

Bio-cementation of sandy soils for improving their hydro-mechanical characteristics

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ABSTRACT: Nowadays, about one ton of carbon dioxide (CO₂) is emitted for every ton of cement produced. Carbon dioxide is the main gas responsible by greenhouse effect, and for this reason it became necessary to find sustainable industrial processes. In case of cement use in Geotechnical applications, it can be interesting to replace traditional techniques of soil stabilization mixing soils with cement, such as jet grouting. Microbial-induced calcite precipitation (MICP) is a green and sustainable technique that improves the geotechnical properties of soil through the precipitation of calcite at soil particles contacts and has the potential to be used as an alternative. This paper presents the main results found in the study on the MICP technique applied to sandy soils, performed in IST for the first time. The main purpose of the study was to define an adequate experimental setup to put into practice this new technique and to identify the best conditions to take a maximum production of calcite. Adding to that, the unconfined compression resistance obtained in tests of specimens treated through MICP treatment and that found for specimens prepared with cement were measured and compared in order to understand the potential use of this new technique. .

KEYWORDS: *Bacillus pasteurii*, Bio-cementation, calcite precipitation, soil improvement

1. INTRODUCTION

Microbial-induced calcite precipitation, MICP, has many applications in civil engineering and in particular in the Geotechnical area. It can be used for reinforcement of foundations and to prevent internal erosion in earth dams (piping) and the destruction of dikes in the occurrence of natural disasters such as floods and storms at sea (Van Paassen, 2011). It also can be a good solution to treat soils with a high potential for liquefaction.

There are also other applications such as the replacement of asphalt in roads (Wang, 2010), the ability to dig tunnels in sand and solidify the ocean floor to facilitate the work of extracting oil and natural gas (Latil et al., 2008).

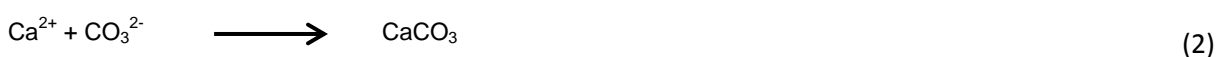
2. BIO-CIMENTATION

For MICP, the most used bacterial specie is *Sporosarcina pasteurii* or *Bacillus pasteurii* (*B. pasteurii*), which is well known for producing urease and hydrolysing urea to form calcite. The calcite (calcium carbonate, CaCO₃) is the bio-cement and is responsible for binding the soil particles which leads to an increase of the resistance.

More specifically, the hydrolysis of urea is a chemical reaction where the urease enzyme, supplied directly into soil or produced in situ by bacteria, decompose urea (CO(NH₂)₂) in the soil through this reaction:



The ammonium (NH₄⁺) released from urea hydrolysis results in local pH rise and commences the precipitation of calcium carbonate. The high pH favours bacterial activity. Calcite is precipitated through the combination of carbonate ions (CO₃²⁻) from the hydrolysis of urea and the calcium ion (Ca²⁺) supplied in as calcium chloride (Ng et al., 2012):



There are some precautions to take to achieve an efficient production of calcite, namely it is necessary to control the soil pH, the temperature, the nutrients supplied to the bacteria, the type of sandy soil used, etc.

All this information and the information about the process of growing the bacteria, the constitution of the liquid with nutrients to feed the bacteria and the application of MICP treatment in laboratory was withdrawn from some scientific papers about the same subject: *An overview of the factors affecting microbial-induced calcite precipitation and its potential application in soil improvement* (Ng. et al., 2012); *Factors affecting efficiency of microbially induced calcite precipitation* (Al Qabany et al., 2012); *Effect of chemical treatment used in MICP on engineering properties of cemented soils*. (Al Qabany et al. 2013); *Dynamic response of liquefiable sand improved by microbial-induced calcite precipitation* (Dejong et al., 2012).

In this work, the MICP treatment consist of mixing the soil with bacteria, then compact the soil a mould and feed the bacteria with nutrients so that they can produce calcite.

3. BIOLOGICAL GROWTH

The process of growth of *B. pasteurii* was performed at the laboratory of Bioengineering of IST. The bacteria were grown to an optimal density of 600nm (OD₆₀₀) on NH₄-YE liquid media at temperature of 37°C under aerobic condition. The liquid media was composed by 20g yeast extract, 10g of ammonium sulphate ((NH₄)₂SO₄) and 20g of agar in 0,13 M Tris buffer in pH 9,0. To perform this work are necessary higher quantities of cells (200ml), for this reason the process of growth of this microorganism takes about three days for a sequential increase in volume. Further details can be found in Pedreira (2014).

4. EXPERIMENTAL SETUP

The soil studied is sand collected between 0.5 and 1.0m depth. This non plastic soil is characterized by having a neutral pH (pH=7,0) and particle density G_s=2,64. For sample preparation it is assumed a dry volumetric weight of $\gamma_d = 17.89$ kN/m³ because is close to the values found *in situ* for this type of soil. Two different grading size distributions were studied to find if grading size has influence in for MICP. The first grading size distribution chosen, designed by G1 is characterized by having a particle size almost uniform, with diameters between 0.425mm and 0.075mm (# 200 <D <# 40). The second, referred as G2, is characterized by having particles larger than 4.75mm (D <# 4) and 12% of fines.

The specimens investigated were prepared with soil and culture medium with known quantity of bacteria, and with soil and culture medium without bacteria. The last are control samples, necessary to understand if bio-cement present in the soil results from bacterial activity, or only from chemical reaction between the reactive of the food fluid. Another type of samples were prepared, only soil prepared with soil and water, and soil-cement mixture, with soil, low cement content (150 kg/m³) and water to get a water/cement ratio of 2, similar to the values used for cement grouts. The comparison between the unconfined compression resistance found for all the types of specimens allowed to compare the effects of the MICP treatment when compared with soil specimens, and if MICP provides similar strength as the mixture with cement.

The specimens were compacted in cylindrical PVC moulds (3.24cm diameter and 9.72cm height). For the cases when *B. pasteurii* were used, and also for control, the moulds were inside a oven at constant temperature of 37°. For the cases of soil-cement mixture, curing was done by submersion, being the mould extracted at the end of the third day. The cure times studied were 3 and 28 days. The samples were kept at the laboratory of Geotechnics of IST. Figure 1 presents a schema of the moulds used, as well as the drainage system. The bottom of the moulds was bonded to a tile to form a collector chamber with 3 cm high for the purge fluids. A silicon drain tube with 6mm diameter was added in that chamber trough hole, connector to a container where volume was controlled. A metallic tweezers was used to regulate the output of the

purging fluid. A filter was included in the drainage system to allow the liquid with nutrient to flow in the sample, without losing soil.

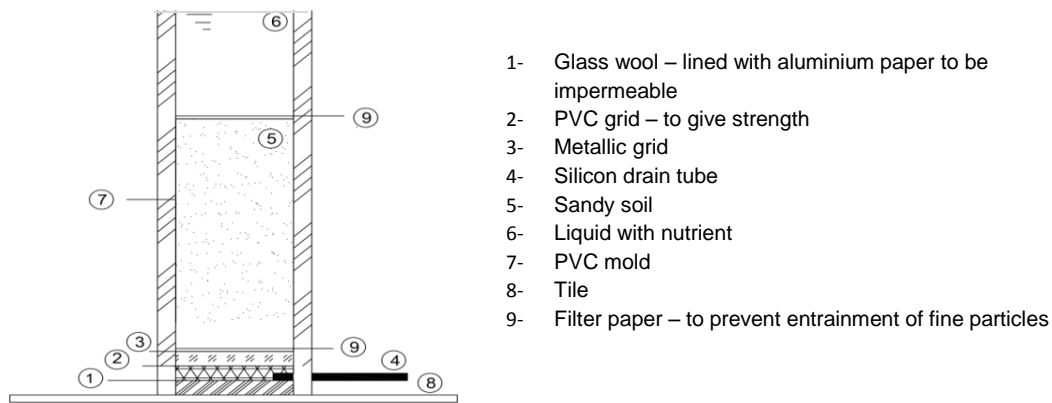


Figure 1 - Schematic vertical section of the specimens with illustration of the drainage system

The conception of the drainage system was improved during this study. As is shown in Figure 1, the drainage system is composed by four layers. Layer 1 is made with glass wool lined with aluminium paper to turn it impermeable. As the drain tube is approximately 3cm from the base, this first layer aims only to fill the void and provide support to the drain. Layer 2 is a PVC grid placed immediately above the first layer. The objective is to create an empty area and ensure that the drain tube is not blocked and the purging liquid can drain out. Layer 3 is a pervious metallic grid and was placed to ensure that there is no loss of soil. Finally, Layer 9 is a filter paper.

Each sample contained 95.87kg of soil mixed with 20ml of medium with bacteria. The bacteria were fed daily, by introducing 20ml of liquid with nutrient in the top of the mould (see Figure 1) and purging 20ml of liquid from the bottom. The same volume of soil was used for the soil and soil-cement specimens, but in this case there was no inclusion of the drainage system.

5. EXPERIMENTAL RESULTS AND DISCUSSION

This study was held in four sequential phases (Phases I to IV), conceived to understand and optimize the process of assembling and testing of the specimens. This process was necessary because this was the first time in IST that the MICP treatment was applied.

Unconfined compression tests were performed in the samples after being subjected to a MICP treatment in order to quantify the unconfined compression strength (UCS) obtained with this treatment. Before the compression test, all the samples were placed in an oven at 100° C with dry heat during 24h, except for Phase I.

Other tests were also carried out with the aim to check the biological activity and understand the UCS results obtained, such as: durability tests, scanning electron microscope (SEM), tests to quantify calcium carbonate (calcite) in the samples, tests for measuring the amount of ammonia in the samples, mercury intrusion porosimetry tests, etc. They will be described as follows.

PHASE I

This was the first test mounted with the MICP technique in IST, as far as the author know. The specimens broken at the end of the test because the samples were too wet and did not support the load cell (initially mounted in a triaxial chamber). However it was possible to learn about the biological processes (manipulation of cells and production of the bacteria food) involved, as well as to learn about samples preparation, demoulding and setup of the compression test.

The broken samples were observed in the SEM microscope in order to detect bacterial activity. The photographs in Figure 2 show the presence of biofilm, which indicates the presence of bacteria in the soil. The biofilm acts as a protective

hydrated barrier between bacterial cells and their environment (Pacheco, 2009). It facilitates the bacteria survival under difficult condition such as ultraviolet radiation, physical and chemical stress, desiccation and insufficient supply. Spherical crystals were also observed, typical of calcite produced by this type of bacteria (Al Qabany et. al., 2013), and for this reason it seems that the bacteria in the biofilm were the *B. pasteurii* responsible for producing bio-cement.

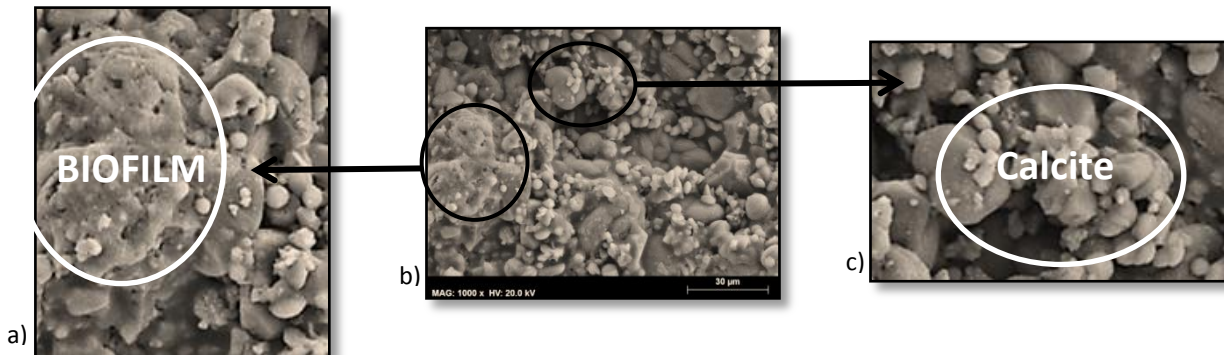


Figure 2 – Images obtained through the microscope SEM corresponding to a sample with particle size G1 with bacteria, a) detail of the biofilm formed by bacteria, b) general photography (1000x), c) detail of the spheres of calcite

PHASE II

The purpose of this step was to compare results on samples with different particle sizes (G1 and G2) and treated under two different temperatures: 20 ° C (in a laboratory) and 30 ° C (oven). The temperature at 20 ° C is more similar to the temperatures in situ and is why this temperature was also analysed.

As in the Phase I, the dismantling system was not good enough and some of the samples were broken. The specimens were placed in an oven at 100° C for few hours to lose humidity and the rest of liquid food that was still inside. It was much easier to remove the samples from the moulds, but suction effect in strength had to be investigated and for this reason the soil specimens were studied after being subjected to the same thermal treatment.

The samples resulting from this process did not have the same sizes and were not exactly vertical, and for this reason a large dispersion of results was found. From visual inspection it was possible to see the presence of calcite in the top of the samples because there was the formation of a white and stiff zone. A larger treated volume was observed for the specimens kept under 30° C and for this reason it was decided for the next phases to only submit the samples to a temperature of 30° C.

A durability test was performed to check if the bio-cement formed in the process was stable after some time of submersion. This is a simple test that consists in put the samples (after the treatment) in water during more or less one month (Figure 3). The samples submerged were taken from the top of the cylinders and had initial sizes between 4 and 5 cm. The base of the sample was crumbling over submersion time and at only the top part with approximately 1.5 cm height remained intact at the end of this period (Figure 4). The hand crushing strength of this part of the sample was maintained high and much higher to that found for the bottom part and for the soil without treatment both for saturated and air dried conditions (Figure 4). What is left of the sample is very durable and has remained unchanged since then.

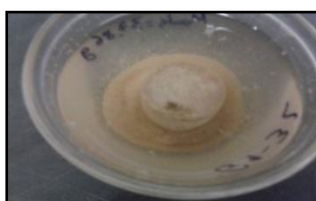


Figure 3 - Submersion test ongoing



Figure 4 - Top of the specimen one month after the test a) section b) plant

The result of this test confirms the results of the previous phase, namely that there is a gradient of bio-cement along the specimen and for this reason there is a significant difference in strength. The top is much more resistant than the bottom. This indicates that this type of treatment may be more efficient in surface areas and must be improved to be more uniform in depth. This can be achieved by improving the injection system and maybe increasing the time of treatment.

PHASE III

Phase III had four steps in which the sample were fed during increasing periods of time: 10days, 20days, 30days and 40days. The objective was to find out the influence of the time of treatment in the efficiency of the bacteria in the production of calcite.

There was no problem with the demoulding system but the percolation of the food fluid and purge was not verified at the end of few days of test. The bacteria were not properly fed during the test because of this drainage problem and the specimens had shown similar look (bio-cement concentrated in the top, along the first 2-3 cm) independently from the feeding time. As the MICP treatment has not elapsed as provided, the results of this phase are perhaps unreliable. Indeed, it was verified that increasing the duration of the treatment has no significant influence on the production of calcite. Therefore, the next phase was held in 10days.

SEM photographs were also taken to samples of these specimens, after being dried in a oven at 100°C for more than 24h. Neither biofilm or bacteria were expected to be observed due to the high temperature applied, being the purpose of this analysis to check the formation of calcite crystals.

As shown in Figure 5, the presence of small holes in the particles of soil was observed. These small holes are called imprints and indicate the presence of bacteria corroding the zone they contact with the solid surface (Figure 5). This proves that had really bacteria in the soil.

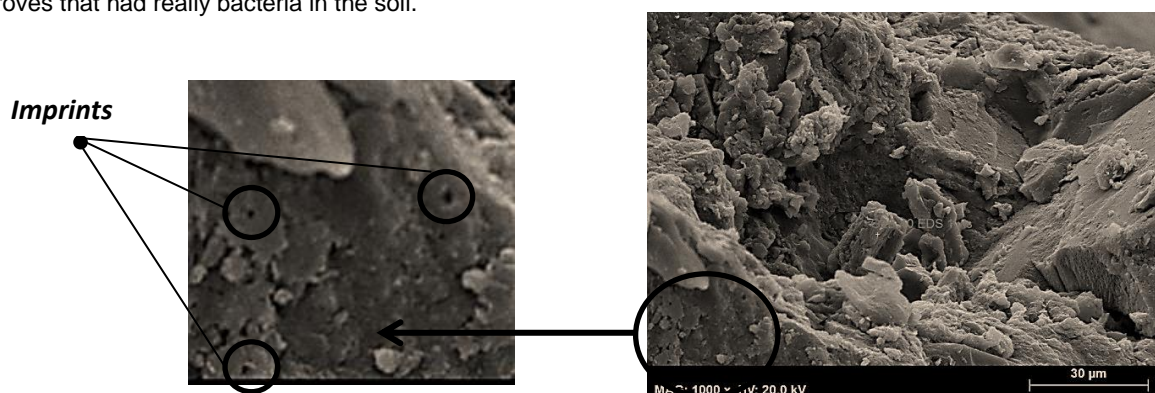


Figure 5 - Identification of imprints in the soil that indicate the presence of bacteria, sample: G2 – 40 days (Phase III)

It was also observed some different forms of calcite, as is shown in the Figure 6. See Pedreira (2014) for further details.

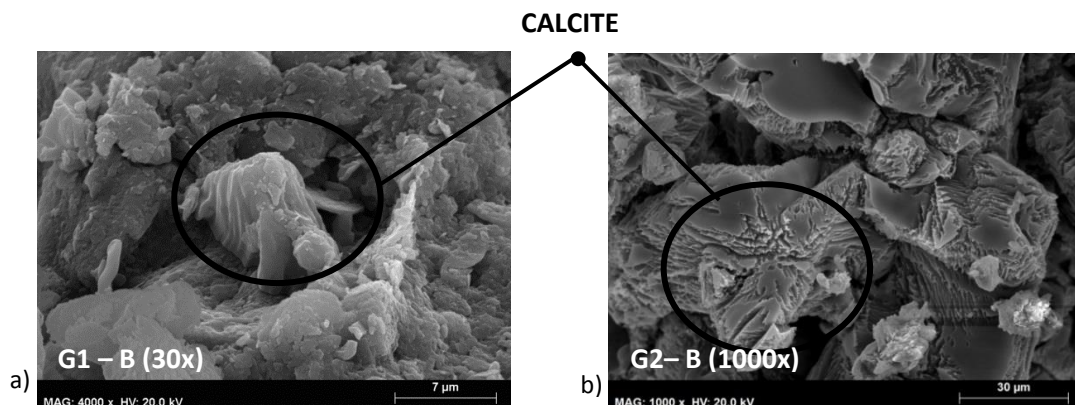


Figure 6 - Different shapes of calcite identified in the samples of Phase III – SEM microscope

PHASE IV

The results obtained in the earlier phases showed that the resistance of control samples was larger than that of the samples with bacteria for several cases, and much higher than the values found for the soil samples prepared only with water. It is thought that the presence of *B. pasteurii* in the soil would increase calcite production. There was the possibility of other bacteria would be present in the soil because it was not sterilized before the tests, and eventually they would be fed during the test in the control samples. As alternative explanation, the resistance of these control samples may also be due to chemical reaction of the reactants introduced in the soil each day, which can also form calcite and consequently increase the resistance of the soil. However the calcite formation rate due to biological activity is much higher than when only chemical reactions take place.

To investigate the first hypothesis, the soil in the samples was sterilized in an oven at 100°C with dry heat during two days in order to eliminate all microorganisms existing naturally in the soil.



Figure 7 - Samples prepared for the MICP treatment (Phase IV)

The presence of white bio-cement was observed in all the samples, but it was more concentrated in the top in the other phases and now it seemed more homogeneous (Figure 8). However during the unconfined compression test the upper zone shown to be much more resistant than the bottom. It was also observed a different colour between the control and the samples with bacteria. Figure 9,10 and 11 shows the results of the compression test performed in selected samples.

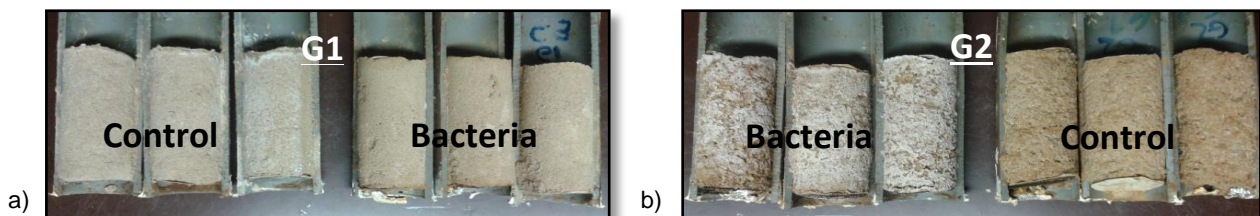


Figure 8 - Samples after going to the oven at 100°C a) particle size G1 b) particle size G2

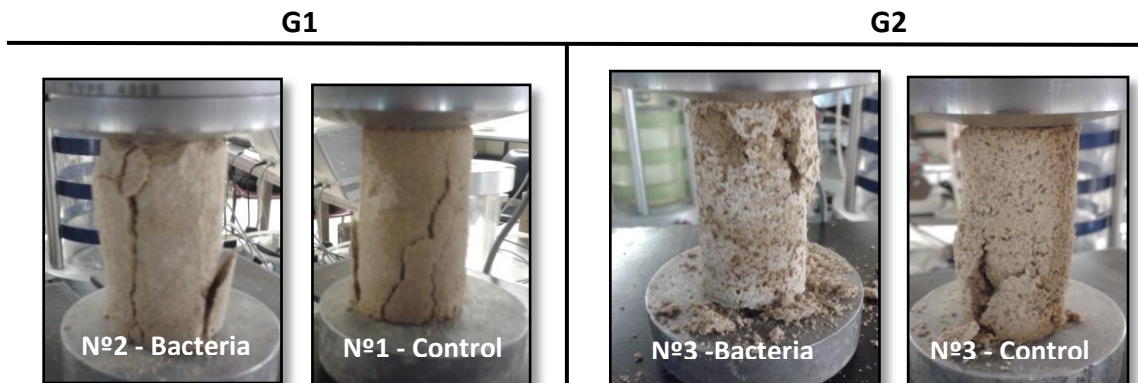


Figure 9 Ruptures observed in the compression test for G1 and G2 (Phase IV)

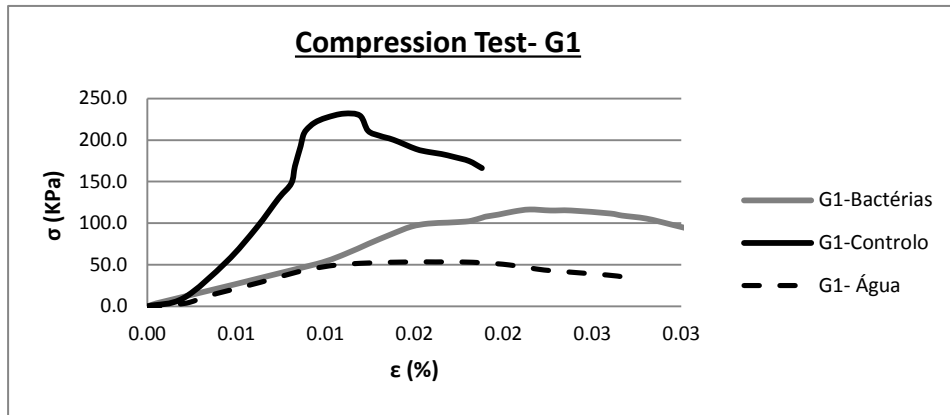


Figure 10 Compression test results, particle size G1 (Phase IV)

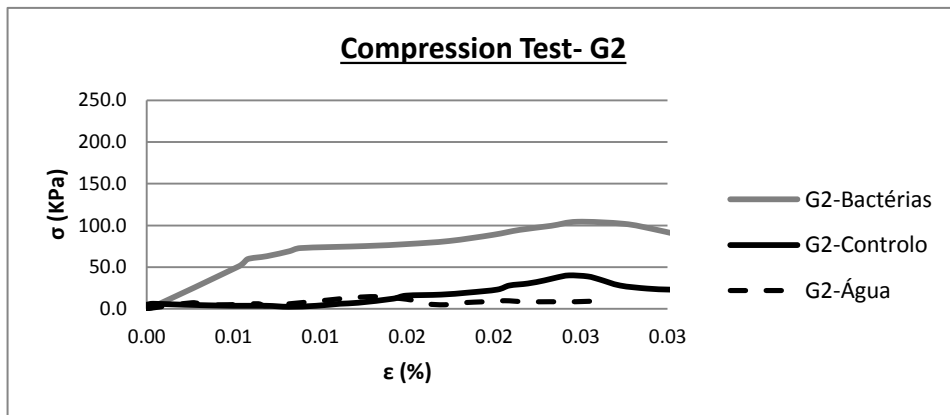


Figure 11 - Compression test results, particle size G2 (Fase IV)

The comparison of the UCS found for the different treatments is presented in Table 1.(B/C: MICP treatment versus control; B/S: MICP treatment versus untreated samples (soil+water); C/S: control versus untreated samples).

Table 1 - Quantification and Comparative analysis of strength gain of the different treatments

		G1			G2		
Sample		1	2	3	1	2	3
UCS (KPa)	Bacteria (B)	58.22	167.37	115.22	24.26	84.90	101.88
	Control (C)	231.66	255.91	172.23	50.94	48.51	40.02
	Soil + Water (S)	53.37	53.37	53.37	15.77	15.77	15.77
Treatment improvement	B/C	0.25	0.65	0.67	-	1.75	2.55
	B/S	1.09	3.14	2.16	1.54	5.38	6.46
	C/S	4.34	4.80	3.23	3.23	3.08	2.54

Data presented in Table 1 for the grading size distribution G1 shows that the resistance of the samples treated with bacteria is lower than the resistance of the control samples (maximum ratio B/C is 0.67). The results obtained were very satisfactory for grading size distribution G2 because the resistance of the samples treated with bacteria is nearly twice the resistance of the controls (min. B/C= 1.75). The results obtained for samples with grading size distribution G1 are higher than the results obtained for the grading size distribution G2. (Maximum values: G1-bacteria =167.37 KPa, G1-control= 255.91KPa; G2-bacteria =101.88 KPa, G2-control= 50.94KPa). However, for G1 and G2 the resistance of the samples

treated with bacteria and the controls is much higher than the untreated samples (B/S: min. value=1,09 and max. value=6,46 ; C/S: min. value=2.54 ; max. value=4,80).

There is a large difference between the maximum and minimum UCS values found for the samples treated with bacteria, but similar values were found for the control samples. This suggests that bacteria are responsible for the discrepancies observed in the results. It may be admitted that when conditions are favourable for the survival of the bacteria in soil they produce calcite naturally, but when something affect them, for any reason, their activity drops. Factors such as pH, temperature, soil particle's size, presence of other bacteria, etc, may affect their activity. This shows that *B. pasteurii* are very sensitive, even if usually they can survive in a relatively hostile environment.

To quantify the presence of calcium carbonate (CaCO₃) in the specimens at the end of the treatment, it was sent 20g of each specimen to the Laboratory of Water Analysis of IST. A leaching test (proportion: 1:10; medium: 5% hydrochloric acid; during 24h with shaking) was carried out to quantify the presence of this mineral. The analysis was performed in similar volumes taken from the top and bottom part of the same specimen, being analysed the two grading size distributions studied and a specimen treated with bacteria and a control. The results are shown in Table 2.

Table 2 - Results of the calcite analysis (Phase IV)

		Bacteria			Control
		Top	Base	Average	Top
G1	g/kg	35,0	8,4	21,7	11,0
	kg/m ³	62,7	15,0	38,9	19,7
G2	g/kg	46,0	24,0	35,0	13,0
	kg/m ³	82,3	43,0	62,65	23,3

The results confirm that, for both grading size distributions, there is a higher production of calcite on the top of the specimens. This result confirms that the bio-cement concentrates in top of the samples. There is a large difference between the quantities of calcite along the height of the specimens treated with bacteria. The heterogeneity of the treated specimens is therefore a problem to be solved because it affects UCS in a very significant manner.

One of the reasons for the concentration of calcite in the top of the specimens may be the decrease in permeability caused by calcite formation entrapping the voids of the soil, and therefore the fluid can no longer circulate and feed the bacteria in the bottom zones. Mercury intrusion porosimetry tests were performed to check changes in pore size distribution caused by the treatment. Control specimens were also analysed. Only grading size distribution G1 was studied because it was almost uniform, and for this reason it was easier to detect differences. Further details can be found in Pedreira (2014). The results are shown in Figure 12. The plot for the natural soil was estimated considering its grading size distribution.

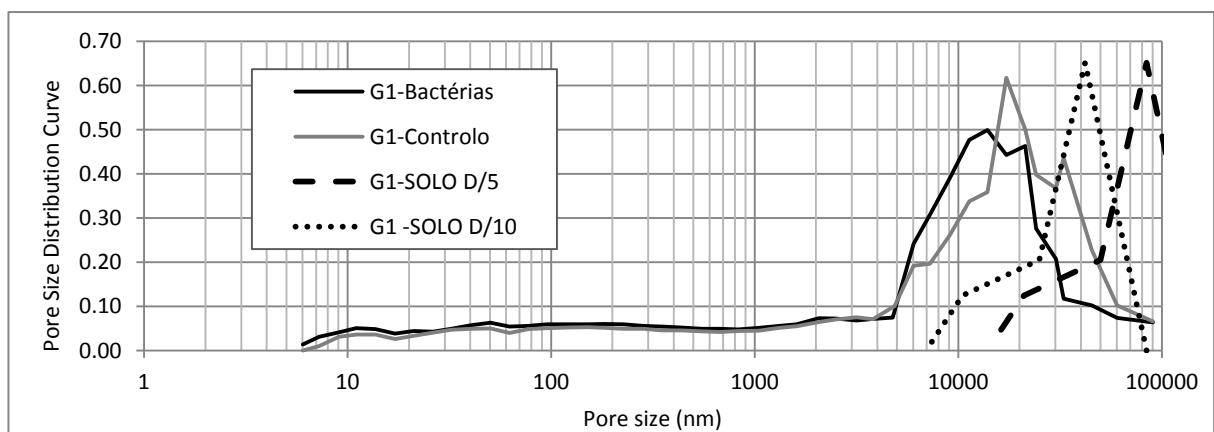


Figure 12 - Results of the Mercury intrusion porosimetry test (Phase IV)

It can be seen that the highest percentage of pores present in the specimen has a diameter of 14.5µm for samples with bacteria. For the control samples, the majority of the pores have a diameter of 19µm, and for the untreated samples (soil+water) the diameter is between 37µm and 73µm. This result shows that there is a real reduction of the pore size in the treated samples and this reduction is higher for the samples with bacteria. This decrease can be explained by the fact that there is a real production of calcite which fills the soil voids over time.

6. COMPARATIVE ANALYSIS: BIO-CEMENTATION VERSUS CEMENT

Finally, the UCS of the specimens treated with MICP was compared with that of the soil-cement mixture considering only three days of curing. The comparison is presented in Table 3, where H refers to the specimens treated with cement.

Table 3 - Comparison between MICP (Fase IV) and cement treatment

		G1	G2
UCS (KPa)	Cement with 3days of curing (H)	606,43	1055,18
	Bacteria (B)	113,60	70,34
	Control (C)	219,93	46,49
	Soil + water (S)	53,37	15,77
Treatment improvement	H/B	5,34	15,00
	H/C	2,76	22,70
	H/S	11,36	66,91

Although the negative impacts for the Enviroment of soil treatment with cement, based in these results it can be concluded that the cement treatment is more efficient than MICP treatment because a much higher UCS was achieved (MICP: max. value=113,60KPa, Cement: min. value=606,43KPa). However the results found for the MICP are not reliable because of the heterogeneity of the treatment, and for this reason new tests must be performed.

7. CONCLUSIONS

MICP is a sustainable and environmentally friendly technique that must be improved both at laboratory and field scales. This technique must be optimized to find the best conditions (pH, soil, temperature, nutrient concentration etc) for bacterial activity, and also to get homogeneous distribution in the soil. It is believed that the conditions for bacterial activity were achieved, and therefore future research must be focused on finding efficient injection systems both for bacteria and nutrients. It is necessary to understand why the control samples also gain strength over time. Only after solving these problems the UCS from this treatment can be compared with that for soil-cement mixtures, however it is expected that UCS of cement may be larger.

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