Addition of sodium alginate capsules containing Lysinibacillus sphaericus for self-healing of cracks in mortars

Adição de cápsulas de alginato de sódio contendo Lysinibacillus sphaericus para autocura de trincas em argamassas

Adición de cápsulas de alginato de sodio que contienen Lysinibacillus sphaericus para la autocuración de grietas en morteros

Received: 01/27/2023 | Revised: 02/15/2023 | Accepted: 02/16/2023 | Published: 02/21/2023

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Abstract
The biomineralization technique of crack remediation in construction mortar can be achieved through the incorporation of calcifying bacteria within the mortar matrix. However, the thermal and autogenous shrinkage associated with the hydration of cement can be detrimental to the survival of the bacterial spores. Encapsulation of the spores in a matrix such as superabsorbent hydrogels, specifically sodium alginate, can provide protection from such detrimental conditions while also serving as a water reservoir for the metabolic activity of the bacterial cells. This paper investigated the addition of sodium alginate capsules prepared through spherification in construction mortars, aiming to preserve and optimize rheological, physical and mechanical properties, and to release bacteria when cracks occur. Mortar formulations with capsules containing Lysinibacillus sphaericus were calculated in order to maintain the granulometric distribution of standard formulation, thus obtaining products with excellent rheological, physical and mechanical properties, and allowing the addition of up to 1% capsules. Multivariate analysis of variance was applied at each age, showing that formulations with capsules kept the same properties as the original formula. After cracking, it was possible to observe the action of the released bacteria, through the formation of crystalline structures at the cracks. The capsules were prepared through a simple and cheap process, and it was proved that not only they do not affect concrete properties, but also are effective in both protecting the bacteria during mixing and hardening and releasing it after cracking.
Keywords: Mortar; Cracks; Biomineralization; Sodium alginate; Spherification.

Resumo
A técnica de biomineralização para remediação de trincas em argamassas de construção pode ser realizada por meio da incorporação de bactérias calcificantes na matriz da argamassa. No entanto, a retração térmica e autógena associada à hidratação do cimento pode ser prejudicial à sobrevivência dos esporos bacterianos. O encapsulamento dos esporos em uma matriz, como hidrogéis superabsorventes, especificamente alginato de sódio, pode fornecer proteção contra essas condições prejudiciais, além de servir como reservatório de água para a atividade metabólica das células bacterianas. Este trabalho investigou a adição de cápsulas de alginato de sódio preparadas por esferificação em argamassas de construção, com o objetivo de preservar e otimizar as propriedades reológicas, físicas e mecânicas e

liberar bactérias quando ocorrem trincas. As formulações de argamassa com cápsulas contendo Lysinibacillus sphaericus foram calculadas de forma a manter a distribuição granulométrica da formulação padrão, obtendo-se assim produtos com excelentes propriedades reológicas, físicas e mecânicas, e permitindo a adição de até 1% de cápsulas. A análise de variância multivariada foi aplicada em cada idade, mostrando que as formulações com cápsulas mantiveram as mesmas propriedades da fórmula original. Após a formação das trincas foi possível observar a ação das bactérias liberadas, por meio da formação de estruturas cristalinas nas trincas. As cápsulas foram preparadas através de um processo simples e barato; além disso a sua adição não afetou as propriedades do concreto e protegeu eficazmente as bactérias tanto durante a mistura e endurecimento, quanto na liberação após a fissuração.
Palavras-chave: Argamassa; Fissuras; Biomineralização; Alginato de sódio; Esferificação.

Resumen
La técnica de biomineralización para la remediación de grietas en morteros de construcción se puede realizar incorporando bacterias calcificantes a la matriz del mortero. La contracción térmica y autógena asociada con la hidratación del cemento puede ser perjudicial para la supervivencia de las esporas bacterianas. La encapsulación de las esporas en una matriz como los hidrogeles superabsorbentes, específicamente el alginato de sodio, puede brindar protección contra estas condiciones nocivas, además de servir como reservorio de agua para la actividad metabólica de las células bacterianas. Este trabajo investigó la adición de cápsulas de alginato de sodio preparadas por esferificación en morteros de construcción, con el objetivo de preservar y optimizar las propiedades reológicas, físicas y mecánicas y liberar bacterias cuando se producen fisuras. Las formulaciones de mortero con cápsulas con Lysinibacillus sphaericus se calcularon para mantener la distribución granulométrica de la formulación estándar, obteniendo así productos con excelentes propiedades reológicas, físicas y mecánicas, y permitiendo la adición de hasta un 1% de cápsulas. Se aplicó análisis de varianza multivariante en cada edad, demostrando que las formulaciones mantuvieron las mismas propiedades que la fórmula original. Luego de la formación de grietas, se pudo observar la acción de las bacterias liberadas, a través de la formación de estructuras cristalinas en las grietas. Las cápsulas se prepararon mediante un proceso sencillo y económico; además, su adición no afectó las propiedades del hormigón y protegió eficazmente a las bacterias tanto durante la mezcla y el endurecimiento como en el desprendimiento después de la fisuración.
Palabras clave: Argamasa; Fisuras; Bacterias; Biomineralización; Alginato de sodio; Esferificación.

1. Introduction

Mortars are composite materials used in construction, formed by the combination of fine aggregates, Portland cement and water (Isaia, 2017). Although they possess good resistance to compression, their low flexural strength may lead to crack formation. Degradation of these materials through time is caused by several mechanisms, such as thermal stress, expansive chemical reactions, and freeze-thaw cycles (Trenson, 2017; Van Tittelboom, et al., 2010). Cracks reduce durability of the mortars, since they allow penetration of water and harmful chemical agents (Cabrera, 1996).

Active self-healing treatments are techniques which can instantaneously seal cracks, regardless of position or orientation (Trenson, 2017; Wu, et al., 2012). The use of bacteria for active treatment involves the production of calcium carbonate through biomineralization, that is, the formation of minerals by living organisms, usually due to their microbial activity (Seifan, et al., 2016).

Lysinibacillus sphaericus, which was previously known as Bacillus sphaericus (Ahmed, et al., 2007), is one of the bacteria most frequently used in active treatments for self-healing. Tests performed by Wang, et al., (2014a) showed that the addition of capsules with spores of L. sphaericus in mortars results in healing rates significantly higher when compared to standard formulations (with variations from 48 to 80%).

Self-healing with capsules is a technique which involves the addition of biocompatible capsules to protect and release bacteria to seal cracks in construction materials. The capsules must be mechanically resistant, do not affect the mechanical properties of the mortar, and be uniformly distributed in the material so that cracks may reach the capsules regardless of position. Besides, the capsules must be activated by cracks, moisture, air of changes of pH in the matrix (Souradeep & Kua, 2016; Lee & Park, 2018).

According to studies performed by Wang, et al., (2014a), the addition of capsules resulted in significant reduction of compression resistance of the mortar, with variation from 22 to 47%. Several materials were investigated as capsules, such as diatomaceous earth, silica gel, polyurethane, melamine microcapsules, graphite nanoparticles, light aggregates and hidrogel capsules. The use of hidrogel as a carrier for bacterial spores has advantages such as their capacity of acting as water reservoirs, supplying water during dry periods and enabling metabolic activity (Lee & Park, 2018).

Alginates are anionic polysaccharides largely used in biomedical applications due to its biocompatibility, low toxicity and low cost (Lee & Mooney, 2012; Scott, 1968; Trenson, 2017). They are obtained through the extraction of brown algae using an alkaline aqueous solution, such as NaOH. To precipitate the alginate, sodium or calcium chloride is added to the filtered solution, using the mechanisms of acid or basic precipitation, respectively (Trenson, 2017).

Spherification is a simple method for creating spheres, vastly used in molecular cuisine, and can be used to prepare sodium alginate capsules with bacteria to be added in mortar formulations. This method consists in moulding a liquid in small spheres through reverse gelation. Basically, it is necessary to add calcium ions in an alginate dispersion to form the spheres (De Farias & Noreña, 2019).

When considering the addition of capsules with bacteria to the formulation of mortars, it is important to evaluate the possible side effects to rheological, physical, and mechanical properties of the material. An adequate mortar formulation is a result of efficient packing of the particles present, and it has direct effect on its final properties. The obtaining of dense packings is related to continuous filling void spaces between particles by others smaller in diameter. It is important not to introduce particles greater than the ones present since it can lead to new voids, and consequently increase porosity. Thus, it is important to control both the size and distribution of the particles, to determine the increase of not of the packing density (Oliveira, et al., 2000). The addition of bacteria in capsules in mortars has been studied in order to improve their properties, as can be seen in Table 1.

Table 1 – Studies performed on the addition of bacteria in capsules in mortars.

Authors	Year of publication
Khalic & Ehsan	2016
Rehman et al	2022
Roy, Rossi, Silfwerbrand, & Jonkers	2020
Salman, Al-Jabbar, & Mahmod	2021
Su, Qian, Rui, & Feng	2021
Van Tittelboom, De Belie, Van Loo, & Jacobs	2011
Wang, De Belie, & Verstraete	2012
Wang, Van Tittelboom, De Belie, & Verstraete	2012
Wang, Soens, Verstraete, & De Belie	2014
Wang, Snoeck, Van Vlierberghe, Verstraete, & De Belie	2014
Wang et al	2015
Wang, Xu, Wang, & Yao	2022
Zhang, Jin, Li, & Qian	2021

Source: Authors (2023).

However, simple addition of capsules may cause problems in rheological and mechanical properties of the mortar, such as reduction of flow, increased porosity and reduction of mechanical resistance. To avoid these problems, it is possible to perform granulometric compensation, that is, as the capsules are added, remove an equivalent amount of the aggregate whose grain size is closest to the sodium alginate spheres. This procedure guarantees the maintenance of grain size distribution of the original formula and minimizes the effects of capsule addition in the mortar.

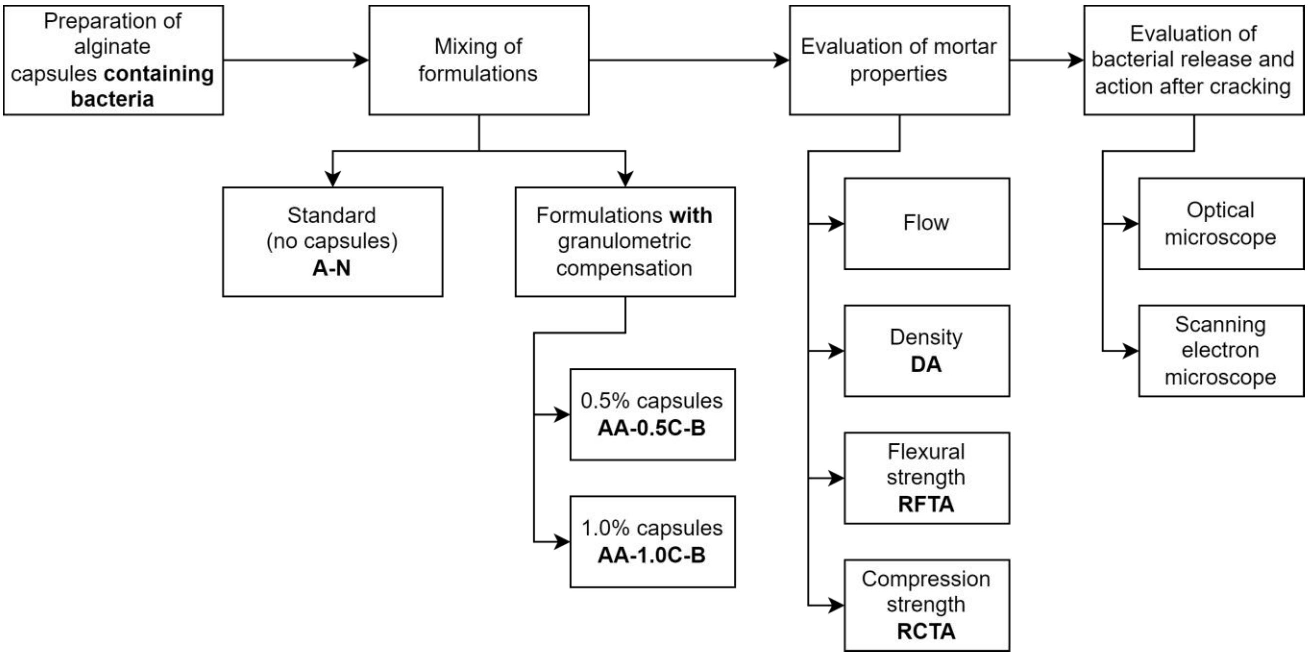
In order to evaluate the influence of the addition of sodium alginate capsules in mortars, it is possible to use multivariate analysis of variance (MANOVA), which allows the comparison of the results in different formulations. The analysis may identify which formulation presents results statistically different than the others. Besides, complementary statistical tests, such as univariate analysis of variance for each property and post hoc tests with correction of Bonferroni and Tukey, may be used to determine whether this influence is positive or negative (Field, et al., 2012).

This study aimed to evaluate the use of sodium alginate capsules, prepared via spherification, as potential carriers of Lysinibacillus sphaericus bacteria in mortar formulations, and their release after cracking. Multivariate analysis of variance and further statistical tests were performed to evaluate the influence of this addition in the properties of the fresh and hardened material, in order to keep properties during application. Furthermore, it was studied the action of the bacteria after its release, its interaction with the cementitious matrix and the production of calcium carbonate as a result of its metabolic process.

2. Methodology

Figure 1 illustrates the procedures used in this experimental study, aiming to select the composition with addition of sodium alginate capsules which brought the best set of results, in order to minimize the effect on the properties and characteristics of standard formulation, as well as the efficacy of the capsules in releasing the bacteria to seal the cracks. The methodology chosen to be applied in this study is comprised of experimental research with quantitative approach, whose main characteristic is the obtention of numerical data by tests in scientific laboratories (Severino, 2018).

Figure 1 – Detailing of methodology. The image shows the complete sequence of procedure adopted in this work to produce and to characterize the mortars.



Source: Authors (2023).

The first step was to grow and sporulate the bacteria to be added in the alginate capsules. Then, the dried capsules were added to the formulation during mixing, obtaining two different formulations containing capsules, to be compared to the standard formulation with no capsules. Then, rheological, physical and mechanical properties were evaluated after 7, 28 and 91 days of curing, and the evaluation of bacterial release and action was performed after cracking. The procedure for each of these steps are detailed subsequently.

Growth and sporulation of bacteria

Lysinibacillus sphaericus ATCC14577 used in the tests was obtained from the Collection of Tropical Cultures of Foundation André Tosello for Research and Technology. The strain was incubated in TSB (Tryptone Soya Broth) for 24 hours under agitation of 130 rpm (Shaker TE-420, Tecnal) at 28°C for reactivation. Then, 150 µL of the inoculum was transferred to 150 mm diameter plates containing nutrient agar (20 g/L), urea (10 g/L) and sodium bicarbonate (2.12 g/L), autoclaved for 15 minutes at 121°C in a procedure similar to Pungrasmi (2019), but with modifications. The plates were incubated at 28°C for 6 days. Formation of spores was accompanied through daily observation of the blades using Wirtz-Conklin coloration (Optical microscope, Quimis).

Posteriorly, the plates were washed with sterile distilled water and the solution containing the spores was transferred to a tube with conical bottom. The tube containing the suspension was centrifuged for 20 minutes at 3500 rpm (Centrifuge CT-5000, Cientec). After centrifugation the supernatant was discarded, and the precipitate was resuspended in sterile distilled water. Then, the tube was incubated in ice bath for 30 minutes, followed by 20 minutes in bath at 80°C and 5 minutes in ice. The suspension was centrifuged again for 20 minutes at 3500 rpm, the supernatant discarded, and the precipitate resuspended in sterile distilled water. The concentration was adjusted to 108 cells/mL (absorbance of 600 nm) (Spectrophotometer DU-800, Beckman) and the tube was stored at 4°C for posterior use.

Synthesis of sodium alginate capsules with bacteria

To prepare the spheres it was used aqueous alginate solution at 2% (m/v) under mechanical agitation at room temperature. After dissolution, it was added 20 mL of the solution containing the spores (108 cells/mL). the solution obtained was dropped in a calcium chloride solution at 5% (m/v). The spheres were washed with distilled water and subsequently dried at 30°C in drying oven with air circulation for 48 h.

Formulations with bacteria-containing sodium alginate capsules

After the capsules were obtained, they were added to the mortar formulation in proportions of 0.5% and 1.0% wt. in relation to the amount of cement, and with granulometric compensation by removing part of the coarse aggregate, which is closest in size to the spheres. The formulations were designated as "-B" since they have capsules with bacteria, and they are detailed in Table 2.

Table 2 – Formulations of mortar with sodium alginate capsules containing bacteria.

Components	A-N	AA-0.5C-B	AA-1.0C-B
Portland cement	22.05%	22.05%	22.05%
Water	11.81%	11.81%	11.81%
Sand			
Coarse	16.54%	16.43%	16.32%
Regular-Coarse	16.54%	16.54%	16.54%
Regular-Fine	16.54%	16.54%	16.54%
Fine	16.54%	16.54%	16.54%
Capsules	-	0.11%	0.22%

Source: Authors (2023).

Evaluation of mortar properties

The evaluation of mortar properties was conducted according to ABNT NBR 7215:2019 and NBR 16738:2019 standards. The flow properties of each formula were determined by placing the mortar in a flow table and compacting it within an aluminium cone with a top diameter of 70 mm and a bottom diameter of 100 mm. The cone was then removed, and 25 taps were applied in 15 seconds. The spread of the mortar was measured using a pachymeter.

Afterward, specimens were moulded with dimensions of 160x40x40 mm and evaluated at three different ages (7, 28, and 91 days). The specimens were demoulded and kept submerged in water until it was time to measure their physical and mechanical properties.

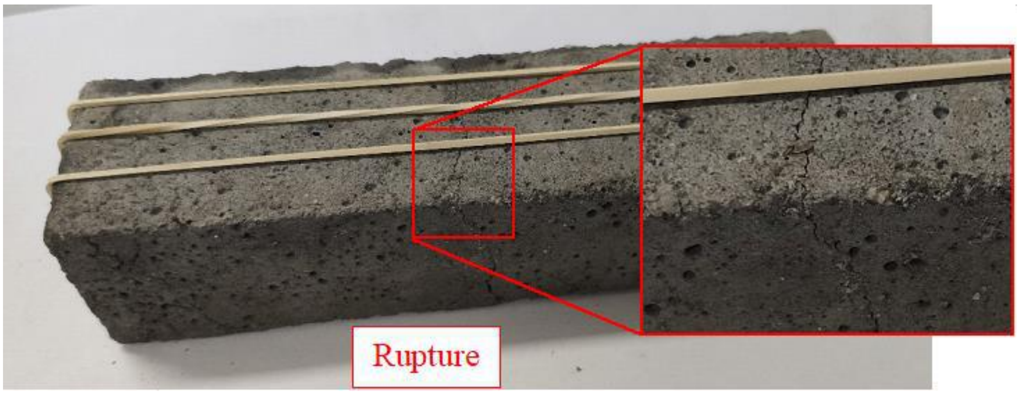
To identify the formula with the best performance, statistical analysis was conducted on the measurements of apparent density (DA), flexural strength (RFTA), and compression strength (RCTA) at each age (7, 28, and 91 days) using multivariate analysis of variance (MANOVA) with $\alpha = 0.05$. This was followed by individual analysis of variance (ANOVA) for each property, and post hoc tests with Bonferroni and Tukey corrections (Field, et al., 2012).

Evaluation of bacterial activity

In addition to the basic tests, it was also performed a visual follow-up to verify bacterial activity in the cracks. Two specimens of each formulation were prepared for evaluation at each age, in the same moulds used in physical and mechanical tests. For each age, one of the specimens was submitted to flexural solicitation until complete rupture, and the other was submitted to flexural solicitation that resulted in a small crack. Both specimens were kept in ware and monitored in the following days, the two halves were kept together using elastic bands longitudinally as can be visualized in Figures 2 and 3.

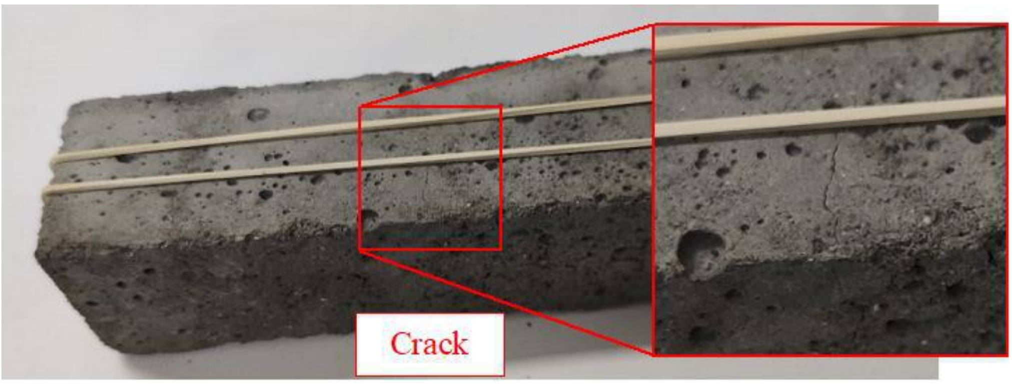
After 20 days, it is expected that the bacteria had already been released and activated, since the peak of activity is 7-10 days (Lee & Park, 2018). The crack was photographed for evaluation. Images of the specimens were obtained in optical microscope with 500x magnification. Then, the same points were evaluated through scanning electron microscope, with 50x, 500x and 5000x magnifications.

Figure 2 - Specimen with complete rupture. The highlighted region in red color shows the main crack that causes the body rupture after flexural testing.



Source: Authors (2023).

Figure 3 - Specimen with partial rupture. The highlighted region in red color shows a small crack created after incomplete flexural testing.



Source: Authors (2023).

3. Results and Discussion

Rheological properties

Table 3 shows the flow results found for each formulation prepared with sodium alginate capsules containing bacteria. It can be noted that all formulations presented similar values of flow, the material remained cohesive and with good workability in all formulations investigated. It proves that the addition of capsules was not detrimental to rheological properties, when granulometric compensation is performed accordingly.

Table 3 - Flow for standard formulation in comparison to formulations with capsules.

Formulation	Flow (mm)
A-N	193.9 ± 0.7
AA-0.5C-B	190.4 ± 1.7
AA-1.0C-B	193.5 ± 2.5

Source: Authors (2023).

Physical and mechanical properties

Figure 4 shows the development of density, flexural strength and compression strength of each formulation, at each age (7, 28 and 91 days). In the graph, we have values of each property for the standard formulation, in comparison to the formulations with 0.5% and 1.0% capsule addition. In all formulations with capsules, it was performed grain size compensation, in order to maintain the original grain size distribution.

Regarding to density, the average values obtained for each formulation, at each age, are similar, and the development of density is constant in all cases evaluated. Equally, it can be observed that the development of flexural and compression strength happens constantly and similarly in all cases, and the growth is more pronounced from 28 to 91 days in the formulas containing capsules. This may indicate that the capsules have a little more influence in the early ages; however, after 91 days the average values of resistance are equal or superior to what was observed for standard formulation.

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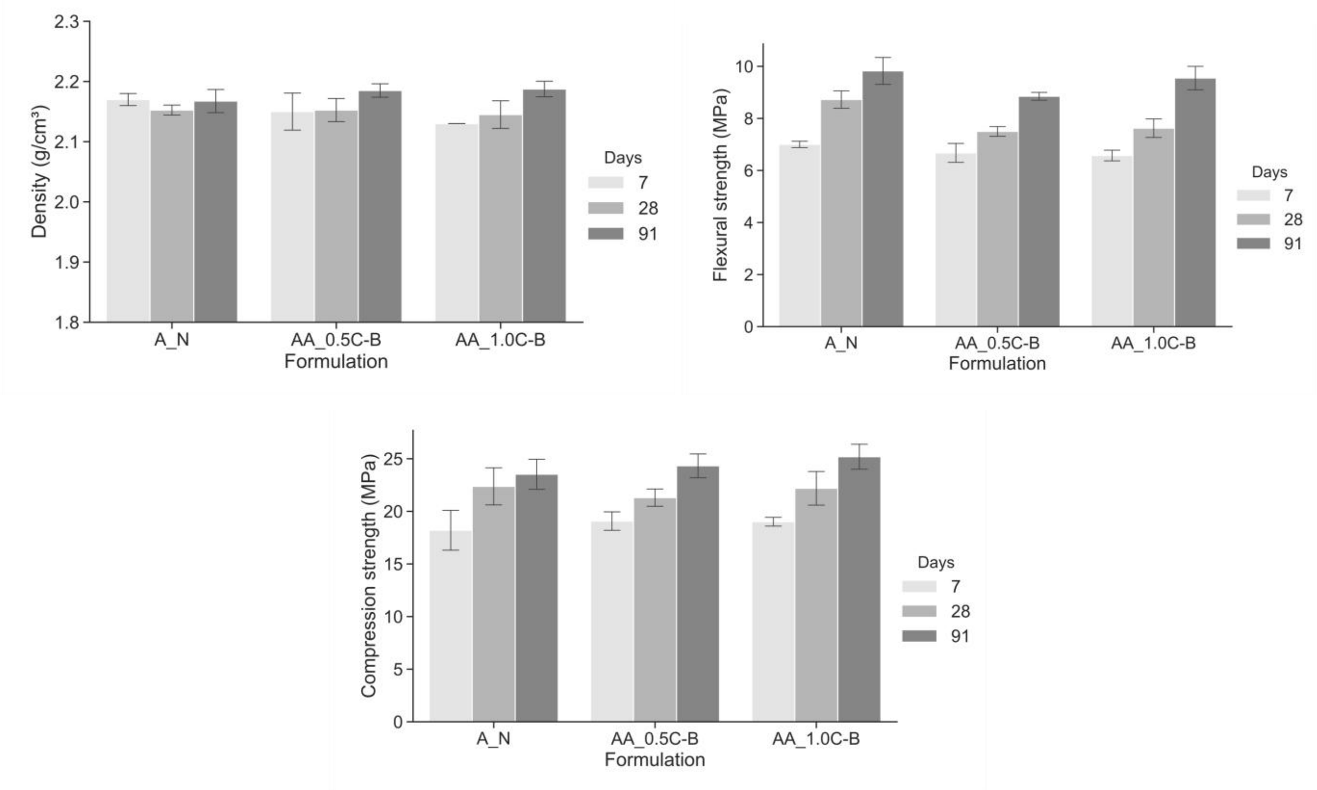
Figure 4 - Physical and mechanical properties of formulations after 7, 28 and 91 days. The figures show that density is constant for all samples investigated; the flexural and compression strength are similar in all cases, and the growth is more pronounced from 28 to 91 days in the formulas containing capsules.

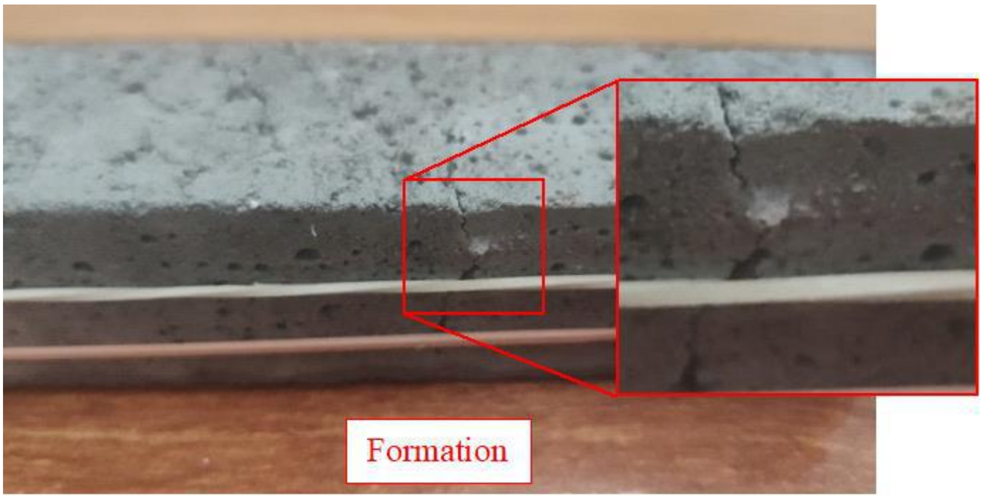


Source: Authors (2023).

Evaluation of bacterial activity

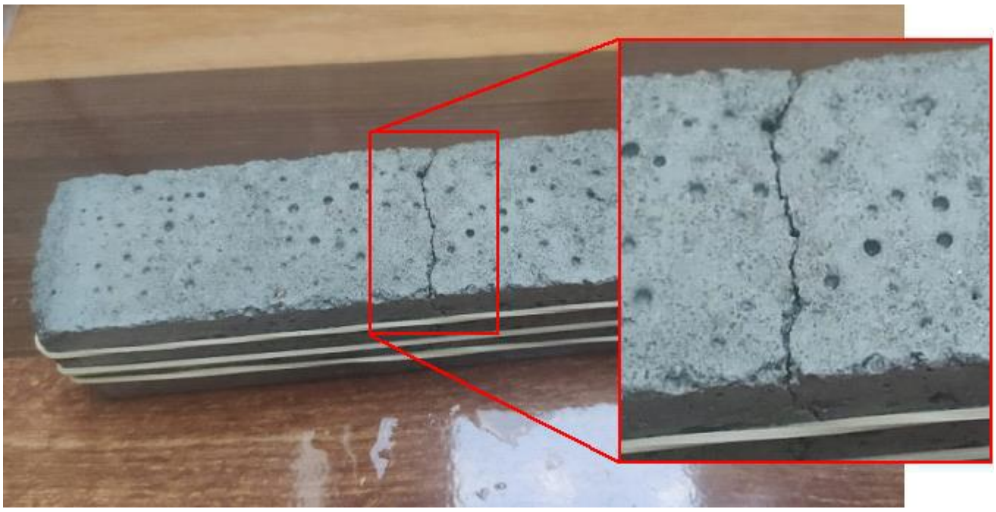
In order to verify if there was bacterial activity after cracking, it was performed the visual follow-up of the specimens after rupture. Through visual inspection, it was noticeable that approximately 70% of specimens presented unknown formations at crack region, as can be seen in Figure 5 As a reference, Figure 6 shows an example of specimen with no formations at crack region. It is important to emphasize that these formations were evenly observed in all formulations with capsules, with 0.5% or 1% capsule concentration.

Figure 5 - Example of formations at crack region after 20 days. The highlighted region in red color shows the presence of an unknown formation at crack region.



Source: Authors (2023).

Figure 6 - Reference specimen with no formations after 20 days. The highlighted region in red color shows only the presence of crack, without the presence of an unknown formation.

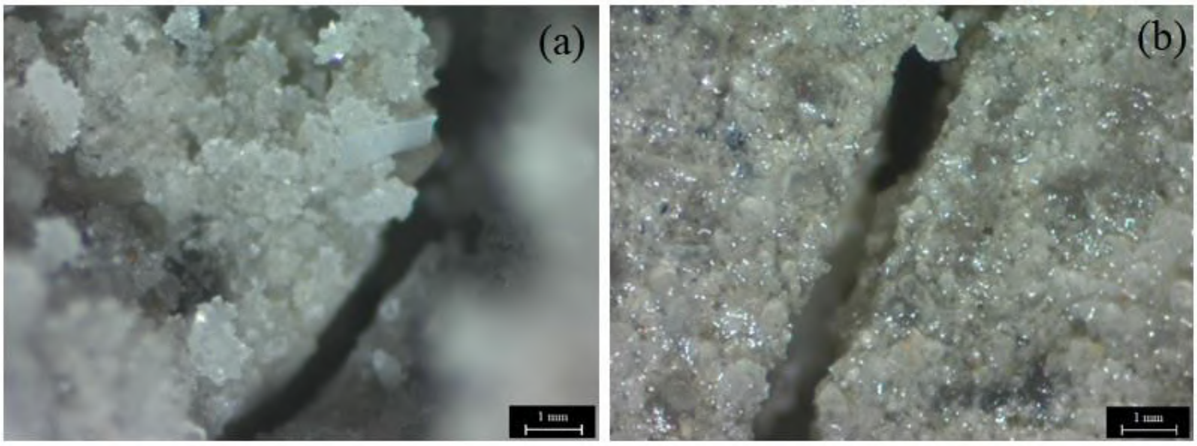


Source: Authors (2023).

To visualize with further detail the formations observed, the specimens were placed in optical microscope with 500x magnification. Figure 7 shows, side by side, the crack region in the specimen with crystalline formation and the reference specimen.

By observing this figure, it is clear the difference between a specimen where a crystalline formation is observed and the reference specimen, showing the result of bacterial activity after cracking. In this study, extreme situations were used, by reaching total rupture of the specimens, so it was possible to visualize bacterial activity. It must be considered that a structure would not have such large cracks, but microcracks which could be easily filled by the calcium carbonate generated by the bacteria.

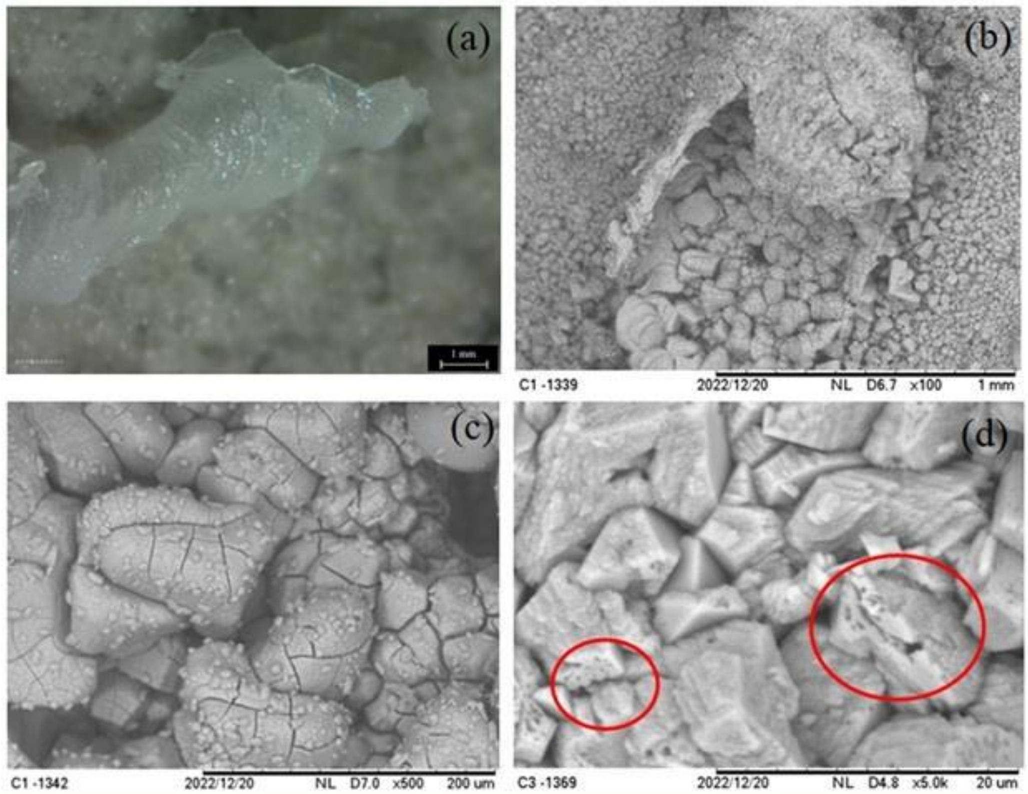
Figure 7 – 500x magnification at crack region of (a) specimen with crystalline formation and (b) reference specimen. The crystalline formation is due to the bacterial activity after cracking; this formation can help to improve the physical and mechanical properties of the mortars.



Source: Authors (2023).

In order to further examine the source of the crystalline formations observed in the specimens, Figure 8 shows the images generated via optical microscope and scanning electron microscope (SEM).

Figure 8 - Formation at crack region with (a) 500x magnification in optical microscope, (b) 100x magnification in SEM, (c) 500x magnification in SEM and (d) 5000x magnification in SEM. The figures highlighted the calcium carbonate formation due to the bacterial activity after cracking in different regions of the mortars.



Source: Authors (2023).

In this figure, it is possible to see in greater detail the action of the bacteria to produce the crystalline formation observed, in addition to the impression left by them in the crystals formed, similar to the ones showed by De Muynck, et al., (2008). Thus, it is clear that such formations were, indeed, caused by bacterial activity after cracking of the specimens.

Statistical analysis

Previous literature has documented the mechanical evaluation of mortar specimens with added capsules containing bacteria (Wang, et al., 2014; Wang et al, 2015; Wang et al, 2018), however, the mixing and evaluation procedures used in those studies differ from the one established by the ABNT NBR 16738 standard used as a reference in this study. As a result, the results obtained in these studies are vastly different from the ones found in this study. The compression strength results for the mortar prepared according to the ABNT standard are around 22 MPa after 28 days, while results found in literature range from 40-70 MPa, which are more similar to what is typically found for concrete.

Since the ABNT standard does not establish minimum values for flexural or compression strength, all statistical evaluations were performed using the standard formulation as specified by ABNT NBR 16738 as a reference. The test formulation was considered satisfactory when the results were statistically equal to or superior to the standard formulation.

The results obtained for MANOVA in all three properties after 7 days using Pillai's trace indicated significant effect of formulation in the set of results, $V = 1.55$, $F(12, 30) = 2$, $p < 0.05$. Individual ANOVAs and post hoc tests with Bonferroni and Tukey corrections shows that there is no significant effect on DA [$F(2, 6) = 2.40$, $p = 0.172$] and RCTA [$F(2, 6) = 3.74$, $p = 0.088$], only for RFTA [$F(2, 6) = 9.56$, $p < 0.05$] where we can see that AA-1.0C-B has a slightly inferior resistance value.

After 28 days, MANOVA using Pillai's trace did not indicate significant effect of formulation in the set of results, $V = 0.90$, $F(12, 30) = 1$, $p = 0.313$, which was confirmed by individual ANOVAs and post hoc tests with Bonferroni and Tukey corrections. At this age, all formulations are statistically equal, which shows that capsule addition is not detrimental to mortar properties.

After 91 days, the results obtained for MANOVA using Pillai's trace indicated significant effect of formulation in the set of results, $V = 1.44$, $F(12, 30) = 2$, $p < 0.05$. Individual ANOVAs and post hoc tests with corrections of Bonferroni and Tukey indicated that there is no significant effect of formulation for DA [$F(2, 6) = 0.176$, $p = 0.842$], however there is significant effect for RFTA [$F(2, 6) = 6.86$, $p < 0.05$] and RCTA [$F(2, 6) = 6.41$, $p < 0.05$], by observing the average values it can be concluded that only formulation A-N presented inferior resistances at this age. All formulations with capsules presented results statistically equal or superior to standard formulation.

4. Conclusion

Through this study, it was possible to prove the efficacy of sodium alginate spheres as capsules for protection and release of bacterial spores in mortar formulations. Here, it was used extreme circumstances, with complete rupture of the specimen. However, in normal situations when microcracks occur it will be sufficient to activate the capsules, releasing the bacteria and sealing the cracks before the situation can become catastrophic.

The capsules were able to remain intact after the mixing of the mortar, fulfilling its role and protecting the spores from the mechanical forces applied during preparation, and from the heat generated by hydration reactions during hardening. After specimen rupture, it was observed after 20 days there are crystalline formations emerging from the cracks which, after examination through optical microscope and scanning electron microscope, were proved to be the result of bacterial activity when in contact with the cementitious matrix.

Furthermore, due care was taken to guarantee that capsule addition did not affect the physical, mechanical, and rheological properties of mortars, which would make their application unfeasible in cementitious structures. By analysing the set of results, at each age, it can be observed that formulations with sodium alginate capsules presented results statistically equal or superior to what was observed for standard formulation. It is clear, according to the tests performed, that compensation is fundamental to keep the properties expected for the mortars.

As for the rheology of the material, through flow evaluation it was observed that the formulations present results statistically equal to what was observed for standard formulation. Thus, it is proved that granulometric compensation aids in the maintenance of mortar grain size distribution and properties.

For future research and articles, it is important to test these capsules in concrete formulations, and their resistance to the coarse aggregates during mixing. Furthermore, it would be of value to understand bacteria release and behavior in larger specimens of mortar and concrete.

Acknowledgments

We thank the partner companies of this study: Togni Materiais Refratários S/A, Master Builders Solutions and Polimix Concreto LTDA, Professor Luiz Antônio dos Reis, of the Pontifícia Universidade Católica de Minas Gerais and engineer Bruno Cesar Lopes Coutinho (Polimix). We also thank FAPEMIG and CNPq for their financial support.

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