

Viability of *Bacillus subtilis* immobilization using silica gel for self-healing of cement based materials

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Concrete, Immobilization, Self-healing.

Abstract— Cracks in concrete's structure may lead it to lose some properties, such as compressive strength. The use of self-healing concretes by the addition of bacteria is a way of sealing these cracks. In this work, 10^5 cells/ml of *Bacillus subtilis* showed favorable to this application. In the analysis of Dispersive Energy Spectroscopy coupled to Scanning Electron Microscopy, the *Bacillus subtilis* bacteria showed CaCO_3 precipitants in mortars samples. By means of the analysis of optical microscopy, the closure of 0.4 mm induced crack was severe observed during 168 days. Furthermore, an increase in compressive strength was observed by the immobilization of *Bacillus subtilis* on silica gel, by the addition of only silica and only to the bacterium.

I. INTRODUCTION

The second most consumed material worldwide is concrete, losing only to water. It is also the most used material in civil construction, which implies in its high consumption and, consequently, in an increase in the consumption of its components, such as cement (Instituto Brasileiro do Concreto, 2009). The durability of concrete is highly related to its permeability, which can provide an easy path for the ingress of potentially harmful liquids and gases that, consequently, causes loss of mechanical properties (Xu & Yao, 2014).

The risk of accidents caused by concrete degradation is recurrent. There are records of several landslides, such as a bridge in Genoa, Italy (2018) and viaducts in São Paulo (2009), Belo Horizonte (2014), Fortaleza (2016) and Brasília (2018). Data indicate that one in five bridges or viaducts needs renovation (Gomes, 2018).

In addition to this concern, there is also the economic issue. In the work of Medeiros, Andrade & Helene (2011), expenses with maintenance of concrete are presented in

developed countries and it can be seen that the cost of repairing these structures is equivalent to the cost for a new construction. Furthermore, it can be impossible to repair affected structures that are in continuous service, such as tunnels and highways (Xu & Yao, 2014).

One way to protect the concrete against this deterioration and increase its durability is to seal cracks and pores that allow degrading elements to enter the material. Nowadays, a wide variety of products are available to provide this protection, among which are: types of coverings, water repellents and pore blockers. However, these means have some disadvantages, such as a difference in the coefficient of thermal expansion, degradation over time, cost for constant maintenance, and these materials may contain solvents that contribute to environmental pollution (Muynck, Cox, Belie & Verstraete, 2008). The use of self-healing concrete is environmentally friendly and prevents future repairs (Huynh, Imamoto & Kiyohara, 2019). This concrete can be made by incorporating calcium carbonate precipitating bacteria (CaCO_3) into its structure. This

precipitation is capable of closing cracks, decreasing the porosity of the material and being able to increase its compressive strength (Wang, Soens, Verstraete & Belie, 2014). Some types of bacteria can be added in different ways to the concrete and one of them involves its immobilization on silica gel, which protects the bacteria from the highly alkaline pH and from cement hydration (Wang, Tittelboom, Belie & Verstraete, 2012).

This article aims to analyze the self-healing of cracks, by CaCO_3 precipitation, in cementitious materials containing the bacteria *Bacillus subtilis* immobilized by silica gel, the immobilization of the *Bacillus subtilis* after the addition of silica to the mortars and the influence of these addition in the compressive strength property.

II. MATERIALS AND METHOD

The methodology used in the study is illustrated in Fig. 1. Each step will be discussed and further detailed below.

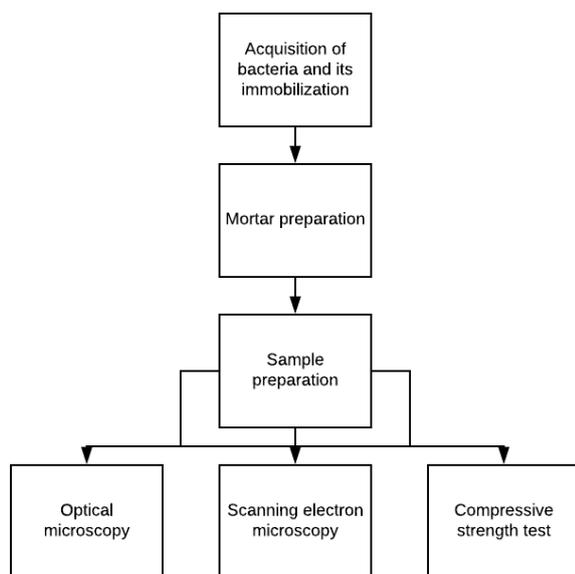


Fig. 1: Work methodology flowchart

2.1 Acquisition of bacteria and its immobilization

The bacterium *Bacillus subtilis* was supplied in association with State University of Londrina. The concentration used was 105 cells/mL in a saline solution.

For the growth of the bacteria that made up the mortar, they were inoculated in the LB (Luria-Bertani) culture medium, according to the parameters presented by Schwantes-Cezario, Nogueira & Toralles (2017).

To immobilize the bacteria, silica gel used was Aerosil® 200 from Evonik.

To prepare the silica gel for the immobilization, a mixture of 30 g of silica powder with 100 mL of water was prepared, as indicated in Tittelboom, Belie, Muynck & Verstraete's (2010) research. In order to verify the bacteria immobilization on silica gel, images were taken on Physis stereoscope.

2.2 Mortars preparation

Due to the fact that the mortar and concrete have similar compositions and the practical use of the mortar, the samples were made of this material. Quartz river sand, Portland cement of high initial strength (CP V – ARI, Brazilian denomination) and PVC molds were used to make the samples.

The mortar was composed of a cement-to-sand ratio of 1:3 (by weight) and a water-to-cement ratio of 0.50. Bacteria without immobilization and immobilized on silica gel were added during mixing mortar.

To check the quantity of materials used, calculations were performed relating the volume of the specimens to the mix proportion, using a mortar density value of 2.4 g.cm⁻³.

To prepare the mortars, four different mixes were made. Their compositions and names are shown in Table 1.

2.3 Sample preparation

The molds used were cut from a four-meter PVC pipe. The cuts were made by a bench saw by the company FUNDISA. Thirty-two molds were molded with dimensions of 50 mm in diameter and 100 mm in height. Eight specimens were produced for each composition. These samples were used for compressive strength tests.

For the first composition (C + S), the cement and sand were mixed, then, the water was added gradually. To receive the mixture, the molds of all compositions received an application of release agent.

Table.1: Mortars preparation – Compositions and nomenclature

Composition	Nomenclature	Cement (g)	Sand (g)	Water (mL)	Water (mL) with Silica gel	Water (mL) with Bacteria	Bacteria (cells/mL)
Cement, sand and water	C + S	942.48	2827.44	471.24	-	-	-
Cement, sand, water and silica gel	C + S + SG	942.48	2827.44	421.24	50	-	-
Cement, sand, water and bacteria	C + S + B	942.48	2827.44	-	-	471.24	1 x 10 ⁵
Cement, sand, water, bacteria and silica gel	C + S + B + SG	942.48	2827.44	371.24	50	50	1 x 10 ⁵

To mold the specimens, the NBR 7215 (Brazilian standard) was adapted: Portland cement - Determination of the compressive strength (ABNT, 1997). The placement of the mortar in the mold was made with the help of the spatula, in four layers of approximately equal height, each layer receiving 30 uniform strokes with the socket, homogeneously distributed. Finally, the top of the specimen was scraped.

For the second composition (C + S + SG), the same procedure was performed in relation to cement and sand. For the formation of silica gel, 10 ml of deionized water with 1.2 g of sodium chloride were added to 40 ml of the previously prepared solution and this mixture was taken to a magnetic mixer until the consistency was similar to a gel. The gel was placed after these materials. For molding, compaction followed the procedure described in NBR 7215/1997 (ABNT, 1997).

For the third composition (C + S + B), the amount of bacteria of 10⁵ cells/mL was placed in the water that had been separated into the mixture, with an addition of 0.85% sodium chloride, to maintain the medium isotonic for the bacteria.

Afterwards, the molding followed the same procedure as the other two mortars.

For the fourth composition (C + S + B + SG), the amount of bacteria of 10⁵ cells / ml and 0.85% sodium chloride were placed in the separate water for this composition. The procedure used in the preparation of

silica gel in composition C + S + SG was repeated, but 50 ml of the water with bacteria was added to the solution.

A small sample of the water with bacteria was analyzed on the PHYSIS stereoscope to check if the immobilization in silica gel has happened.

At the end of each impression, a piece of film paper was placed over the sample so that water would not evaporate and cause porosity.

Twenty-four hours after the completion of the moldings, the specimens were placed in four buckets (one for each composition) containing water saturated with calcium oxide and remained this way until the test ages.

2.4 Optical microscopy

To perform the crack analysis, four samples were made with a crack in each one. A CAP was cut to serve as a template and the height obtained was approximately 2 cm. With the help of a spatula, a certain amount of the composition was placed and a few strokes were made with the socket until the mold was filled. Galvanized plates measuring 2 cm x 2 cm with a thickness of 0.43 mm were used to generate the crack in the mortar. The same procedure was performed for the four compositions.

The crack analysis was carried out on an optical microscope with attached camera Axio Scope.A1 at the ages of 2, 14, 28 and 168 days.

2.5 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) was performed at the Electron Microscopy and Micro Analysis Laboratory - LMEN, of the State University of Londrina, using the FEI Quanta 200 Microscope.

The samples for the analysis were taken from the compressive strength test performed on the seventh day of cure. The samples were then kept in water saturated with calcium oxide until they were taken to the laboratory (in order to be analyzed at the twenty-eighth day of cure) to be covered with gold. The sample size was approximately 0.5 cm x 0.5 cm x 0.5 cm.

2.6 Compressive Strength

The compressive strength test was carried out according to the NBR 7215 standard: Portland cement - Determination of compressive strength (Brazilian Standard), with the exception of the mold material and capping. The specimens were broken at the ages of 7 and 28 days, in the universal testing equipment, model WDW-100E. To smooth the surface, a piece of neoprene was used. Thus, there was a better distribution of the load application. Such application was carried out with a speed of 0.5 kN/s and a preload of 0.3 kN. For each composition, four specimens were tested at each age of analysis.

III. RESULTS AND DISCUSSION

In order to verify whether the bacterium was immobilized on silica gel, the image taken on the Physis stereoscope was observed in Fig. 2.

It is known that it is possible to identify the spores in a stereoscope by bright points (Figueiredo, Pedro & Barroso, 1989). In the center and on the left side of the photo, it's seen two clusters of material that appear to form a network. This characteristic is the same that silica obtains when it becomes silica gel. Within these clusters, it is possible to observe bright spots, which may be the spores. It is possible that the silica gel was not yet fully formed and that is why you can see a bright spot without being involved in this material at the top of the image. In Fig. 2, bacteria are pointed out by arrows and silica gel by asterisks.

From this analysis, it is likely that the bacterium was immobilized by silica gel upon contact with the same.

3.1 Optical microscopy

In Fig. 3, it's seen the images taken from the cracks at 2, 14, 28 and 168 days after molding. A comparison is made between the cracks in the same composition.

In compositions C + S and C + S + SG, no crack closure was observed over the days, while in compositions

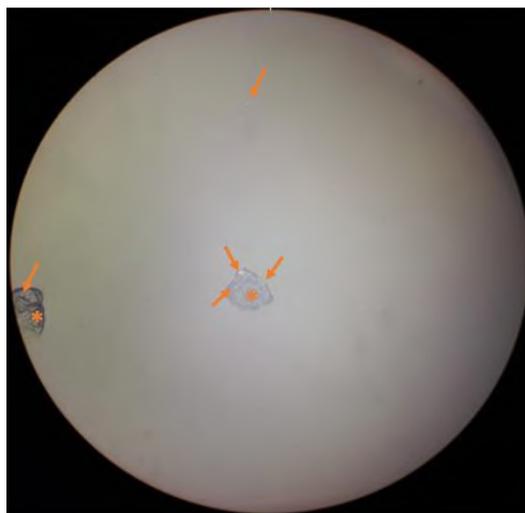


Fig. 2: Bacteria immobilized on silica gel. Bacteria are pointed out by arrows and the silica gel by an asterisk

C + S + B and C + S + B + SG, partial crack closure occurs at 14 days and certain regions close almost completely at 28 days. At 168 days, it can be seen that more regions have been closed and this may be an indication that, over time, the closure of cracks is increasingly greater.

Compositions C + S + B and C + S + B + SG contain CaCO_3 precipitating bacteria, which is probably why the fissure is being closed over the days.

Other researchers studied the healing of cracks by the addition of bacteria.

Wang, Soens, Verstraete & Belie (2014) verified the incorporation of *B. sphaericus* spores microencapsulated by a polycondensation reaction over 8 weeks. In the bacterial series, the cracks healed from 48% to 80%, but the fissures weren't totally closed.

Rong et al. (2020) studied the effect of addition of *B. pasteurii*, a type of urease-producing bacteria, on the self-healing of cement mortar. The most significant crack healing was verified at the end of 50 days and for cracks with a width of 0.2 – 0.3 mm.

In the studies of Luo, Qian & Li (2015), spore-forming alkali-resistant bacteria were used to analyze the factors affecting its crack repairing capacity. They verified that when the crack of age was more than 60 days, the crack healing ratio was very small, despite of this study in which the crack repair was significant noticed over the 168 days. After 100 days, bacteria *alkalinitrilicus* impregnated in expanded clay with a culture medium also showed favorable to healing cracks in the studies of Wiktor & Jonkers (2011).

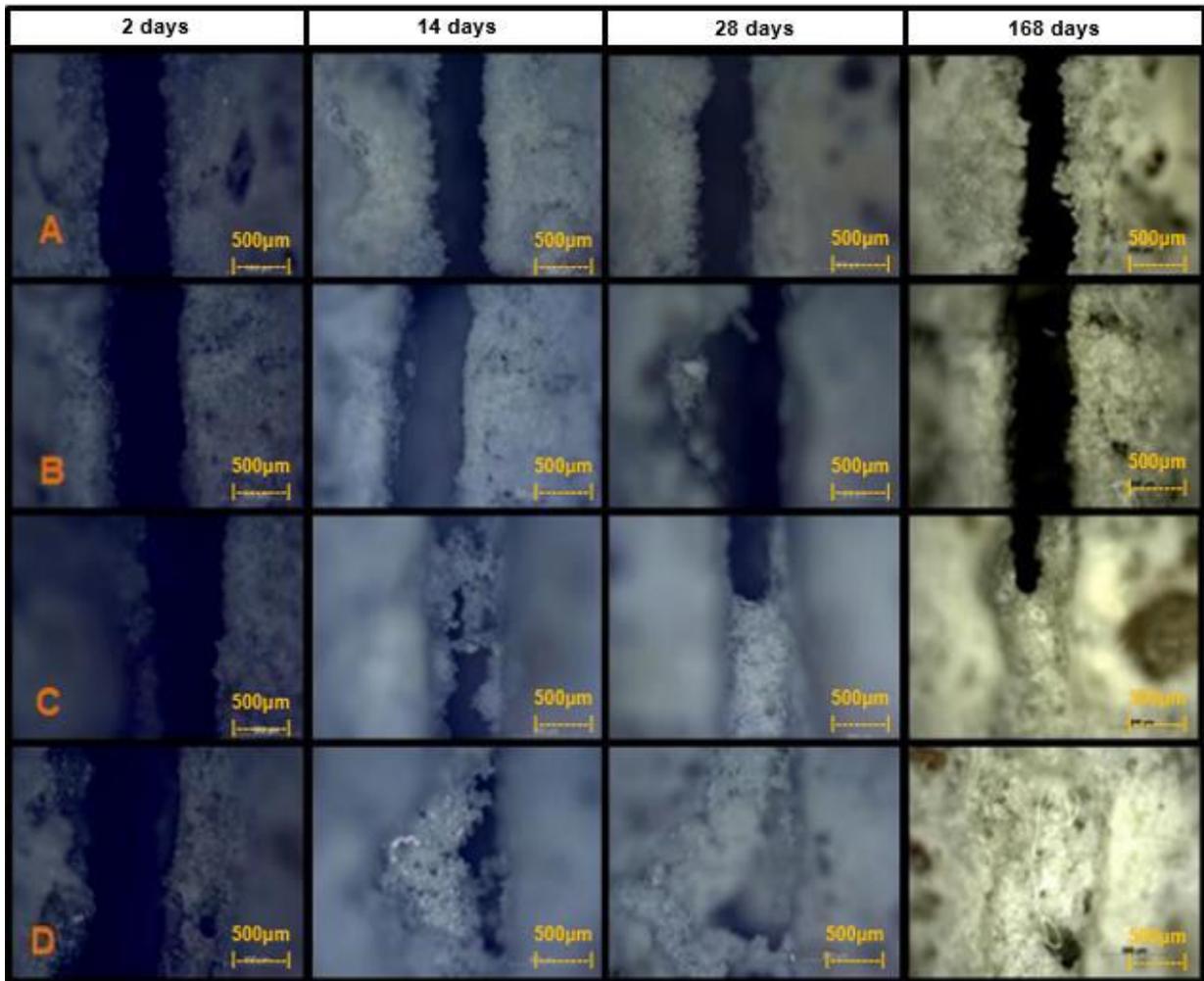


Fig. 3: Monitoring of cracks at 2, 14, 28 and 168 days for (A) composition C + S, (B) composition C + S + SG, (C) composition C + S + B and (D) composition C + S + B + SG

Liu, Xu, Lv & Xing (2020) studied the self-healing capacity of *B. pasteurii* carried by recycled aggregate. It was verified that specimens that were directly introduced bacteria without protective carrier had their cracks slightly decreased at 14 days of healing. However, this decrease wasn't observed at 28 days. The authors attributed that to the difficulty of bacteria to survive in high alkaline environment without protection.

In the addition of *B. subtilis* in contact with urea and calcium chloride, Kalhori & Bagherpour (2017) verified the self-healing of cracks after 28 days. As mentioned before, the addition of calcium chloride as a source of calcium can be harmful to concrete's structure. The bacteria *B. subtilis* added without chlorides to mortar in this study also promoted self-healing of cracks at the first seven days of cure.

In reinforced concrete, the structure is protected by a thin oxide layer promoted by the concrete alkalinity. That layer can be destroyed by the carbonation of concrete or by

the presence of chlorides. The destruction of that layer by chlorides causes pitting corrosion (Ormellese, Berra, Bolzoni, & Pastore, 2006). At the study of Tittelboom, Belie, Muynck & Verstraete (2010), calcium chloride was added with the bacteria immobilized by silica gel and analyzed. The influence of the calcium source is related only to the morphology of the crystals and not to its efficiency, therefore it was suggested alternative calcium sources. Considering the harmfulness of chlorides to the reinforced concrete and its influence limited only to the morphology, in this study there was no use of calcium chloride and the calcium source was limited to the water saturated with calcium oxide and calcium hydroxide from cement hydration reactions. As observed at the studies of Tan et al. (2020), for cementitious composites that do not carbonate prior to cracking, this calcium hydroxide is sufficient to provide an efficient level of healing. Besides that, the production of ammonium is concerning because of its detrimental effects on concrete. That production is

similar to an acid attack and it contributes to calcium hydroxide leaching (Kaur & Mukherjee, 2012).

At Tittelboom, Belie, Muynck & Verstraete's (2010) studies, the bacteria immobilized with silica gel was added to the cracks by the means of a syringe. Although their results can't be compared to the immobilization of bacteria with silica gel during mixing mortar, this mean of addition was chosen because of its applicability. It would be more functional not to have to fill in the cracks with the bacteria immobilized when repairing a structure.

Thiyagarajan et al. (2016) concluded that the supply of nutrients plays a significant role in the bacterial activity in cement mortar. Alazhari, Sharma, Heath, Coope & Paine (2018) incorporated in mortars *B. pseudofirmus* encapsulated in expanded perlite with following nutrients: yeast extract and calcium acetate. The healing of cracks occurred after 165 days of analysis. Despite of what was done in this study, the addition of nutrients may lead to a decrease in compressive strength of the concrete. This fact was observed when Joshi, Goyal & Reddy (2018) studied the effect of nutrient components of media on structural properties of concrete during biocimentation.

3.2 Scanning Electron Microscopy

From the Scanning Electron Microscopy (SEM), performed in the Microscope model FEI Quanta 200, the images of Fig. 4 were obtained. Coupled to the SEM, using the INCA software the performance of Dispersive Energy Spectroscopy (EDS) in certain regions were obtained (Fig. 4).

With the SEM analysis, it was possible to verify, for the first composition (C + S), the presence of one of the cement hydrations forms: the ettringite (needle shape), which is indicated by "ETT" in Fig. 4. In the EDS, the presence of calcium is observed, which is one of the components of cement hydration products and is present in ettringite.

For the second composition (C + S + SG), it is also possible to observe the presence of ettringite (also indicated by arrows), by microscopy and calcium, in the analysis made by EDS.

In the third composition (C + S + B), it is possible to identify CaCO_3 precipitation (indicated by "CC", a structure similar to that of vaterite, and also by observing the EDS, it is possible to verify the presence of the elements of calcium carbonate. Ettringite is also observed. Similar morphologies of CaCO_3 were seen in previous researches (Daskalakis et al., 2015; Dupraz, Parmentier, Ménez & Guyot, 2009; Ercole, Cacchio, Botta, Centi & Lepidi, 2007; Hammes, Boon, Villiers, Verstraete & Siciliano, 2003; Park, S. J., Park, J. M., Kim & Ghim,

2012). In the study of Muynck, Cox, Belie & Verstraete (2008), the treatment of mortar with bacteria and a calcium source resulted in the presence of a crystalline layer on the surface. The X-Ray Diffraction Spectroscopy results of that study indicated the presence of calcite and vaterite. The last one was related to the spherical particles.

In the fourth composition (C + S + B + SG), CaCO_3 precipitation is also observed, whose elements are also present in the EDS analysis. However, Ettringite is not seen in the analyzed region. In Fig. 4 (D), it's possible to see that the particles have a regular cubic shape and are homogeneously distributed. Wang, Tittelboom, Belie & Verstraete (2012) studied the addition of *B. sphaericus* (grown in a medium of yeast extract and urea) immobilized into silica gel and into polyurethane. In their study, the CaCO_3 particles from immobilized silica gel also had regular cubic shape, while the particles from polyurethane immobilization showed an irregular block shape. Observing another region in the same sample, another hydration product can be observed: CSH (hydrated calcium silicate). The presence of silica in this composition may have been a factor that influenced this structure. The CSH (indicated by "CSH") can be observed in the SEM of the fourth composition, for an increase of 4000 times, in Fig. 5.

3.3 Compressive Strength test

The data obtained for the compressive strength on the seventh and twenty-eighth day are shown in Fig. 6, as well as the average between the four specimens of each composition, the standard deviation and their error. According to some studies (Park, S. J., Park, J. M., Kim & Ghim, 2012; Pei, Liu, Wang & Yang, 2013; Schwantes-Cezario, Nogueira & Toralles, 2017), the compressive strength values obtained were between 20 and 65 MPa.

Lower compressive strength values (between 7 MPa and 10 MPa) than those of the references were expected due to the conditions of sample preparation and testing. To perform the analysis of the compressive strength graph, there are some factors common to all very important compositions to consider. The first is the surface of the specimens that were tested. Its irregularity does not allow a uniform load to be applied and this can significantly influence the compressive strength values. Small irregularities in the surface are enough to reduce the final strength. In order to minimize this problem, it is recommended to cover the bases of the specimens with a thin layer (less than 3 mm) of appropriate material, that is, capping. The most efficient material found is the neoprene pad confined by a metallic base that restricts the lateral deformation of the elastomer (Tres, Balz, Bieger &

Pedrozo, 2018). This material was used in this work, but it was not confined to the metallic base.

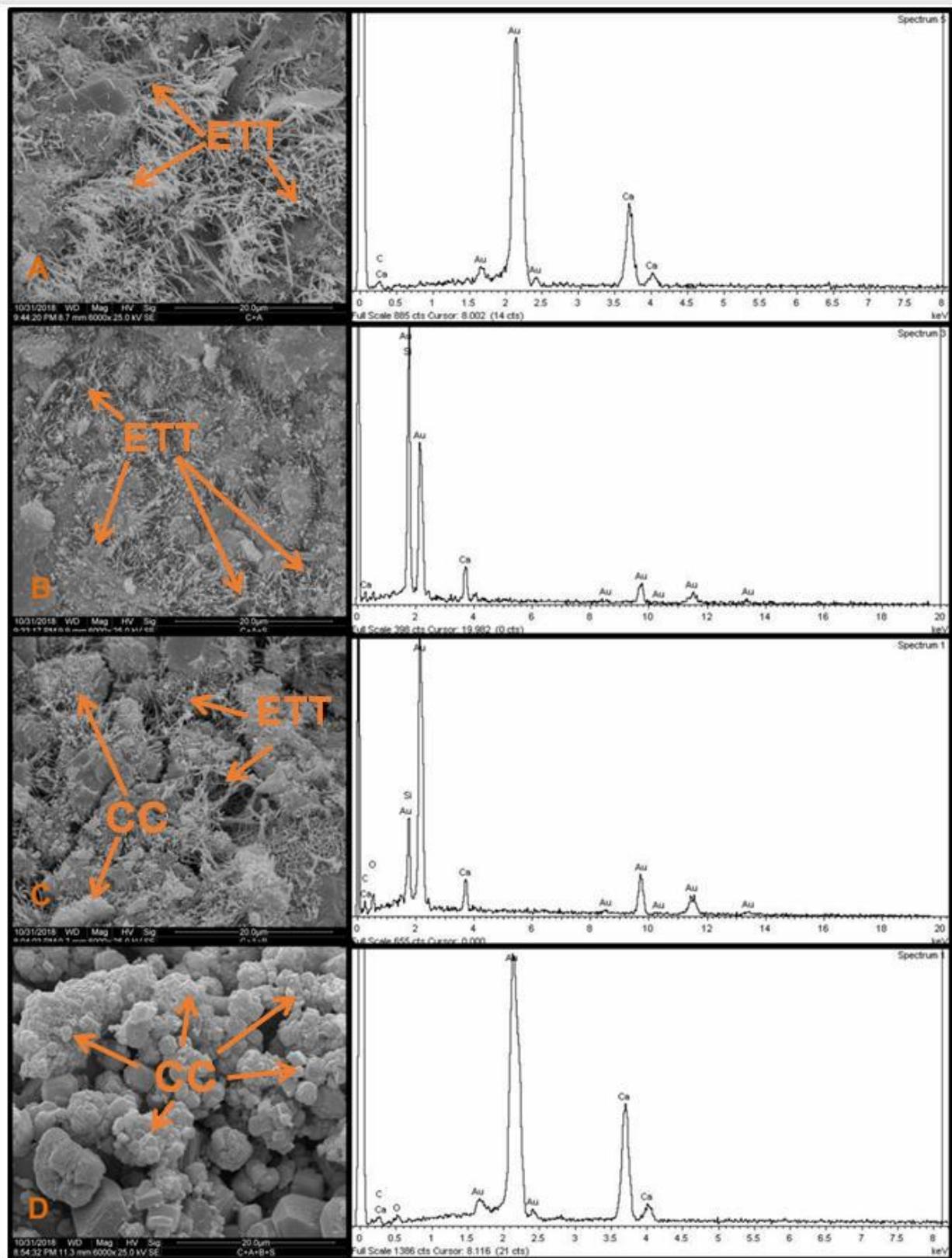


Fig. 4: SEM and EDS of (A) composition C + S, (B) composition C + S + SG, (C) composition C + S + B and (D) composition C + S + B + SG, 20 μm scale, 6000-fold magnification. Ettringite is indicated by “ETT” and CaCO₃ precipitation is indicated by “CC”.

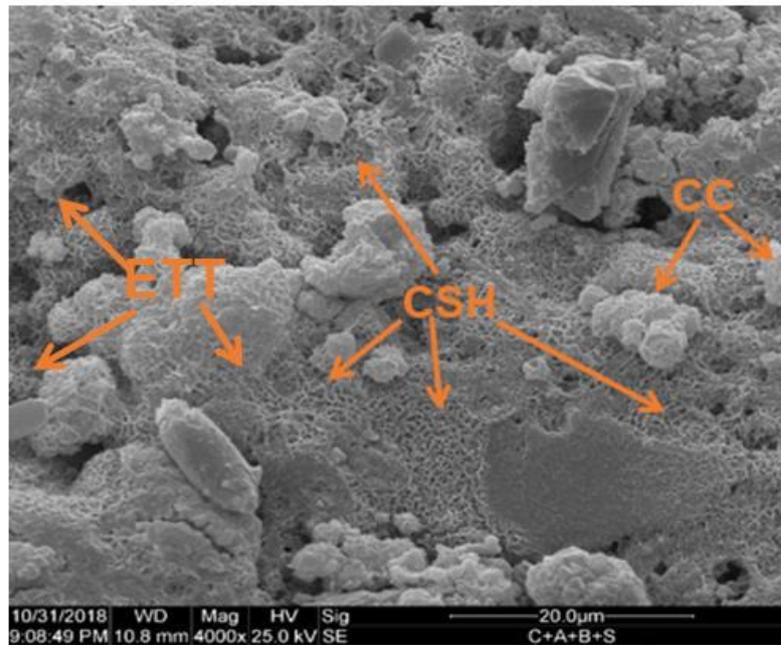


Fig. 5: SEM of the composition C + S + B + SG sample, 20 μm scale. Hydrated calcium silicate is indicated by “CSH” and CaCO_3 precipitation is indicated by “CC”

Other factors that may have influenced were noticed during the molding, which is done manually and consists of applying blows to a certain amount of material. The lack of experience and consistency during molding may have led to a non-standardization of these specimens, varying, for example, in the amount of material that received the blow and in the intensity with which these blows were applied. In addition, the molds were adapted. According to NBR 7215: Portland Cement - Determination of compressive strength (ABNT, 1997), the molds should be made of non-corrosive metal and were made of PVC pipe, due to the high cost. To remove the sample from the mold, it was necessary to cut them with a hand saw and part of the specimens were damaged.

Although the values themselves were lower than expected, there was an increase in the compressive strength values by adding only silica, only bacteria and bacteria immobilized on silica, for the two test ages. When carrying out the 7-day trial, in relation to composition C + S, the increase verified was 3.25% in composition C + S + SG, 11.18% in composition C + S + B and 13.90% in composition C + S + B + SG. Regarding the composition C + S + SG, the increase verified was 10.32% in the composition C + S + B + SG. In their studies, Schwantes-Cezario, Nogueira & Toralles (2017) found an increase of 10% when adding the bacteria to the composition of cement, sand and water. In this study, the increase found was 11.18%.

When carrying out the 28-day test, in relation to composition C + S, the increase verified was 14.22% in composition C + S + SG, 17.40% in composition C + S + B and 25.29% in composition C + S + B + SG. Regarding the composition C + S + SG, the increase verified was 9.69% in the composition C + S + B + SG.

When adding bacteria to the mortar, Park, S. J., Park, J. M., Kim & Ghim (2012) found an increase of 19.5% and Pei, Liu, Wang & Yang (2013), an increase of 15.6%. The increase found in this work was 17.4%.

The compositions that could be compared to the literature brought values of compressive strengths compatible with those already studied.

Regarding the addition of only silica gel and bacteria immobilized on silica gel, there are no comparisons in the literature, as the references used did not use the same methodology as this work and the results are restricted to the conditions in which the study was developed.

The increase verified by the addition of silica gel to the mortar can be explained by Pinheiro (2015). According to her, silica gel may have accelerated the hydration reactions of the cement, increasing its performance. The increase verified by the addition of bacteria to the mortar can be explained by the precipitation of calcium carbonate and, consequently, the closing of cracks and pores in the structural material. The greater increase verified by the simultaneous addition of silica gel and bacteria can be explained by the immobilization of the bacteria in the

silica grid. In this way, bacteria would be protected during cement hydration, acting more efficiently.

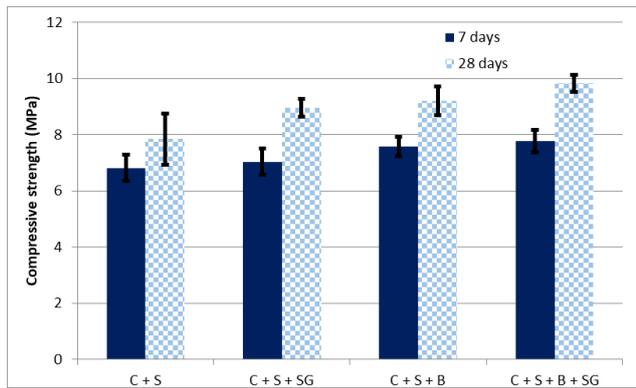


Fig. 6: Results of the compressive strength tests at 7 and 28 days

IV. CONCLUSION

In this study, the viability of *B. subtilis* immobilization using silica gel for self-healing of cement mortars was analyzed. By the means of the analyzes made from an Optical Microscopy to verify the closure of cracks, the analyzes made by Dispersive Energy Spectroscopy coupled to Scanning Electron Microscopy and the compression tests performed, some conclusions can be made:

- *Bacillus subtilis* bacteria were found to precipitate CaCO_3 in cementitious materials, which can be observed in the Scanning Electron Microscopy and Dispersive Energy Spectroscopy analyzes.
- The CaCO_3 precipitation influenced the 0.4 mm crack closure, which increased with the passing of the days and was verified using the optical microscope with attached camera. It is important to highlight that with the immobilization the complete closure of the crack was observed during the analyzed time.
- The immobilization of *Bacillus subtilis* bacteria on silica gel was observed, influencing the increase in resistance to compression (13.90% in the 7-day trial and 25.29% in the 28-day trial). This increase was also due to the addition of only silica (3.25% in the 7-day trial and 14.22% in the 28-day trial) and only bacteria (11.18% in the 7-day trial and 17.40% in the trial) 28 days). The highest values of compression resistance are related to the protection of bacteria from the high pH of concrete and from the cement hydration reactions.

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