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Production and addition of encapsulated biomineralizing bacteria in construction concrete

Produção e adição de bactérias biomineralizadoras encapsuladas em concretos de construção civil

Producción y adición de bacterias biomineralizantes encapsuladas en hormigón de construcción

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Abstract
The use of calcium carbonate for closing cracks in concrete by the action of biomineralizing bacteria has been investigated. However, these bacteria are fragile and susceptible to the reaction medium, and they must be protected by encapsulation, until the moment they must carry out the biomineralization process. This research covered the study and optimization of the production of sodium alginate capsules for subsequent encapsulation of biomineralizing bacteria. The research also investigated the effect of these capsules (added in different percentages) in concrete masses using a CP II – E Portland cement (ABNT NBR Standard), formulated from the Andreassen equation. The samples were characterized in their fresh and hardened state. The swelling tests indicated that the sodium alginate capsules provided good conditions to receive the bacteria, to keep them alive and to be mixed in the concrete, presenting enough mechanical strength. Among the investigated conditions, the composition formulated using the Andreassen coefficient equal to q=0.37 and with the addition of 1.5% of sodium alginate capsules was the one that presented the most promising results; and after 28 days of curing, the mechanical strength to compression was 45.4 MPa, with the value within ABNT NBR 11578, since it establishes a minimum of 32 MPa.
Keywords: Calcium carbonate; Sodium alginate; Concrete; Biomineralization; Mechanical strength.

Resumo
A produção de carbonato de cálcio para o fechamento de fissuras em concretos por meio da ação de bactérias biomineralizadoras vem sendo investigada; todavia, essas bactérias são frágeis e suscetíveis ao meio reacional, devendo-se então protegê-las através de encapsulamento, de modo que possam realizar o processo de biomineralização quando solicitadas. Esta pesquisa abrangeu o estudo e otimização da produção de cápsulas de alginato de sódio para posterior encapsulamento de bactérias biomineralizadoras. A pesquisa investigou ainda o efeito dessas cápsulas (adicionadas em diferentes porcentagens) em massas de concretos utilizando cimento Portland do tipo CP II – E (norma ABNT NBR) e formuladas a partir da equação de Andreassen, caracterizando os corpos obtidos através de ensaios no seu estado fresco e endurecido. Os testes de intumescimento indicaram que as cápsulas de alginato de sódio propiciaram um ambiente adequado para receber as bactérias e nutri-las até o seu uso, além de uma resistência mecânica adequada para sua inserção (e mistura) no concreto. Dentre as condições investigadas, aquela

onde foi utilizado o coeficiente de Andreassen igual a q=0,37 e com adição de 1,5% de cápsulas de alginato de sódio foi a que apresentou os resultados mais promissores da pesquisa; sendo que após 28 dias de cura, a resistência mecânica à compressão encontrada foi de 45,4 MPa, estando com o valor dentro da ABNT NBR 11578, uma vez que a mesma estabelece uma resistência mínima de 32 MPa.
Palavras-chave: Carbonato de cálcio; Alginato de sódio; Concreto; Biomineralização; Resistência mecânica.

Resumen
Se ha investigado la producción de carbonato de calcio para el cierre de fisuras en el hormigón mediante la acción de bacterias biomineralizantes; sin embargo, estas bacterias son frágiles y susceptibles al medio de reacción, por lo que deben ser protegidas mediante encapsulación, para que puedan realizar el proceso de biomineralización cuando se le solicite. Esta investigación abarcó el estudio y optimización de la producción de cápsulas de alginato de sodio para su posterior encapsulación de bacterias biomineralizantes. La investigación también investigó el efecto de estas cápsulas (agregadas en diferentes porcentajes) en masas de concreto utilizando cemento Portland tipo CP II – E (norma ABNT NBR) y formuladas a partir de la ecuación de Andreassen, caracterizando las pastas obtenidas mediante ensayos en estado fresco y endurecido. Los ensayos de hinchamiento indicaron que las cápsulas de alginato de sodio proporcionaron un ambiente adecuado para recibir las bacterias y nutrirlas hasta su uso, además de una adecuada resistencia mecánica para su inserción (y mezclado) en el concreto. Entre las condiciones investigadas, aquella donde se utilizó el coeficiente de Andreassen igual a q=0,37 y con la adición de 1,5% de cápsulas de alginato de sodio fue la que presentó los resultados más promisorios de la investigación; y después de 28 días de curado, la resistencia mecánica a la compresión encontrada fue de 45,4MPa, con el valor dentro de la ABNT NBR 11578, ya que establece una resistencia mínima de 32MPa.
Palabras clave: Carbonato de calcio; Alginato de sodio; Hormigón; Biomineralización; Resistencia mecánica.

1. Introduction

The use of concrete is common in civil construction; world production exceeds 40 billion tons per year (Global Cement and Concrete Association, 2019). The high mechanical resistance after drying of these materials is attributed to a well-founded formulation in the principles that involve the packing of the particles, guaranteeing a better characteristic to finished product. In addition, the granulometric distribution must be controlled so that particles larger than those already belonging to the mixture are not introduced, which could lead to the appearance of new voids, increasing the final porosity (Oliveira et al., 2000; Fusco, 2008).

Properties such as durability, fire resistance, high compressive strength, and others, are directly related to the quality of particle packaging (Meyer, 2009), as the type and content of special additives used, such as superplasticizers (Neville, 2016; Mehta & Monteiro, 2008). Thus, many factors affect this packing, such as morphology, density, type of dispersion and models and techniques to pack the particles (Oliveira et al., 2000). With so many factors, the existence of several models, both practical and theoretical, are beginning to be studied and used to determine the best particle distribution during the concrete formulation process. Among them, Andreassen's model stands out in the ceramist sector (Oliveira et al., 2000).

The continuous distribution of particles is the starting point for creating the Andreassen model. According to this model, to create an ideal packing, the particles must in turn fit together in the best possible way to occupy the maximum available spaces by sizes, as indicated in equation 1 (Oliveira et al., 2000). Within the Andreassen model, the best possible packing, theoretically, is the one that uses q=0.37 (Oliveira et al., 2000).

$$CPFT = \left(\frac{D_p}{D_L}\right)^q x100 \quad \text{(Eq.1)}$$

Here, CPFT is the percentage of accumulated particles smaller than *Dp*, where *Dp* is the particle diameter value, *DL* is the largest particle diameter value, and *q* is the distribution coefficient.

Concrete Concrete is often used in situations where its mechanical properties are very demanding as bridges, buildings. Although it is resistant to mechanic compressive, the tensile strength ends up being low throughout its useful life, with the formation of cracks that will initiate a process of internal pathology, mainly generating the oxidation of its steel reinforcement, causing a deterioration and a decrease in its durability (Seifan et al., 2016; Wu, et al., 2019). Due to this type of pathology, many studies have been focused on the recovery and self-healing of concrete, since the so-called as conventional methods for repairing structures are laborious, slow and costly (Wang et al., 2018). These inconveniences contributed to the development of new means for repairing concrete; among them, the study of the addition of some species of bacteria in spores within the concrete formulation (Seifan et al., 2016).

A spore is a dormant and mechanically resistant structure, produced by bacteria so that they can live in an environment under extreme conditions, such as desiccation, high temperature, among others (Seifan et al., 2016). The addition of bacteria *Lysinibacillus sphaericus* can carry out biomineralization, which consists of the synthesis of inorganic minerals by living organisms. The process occurs when bacterial capsules encounter an external environment or even by changing the pH of the concrete, through a fissure that occurred at the site, causing these bacteria, through microbial activity, to begin to form minerals (Seifan et al., 2016). From the reaction of external elements such as oxygen, humidity caused by water, CO_2, different types of requested efforts, or even climate changes associated with the metabolic activities of the organism, the precipitation of calcium carbonate is observed. Calcium from this process is considered one of the best fillers because it is compatible with concretesupporting the closure the crack when requested. Thus, when the cracks are filled, the bacteria enter their hibernation state again (Tebo, et al., 2015; Seifan et al., 2016; Zhu et al., 2021).

When working with bacteria in a very reactive environment, where there is a high alkaline content and a strong chemical reaction in the early ages of the concrete, the survival rate of the biomineralizing bacteria becomes small. Based on the need to protect these bacteria in such an inhospitable environment, studies have been carried out on encapsulation, creating a protective barrier in which the bacteria remain in a state of hibernation until the capsule ruptures, causing them to react with the external environment (Jonkers, 2007; Khalic & Ehsan, 2016; Tittelboom, et al., 2011; Wang, et al., 2012; Wang et al., 2012, 2014a, 2014b, 2015). Thus, although there are works evaluating the protection of bacteria by capsules prepared with different types of materials (including those made of alginate), limited information is available regarding the mechanical resistance of these capsules in the mixture and influence on the production of concrete (Jonkers, 2007).

Alginates form gels with relative ease, showing good mechanical properties. Research related to sodium alginate reveals that, due to its biocompatibility and relatively low cost, this compound becomes viable for applications in different areas, ranging from the medical field to civil construction. The treatment of brown seaweed with an aqueous alkaline solution (NaOH) is one of the ways to extract alginate for its production. This extraction takes place due to the addition of sodium chloride to the filtrate, to carry out acid precipitation, or else calcium chloride by the calcium precipitation method (Farias & Noreña, 2019; Lee & Mooney, 2012; Trenson, 2017).

In this research, spheres (capsules) of sodium alginate were obtained through spherification, a technique that consists of molding a liquid through reverse gelation, dripping a dispersion containing alginate into a solution of calcium ions to form spheres (Farias & Noreña, 2019) that will be used *a posteriori* in the containment and protection of bacteria of the *Lysinibacillus sphaericus* type. In this work, the effect of the presence of these capsules in concrete masses formulated from the Andreassen equation was also evaluated, seeking the optimization of the physical, mechanical, and rheological properties, such as slump, density, water absorption and mechanical resistance.

2. Methodology

For the production of sodium alginate capsules (spheres), the following reagents were used: Sodium alginate (Dinâmica), with a purity of 90% and Calcium chloride (Vetec, PA), with a purity of 99.99%; for the production of concrete specimens, the following were used: gravel powder (less than 1.7 mm) as fine aggregate, from the region of Poços de Caldas/MG; gravel 1 (between 4.8 mm and 1.7 mm); commercial cement type CP II-E 32 from the manufacturer Votorantim of the Itaú line, in accordance with the requirements of the ABNT NBR 16697 standard (ABNT, 2018); water made available by the supply network of the city of Poços de Caldas-MG following the NBR/159000-09 standard, which considers it suitable for use in kneading and as an additive the third generation superplasticizer, with the trade name Glenium 51® from the manufacturer BASF S.A., classified in relation to the chemical base as polycarboxylic ether, with variable density from 1.067 to 1.107 g/cm³. This superplasticizer was used as a deflocculant during the preparation of the concrete mass.

This research was divided into two steps, which will be referred to as Step 1 and Step 2 in this article. In Step 1, the sodium alginate capsules were obtained and characterized; Step 2 consisted in the production and evaluation of the properties of concrete produced from the variation of the Andreassen packing coefficient (q) at 0.37 and 0.30 and the content of alginate capsules added to the formulation.

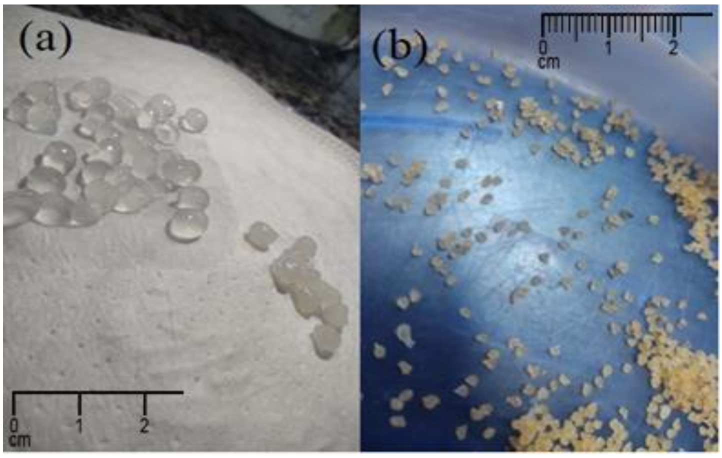
Step 1

At this stage of the research, sodium alginate capsules were produced for use in concrete, evaluating their swelling characteristics, a process in which the amount of water absorbed by the capsule is defined.

The concentration of sodium alginate in aqueous solution was set at 2% (w/v) after a previous study (Cruz, 2023) to adapt the viscosity to the dripping process. 0.0001 m^3 of distilled water was added to a beaker, kept under constant agitation (60 rpm) by means of a magnetic stirrer. Then, the sodium alginate was gradually added, so that there was no formation of lumps. Stirring was maintained for about 1 h until complete solubilization. An analogous process was carried out separately to obtain an aqueous solution of calcium chloride (5% (w/v), using 0.0005 m^3 of distilled water in a beaker and adding 25 g of calcium chloride until complete solubilization (constant agitation at 60 rpm) and homogenization (about 10 minutes) at room temperature.

By using a peristaltic pump at a flow rate adjusted between 30 and 40 drops per minute, the alginate solution was dropped into the $CaCl_2$ solution, in a shaker with agitation at 60 rpm, at room temperature. Then, with the aid of a paper towel, the newly prepared spheres were dried and placed in a specific container covered with TNT (non-woven fabric). The material was then dried in an oven with air circulation and a temperature of 30°C for 48 h. Figure 1a illustrates the alginate spheres just dropped in the calcium chloride solution, they have a more swollen appearance due to the high water content present in Figure 1b, we have the spheres (with a diameter of 5 mm approximately) after the drying process, with a more yellowish color, as well as a less gelatinous and more rigid appearance.

Figure 1 – Sodium alginate capsules (a) recently produced and (b) after drying.



Source: Authors (2023).

Three different tests (methodologies) were performed to evaluate the swelling of the sodium alginate. In the first methodology (called the simple test) 0.2 g of dry spheres of sodium alginate were added to a beaker with 30 g of distilled water. Then, the beaker was covered with a PVC plastic film so that there was a barrier to prevent water evaporation. The spheres were immersed in distilled water for 24 hours. After this period, a paper filter was saturated with distilled water, filtering the sample, so that the water drained into a container with a known weight. The test was performed in triplicate and the swelling capacity (swelling) of sodium alginate (I) was calculated from equation (2):

$$I(\%) = \frac{m_0 - m_f}{m_{gel}} \cdot 100\% \qquad \text{(Eq.2)}$$

Where:

m_0 is the weight of water added to the beaker

m_f is the weight of water obtained after filtration

m_{gel} is the weight of the alginate spheres added to the beaker

The second (tea-bag method) and third tests (filtration method) were performed according to Snoeck et al., (2018), who describe two distinct methodologies for characterizing capsules in cementitious-based materials, generating a greater understanding of how the capsules react when mixed with the concrete formulation in its fresh state.

Step 2

For the conformation of the test specimens, all the component materials of the formulation were previously dried in an oven with air circulation and renewal, model SL-102 - Solab, for a period of 24 h at 60 °C for subsequent granulometric separation. At the end of drying, the aggregates (coarse: gravel 1 and fine: gravel powder) were passed through the sieves (keeping the granulometry for fine aggregates between 0.15 mm and 9.5 mm and for coarse aggregates of 9.5 mm to 25 mm) until reaching the appropriate granulometry, where a suspended mechanical granulometric sieve by Solotest was used. After the granulometric separation, the materials were weighed according to the 4 concrete formulations to be investigated in the research, varying the Andreassen packing coefficient at q=0.30 and q=0.37 and the alginate contents of sodium in 1 and 1.5% by weight in relation to the weight of cement.

Consistency was evaluated by slump test (ABNT NBR NM 67 (1998) standard) for the investigated compositions. The masses used in this test were used later in the conformation of the test specimens.

Forty-eight specimens were produced, 24 of which were formulated using q=0.30. Of these, 12 bodies had the addition of 1% by weight of sodium alginate and the remaining 12, 1.5 % by weight. The production of the 24 remaining specimens was formulated with q=0.37, divided into two percentages of addition of capsules as well (being 1.0 % and 1.5 %). The choice of q values and sodium alginate content was based on previous studies by the research group.

The molding and curing of the concretes were carried out in accordance with the ABNT NBR 5738 (ABNT 1934) standard. For the molding process of the specimens that were tested in compression, a cylindrical mold measuring 100 x 200 (mm) was used, greased with vegetable oil to facilitate unmolding. The filling was done in two layers, each layer being compacted with 12 strokes. The last layer was molded with excess concrete, so that, when compacted, it completes the entire volume of the mold, posteriorly eliminating all excess concrete. After 24 hours of molding, curing began, with the specimens being demolded and immersed in a tank with water saturated with calcium hydroxide, aiming the continuous hydration of the cement, in order to avoid premature loss of water at a temperature around 21°C, thus preventing the appearance of future cracks (Figure 2).

Figure 2 – Concrete specimens immersed in saturated water for cement hydration.

Source: Authors (2023).

After 28 days, according to the ABNT NBR 9778 standard (ABNT, 2005), the water absorption, the void index and the dry and real specific mass of the specimens were determined. The results obtained were analyzed considering the different *q* values and investigated sodium alginate contents. Compressive strength measurements were carried out in accordance with ABNT NBR 5739 (ABNT, 2018) standards, which establishes the method of preparation and compression testing for cylindrical concrete specimens and ABNT NBR 5738 (ABNT, 2015), which determines the ages at which the bodies were tested.

For the mechanical compression tests, there is a need for a rectification process, according to the ABNT NBR 5738 standard (ABNT, 2015), which was carried out in a cutting machine with a Bel Air pneumatic actuator, with a maximum pressure of 1.03 MPa, at a speed of 400 rpm, with cooling of the cutting disk and the blade with water. After the grinding process, the specimens were subjected to a compression test using a digital electro-hydraulic concrete press, brand A.M.C, model 10014, maximum capacity of 980.6 kN and manual control of application speed of load, with an accuracy of 0.098 kN. The tests were carried out at the company Polimix Concreto Ltd., located in the city of Poços de Caldas/MG and the specimens were ruptured after 7, 14 and 28 days after molding.

3. Results and Discussion

Step 1

Tables 1 to 3 indicate the results obtained for the swelling test using, respectively, the methods called simple test, tea bag test and filtration test according to Snoeck et al., (2018).

Table 1 – Swelling measurements obtained by the simple test.

Samples 1,2 and 3	**I (%)**
Average	203.3 ± 1.3

Source: Authors (2023).

Table 2 – Swelling percentage (I%) obtained by tea-bag method as a function of time (min).

Time (min)	**1**	**5**	**10**	**30**	**60**	**180**	**1440**
Average (g)	4.24 ± 0.25	4.28 ± 0.25	4.53 ± 0.06	5.58 ± 0.03	5.84 ± 0.03	6.32 ± 0.03	6.56 ± 0.04
I (%)	423.57	428.05	453.01	558.28	583.88	631.71	656.44

Source: Authors (2023).

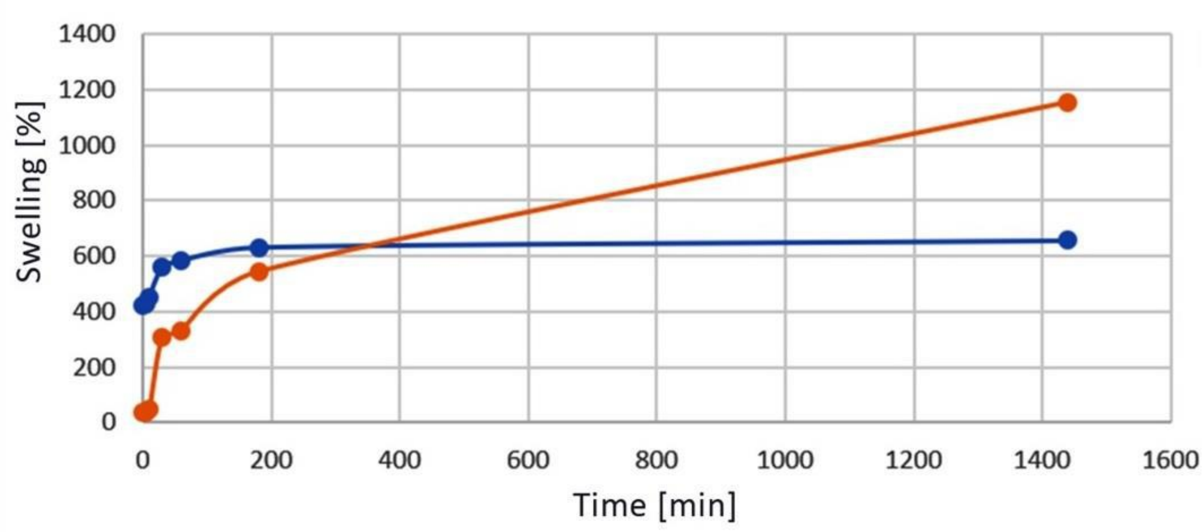
Table 3 – Swelling percentage (I%) obtained by filtration as a function of time (min).

Time (min)	**1**	**5**	**10**	**30**	**60**	**180**	**1440**
Average (ml)	0.36 ± 0.10	0.39 ± 0.14	0.48 ± 0.12	3.05 ± 0.17	3.32 ± 1.09	5.44 ± 0.12	11.53 ± 2.50
I (%)	36.03	39.27	48.23	305.43	331.67	544.43	1153.03

Source: Authors (2023).

When carrying out the swelling analysis by the tea-bag method, a constant is observed after 180 minutes, where the exponential curve reaches its threshold for a growth line. (Figure 3) The same occurs when observing the swelling test of the filtration method, this constant appears after 180 minutes (Figure 3); so, the difference is due to the high change in swelling when it is inside a tea bag, since the shape of the bag offers a certain resistance in relation to its growth. Due to the physical barrier that exists in the tea-bag method, the capsules grow less when compared to the filtration method, in which the capsule is loose inside the liquid.

Figure 3 *– Swelling test of the tea-bag and filtration method.*



Source: Authors (2023).

Studies prove (Reis, 2019) that when the capsule reaches a very high swelling, its material dissolves more easily, not being interesting for use in cementitious media due to the mixture of coarse and fine aggregates that can break the capsule more easily, as well as the alkaline mixture can influence its interior. When subjected to swelling tests, some materials (such as alginate or chitosan gels) have a lower apparent resistance, demonstrating a fragility at 10 minutes, since it is from this time that it begins to dissolve, while the Sodium alginate supports a greater degree of swelling without dissolving in the triplicate test (Reis, 2019).

Step 2

Table 4 shows the results of the slump test for the investigated compositions varying *q* (Andreassen packing coefficient) at 0.30 and 0.37 and the sodium alginate content at 1 and 1.5 % in relation to the weight of cement.

Table 4 – Slump values of the investigated compositions according to NBR NM 67 standard.

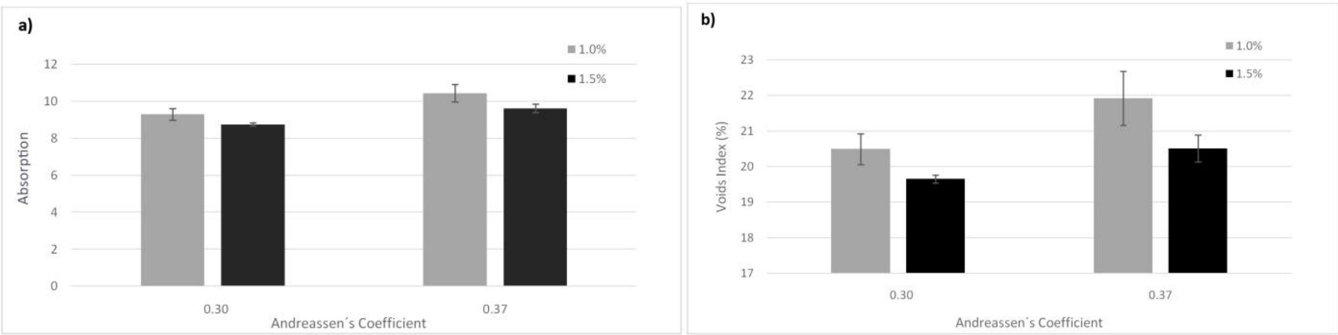
Composition	Slump value (cm)
q=0.30; sodium alginate:1.0 %	24.0
q=0.30; sodium alginate:1.5 %	23.7
q=0.37; sodium alginate:1.0 %	20.0
q=0.37; sodium alginate:1.5 %	21.0

Source: Authors (2023).

All the investigated formulations obtained slump values between 18 and 25 cm, reaching a fluid consistency, according to the NBR NM 67 standard (ABNT, 1998), indicating that fluidity, the investigated formulations are suitable for molding. Furthermore, it is observed that the presence of sodium alginate does not interfere with the good consistency results obtained.

Figures 4(a) and 4(b) show, respectively, the average values of water absorption and voids index for the different formulations investigated.

Figure 4 – (a) Water absorption (%) and (b) average voids index (%) obtained for the investigated samples, varying the sodium alginate content and the *q* value of the Andreassen equation.

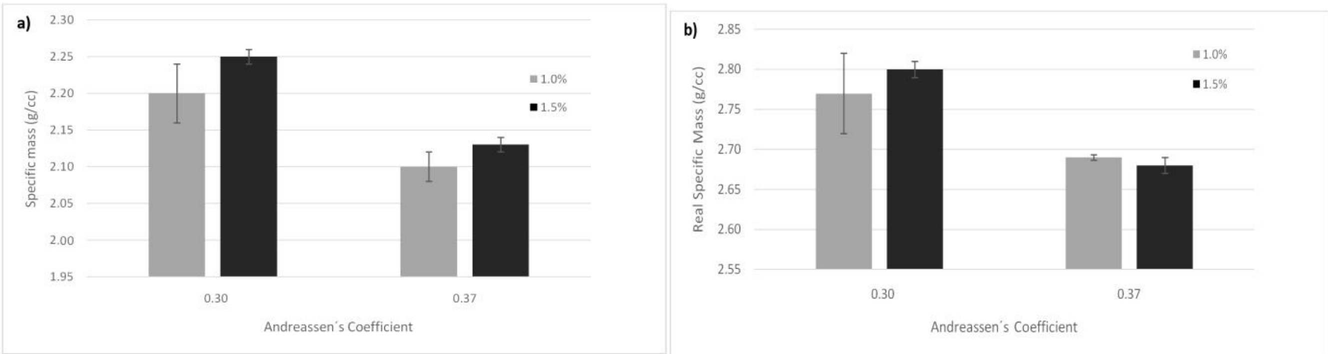


Source: Authors (2023).

It is observed that the formulation using the Andreassen coefficient equal to 0.37 provided a higher water absorption and that samples containing higher additions of sodium alginate capsule showed lower absorption rates for both coefficients. The results also show that the samples presented good conformation, since the deviation of the measures obtained is slim, indicative of good homogeneity. The greater use of coarse aggregates for the Andreassen formulation with q=0.37 may have generated a greater number of voids (spaces) not filled in the mass by packing), resulting in higher levels of water absorption in relation to the formulation where q =0.30. The presence of sodium alginate capsules may contribute to the partial filling of small voids left in both formulations, causing a decrease in absorption values. These results are corroborated by the behavior observed in the void ratio.

Figures 5(a) and 5(b) show the results of the average specific mass of the dry samples and the real specific mass, respectively, for the investigated formulations. A slight influence of the added sodium alginate content was observed for compositions with q=0.30 or q=0.37; the presence of a higher content of sodium alginate seems to cause a increase in specific mass values, which agrees with the previously discussed results. The influence between the compositions is due to the variation in the *q* value; that is, the granulometric distribution used in the investigated formulations. The formulation with q=0.30 has a greater number of fines compared to q=0.37, which affected the degree of final packing, providing higher levels of specific mass (or density) both dry - which considers the presence of permeable and impermeable pores – as well as the actual specific mass, where, by the equation used, permeable pores are excluded. It should also be noted the good reproducibility of the results, indicating that the samples are homogeneous, resulting in low values of deviation in the measurements obtained.

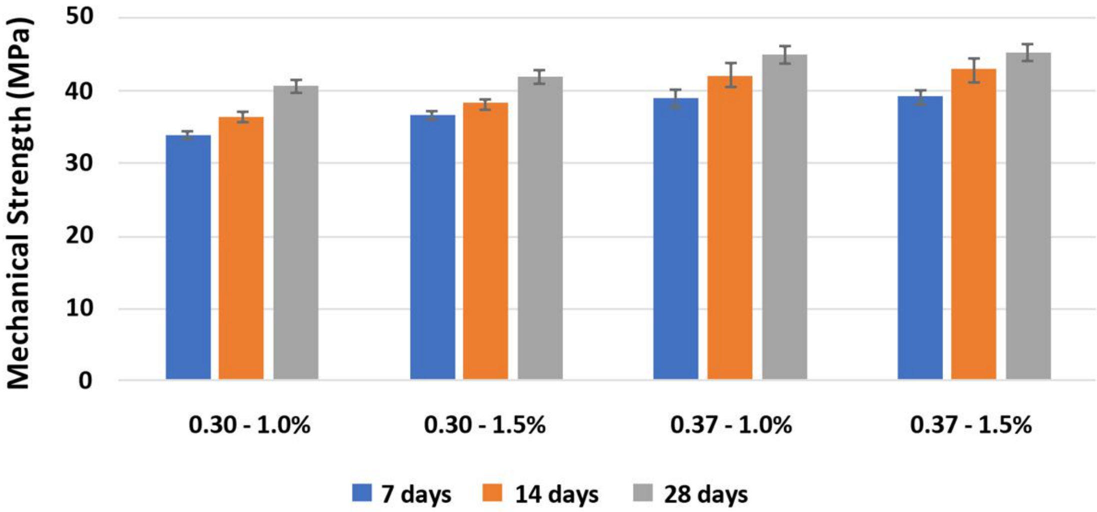
Figure 5 - (a) Specific mass of dried samples and (b) real average specific mass for the investigated samples, varying the sodium alginate content and the *q* value of the dry Andreassen equation for the investigated formulations.



Source: Authors (2023).

Figure 6 indicates the results obtained from the compression tests carried out for the different compositions. All specimens reached minimum values close to 35 MPa, or higher. A progressive increase in mechanical strength was also observed by the age of the concrete, which is due to the hydration and curing process of the concrete.

Figure 6 – Mechanical compression resistance of investigated compositions (MPa).



Source: Authors (2023).

The influence of the sodium alginate content is also proven for all investigated samples, varying *q* as well as the age of the concrete. For all compositions, an improvement in the mechanical properties of concrete with a presence of sodium alginate was observed, a fact previously discussed in the previous paragraph. This result was expected, since samples with lower void ratios, lower levels of water absorption and higher values of dry and real specific mass tend to have higher mechanical strength since critical defects tend to be minimized.

When comparing the results obtained for the formulations with different packing coefficients the mechanical properties of the compositions with q=0.30 were lower than those where q=0.37 was used, regardless of the sodium alginate content added. Although the compositions with q=0.30 present a greater degree of packing due to the greater presence of fine particles, which results in a specific mass increase and lower voids index, it's noted that the greater presence of coarse aggregates (which occurs in the formulations where q=0.37) allow greater anchoring between particles during the hydration process. In addition, the greater presence of coarse aggregates tends to increase resistance to catastrophic crack propagation, since cracks need to bypass these coarse aggregates, causing an increase in compressive strength.

4. Conclusion

The methodology adopted for obtaining sodium alginate capsules proved to be adequate for the insertion process in the test specimens. The control of processing parameters as concentration of the alginate solution, dripping speed of constant slow rate and agitation of 60 rpm during dripping at room temperature was feasible for the production of spheres of 5 mm on a larger scale.

The greater presence of sodium alginate capsules positively affected the physical and mechanical properties of the concretes and with regard to the variation in the packing coefficient (and what this variation implies in the formulation), it was observed that, despite q=0.30 providing a lower void ratio and higher specific mass for both capsules addition, lower anchoring of raw materials and a lower amount of coarse aggregates seem to be factors of greater influence regarding the mechanical strength. Thus, the use of an Andreassen coefficient equal to 0.37 along with the addition of 1.5% alginate capsules provided the best results when the rheological, physical and mechanical properties were evaluated together.

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References

Ahmed, I., *et al*. (2007) Proposal of Lysinibacillus boronitolerans gen. nov. sp. nov., and transfer of Bacillus fusiformis to Lysinibacillus fusiformis comb. nov. and Bacillus sphaericus to Lysinibacillus sphaericus comb nov. *International Journal of Systematic and Evolutionary Microbiology*, 57 (5), 1117-1125. http://dx.doi.org/10.1099/ijs.0.63867-0.

Associação Brasileira de Normas Técnicas. (2010). *Concreto – Determinação da resistência à tração na flexão de corpos de prova prismáticos.* (ABNT NBR No. 12142)

Associação Brasileira de Normas Técnicas. (1996). *Concreto - Preparo, controle e recebimento.* (ABNT NBR No. 12655).

Associação Brasileira de Normas Técnicas. (1998). *Concreto - Amostragem de concreto fresco.* (ABNT NBR NM No. 33).

Associação Brasileira de Normas Técnicas. (1998). *Concreto – Determinação da consistência pelo abatimento do tronco de cone.* (ABNT NBR NM No. 67).

Associação Brasileira de Normas Técnicas. (2015). *Moldagem e cura de Corpos de Prova cilíndricos ou prismáticos de concreto.* (ANBT NBR No. 5738).

Associação Brasileira de Normas Técnicas. (2018). *Concreto - Ensaio de Compressão de Corpos de prova cilíndricos.* (ABNT NBR No. 5739).

Associação Brasileira de Normas Técnicas. (2014). *Projeto de estruturas de concreto - Procedimento.* (ABNT NBR No. 6118).

Associação Brasileira de Normas Técnicas. (1992). *Concreto – para fins estruturais.* (ABNT NBR No. 8953).

Associação Brasileira de Normas Técnicas. (2005). *Argamassa e concreto endurecidos – Determinação de absorção de água, índice de vazios e massa específica.* (ABNT NBR No. 9778).

Associação Brasileira de Normas Técnicas. (2018). *Cimento Portland Composto.* (ABNT NBR No. 16697).

Biasi, L. A. (2022). Adubação orgânica na produção, rendimento e composição do óleo essencial da *alfavaca quimio tipo eugenol*. ***Horticultura Brasileira***, 1 (27), 35-39.

Bissonnette, B., Pierre, P., & Pigeon, M. (1999). Influence of key parameters on drying shrinkage of cementitious materials. *Cement and Concrete Research*, 29 (10), 1655-1662. http://dx.doi.org/10.1016/s0008-8846(99)00156-8.

Boquet, E., Boronat, A., & Ramos-Cormenzana, A. (1973). Production of Calcite (calcium carbonate) Crystals by Soil Bacteria is a general Phenomenon, Nature. *Springer Science and Business Media LLC*, 246 (5434), 527-529. http://dx.doi.org/10.1038/246527a0.

Brancalhão, R. M. C., & Cavéquia, M. C. *Microscópio Óptico - microscópio de luz.* https://projetos.unioeste.br/projetos/microscopio/index.php?option=com_phocagallery&view=category&id=76&Itemid=140.

Brunauer, S., & Copeland, L. E. (1964). The chemistry of concrete. *Scientific American: a division of Nature America*, 4 (210), 80-93.

Cabrera, J. G. (1996). Deterioration of concrete due to reinforcement steel corrosion. *Cement and Concrete Composites*, 18 (1), 47-59. https://doi.org/10.1016/0958-9465(95)00043-7.

Carmona, J. P. S. F. (2016). *Utilização da Biotecnologia para a estabilização de solos: Precipitação de $CaCO_3$ por via enzimática*. [Dissertação de Mestrado, Faculdade de Ciências e Tecnologia]. Universidade de Coimbra. https://estudogeral.uc.pt/handle/10316/98908.

Castro, A. L., Liborio, J. B. L., & Pandolfelli, V. C. Reologia de concretos de alto desempenho aplicados na construção civil - Revisão. *Cerâmica*, 57, 63-75.

Cruz, C. M. (2023). *Estudo da biomineralização aplicada a concretos de construção civil e sua viabilidade tecnológica*. [Dissertação Mestrado, Curso de Engenharia e Ciências de Materiais]. Universidade de São Paulo Faculdade de Zootecnia e Engenharia de Alimentos.

Farias, Y. B., & Noreña, C. P. Z. (2019). Reverse encapsulation using double controlled gelification for the production of spheres with liquid light soy sauce-core. *International Journal of Gastronomy and Food Science*, 16 (100137), 1-7. http://dx.doi.org/10.1016/j.ijgfs.2019.100137.

Faria, G. C., & Silva, D. S. (2019). *Análise da evolução da profundidade de carbonatação em estruturas de concreto ao longo do tempo*. [Trabalho de conclusão de Curso de Graduação, Curso de Engenharia Civil]. Universidade Estadual de Santa Catarina. http://repositorio.unesc.net/handle/1/7132.

Filho, L. A. E., Alves, T. R., & Fernandes, V. A. (2017). *Bioconcreto: Estudo exploratório de concreto com introdução de Bacillus Subtilis, Bacillus Licheniformis, acetato de cálcio e ureia*. [Trabalho de Conclusão de Curso, Graduação em Engenharia Civil]. Universidade Federal de Goiás. https://files.cercomp.ufg.br/weby/up/140/o/ESTUDO_EXPLORAT%C3%93RIO_DE_CONCRETO_COM_INTRODU%C3%87%C3%83O_DE_BACILLUS_SUBTILIS__BACILLUS_LICHENIFORMIS__ACETATO_DE_C%C3%81LCIO_E_UREIA..pdf.

Freitas, A. Á., Romão, E. M., Anício, S. O., & Barros, A. J. (2021) Bioconcrete: A review of its application in civil construction. *Research, Society and Development*, 10 (4). https://doi.org/10.33448/rsd-v10i4.14270.

Fusco, P. B. (2012). *Tecnologia do concreto estrutural: tópicos aplicados*. Editora PINI.

Garcia, A., Spim, J. A., & Santos, C. A. (2012). *Ensaio dos Materiais.* Editora LTC.

Gato, M. C. S., Muniz, W., Silva, K. B., & Sá, M. S. (2021). Self-regeneration of cracks in concrete from a bacteria culture. *Research, Society and Development*, 10 (6), 1-13. http://dx.doi.org/10.33448/rsd-v10i6.15734.

Gonçalves, E. A. B. (2015). *Estudo de patologias e suas causas nas estruturas de concreto armado de obras de edificações*. [Disssertação de Doutorado, Curso de Engenharia Civil]. Universidade Federal do Rio de Janeiro. http://repositorio.poli.ufrj.br/monografias/monopoli10014879.pdf.

Global Cement and Concrete Association. (2019). *Key Facts. Londres: GCCA.*

HammeS, F., & Verstraete, W. (2002). Key roles of pH and calcium metabolism in microbial carbonate precipitation. *Reviews in Environmental Science and Biotechnology*, 1, 3-7. http://dx.doi.org/10.1023/a:1015135629155.

Jonkers, H. M. (2007). Self-Healing Concrete: a biological approach. *Springer Series in Materials Science*, 195-204. http://dx.doi.org/10.1007/978-1-4020-6250-6_9.

Jonkers, H. M., & Schlangen, E. (2007). Self-healing of cracked concrete: A bacterial approach. *Fracture mechanics of concrete and concrete sructures, Catania*, 17 (22), 1821–1826. http://framcos.org/FraMCoS-6/164.pdf.

Jonkers, H. M., *et al.* (2010). Application of bacteria as self-healing agent for the development of sustainable concrete. *Ecological Engineering*, 36 (2), 230-235. http://dx.doi.org/10.1016/j.ecoleng.2008.12.036.

Khalic, W., & Ehsan, M. B. (2016). Crack healing in concrete using various bio influenced self-healing techniques. *Construction and Building Materials*, 102 (1), 349-357. http://dx.doi.org/10.1016/j.conbuildmat.2015.11.006.

Lee, K. Y., & Mooney, D. J. (2012). Alginate: Properties and biomedical applications. *Progress in Polymer Science*, 37 (1), 106-126. http://dx.doi.org/10.1016/j.progpolymsci.2011.06.003.

Lee, Y. S., & Park, W. (2018). Current challenges and future directions for bacterial self-healing concrete. *Applied Microbiology and Biotechnology*, 102 (7), 3059-3070. https://doi.org/10.1007/s10295-008-0514-7.

Li, C. V., & Herbert, E. (2012). Robust self-healing concrete for sustainable infrastructure. *Journal of Advanced Concrete Technology*, 10 (6), 207-218. http://dx.doi.org/10.3151/jact.10.207.

Li, M., & Li, V. C. (2011). Cracking and healing of engineered cementitious composites under chloride environment. *ACI Materials Journal*, 108 (3), 333-340. https://doi.org/10.14359/51682499.

Mehta, P. K., & Monteiro, P. J. M. (2008) *Concreto: Microestrutura, propriedades e materiais.* São Paulo: Ibracon.

Menezes, C. R., *et al.* (2013). Microencapsulação de probióticos: avanços e perspectivas. *Ciência Rural*, 43 (7), 1309-1316. http://dx.doi.org/10.1590/s0103-84782013005000084.

Meyer, C. (2009). The greening of the concrete industry. *Cement and Concrete Composites*, 31, 601-605. https://doi.org/10.1016/j.cemconcomp.2008.12.010.

Muynck, W., Belie, N., & Verstraete, W. (2010). Microbial carbonate precipitation in construction materials: a review. *Ecological Engineering*, 36 (2), 118-136. http://dx.doi.org/10.1016/j.ecoleng.2009.02.006.

Neville, A. M., & Brooks, J. J. (2010). *Tecnologia do concreto.* Bookman Editora Ltda.

Neville, A. M. (2016). *Propriedades do concreto.* Bookman Editora Ltda.

Oliveira, I. R., *et al.* (2000). *Dispersão e empacotamento de partículas.* Fazendo Arte.

Park, S-J., Park, J-M., Kim, W-J., & Ghim, S-Y. (2012). Application of Bacillus subtilis 168 as a multifunctional agent for improvement of the durability of cement mortar. *Journal of Microbiology and Biotechnology*, 22 (11), 1568-1574. http://dx.doi.org/10.4014/jmb.1202.02047.

Peruzzi, A. P. (2002). *Comportamento das Fibras de Vidro Convencionais em matriz de cimento Portland modificada com látex e adição de sílica ativa*. [Dissertação Doutorado, Curso de Arquitetura]. Escola de Engenharia de São Carlos da Universidade de São Paulo. https://www.teses.usp.br/teses/disponiveis/18/18131/tde-13112002-180613/publico/AntonioPPeruzzi.pdf.

Pró Lab - Materiais para Laboratório. *Entenda como funciona um microscópio óptico.* https://www.prolab.com.br/blog/equipamentos-aplicacoes/entenda-como-funciona-um-microscopio-optico/.

Reis, J. F. A. (2008). *Determinação de parâmetros reológicos de concretos através do ensaio de abatimento de tronco de cone modificado: estudo de caso.* [Dissertação Mestrado, Faculdade de Engenharia Mecânica]. Universidade Estadual Paulista. https://repositorio.unesp.br/bitstream/handle/11449/94495/reis_jfa_me_ilha.pdf?sequence=1.

Reis, T. (2019). *Síntese de hidrogéis de alginato reticulados com nanofibras de lactato de cálcio/poli(óxido de etileno) obtidas por eletrofiação*. [Tabalho de Conclusão de Curso Graduação, Curso de Química] Universidade Federal de Santa Catarina. https://repositorio.ufsc.br/bitstream/handle/123456789/202624/TCC%20II%20-%20Tamara%20Reis.pdf?sequence=1.

Rocha, L. N., *et al.* (2019). Estudo comparativo de desempenho entre concreto convencional e o concreto com adições de fibra de aço. *Revista de Engenharia e Tecnologia*, 11(4), 267-276. https://revistas2.uepg.br/index.php/ret/article/view/14275.

Scott, J. E. (1968). Periodate oxidation, pKa and conformation of hexuronic acids in polyuronides and mucopolysaccharides. *Biochimica et Biophysica Acta (BBA) – General Subjects*, 170 (2), 471-473. https://doi.org/10.1016/0304-4165(68)90040-8.

Seifan, M., Samani, A. K., & Berenjian, A. (2016). Bioconcrete: next generation of self-healing concrete. *Applied Microbiology and Biotechnology*, 100 (6), 2591-2602. https://doi.org/10.1007/s00253-016-7316-z.

Shinano, H. (1972). Studies of Marine Microorganisms Taking Part in the Precipitation of Calcium Carbonate-II. *Nippon Suisan Gakkaishi*, 38 (7), 717-725. http://dx.doi.org/10.2331/suisan.38.717.

Snoeck, D., *et al.* (2018). Recommendation of RILEM TC 260-RSC: testing sorption by superabsorbent polymers (sap) prior to implementation in cement-based materials. *Materials and Structures*, 51 (5), 1-7. http://dx.doi.org/10.1617/s11527-018-1242-8.

Souradeep, G., & Kua, H. W. (2016). Encapsulation technology and techniques in self-healing concrete. *Journal of Materials in Civil Engineering*, 28 (12). http://dx.doi.org/10.1061/(asce)mt.1943-5533.0001687.

Tebo, B. M., Johnson, H. A., Mccarthy, J. K., & Templeton, A. S. (2005). Geomicrobiology of manganese (II) oxidation. *Trends In Microbiology*, 13 (9), 421-428. http://dx.doi.org/10.1016/j.tim.2005.07.009.

Trenson, G. (2017). *Application of pH responsive hydrogel encapsulated bacteria for self-healing concrete*. [Dissertação Mestrado, Engenharia Civil]. Ghent University, Gante.

Van Tittelboom, K., *et al.* (2010). Use of bacteria to repair cracks in concrete. *Cement Concrete Research*, 6 (1), 157-166. http://dx.doi.org/10.1016/j.cemconres.2009.08.025.

Van Tittelboom, K., *et al.* (2011). Self-healing efficiency of cementitious materials containing tubular capsules filled with healing agent. *Cement and Concrete Composites*, 33 (4), 497-505. http://doi.org/10.1016/j.cemconcomp.2011.01.004.

Van Tittelboom, K., *et al.* (2013). Self-Healing in Cementitious Materials—A Review. *Materials*, 6 (6), 2182-2217. http://dx.doi.org/10.3390/ma6062182.

Velloso, F. T. (2008). *Desenvolvimento e caracterização de microcápsulas de alginato/quitosana contendo ácido retinóico e óleo de babaçu.* [Dissertação Doutorado, Curso de Ciências Farmacêuticas]. Universidade Federal de Pernambuco. https://repositorio.ufpe.br/handle/123456789/2946.

Wang, J. Y., De Belie, N., & Verstraete, W. (2012). Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete. *Journal of Industrial Microbiology and Biotechnology*, 39 (4), 567-577. https://doi.org/10.1007/s10295-011-1037-1.

Wang, J. Y., *et al.* (2012). Use of silica gel or polyurethane immobilized bacteria for self-healing concrete. *Construction and Building Materials*, 26 (1), 532-540. https://doi.org/10.1016/j.conbuildmat.2011.06.054.

Wang, J. Y., *et al.* (2014). Application of hydrogel encapsulated carbonate precipitating bacteria for approaching a realistic self-healing in concrete. *Construction and Building Materials*, 68, 110-119. http://doi.org/10.1016/j.conbuildmat.2014.06.018.

Wang, J. Y., *et al.* (2014). Self-healing concrete by use of microencapsulated bacterial spores. *Cement and Concrete Research*, 56, 139-152. http://doi.org/10.1016/j.cemconres.2013.11.009.

Wang, J., *et al.* (2015). Application of modified-alginate encapsulated carbonate producing bacteria in concrete: a promising strategy for crack self-healing. *Frontiers in Microbiology*, 6 (1088), 1-14. http://doi.org/10.3389/fmicb.2015.01088.

Wang, J., *et al.* (2018). A chitosan-based pH-responsive hydrogel for encapsulation of bacteria self-healing concrete. *Cement and Concrete Composites*, 93, 309-322. http://doi.org/10.1016/j.cemconcomp.2018.08.007.

Wu, M., Johannesson, B., & Geiker, M. (2012). A review: self-healing in cementitious materials and engineered cementitious composite as a self-healing material. *Construction and Building Materials*, 28 (1), 571-583. http://dx.doi.org/10.1016/j.conbuildmat.2011.08.086.

Wu, M., Hu, X., Zhang, Q., Xue, D., & Zhao, Y. (2019). Growth environment optimization for inducing bacterial mineralization and its application in concrete healing. *Construction And Building Materials*, 209, 631-643. http://dx.doi.org/10.1016/j.conbuildmat.2019.03.181.

Zhu, X., *et al.* (2021). Viability determination of *Bacillus sphaericus* after encapsulation in hydrogel for self-healing concrete via microcalorimetry and in situ oxygen concentration measurements. *Cement and Concrete Composite***s**, 119, 104006-104015. http://dx.doi.org/10.1016/j.cemconcomp.2021.104006.