Comparison of experimental techniques for biocimentation of sands considering homogeneity and its effects on uniaxial compression strength

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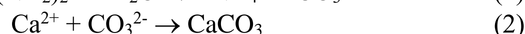
The purpose of this work was to define an efficient method for sandy soil treatment using biocimentation. An experimental device was developed for cylindrical soil samples with 7.2 cm diameter and 12 cm height. Two sample preparation techniques were tested: (i) pre-mixing the soil with bacteria, then inject feeding solution; (ii) inject bacteria followed by feeding solution. In both, the injection conditions varied in two distinct ways: (i) injecting from the top, like a ''shower'' and (ii) injecting through a central perforated tube. The homogeneity of the treatment was evaluated in samples extracted from different zones of the specimens using X-Ray diffractometry and scanning electron microscope SEM images. Mercury Intrusion Porosimetry tests (MIP), $CaCO_3$ dissolution using HCl and NH_4^+ concentrations determination through Nessler reagent were also performed. Results showed $CaCO_3$ content varying between 1.8% to 4.6%, unevenly distributed between the top and bottom sections, as well as along radial direction. Homogeneity studies showed less amount of precipitate near the bottom sections (far from the inlet). The most homogeneous and cemented samples were found when pre-mixing the bacteria with soil. Finally, unconfined compression tests (UCS) were performed. In the comparison between the values measured in the control (specimens prepared using only feeding solution and no bacteria) and in the specimens treated with bacteria, strength increments from 18 kPa to 150 kPa were observed, showing that treatment worked independently from the procedure adopted for its application.

KEYWORDS: biocementation, calcium carbonate, homogeneity, MIP, UCS

1 Introduction

Biocementation is a process that allows for the formation of calcium carbonate precipitate ($CaCO_3$) using ureolytic bacteria (Mitchell e Santamarina, 2005). The process involves the hydrolysation of urea ($CO(NH_2)_2$) into carbonate ions (CO_3^{2-}), which when in presence of calcium ions (Ca^{2+}) precipitates into calcium carbonate (Eq 2). The hydrolysis of urea forms as well ammonium ions (NH_4^+) (Eq 1), increasing the pH level of the medium to basic values (Stocks-Fischer *et al.*, 1999; Yasuhara *et al.*, 2012).

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad (1)$$



$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \quad (2)$$

Calcium ions come usually from fed calcium chloride ($CaCl_2$) because it is easy to obtain and dissolve in aqueous medium (Eq 3) (Whiffin, 2004)

$$CaCl_2 \rightarrow Ca^{2+} + 2Cl^- \quad (3)$$

The enzyme urease acts as a catalyst of the urea hydrolyzation, being present in several bacteria. The species *Sporosarcina Pasteurii*, also known as *Bacillus Pasteurii* is the most used bacteria in biocementation studies (Wei-Soon *et al.*, 2012), mostly due to its high enzyme activity and known adaptability to hostile environments (Hammes e Verstraete, 2002). The enzyme urease is also found in powder form, extracted from the plant *Canavalia Ensiformis* (Jack Bean). Its usage is mostly justified for allowing to avoid slow bacteria growth stage (Carmona *et al.*, 2016).

The interest in biocementation application in soils is justified by its ability to change and improve their hydro-mechanical properties. These changes vary from a wide range: (i) reduction of permeability of the porous media because of accumulation of precipitate in the soil pores, also known as clogging (Gollapudi *et al.*, 1995; Nemati e Voordouw, 2003), (ii) strength and stiffness improvement due to bonding generation between soil particles (van Paassen *et al.*, 2010; Whitaker *et al.*, 2018). It is also considered as an environmentally friendly technique and a future substitute to some other more aggressive existing soil improvement methods like *jet-grouting* (DeJong *et al.*, 2006; Van Paassen *et al.*, 2009).

Nonetheless, the biocementation technique is a complex process which requires a deep understanding of the factors controlling the enzyme and bacteria ability to hydrolyze the urea. As such, chemical parameters like the concentration of reagents (Whiffin, 2004; Al Qabany *et al.*, 2012) or the conditions of the medium such as its alkalinity and temperature (Sahrawat e Patancheru, 1984; Stocks-Fischer *et al.*, 1999) may be optimized in order to

assure high enzyme activity and increase the amount of precipitate. The geometric compatibility of soil particles with the size of the bacteria is also a defining factor, since an excessive amount of silts and clay in the treated soil may hinder the transportation of the micro-organism and their access to the cementation reagents (Mitchell e Santamarina, 2005; Wei-Soon *et al.*, 2012).

Moreover, the distribution of calcium carbonate precipitate is another factor that impacts the range and quality of the treatment (Whiffin *et al.*, 2007). In 2009, in a scale-up experiment, Van Paassen *et al* determined a heterogeneously distribution of $CaCO_3$ content over 7 m of treated sand, ranging from 0% to 24%. They also observed a significant reduction in cementation the further he would extract the samples from the injection points. Consequently, and since the increases in strength and stiffness are connected to the amount of $CaCO_3$ content in the soil (Yasuhara *et al.*, 2012), heterogeneity leads to irregular results which need to be predicted to apply successfully the technique (Whiffin *et al.*, 2007).

In this work, the effect of several methods of bacteria and feeding solution (or cementation solution) injection were studied aiming to characterize the homogeneity of the distribution of the precipitate. Three kinds of samples were prepared: (i) to be treated with enzyme (named E), (ii) to be treated with bacteria (named B) and (iii) for control (named C), prepared only using feeding solution. The methods included injecting enzyme or bacteria as an infiltration column or through a perforated central tube, or by pre-mixing bacteria with the soil before packing. The feeding solution was injected at each day, for 4 days using or the central perforated tube or by infiltration from the top as an infiltration column. In total, seven specimens were evaluated (Table 1).

Table 1 - Samples prepared and injection methods tested

Specimen Name	Form of bacteria/enzyme injection	Form of feeding solution injection
E4ct (enzyme)	Injection tube	Injection tube
B2st	Infiltration column	Infiltration column
B2ct	Injection tube	Injection tube
B3st	Pre-mixed	Infiltration column
B3ct	Pre-mixed	Injection tube
C1 (control)	Culture medium with no bacteria	Infiltration column
C2 (control)	Culture medium with no bacteria	Injection tube

Samples were taken from cylindrical shaped treated columns with 12.0 cm height and 7.2 cm diameter, along its length as well as in radial direction. X-ray tests and SEM images with EDS analysis were performed to identify and verify the presence of $CaCO_3$ in form of calcite from the biocementation reactions. In addition, these samples were then analyzed and compared according to their $CaCO_3$ content, and their pore size distribution to consider clogging effect. At the same time, ammonium concentration distribution analysis from the effluent allowed to understand the distribution of the enzyme activity along the length of the specimens studied. Finally, UCS tests, performed on treated specimens, allowed to compare the gains in strength between methods of injection.

Homogeneity tests were also performed in enzyme treated specimens as this were the specimens used during the development of the experimental chamber that allowed the application of the biocementation treatment in soil columns.

2 Materials and methods

2.1. Soil properties and sample preparation

The specimens treated were prepared using a commercial sand river, containing mostly silica minerals according to X-ray tests, but also some clay minerals (Kaolinite). The soil's particle density was determined as Gs= 2.64. Grading size distribution of the sand (Fig. 1) showed D_{10}= 0.25 mm with a reduced portion of fine particles (3.8%) and rest sand material (96.2%) according to the Unified Classification of Soils (Fig. 1). It is classified as a well graded sand (SW) (C_U= 6.25 and C_C=1.00).

For the preparation of all specimens E, B or C (Table 1), the sand was pre-heated in the oven at 105ºC for 48h to ensure its proper sterilization. The specimens were prepared in a cylindrical chamber (7.2 cm diameter and 14 cm in length) with void ratio varying between 0.55 and 0.60 (or 35% to 37.5% porosity), corresponding to dry unit weight varying between 16.6 and 17.1 kN/m^3. These values are similar to those obtained in other studies where biocementation was applied in similar sands (Harkes *et al.*, 2010; Yasuhara *et al.*, 2012).

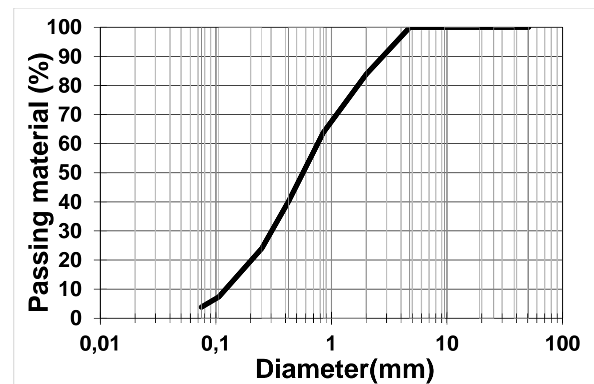


Fig 1 - Grading size distribution of the sand soil

2.2 Bacteria, enzyme and feeding solution

Two different feeding solution application were tested for the samples treated with bacteria: (i) Injection using the perforated tube; (ii) pre-mixed with bacteria. For the pre-mixed bacteria specimens, 200 ml of bacteria liquid medium (or 1 pore volume) was mixed with the sand prior to packing.

The bacteria species used is *Sporosarcina Pasteurii*, provided by the American Type Culture Collection (ATCC). Bacteria were grown in a medium containing 20 g/l of yeast extract, 10 g/l of ammonium sulfate ($(NH_4)_2SO_4$) and Tris (concentration 0,13M) with pH adjusted to 9.0 for optimum enzyme activity conditions, at 30ºC in an incubator. Growth stage took up to 3 days in order to obtain one void volume of inoculated medium (200 ml), with cell concentration of ~10^9 cells/mL, corresponding to an optical density of 1 at 600 nm.

The enzyme urease used, extracted from *Canavalia ensiformis* (Jack Bean), was purchased from Sigma Aldrich, detaining an activity of 1kU/g (1U = 1 µmol/min). For each of the experimental specimens, 200 ml of distilled water were mixed with 1 g of enzyme in order to achieve a final concentration of 5 g/l and frozen at -18ºC waiting for the treatment.

Finally, the feeding solution was prepared using equimolar urea and calcium chloride (source of calcium) with concentration 0.5M and 1:10 diluted growth medium. As a mean to control the pH levels of the medium during treatment (due to the production of ammonium ions from urea hydrolyzation), 2.12g/l of sodium bicarbonate and 10g/l of ammonium chloride were added.

2.3 Experimental setup

When using this technique, one of the challenges was to ensure homogeneous treatment in the entire soil volume. Two different sample preparation techniques were tested: (i) pre-mixing the soil with bacteria, and then inject the feeding solution; (ii) inject bacteria followed by injecting the feeding solution. In both, the injection conditions varied in two distinct ways: (i) injecting from the top, like a ''shower'' (Fig 2.b) and (ii) injecting through a central tube perforated along its length (Fig 2.a).

Due to high pressures necessary to apply during the injection stages, a layer of approximately 1 cm of drain gravel and filter geotextile was added to the top and bottom sections of the chamber, similar to the solution adopted by Harkes *et al* (2010) and presented in Fig 2. The length of the soil specimens had to be reduced from 14 cm to around 12 cm. The combination of this drainage solution and injection methods allowed to compare the precipitate distribution patterns between the bacteria and feeding solution techniques.

As presented in Fig. 2, the chambers had valves in their top and bottom to allow the inlet and outlet of feeding solution and fluid circulating through the soil during the injection.

The injection of the bacteria/enzyme was done using a 20 ml syringe from the top of the chamber. The infiltration tube, when used, was connected to this valve. Without the tube, the valve would feed the upper drainage layer and the system worked as an infiltration column.

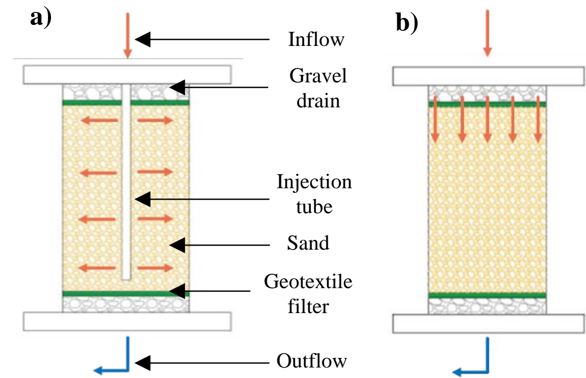


Fig 2 - illustration of the interior of the inoculation chamber and injection methods tested

The injection of the feeding solution was done from the top valve as well, using a pressure/volume controller GDS working as a pumping station. This controller was connected to an interface made by a balloon inside a chamber filled with the feeding solution. This balloon was necessary to separate the clean water inside the controller and the feeding solution. As water was pumped by the controller into the chamber, the volume gained by the balloon allowed to displace a same amount of volume of feeding solution into the inoculation chamber, performing the injection. The photograph of the experimental setup is in Fig 3.

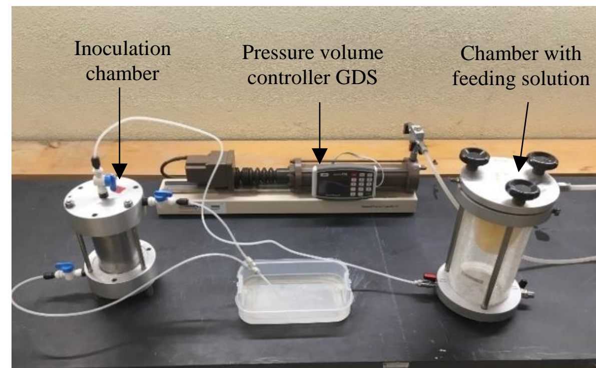


Fig 3 - Experimental setup

A volume of 200 cm^3 of culture medium containing bacteria (or 1 pore volume) was added to the soil at the beginning of the treatment, whether by pre-mixing it or by injection. 4 injections of feeding solution (0.5 M urea-calcium concentrations) were performed, in intervals of 24h. At the end of each injection day, the inoculation chamber would rest at 35±3ºC for an improved enzyme activity (Sahrawat e Patancheru, 1984; Nemati *et al.*, 2005). The flow rate, controlled by the controller GDS, was kept constant at 360 ml/h (or 1.7 pore Volume/h) with the objective of maintaining the pressure inside the chamber low to avoid leakage and, at the same time, avoid the beginning of biocementation reactions during the injection phase.

2.4 Monitoring biocementation treatment

The presence of the reaction products (Eqs. 1 and 3), such as NH_4^+ and Cl^- ions bring important information concerning the unroll of the process of biocementation. At the same time, pH rising is expected. Therefore, the monitorization of the treatment was done using colorimetric strips containing information about the products concentration from the chamber effluent at the end of each daily treatment. At the same time, the injection pressure was monitored in real time because the injections were performed to evaluate occurrences of clogging in the sand but also in inside the chamber.

Ammonium concentrations were also taken using Nessler reagent colorimetric method. Samples were taken from the effluent and immediately frozen at -18ºC waiting for further analysis. The samples were then centrifuged at 6000 rpm at 20ºC, for 3 min, to separate liquid and solid phases. 25 µl of supernatant were then transferred to 50 ml of distilled water to dilute into the 0-0.5 mM NH_4^+ range where the Nessler reagent readings are deemed to be more precise (Harkes *et al.*, 2010). Finally, 100 µl of Nessler reagent were added to 2 ml of the sample, leaving it to react for 1 min before reading its optical density at 425 nm. Optical density readings were calibrated using samples from the control effluent with different NH_4Cl concentrations to mimic the readings conditions of samples.

The samples were taken from the effluent on the second day of injection every 25 ml of volume, measured in the GDS controller (or every 25 ml of effluent). The objective was to determine the distribution of the ammonium concentration along the length of the specimens. It was assumed that the water flow would have little dispersion. The first 25 ml represented the feeding solution already reacted, located near the outlet (at the bottom section), while the last volumes would consist of the upper section of the specimens, at the top.

2.5 Evaluation of mechanical properties

UCS tests on the specimens treated with bacteria (B2st, B2ct, B3st and B3ct), as well as control samples (C1 and C2) were performed to view the mechanical improvements due to the bonding effect between particles due to $CaCO_3$ precipitate presence. The specimens were covered with a latex membrane to minimize material lost during the test and specimen collapse at the end. Axial loading velocity was 0,5 mm/min. Failure criteria adopted was when a resistance peak was measured. Because this has occurred for very low deformations, the stop criteria varied between 5 to 6% for the treated specimens and 11% for C1 and C2. The peak resistances (q_u) and correspondent axial strain were recorded, as well as the tangent Young's modulus in the axial load-axial deformation curve for 50% of the peak value (E_{50}).

2.6 Homogeneity evaluation

The distribution of the $CaCO_3$ precipitate in the treated specimens volume was investigated in order to evaluate homogeneity and therefore compare the different techniques. For each specimen small samples were extracted from different positions along radial and longitudinal direction. The comparison allowed to understand the volumetric impact on the distribution of the precipitate and range of the injection and preparation methods.

The presence of $CaCO_3$ and its different mineral forms was investigated using scanning electron microscope (SEM) and using X-ray diffraction test (XRD). At SEM it was possible also to get information about the chemical composition of the crystals observed through X-ray analyser (EDS). The treated samples investigated were extracted from the top and bottom sections of the injected bacteria specimens (B2st and B2ct), untreated sand (control) and enzyme treated specimen (E4ct).

$CaCO_3$ content was determined by dissolving cemented samples from the enzyme and bacteria treated specimens on top of the control specimen C1, with HCl (0,5M). In this test, the weight of the dried samples was recorded, and acid was added until the liberation of CO_2 would stop (bubbles would stop from coming to the surface of the acid). Samples were then washed using distilled water, while using filter paper to avoid losing material before oven drying at 105ºC for 24 h. The final weight was measured. The difference between both weights was the weight of the carbonates present in the original sample. The $CaCO_3$ content is the ratio between mass loss and the final weight.

Finally, mercury intrusion porosimeter tests (MIP) were performed on the bacteria and enzyme injected specimens (B2st, B2ct, E4ct and E3t), in order to determine if pore dimension reduction due to clogging with precipitate had occurred. For this purpose, the samples taken from the previously mentioned positions, were cut into cubic shape with 1 cm^3 volume before being sent to the laboratory.

3 Results

3.1 Mineral composition

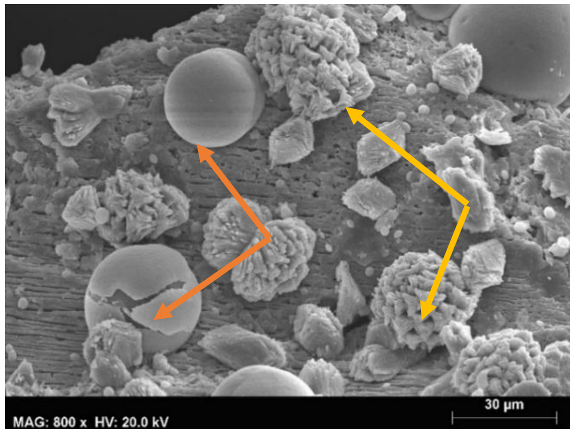
Minerals present in the form of $CaCO_3$ forming bonds was investigated through SEM images and XRD analysis. The comparison between SEM images from the untreated and treated sand showed the appearance of precipitate under three different forms:

i) Crystalline structure, like a rosette, present on all samples and predominant on bacteria treated specimens. The mineral dimensions varied between 20 and 40 µm (Fig 4 – yellow arrows, sample E4ct and figure 5 – sample B2st);

ii) Spherical structure, or amorphous, only observed in samples taken from enzyme treated specimen E4ct, with dimensions varying between 30 and 60 µm (Fig 4 – orange arrows);

iii) Cubic form with reduced size (5 to 20 µm) and less frequent (Fig 4 – blue arrows, sample E4ct).

Chemical X-ray (EDS) analysis of these minerals confirmed the presence of carbon (C), oxygen (O) and calcium (Ca), all present in calcium carbonate.



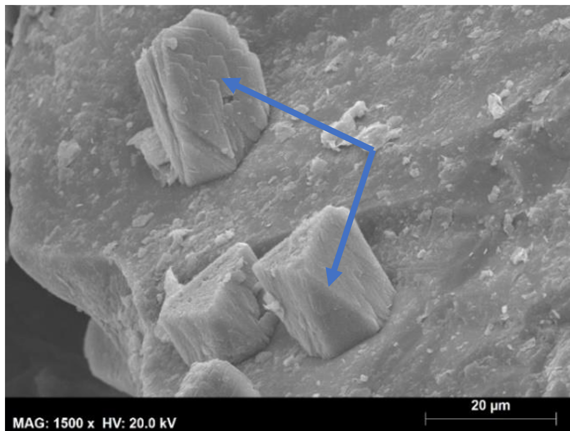


Fig 4 - SEM images of sample E4ct (top and bottom) showing different $CaCO_3$ forms found: rosette (yellow arrows), spherical (orange) and cubic (blue)

As observed in Fig. 5, an image from sample B2st, it was possible to visualize the bonds between particles (active bonds), which contribute to the mechanical improvements observed.

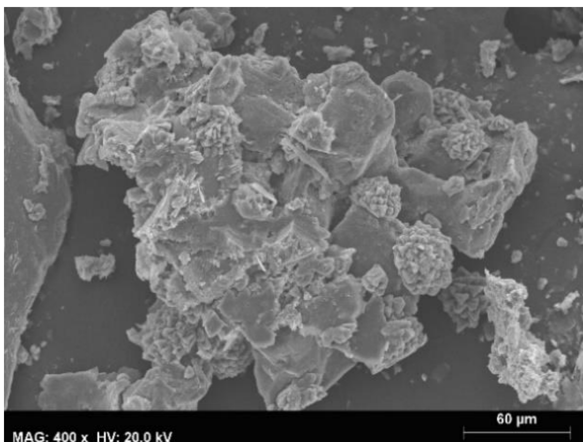


Fig 5 – Sand particles bonded by $CaCO_3$ minerals on sample B2st.

Concerning mineral analysis, XRD results according to the section and position from the specimen where tested samples were removed, are presented in Table 2. It was detected the presence of calcium carbonate in the form of calcite mineral in specimens treated with bacteria B2st and B2ct. The highest scores found for this mineral were obtained at the top sections (the higher the score value, the most probable is the mineral to be present). This can be explained by the fact that this are the zones closest to the inlet, especially for specimens treated without tube. XRD analysis also detected the presence of calcium residues in the enzyme treated sample (E4ct), although the calcium carbonate mineral could not have been identified. These residues were associated with the presence of calcium salts from feeding solution that were not dissolved and therefore did not react during the treatment. The samples from the enzyme treated specimen were extracted from the lateral zone and this may also justify the absence of cementation.

Table 2 - Minerals identified by the XRD analysis

Specimen	Properties	Name of the minerals (score)
Untreated sand	Blank	Quartz (53), Microcline (18), Muscovite (26), Kaolinite (14)
E4ct	Lateral position	Untreated sand minerals, **Albite and Calcium residue (12)**
B2ct	Top section	Untreated sand minerals, **Calcite (27)**
B2ct	Bottom section	Untreated sand minerals, **Calcite (27)**
B2st	Top section	Untreated sand minerals, **Calcite (32)**
B2st	Bottom section	Untreated sand minerals **Calcite (10)**

3.2 Uniaxial compression tests (UCS)

Both the specimens injected with bacteria (B2st and B2ct) presented volumetric deformations, with signs of failure by crushing at the bottom section, as well as some vertical cracks along the entire length (Fig 6.a). The specimens pre-mixed with bacteria (B3st and B3ct) exhibited a shear failure surface, more characteristic of fragile behaviour (Fig 6.b and c). Nonetheless, specimen with tube B3ct showed likewise volumetric deformations and signs of crushing near the bottom section as in the injected specimens (Fig 6.c)). During loading, control specimens C1 and C2 showed volumetric deformation at the middle section with no visible failure surfaces.

The comparison between control specimens and treated sands peak resistance show clearly strength increment up to nine times: Control specimens (C1 and C2) display a resistance of 18 kPa, while the maximum 150 kPa measured in the tests on treated specimen was found in B3st (Fig 7). For the injected specimens, 120 kPa was measured in the specimen with tube B2ct, better than the value of 100 kPa found for the equivalent solution with no tube B2st.

Contrary to what was predicted, the UCS lowest value, 60 kPa, was recorded for specimen B3ct pre-mixed with bacteria. This can be due to the tube inside the specimen, which might had become unstable during axial load due to column buckling type behavior, breaking parts of the bonds that strengthen the soil. Specimen B2ct also had a

tube, but it was partially removed before the UCS test and therefore its interference in the UCS results was avoided.

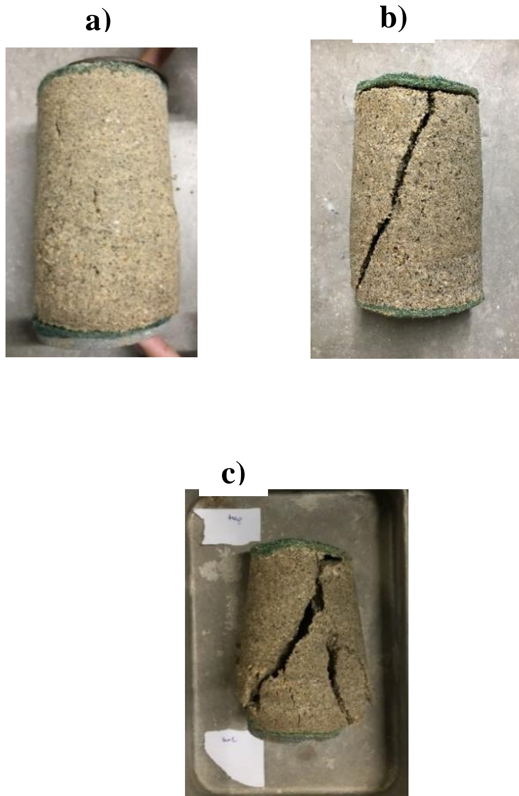


Fig 6 - Failure figures of injected specimen a) B2ct and pre-mixed b) without tube B3st and c) with tube B3ct

Concerning the shape of the stress-deformation curves, the axial strain at peak was different depending on the treatment. For the control samples this peak was observed between 4.5% and 5.7% axial deformation, while it occurred between 3.0% and 4% for the treated specimens tested (Table 3, Fig. 7). An abrupt drop after attaining peak was observed in specimen B3st, which confirms the fragile behavior of the treated soil (Fig 7).

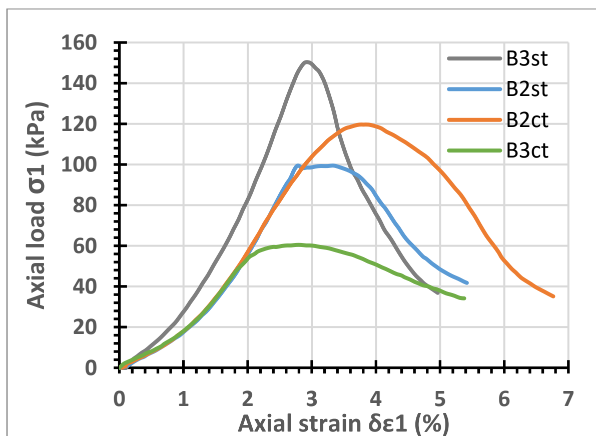


Fig 7 - Stress-strain curves obtained from the UCS tests in biocemented specimens

Stiffness, measured by E_{50}, increased after treatment from 0.35 MPa (C1 and C2) to a maximum of 6.9 MPa found in the pre-mixed specimen B3st. The lowest stiffness measured of 3.5 MPa was found for specimen B3ct, which can be explained by the interruption of the test because of the interference of the inner tube. Concerning the bacteria injected specimens, both B2ct and B2st had similar tangent Young modulus, around 5,0 MPa (Table 3).

It can be observed in Figure 7 that, for the pre-mixed specimen B3ct, the stress-strain curve shares the same path as those found for the specimens injected with bacteria (same evolution of stiffness). In addition, it was expected that the tube would not interfere, at the beginning of the test trial since it would not be loaded until some deformation occurred.

Table 3 - Summary of the results from UCS tests

	Specimen	q_u (kPa)	$\delta\varepsilon_{peak}$ (%)	E_{50} (MPa)
Control	C1	18	4,5	0,35
	C2	18	5,7	0,36
Injected	B2st	99	2,85	4,96
	B2ct	120	3,7	4,79
Pre-mixed	B3st	150	2,9	6,89
	B3ct	60	2,76	3,48

3.3 $CaCO_3$ content

Results from the HCl dissolution tests performed to quantify the amount of calcium carbonate precipitate showed that the $CaCO_3$ contents varied between 1.8% and 4.2% for the injected specimens (B2ct and B2st) (Fig 8).

Overall better results were found when the tube was used. Radial homogeneity measured through the difference in content between lateral and center positions was almost null. Longitudinally, the maximum difference was found between top and bottom sections, but inferior to 0.9% (Fig 8). On the other hand, specimen B2st showed larger differences in radial direction in the top and bottom sections (respectively 0.6% and 1.2%), as well as along axial length. The presence of the tube appeared to have contributed to homogenize the distribution of precipitate.

However, from the comparison of both experiments, it is visible a decrease in the amount of precipitate the further from the inlet zone (top section) we analyze the results. This is explained by the longer distance bacteria and feeding solution must travel to arrive at the bottom sections.

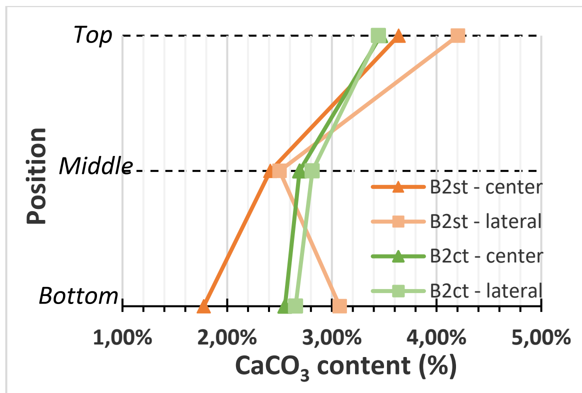


Fig 8 – $CaCO_3$ content distribution along radial and longitudinal directions in the injected specimens

For the pre-mixed specimens, $CaCO_3$ content varied between 3.0% and 4.5%. The tube seemed to not have a greater impact on the homogeneity when the results found for these specimens are compared to those found in the injected specimens. Indeed, for the radial distribution the difference was 1.0% (Fig 9) and for the longitudinal direction the difference between top and bottom sections are around 1,0%, similar to the values found in the specimen injected with tube.

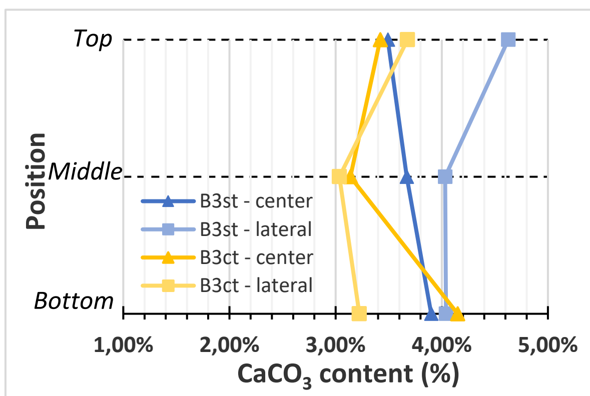


Fig 9 – $CaCO_3$ content distribution along radial and longitudinal directions in the pre-mixed specimens

Considering now the results from the enzyme treated specimens, the contrast in cohesion observed from the lateral and center positions during the removal from the chamber of the specimens was confirmed with dissolution tests. In both E3t and E4t specimens, the sections near the tube, at the center positions, had $CaCO_3$ contents ranging from 7.3% to 12.6%, as opposed to lateral sections that showed almost null values (0.5%). This apparent absence of treatment could explain the discrepancy of $CaCO_3$ content between sections that are closer and further away from the tube (inlet). It is uncertain, nonetheless, whether this occurred because injected enzyme might had not had the capability to travel to the lateral positions, added to the fact that the biocementation reactions occur very fast when enzyme is used.

Finally, control specimens C1 and C2, treated only with feeding solution, revealed $CaCO_3$ content between 1.3% and 1.4%. Although no bacteria was used to catalyze biocementation reactions, some calcium carbonate precipitate was found, probably from the natural reactions and undissolved carbonates. Yet, this precipitate did not affect the specimen's mechanical properties as it did not act as active bonds between sand particles.

3.4 Mercury intrusion porosimetry tests (MIP)

Mercury intrusion porosimetry tests were performed on samples extracted from the radial and longitudinal positions at specimens B2st and B2ct, as well as in enzyme treated specimens E3ct and E4ct. There is no data on the untreated samples because it was not possible to extract undisturbed samples in non-cohesive sand, and therefore the treated samples can only be analyzed by comparison between themselves and also from different locations in the same specimen.

A monomodal porous material was found for all cases studied. Pore clogging (or the presence of precipitate) can be detected if there is a reduction of the frequency of the large pores when comparing different specimens. Figure 10 presents the results found for specimen B2st, where the value of the dominating pore size (corresponding to the highest peak) of the center sample is displaced to the left when compared with the lateral sample. This means that pore clogging was higher at the centre. This is not in accordance with the results from acid dissolution tests, but the amounts of calcium carbonate found in both positions is not significantly different.

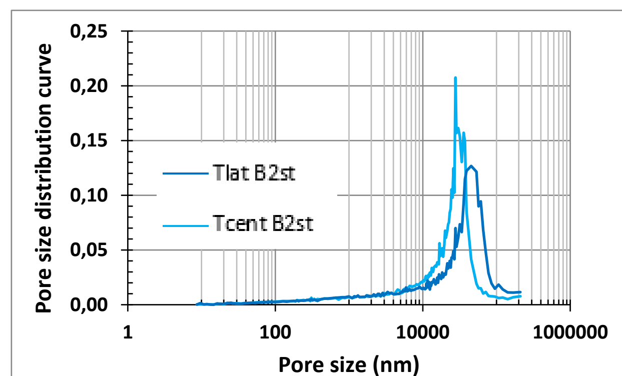


Fig 10 - Comparison of pore size distribution curves between radial positions of the top section

In the case of injected specimen with tube, B2ct, a better radial homogeneity was found because the differences in MIP tests were not significant, yet longitudinal clogging differed between the central and lateral axis, with the last one showing more heterogeneity.

Figure 11 presents the dominant pore diameter found in the different MIP tests performed. The most significant differences were found when comparing the pore size distribution curves of the bacteria and enzyme samples. The samples to which enzyme was injected showed peak sizes around the 20000 nm, while the minimum 29000 nm and maximum 55000 were found for specimens B2st and B2ct. This difference is also consistent with the results of acid dissolution tests.

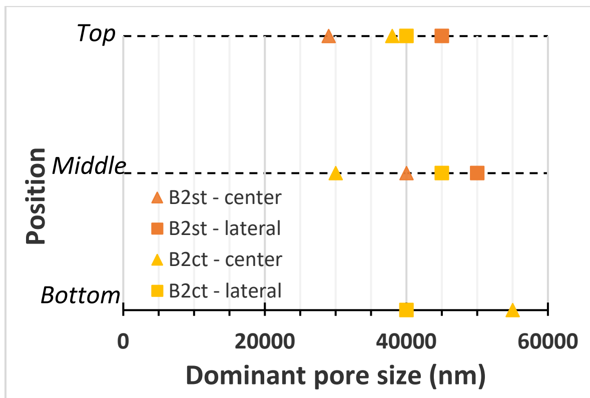


Fig 11 – Distribution of dominant pore size radial and longitudinally

3.5 Distribution of ammonium concentrations

The determination of the ammonium concentrations measured in the flushed volume allowed to characterize the distribution of urea hydrolyzation along the length of the bacteria treated specimens as seen in Figure 12. The results present a general increase in concentrations in the first 75 to 100 mL flushed. These volumes correspond to the fluid from the bottom section which indicates an increase in precipitate near the inlet (top section).

For the samples treated with bacteria, maximum values for the pre-mixed and the injected with tube specimens ranged between 800 and 900 mM. The smaller hydrolyzation values along length were found for specimen B2st, corresponding to 600 mM (Fig 12)

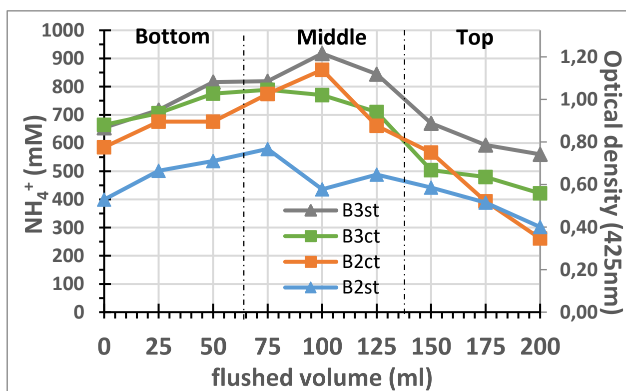


Fig 12 - Distribution of the ammonium concentration and optical density through flushed volume

The last 100 ml of flushed volume correspond to the top sections of the specimens. There is a decrease in concentrations for all cases. This decrease is larger for the injected specimens with tube (50% and 70% for B3ct and B2ct) when compared to the infiltration columns B3st and B2st (33% and 45%). This decrease can be related to the dilution occurring between the reacted feeding solution and the feeding solution being injected.

4 Discussion

Pre-mixing the bacteria appeared to improve homogeneity and overall cementation of the soil. This may be explained by a smoother distribution of the bacteria in the soil and thus a better volume distribution of potential sites for formation of calcium carbonate.

On the other hand, the injected specimen with no tube B2st revealed a high heterogeneity of $CaCO_3$ content, but also the lowest concentrations of NH_4^+ among all the other specimens. This lower indicators of biocementation match with the lower mechanical properties seen in the UCS tests. Yet, the other injected specimen B2ct displayed better indicators of homogeneity along longitudinal (difference between top and bottom sections only 0.9% as opposed to 2.4% of the B2st) and radial directions, where no difference between lateral and center position was noticed. Nessler results also presented higher $NH4^+$ concentrations, being the difference between B2st and B2ct around 400 mM at 100 mL of flushed volume (Fig 12). As a result, B2ct showed an improvement of 20% of the peak resistance when compared to B2st. Thus, the perforated tube in the injected specimens allowed for more homogeneous distribution and, consequently, to find better mechanical results when comparing these specimens with those treated as infiltration columns.

In the case of pre-mixed specimens, the infiltration column B3st showed better results than when feeding solution was injected with tube, B3ct. This could be seen in the overall cementation state (4.1% for B3st versus 3.4% for B3ct), but also in the ammonium concentrations of 150 mM after 100 mL of flushed volume.

This differences in advantages in using the injecting tube between injected and pre-mixed specimens is justified by additional feeding and bacteria distribution provided by the tube. Concerning the renovation of feeding solution, the tube allows the irrigation of the lower sections of the chamber. The reduction of ammonium concentrations in the final flushed volumes, seen in the specimens with tube (50% and 70%) when compared to those of the infiltration columns specimens (33% and 45%) prove the difficulty of the tube in renovating the feeding solution. One of the reasons may be in the change of the unidirectional flow lines to bidirectional with the introduction of the tube (Fig 13) as it can have a negative impact in the renovation of the cementation fluid. This phenomena may explain the better results seen in the infiltration column specimen B3st (cementation wise), compared to the tube one, since the tube was only used to distribute the cementation fluid contrary to the bacteria injected specimen (B2ct) where it helped to additionally homogenate the bacteria in the soil. Thus, the usage of tube in pre-mixed soils has no real advantage when compared to the injected method.

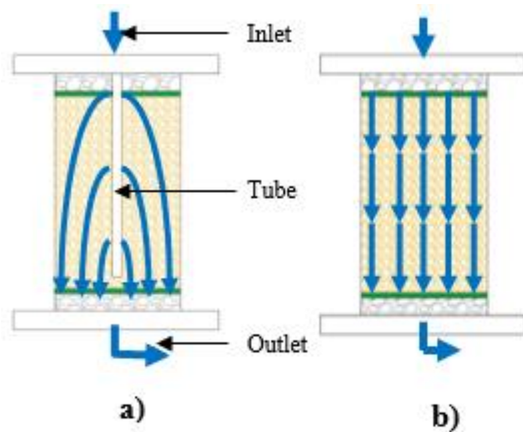


Fig 13 – Flow lines illustration of the specimen with tube a), and with infiltration column b)

Finally, failure modes obtained in the UCS tests show a transition from crushing failures in the bottom sections, to a more fragile failure in which shear surfaces are formed. The first was observed in the injected specimens, while the last was found for the specimens pre-mixed with bacteria. This transition was also related to strength increment (150 kPa for pre-mixed B3st comparing to 100 kPa and 120 kPa from B2st and B2ct) and also stiffness increment (6.9 MPa for B3st versus 5 MPa for injected ones). These differences can be explained by the difference in precipitation distribution achieved by each preparation method:

i) Crushing failure near the bottom may be explained by the lack of cementation compared to other sections in the injected specimens. This was observed in the dissolution tests (B2st showed a difference of almost 2.5% between top and bottom section in the center axis) and in the MIP tests, where pore sizes are small in the bottom sections (Fig 12). The results are consistent with those found when analyzing ammonium concentrations in the first flushed volume (Fig 11).

(ii) The transition into a shear failure is justified by the homogenization of the $CaCO_3$ content in the entire volume, and also by an increase of the overall cementation. In fact, a larger amount of $CaCO_3$ content was found for the pre-mixed specimens when compared with the injected specimens (3.4% for B3ct and 4.1% for B3st, and 3,0% for B2ct and B2st, respectively).

5 Conclusion

An experimental device, able to apply biocementation treatment in cylindrical soil samples, was developed. The inclusion of a perforated tube in the interior had a large impact on injected specimens because it helped homogenizing bacteria distribution in the soil. When the injection was done as infiltration column, therefore without this tube, the amount of precipitate decreased, in particular in the sections far from the inlet.

Pre-mixed specimens showed the best homogeneity and highest overall cementation indicators. In this case, the usage of tube hindered the renovation of the feeding solution inside the chamber. Nevertheless, the amount of precipitate formed was smaller than when feeding was done as infiltration column.

Finally, strength increments were observed in the all treated specimens, showing that treatment worked independently from the biocementation procedure adopted.

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