# Self-healing of concrete containing commercial bacteria by means of water and chlorides permeability

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**Abstract.** Microbial-induced calcium precipitation (MICP) has shown adequate potential to act as a healing product through Calcium Carbonate ($CaCO_3$) precipitation inside cracks. This work studies the self-healing capability of conventional concrete incorporating two dosages of *Bacillus subtilis* encapsulated in diatomaceous earth and a liquid solution consisting of a combination of Bacillus, denitrifying, and photosynthetic bacteria. The two bacterial agents used are commercial or industrial products from other sectors. For these mixes, disks of size ϕ100×50 mm were pre-cracked at the age of 21 days by splitting test until reaching residual cracks of 100 to 450 µm. At the age of 28 days, self-healing was promoted during 28 days in three exposures, continuous water immersion at 20°C, a high humidity environment at 20°C and 95% of relative humidity, and 7 days immersed in water at 20°C and 21 days in the high humidity environment. Self-healing was analyzed with water permeability by comparing the results before and after healing. Afterward, chlorides' penetration was performed to study the possible healing protection on cracked disks compared to uncracked reference disks. As a result, after 7 days of immersion in water, the mixes with bacteria presented acceptable healing results. Some healed cracks could also significantly reduce the penetration of chlorides towards the interior of the concrete matrix.

**Keywords**: Bacillus Bacteria, MICP, Self-healing, Water Penetration, Chlorides, Autogenous Healing

## 1 Introduction

Recently, microbially-induced carbonate precipitation (MICP) has been presented as an environment-friendly technique to improve the durability of cementitious materials [1]. The precipitation happens by metabolic activities of bacteria by converting precursors into calcium carbonate [2]. Bacterially induced $CaCO_3$ precipitation produces crack healing or sealing, which causes a decrease in permeability and penetration of corrosion promoters, which improves durability [3]. Autogenous healing of cracks in traditional concrete is reported to have limited crack healing capability, healing cracks until 200-250 µm [4,5], whereas concrete mixes using microencapsulated bacteria (in melamine-based microcapsules) presented higher healing of cracks, healing cracks until 970 µm in samples immersed in water [5]. Moreover, no healing was observed in samples stored in 95% relative humidity which shows the critical influence of the presence of water for the healing reactions [5]. In the case of permeability, samples incorporating bacteria were reported to have 10 times lower water permeability compared to non-bacterial series [5]. Other studies using bacteria reported improvements [6] and no effects [7] in the strength of mortar and concrete samples; for example, Jonkers et al [8] observed an increase in the compressive strength when using *Bacillus pseudofirmus* spores, which were added directly without encapsulating component to cement mortars.

Many studies have focused on using alkaliphilic and ureolytic bacteria like *Bacillus pasteuri* incorporated in concrete and mortars. Precipitation of calcium carbonate in concrete mixes using the *Bacillus pasteuri* caused them to have lower water absorption, lower chloride penetration, and higher compressive strength [9]. Using *Bacillus pasteuri* in three different growth media presented higher compressive strength if compared to reference samples [10].

In the case of adding bacteria directly in environments with a pH higher than 12, the bacterial activity will decrease [11]. Furthermore, the continuous decrease in pore size diameter during cement setting and hydration can limit the life span of bacteria [8,11,12]. Consequently, researchers adopted various methods to immobilize the healing agent in a carrier that could preserve microbial organisms and could be mixed with concrete.

Diatomaceous Earth (DE) can be used to protect bacteria from the high-pH environment of concrete. DE is a siliceous sedimentary rock, a product of fossilized diatoms, which has a low density and high porosity. DE has been used for in-situ bioremediation of contaminated soil and groundwater [13]. In the self-healing field, DE

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has been studied as a carrier of bacteria [11] (in this case, *Bacillus sphaericus*) to produce a self-healing agent that can be activated after cracking puts it in contact with the bacteria with air and water. The study from Wang *et al.* [11] showed that in mortars with DE immobilized bacteria, cracks with a width of 150 to 170 μm were filled partially or entirely depending on the healing conditions, better in a urea-based depositing medium, and showed an increment in water penetration resistance for specimens with bacteria up to 30% more than reference samples [11].

This study compares the self-healing capability of conventional concrete (C30/37) using bacteria contained in two different mediums, which were obtained as products from other sectors. Self-healing was evaluated by observing crack closure and water permeability. Additionally, chloride penetration protection provided by healed cracks is also evaluated.

# 2 Materials and methodology

## 2.1 Materials and mix design

Four concrete mixes were studied. One reference mix of conventional concrete (C30/37), labeled CC, two mixes with Serenade® Max bacterial product using two dosages, and one mix with a liquid bacterial solution provided by SerBiotec (Table 1).

The cement used in this study was CEM I 42.5 R-SR5 from LAFARGE. Superplasticizer Sika Viscocrete 5970 was used to obtain high workability. The aggregates used in the mixes were 0/2 and 0/4 natural sands ($d_{min}/D_{Max}$) and gravel with a $D_{Max}$ of 16 mm. Moreover, in order to maintain crack opening during the pre-cracking and healing stages, all mixes incorporated 65/35 3D steel fiber from Dramix (L=35 mm, Φ =0.55 mm, and L/Φ=65).

**Table 1.** Mix designs of conventional concrete samples

| kg / m$^3$ | CC | CC7.5DE | CC15DE | CC25LB |
|---|---|---|---|---|
| Cement I 42,5 R-SR | 280 | 280 | 280 | 280 |
| Effective water | 185 | 187 | 188 | 139 |
| Sand 0/2 | 449 | 442 | 434 | 449 |
| Sand 0/4 | 535 | 535 | 535 | 535 |
| Gravel 8/16 | 852 | 852 | 852 | 852 |
| Plasticizer | 2.3 | 5.0 | 10.0 | 2.3 |
| Dramix 65/35 | 40 | 40 | 40 | 40 |
| Serenade Max | 0 | 7.5 | 15.0 | 0 |
| SerBiotec | 0 | 0 | 0 | 46.3 |
| w/c | 0.66 | 0.66 | 0.67 | 0.66 |

Serenade® Max from BAYER is *Bacillus subtilis* encapsulated in diatomaceous earth, formulated as a wettable powder of natural origin containing 15.67% p/p (g/g) bacteria, and bacterial content of $5.13 \times 10^{10}$ CFU/g (colony forming units). Since bacteria are contained in a powder material, the incorporation of this product was performed in substitution of the sand of a smaller fraction (0/2 mm). Serenade® Max was added in the dosage of 7.5 kg/m$^3$ in the mix named CC7.5DE and 15 kg/m$^3$ in the mix named CC15DE. Due to the high DE absorption, 20% of the weight of the bacterial material was added as extra water, as well as additional plasticizer content.

SerBiotec solution is a biodegradable, non-toxic, natural, and ecological liquid solution consisting of a combination of 66.3% Bacillus bacteria, 26.2% denitrifying bacteria, and 7.5% photosynthetic bacteria, containing in total $6.81 \times 10^5$ CFU/l bacteria. This type of liquid bacterial solution is used in water treatment and soil remediation. Since bacteria is contained in the liquid solution, the incorporation of this product was performed in substitution of the water content of the reference mix. SerBiotec solution was added in substitution of 25% of the water content of the mix named CC25LB. The mix with this solution was produced with the same plasticizer content than the reference mix.

Each mix was characterized by its slump test (EN 12350-2) and its compressive strength at the age of 28 days following EN 12390-3, tested on three cubes per mix of side 150 mm.

To evaluate the self-healing capability of each mix, 8 cylinders per mix were prepared of size ϕ100×200 mm. After 21 days stored in a humidity chamber at 20ºC and Relative Humidity of 95%, these cylinders were cut in ϕ100×50 mm disks. Each cylinder was cut into three disks and the ends were discarded (Figure 1). These disks were used to evaluate self-healing properties and capability by crack closure and the water permeability and chloride penetration tests. The methodologies followed are based on those proposed in the COST SARCOS Interlaboratory test groups, among which some studies have finished but others are still ongoing [14,15].

With the purpose of having a better comparison of the involved phenomena, additional uncracked disks as a reference stored in a humidity chamber having the same age of samples were tested for chloride penetration, and these disks were named R specimens.

## 2.2 Self-healing methodology

### *2.2.1 Pre-cracking and crack width measurement*

To evaluate the self-healing capability of the four mixes, the 21-day-old disks of ϕ100×50 mm were pre-cracked by splitting test to have a residual crack between 100 and 450 μm. The crack width was measured on the same day after pre-cracking, and after healing for 28 days in various healing conditions.

All disks were observed with a YINAMA wireless optical microscope. To obtain the representative value of crack width before and after healing, each surface of a disk was measured at three points located on the crack at intervals of 2.5 cm. These six values were averaged to obtain the representative crack width of a disk (Figure 1).

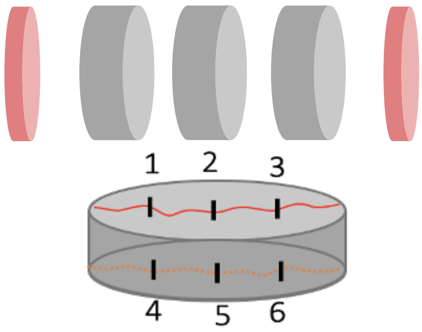



**Fig. 1.** Cut of cylinders to obtain disks (top) and crack measurements after pre-cracking (bottom)

The crack Closure Ratio (CR) is obtained using the measures of crack width before and after healing, following the expression in Eq.1

$$CR = 1 - \frac{Crack\ width\ after\ healing}{Crack\ width\ before\ healing} \quad \text{Eq 1}$$

### 2.2.2 Healing conditions

After pre-cracking the disks, they were divided into groups depending on their assigned healing condition. Three healing conditions were studied: 28 days immersion in water at 20°C (WI), 28 days in a humidity chamber at 20°C and relative humidity of 95% (HC), and 7 days in water immersion followed by 21 days in the humidity chamber (RH=95%, 20°C) (WH). The aim of this last condition was to evaluate if the mixes with bacteria (liquid solution or contained in DE) were able to produce better self-healing than the reference, having only a reduced time under water immersion that is followed by a high humidity ambient that allows keeping the saturation level inside the matrix.

### 2.2.3 Low-pressure Water Permeability Test

The water permeability test was done on pre-cracked disks after measuring the crack width. To avoid leakage of water through the lateral cracks, they were sealed with the resin Sikaflex 11 FC. Afterwards, tubes of ϕ100 mm with a height of 250 mm were glued on the disk using the same resin (Figure 2).

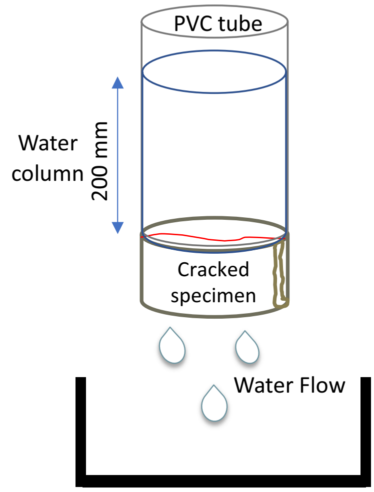



**Fig. 2** Water permeability test setup

After preparing the test setup, the tubes were filled with a 200 mm water column, and the water head level was measured after 0, 1, 5, 10, 15, 20, 25, 30, and 60 minutes. Also, to prevent errors when measuring the water head level at time 0, the bottom crack of the disk was sealed with tape. This tape was removed exactly when the timer started. To evaluate the self-healing capability of the samples, this test was done on the samples before and after healing in the aforementioned conditions.

The water head level reduction after 60 minutes was the value used in this study to evaluate self-healing capability in terms of water permeability. The Healing Ratio (HR) was obtained using the water head level reduction after 60 minutes obtained with a disk before healing and the same parameter after the disk had healed in one of the healing conditions, following the expression in Eq.2.

$$HR = 1 - \frac{Water\ Head\ Reduction\ (60')\ after\ healing}{Water\ Head\ Reduction\ (60')\ before\ healing} \quad \text{Eq 2}$$

### 2.2.4 Chloride Penetration Test

After healing for 28 days in different conditions, and after finishing the final water permeability test, chloride penetration through the healed cracks was evaluated. Hereafter, a modified chloride permeability test presented in previous studies [16,17] was performed. This test not only gives data about the permeability through the healed crack but also about the permeability of the concrete matrix, which provides information about the crack healing produced as well as variations in the matrix.

For this test, the same disk tested in the water permeability test with the same tube was used. This time, the tube was filled with a 20 cm water column containing 33g NaCl/liter, and the saline water was kept inside the tubes for 3 days. After 3 days, the tubes were removed, and the disks were sawed perpendicular to the direction of the cracks on the next day (Figure 3 top). To decrease potential contamination by sawing, this process was done in a dry condition, without water. After sawing the disks, an $AgNO_3$ solution with a concentration of 0.1 mol/liter was sprayed on both surfaces of the cut disks.

As can be seen in Figure 3 (bottom) the chloride penetration pattern emerged after pigmentation. The pattern obtained presents two main areas of penetration: the penetration depth through the crack, which is labeled as W, and penetration through the matrix which is labeled as $P_0$. In order to measure $P_0$, four points with intervals of 2 cm presenting the depth of penetration on both faces of a disk were measured at the positions indicated in Figure 3 bottom. Likewise, for the penetration produced along the crack path, three points were measured on each surface, ensuring enough distance from the surface (2 cm) to avoid the influence from the penetration from the matrix $P_0$.

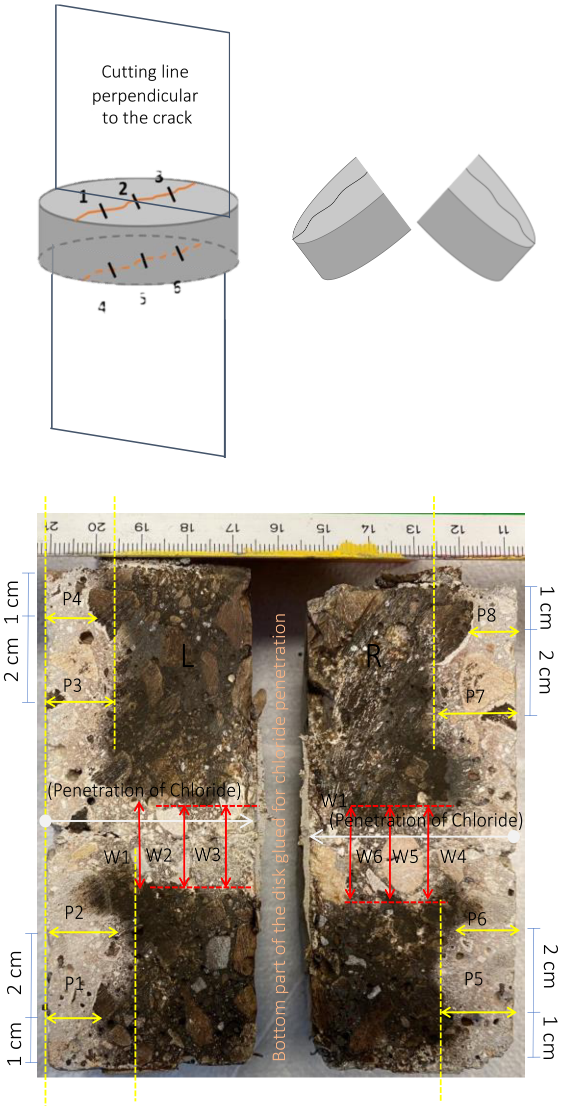



**Fig. 3.** Diagram of the sawing plane of the pre-cracked disks (top) and measurement points for the penetration of chlorides through the matrix and through the crack.

# 3 Experimental results

## 3.1. Characterization results

The compressive strength at 28 days of each mix is presented in Figure 4. The reference mix obtained an average compressive strength of 42 MPa, and the rest of the mixes had values between 36 and 47 MPa. The mix using 7.5 kg/m$^3$ of bacteria in diatomaceous earth, obtained a higher (10.56%) compressive strength than the reference mix. However, introducing a higher amount of the bacteria, 15 kg/m$^3$, reduced the compressive strength by 13.45% if compared with the reference mix. Likewise, samples substituting 25% of water by the bacterial solution produced a similar decrease in strength, obtaining a 13.95% lower strength when compared to the reference mix.

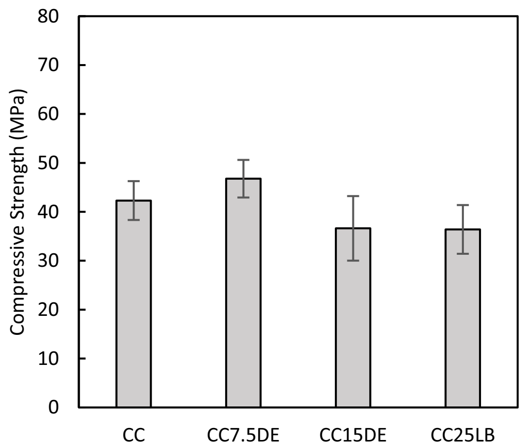



**Fig. 4.** Compressive strength of the mixes at 28 days (average and standard deviation)

In terms of workability, the slump test for all the mixes was practically the same, around 20±3 cm, which presents adequate workability even when incorporating the bacterial agents

## 3.2. Crack closure

The self-healing efficiency in terms of visual closure was analyzed with the crack Closure Ratio, in Eq.1. Figure 5 presents the crack closure ratio (vertical axis) vs the initial crack width measured right after pre-cracking, separated depending on the healing condition and their mix.

The results of Figure 5 represent noteworthy closure for samples that healed in a water immersion condition, which experienced almost complete crack closure in cracks narrower than 200 µm. Cracks below 200 µm had between 80 and 100% of crack closure ratio healing under water immersion. Only a couple of specimens healing in the WH condition, with cracks around 100-120 µm had a similar crack closure efficiency. The rest of the specimens that healed in the WH condition had crack closure values between 40 and 100% with a clear decreasing trend when crack width increased. For specimens that were stored in the humidity chamber conditions, crack closure values obtained were very low, between 0 and 60%, with most of the value below 50%. This shows the critical effect of the presence of water on the healing reactions in both, reference mixes and mixes with the two types of bacteria.

Regarding the efficiency of bacteria, the CC7.5DE samples presented notable crack healing up to 300 μm, especially when healing in water immersion. The rest of the groups had no clear differences because of the presence of bacteria.

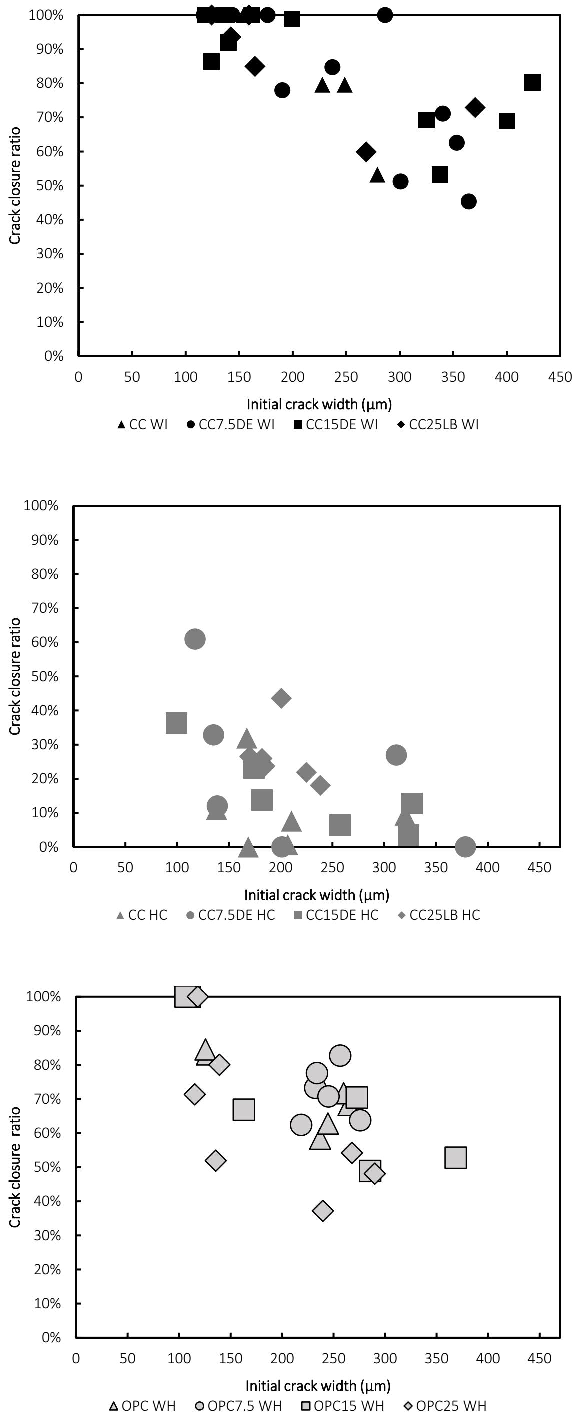



**Fig. 5.** Crack closure ratio of each mix and healing conditions depend on the initial crack width. The first row for samples healed in water immersion condition, the second row for samples stored in a humidity chamber, and the third samples healed 7 days inside water and 21 days in humid condition

### 3.2 Self-healing in water permeability

The self-healing ratio obtained by means of water permeability of the sample depends on the initial crack width obtained for each disk before healing. Figure 6 displays the healing ratio obtained using Eq. 2, depending on their initial crack width in the three healing conditions.

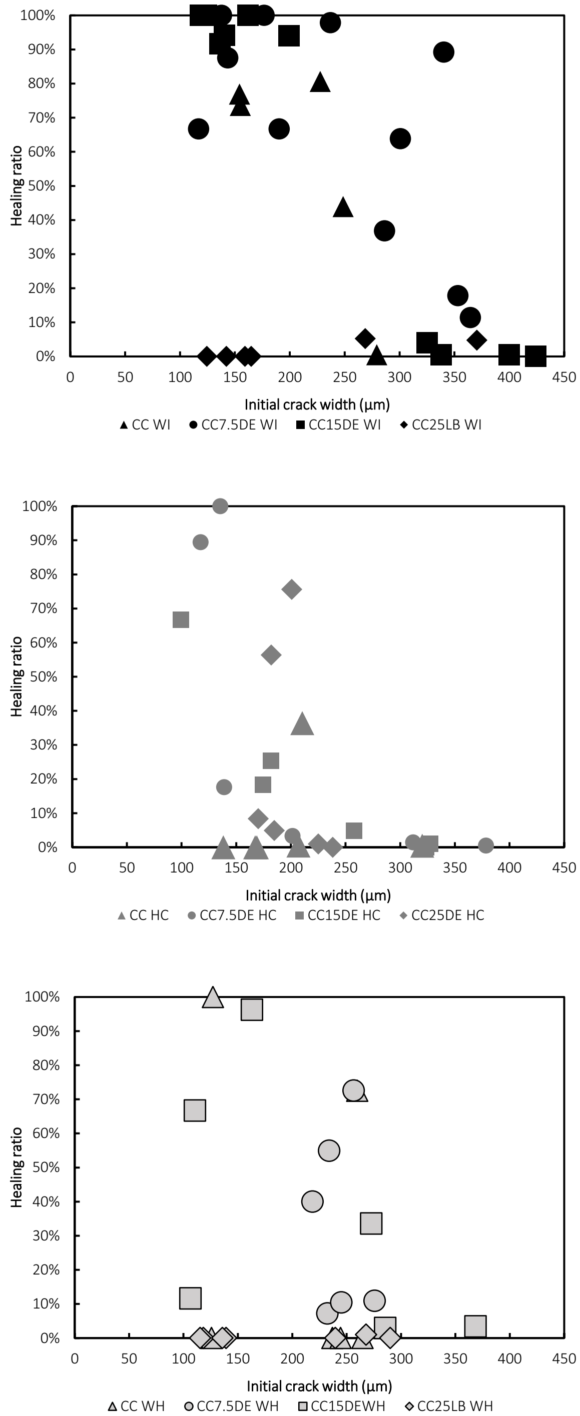



**Fig. 6.** Healing ratio of each mix and healing conditions depend on the initial crack width. The first row for samples healed in water immersion condition, the second row for samples stored in a humidity chamber, and the third samples healed 7 days inside water and 21 days in humid condition

The results show that healing in terms of water permeability is improved when extending the time in water immersion. Figure 6 graphs show a quasi-vertical line indicating a limit in the healing efficiency for the time used in this study. Disks healed in a humidity chamber (HC) can experience improvements in terms of water permeability for cracks up to 180 µm, disks healed in the WH condition for cracks up to 250 µm, and disks healed in water immersion, for cracks up to 300 µm.

The results show better healing ratios in the samples incorporating bacteria in diatomaceous earth immersed in water. This can be seen in Figure 6 (top) for the samples incorporating DE, whose healing ratios are higher than those of the rest of the mixes. In contrast, specimens with liquid bacteria healing under water immersion conditions (WI and WH), had no self-healing in terms of water permeability. While the crack closure ratio of specimens that healed for one week in the immersion condition for 7 days and 21 days in the humidity chamber (WH) presented over 50% crack closure in samples of even 400 µm, the healing in terms of water permeability for most of the disks was very variable, with values between 0 and 100% in all the healing conditions.

### 3.3 Chloride penetration of healed cracks

By analyzing the chloride penetration data, the average value of $P_0$ and W for each disk of a mix were collected.

CC uncracked disks had an average penetration of chlorides of 12.8 mm. Two of the mixes with bacteria had better results with lower penetration, CC25LB and CC7.5DE disks had 6.38 and 7.75 mm as the average penetration respectively. Figure 7 compares, as an example, the penetration through the matrix ($P_0$) of the reference disks (uncracked) for the reference mix CC and the CC7.5DE mix with bacteria in DE. However, disks incorporating 15 kg/m$^3$ of DE bacteria had an average matrix penetration of 21.19 mm, higher than the reference. This last result might be caused due to the large contents of diatomaceous earth, but this point needs further research.

Regarding the pre-cracked disks, as in the uncracked disks, the healing condition had a significant effect on the $P_0$ values obtained. Those healed in the humidity chamber presented a similar $P_0$ value to the reference samples which shows that humidity chamber conditions are not able to activate healing reactions nor reduce the penetrability of the matrix. In contrast, disks healed in WI and WH conditions experienced lower penetration through the matrix than reference and HC samples. Similar to the results obtained in a previous study [18], no relationship was detected between the initial crack width (nor crack width after healing) and the penetration through matrix values $P_0$.

Additionally, the penetration width through the crack (W), was also evaluated. Figure 8 depicts, as an example, two patterns of penetration for mixes CC and CC7.5DE disks healed under water immersion for 28 days. The CC samples presented usually higher W values than the mixes with bacteria. The lower W penetration was obtained in the mixes CC25LB and CC7.5DE.

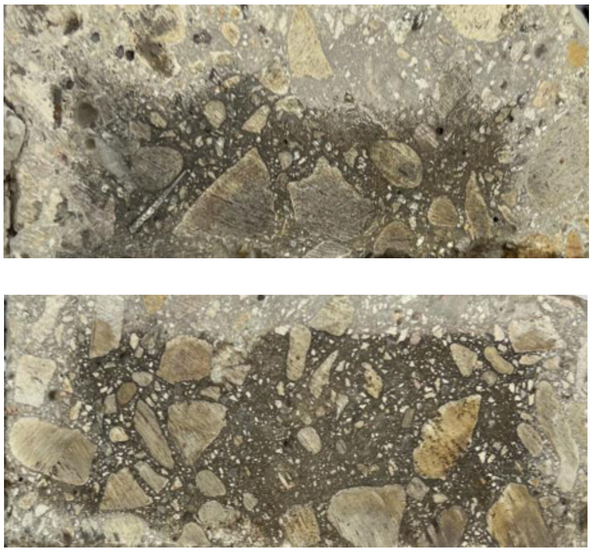


**Fig. 7.** Penetration of the chloride through the matrix in CC (top) and CC7.5DE (bottom) (Direction of the penetration of the chloride is top to bottom)

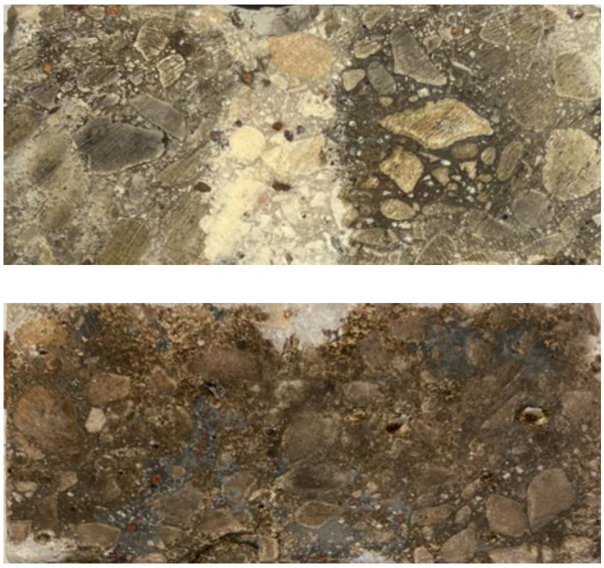
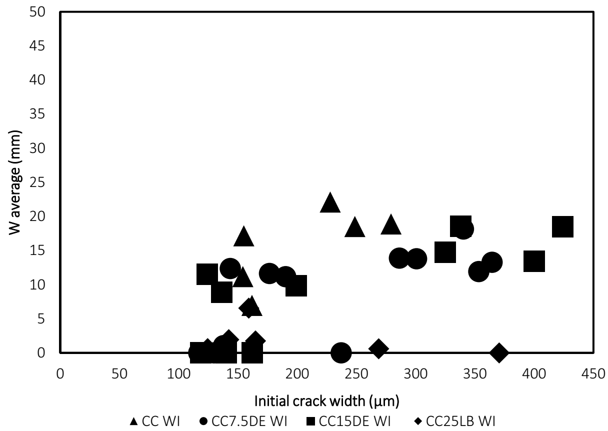
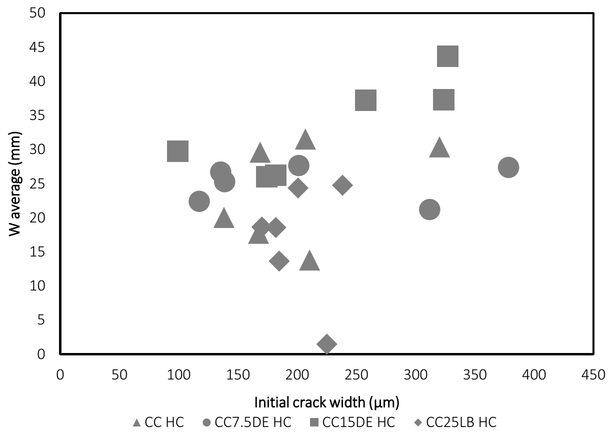


**Fig. 8.** Penetration of the chloride through the matrix and crack in CC with 227µm (top) and CC7.5DE with 237µm crack width (bottom), both healed in water immersion conditions (Direction of the penetration of the chloride is top to bottom)

The penetration width through the crack (W), was also analyzed depending on the initial crack width of each disk (Figure 9). The mix without bacteria (CC) had a higher penetration (25 to 30 mm) to chlorides in samples healed in water immersion conditions. Those samples that incorporated bacteria and healed in water immersion conditions provided acceptable protection from chloride penetration, which can be seen in the lower chloride penetration through the crack obtained (below 20 mm). This result was more notable for samples incorporating liquid bacteria (CC25LB mix), which showed almost complete protection after healing.

In contrast, those disks healed in the humidity chamber, had high penetration of chlorides, independently of the initial crack width value, which indicated a negligible effect of the humidity chamber in reducing the chloride penetration after healing.



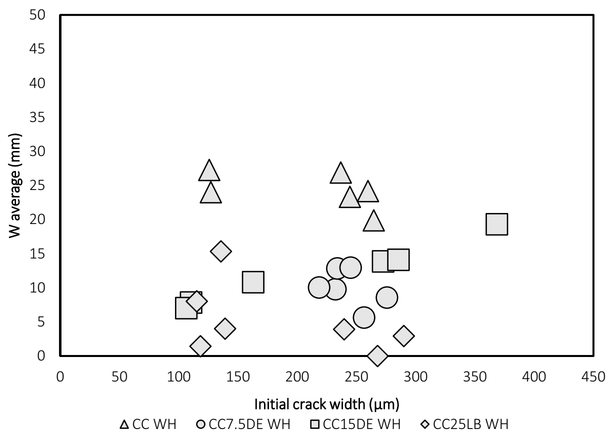




**Fig. 9.** Comparison of the chloride penetration along the crack path (W) after healing depending on their initial crack width separated by their healing condition

## 4. CONCLUSIONS

The conclusions that can be drawn are:

- The results of crack closure show the importance of the presence of water for the healing reactions to fill the cracks. Disks healed in water immersion conditions for 28 days presented a higher closure ratio (almost complete closure) for all samples with cracks up to 200 µm. Additionally, the samples immersed for a week in water immersion and then stored in a humidity chamber presented better results than those that were just healed in the humidity chamber.
- For the water permeability test, the best results were obtained for all mixes when healing in water immersion conditions. Samples incorporating 7.5 kg/m$^3$ of bacteria in DE presented better healing, while the samples incorporating liquid bacteria presented a lower healing ratio. Furthermore, a lower water healing ratio in terms of permeability was obtained for all samples that healed in the humidity chamber, especially for reference samples without bacteria.
- Penetration of chlorides through the matrix in samples healed in the humidity chamber presents a similar value to penetration of the samples without cracks. Similarly, samples using bacteria that healed in water immersion conditions presented lower penetration, which demonstrates the effect of healing condition as a most effective value to have a lower penetration through the matrix.
- Regarding the penetration of chlorides through the crack, the samples incorporating liquid bacteria presented noticeably lower penetration width for those disks healed in water immersion conditions. Furthermore, the higher chloride penetration of reference samples healed in water immersion conditions compared to those containing bacteria shows the effectiveness of using bacteria to reduce chloride penetration.

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