finally, the samples with a crack opening of 10 mm - Samples 7, 8 and 9 - which did not even allow the passage of the wave signal at first due to the space between the two parts, had their propagation times of the waves resumed for values of 48.90, 50.63 and 48.73 μ s, respectively. These numbers are also similar to the reference numbers, reflecting the effectiveness of the treatment.

A point of significant importance is the fact that the measurements before and after the watertightness tests showed little changes in obtained values, which corroborates the fact that there is no washing or excessive detachment of solid particles after contact with water. This may be related to the calcite form of deposition of calcium carbonate identified in mineralogical analysis with higher durability and lower solubility.

The results from this test confirm the results of the previous tests because the wave propagation time for the different treatment rounds was reduced due to the production of biocement.

3.3. Other tests

The presence of biocement precipitated in the cracks was confirmed by detecting vaterite and calcite in the mineralogical analysis. From the bending tests after the biocementation treatment it was possible to conclude that this treatment was not capable of restoring the mechanical properties of the broken samples. No significant strength was measured for Samples 4-9, however Samples 1, 2 e 3 had a better performance, recovering about 90% of the initial resistance. Further details can be found in [11].

3.4. Recommendation of this treatment for practical cases

Some relevant recommendations when considering promoting biocementation treatment to improve crack watertightness in water storage tanks are presented in the following paragraphs.

(i) Concerning the number of treatment applications, at least three inoculation rounds should be carried out for larger cracks (i.e., with widths larger than 5 mm) and only two are sufficient for the smaller ones (i.e., with widths smaller than 5 mm). Each round must have at least 10 applications, the first five with bacteria and feeding solution and the last five with feeding solution only.

(ii) Larger cracks with more than 5 mm require the use of sand to fill the crack and to improve the efficiency of the treatment

(iii) At least a 2-hour-break must be given between two consecutive applications in order to respect a certain period of curing and reaction of the compounds.

(iv) After completing the treatment in each round, tests should be carried out to check the quality of the seal, aiming at continuously monitor the evolution of the treatment. Non-destructive techniques are advisable, such as large-scale thermography, adapted watertightness tests and computed tomography.

4. CONCLUSIONS

The treatment by biocementation to promote watertightness of the concrete cracks was proven to be efficient, however with different behaviours that varied according to the crack width. Above all, the samples with 0.1 mm width cracks - Samples 1, 2 and 3 - showed the best behaviour because watertightness was achieved.

Filling with sand before the treatment was very efficient for larger cracks (i.e., with 10 mm widths). This was because, after the treatment rounds, a very solid conglomerate was formed between the biocement and the sand, which stuck to the rough concrete walls.

It was possible to verify that the biocementation technique was efficient to restore cracks the watertightness of the sample, however it was not adequate to reconstitute the structural capacity of the concrete because of the poor results of the bending tests performed after the completion of the treatment, suggesting it is necessary to combine this technique to another one such as metallic stapling when the structural resistance recovery is requested.

As future studies, first it will be important to define the number of rounds necessary to seal each opening as function of the respective width. Thus, it would be possible to establish the number of applications according to the characteristics of the crack, in addition to realizing whether it would be possible to completely seal it by extending the treatment period. Second, it is crucial to study different water column heights and to determine the maximum pressure (water heights greater than one meter) that the cracks would withstand. Third, further studies on the risk of biocement dissolution are also particularly important. For this, the watertightness tests could be kept longer to see whether the biocement dissolves after a certain period of contact with water. Fourth, diagnostic tests of water quality are also important to be studied, particularly because there is a lack of knowledge on the risk of water contamination in cases of drinking water storage tanks. Fifth and final, it is recommended to deepen the studies aimed to optimize resources, time and money and seeking to improve the performance of this alternative technique and to study ways to make it more competitive and viable for application to a wider range of different needs.

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5. REFERENCES

- [1] Siddique, R., Chahal, N. K., (2011), "Effect of ureolytic bacteria on concrete properties", *Construction and Building Materials*, Elsevier, vol. 25, pg. 3791-3801.
- [2] Rodriguez-Navarro, C., Rodriguez-Gallego, M., BenChekroun, K. Gonzalez-Munoz, M.T., (2003), "Conservation of ornamental stone by *Myxococcus xanthus*-induced carbonate biomineralization", *Appl. Environ. Microb.*, vol. 69, pg. 2182–2193.
- [3] Dick, J., Windt, W. D., Graef, B. D., Saveyn, H., Meeren, P. V. D., Belie, N. D., Verstraete, W., (2006), "Bio-deposition of a calcium carbonate layer on degraded limestone by *Bacillus* species", *SpringerLink*, vol. 17, pg. 57-67.
- [4] Le Metayer, G., Orial, S. C., Loubière, J. F., Perthuisot, P., (1999), "Applications of bacterial carbonatogenesis to the protection and regeneration of limestones in buildings and historic patrimony", *Sedimentary Geology*, Elsevier, vol. 126, pg. 25-34.
- [5] Tiano, P., Cantisani, E., Sutherland, I., Paget, J.M., (1995-2006), "Biomediated reinforcement of weathered calcareous stones", *J. Cult. Herit.*, vol. 7, pg. 49–55.
- [6] Bonshor, R., Bonshor, L., (2001), "Cracking in buildings", Routledge, London.
- [7] Flores-Colen, I., e Brito, J., (2005), "Diagnóstico, Patologia e Reabilitação de Construção em Betão Armado", Mestrado em Ciências da Construção da Universidade de Lisboa (in Portuguese).
- [8] Von Fay, K.F., (2015), "Guide to Concrete Repair", Second Edition, United States Department of the Interior, Bureau of Reclamation, Books for Business.
- [9] Gaspar, P. L., Flores-Colen, I, Brito, J., (2006), "Técnicas de Diagnóstico e Classificação de Fissuração em Fachadas Rebocadas", Instituto Superior Técnico, Universidade de Lisboa (in Portuguese).
- [10] Brito, J., (2021), "Reabilitação de estruturas e elementos de betão", Tecnologia da Construção de Edifícios, Mestrado Integrado em Engenharia Civil do Instituto Superior Técnico (in Portuguese).
- [11] Bonetti, L. S. F., (2022). " Biocimentação como técnica para selagem de fissuras em reservatórios de água em betão ", MSc Thesis. Instituto Superior Técnico, Universidade de Lisboa (in Portuguese).