Proceedings of the 7th World Congress on Civil, Structural, and Environmental Engineering (CSEE'22)
~~Lisbon, Portugal~~ Virtual Conference – April 10 – 12, 2022
Paper No. ICGRE 240
DOI: 10.11159/icgre22.240

Relationship between Enzyme Concentration and Carbonate Precipitation in a Sand Treated By Biocementation Using Enzyme

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Abstract **–** Biocementation is a relatively recent soil improvement technique which consists in using biological agents to promote the precipitation of calcium carbonate (biocement) that works as a cement binding the grains. Some of the main difficulties when using this technique are how to predict the amount of biocement precipitated by a fixed dosage of enzyme, and how to quantify the soil improvement for that amount. In this paper biocementation was applied to a uniform-graded fine sand using enzyme, to promote enzymatically induced carbonate precipitation (EICP). Three different enzyme concentrations (20, 40 and 60 mg/mL) were used on each sample and the amount of calcium carbonate precipitated, load penetration strength (measured using a pocket penetrometer) and electrical resistivity were measured. It was found out that carbonate production is proportional to the enzyme concentration, as precipitated calcium carbonate content increases from 4.8 to 6.6% when the enzyme concentration increased from 20 to 60 mg/mL. Good relationships were found between the amount of calcium carbonate and electrical resistivity, and also between calcium carbonate and penetration strength, showing that it is possible to quantify soil improvement as function of the dosage of enzyme used in the treatment as long as the amount of precipitated calcium carbonate is known.

Keywords**:** EICP, urease enzyme, biocementation, electrical resistivity, pocket penetrometer.

1. Introduction

Biocementation is an eco-friendly solution for soil improvement [1]. When enzyme is used, it is called enzymatically induced carbonate precipitation (EICP). This treatment can become a consistent alternative to conventional techniques, having the potential to be used for a wide range of applications [2]. As a consequence, in recent years, more and more examples of the application of this technique can be found, e.g. for cementing desert sands for sandstorm control [3], to prevent coal dust pollution in open-pit coal mines [4], or to create biocemented soil columns for ground improvement purposes, therefore increasing the load bearing capacity of the soil [5].

Basically, urease enzyme added to the soil catalyse the hydrolysis of the urea ($CO(NH_2)_2$) Eq. (1) present in a feeding solution also supplied during its treatment. Calcium carbonate ($CaCO_3$) precipitates in the presence of calcium ions, also supplied in the feeding solution, Eq. (2).

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

$$C_a^{2+} + CO_3^{2-} \rightarrow C_aCO_3 \quad (2)$$

The properties of the soil (composition, porosity, degree of saturation, etc.) must be analysed before the treatment in order to optimise the quantities used, both in terms of volume and enzyme concentration. As a result, each location requires a specific recipe. Particularly relevant is the fluid movement paths through the soil, which is directly dependent on its pore space distribution (tortuosity) and pore size [6]. Understanding this relationship is necessary to ensure a uniform treatment along the interest area and guarantee the durability of the treatment [7].

In this paper, EICP treatment was applied to three cylindrical sand samples using different enzyme concentrations. The production of carbonate in each sample was analysed and related with the concentration of enzyme. The presence of the precipitated carbonate in each case was also related with electrical resistivity and penetration strength, both properties depending on the degree of cementation of the treated soil. In the first case, the presence of the biocement bonding the grains affects porosity, therefore reduces the amount of interstitial water and interferes with the path of electrons in the liquid phase.

In the second case, these bonds are responsible for increasing soil load bearing capacity, in this case measured by penetration strength.

2. Materials and Methods

Three cylindrical samples were prepared with the same void ratio, using dry uniform graded sand (D_{50}=0.3mm) carefully placed to ensure homogeneity. The sand, mainly silicate minerals, was dried in the oven before being poured into PVC moulds with a diameter of 7 cm and 4 cm height. Final void ratio was 0.78, which corresponds to a void volume of 67.5 cm^3. The samples were identified as A, B and C.

Powdered enzyme from the Canavalia ensiformis (Jack bean) plant were dissolved in distilled water and a volume equal to the sand void volume was injected into each sample. Different enzyme concentrations were adopted: 20 mg/mL for sample A, 40 mg/mL for sample B, and 60 mg/mL for sample C.

The feeding solution was injected to all samples after the injection of the enzyme solution. The composition of the feeding solution was 0.5M of urea ($CO(NH_2)_2$), 0.5M of calcium chloride ($CaCl_2$,) 10 g/L of ammonium chloride (NH_4Cl), 2.12 g/L of sodium bicarbonate ($NaHCO_3$), 2 g/L of yeast extract and 1g/L of ammonium sulfate ($(NH_4)_2SO_4$). The amount of feeding solution necessary to react with enzyme was investigated in a previous study [8], being determined that one void volume was enough to react with the 20 mg/mL enzyme solution. Therefore, one void volume of feeding solution was applied in sample A, while two and three void volumes were added to samples B and C, respectively. In summary, the treatment steps were: (i) injection of one volume of enzyme solution with a known concentration in all samples (ii) injection of one volume of feeding solution in all samples, (iii) injection of one volume of feeding solution in samples B and C (iv) injection of one volume of feeding solution in sample C. All tests were performed at laboratory environment, where temperature was kept constant and equal to 22ºC.

The experimental setup is presented in Figure 1, where can be seen the PVC container with the sand, and also a plastic grid at the bottom to allow the drainage of the fluids. In this way, when the sample is saturated, the further injection of the enzyme solution allows to distribute the enzymes homogeneously throughout the sample. The injection of the feeding solution in the further steps allows replacing the previously existing fluid. For this reason, the injection of the feeding solution was performed 24 hours after the injection of the enzymes, thus allowing time for the enzymes to become attached to the sand particles before the effluent is washed out.

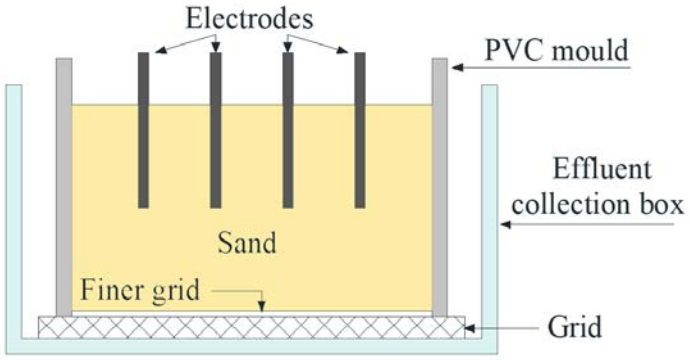

Fig. 1: Samples configuration.

The electrical resistivity was measured 24 hours after the end of the treatment in each sample, using the Wenner method and applying a constant voltage of 35 V. As presented in Figure 1, 4 steel pins were used as electrodes, spaced 1.4 cm, and nailed to a depth of 2 cm.

In addition, load tests were carried out with a pocket penetrometer (CONTROLS Geopocket dial penetrometer 16-T0161). The manufacturer recommends using a plunger diameter of 20 or 25 mm for sands and 10 or 15 when using in

cohesive soils. In this study, a plunger diameter of 10 mm was used as it is considered that, once the treatment is finished, the samples have cohesion due to the action of the biocement binding the particles of sand.

Finally, the amount of calcium carbonate in each sample was determined using the washing method. It consists of mixing mixing the samples with hydrochloric acid (HCl) to dissolve the carbonate. Then, the samples are washed with distilled water water to remove all soluble calcium from the sand particles and, finally, they were dried in an oven. Calcium carbonate content (CCC) was calculated using Eq. (3), where $m_{w/CaCO3}$ and $m_{wo/CaCO3}$ are the dry mass after and before the test, respectively.

$$CCC = \frac{m_{w/CaCO_3} - m_{wo/CaCO_3}}{m_{w/CaCO_3}} \quad (3)$$

3. Results

The relationship between the concentration of enzyme used during sample treatment and the calcium carbonate content (CCC) found is presented in Figure 2. The adjustment achieved is not linear, indicating that there must be an optimum concentration beyond which it is not worth continuing to increase the amount of enzyme. Indeed, when the enzyme concentration is increased from 20 to 40 mg/mL, the increase in carbonate is 1.2%. However, when the same increase in enzyme concentration is made from 40 to 60 mg/ml, the increment in carbonate content is almost half that of the previous case (0.7%).

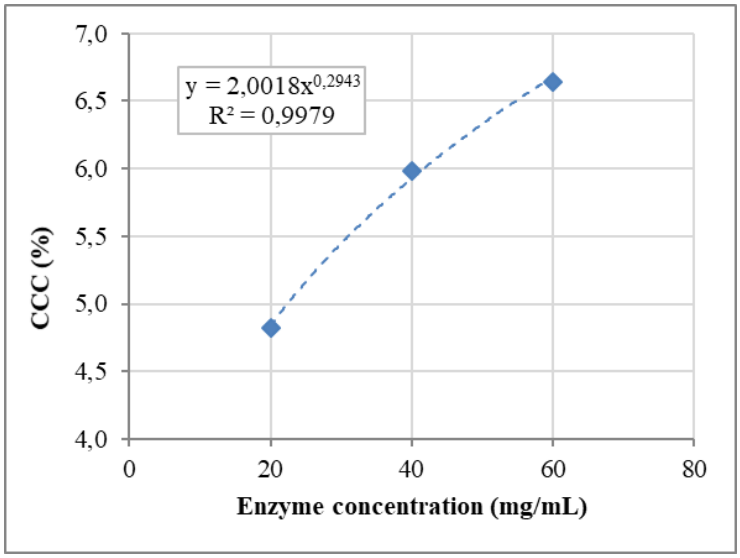

Fig. 2: Relationship between enzyme concentration and calcium carbonate content.

The penetration strength (Qrd) determined using a pocket penetrometer can be related to CCC. As expected, the value of this parameter increases with the increment of carbonate content in the sample (see Figure 3a). This is because carbonate acts as cement binding grains and improving soil strength. However, although a good exponential correlation was obtained, it is not realistic because both CCC and Qrd cannot increase indefinitely.

The same applies to the electrical resistivity measurements (see Figure 3b). In this case, the increase in resistivity values must be attributed to the reduction in porosity as consequence of carbonate precipitation. Since the electrical current is mostly conducted in the liquid phase through the interconnected porosity, a reduction of the porosity hinders the electrical conductivity and thus increases the resistivity.

The results suggest that both techniques (electrical resistivity measurements and pocket penetrometer load tests) allow the detection of changes in the physical properties of the soil with respect to its initial state as a consequence of the treatment performed. Consequently, they have the potential to be used as indirect methods to estimate the amount of biocement precipitated in a soil after its treatment. In addition, by knowing that calcium carbonate is associated to a given amount of enzyme, it is possible to quantify soil improvement as function of the dosage of enzyme used in the treatment. However, further testing is needed to establish reliable correlations between all these parameters.

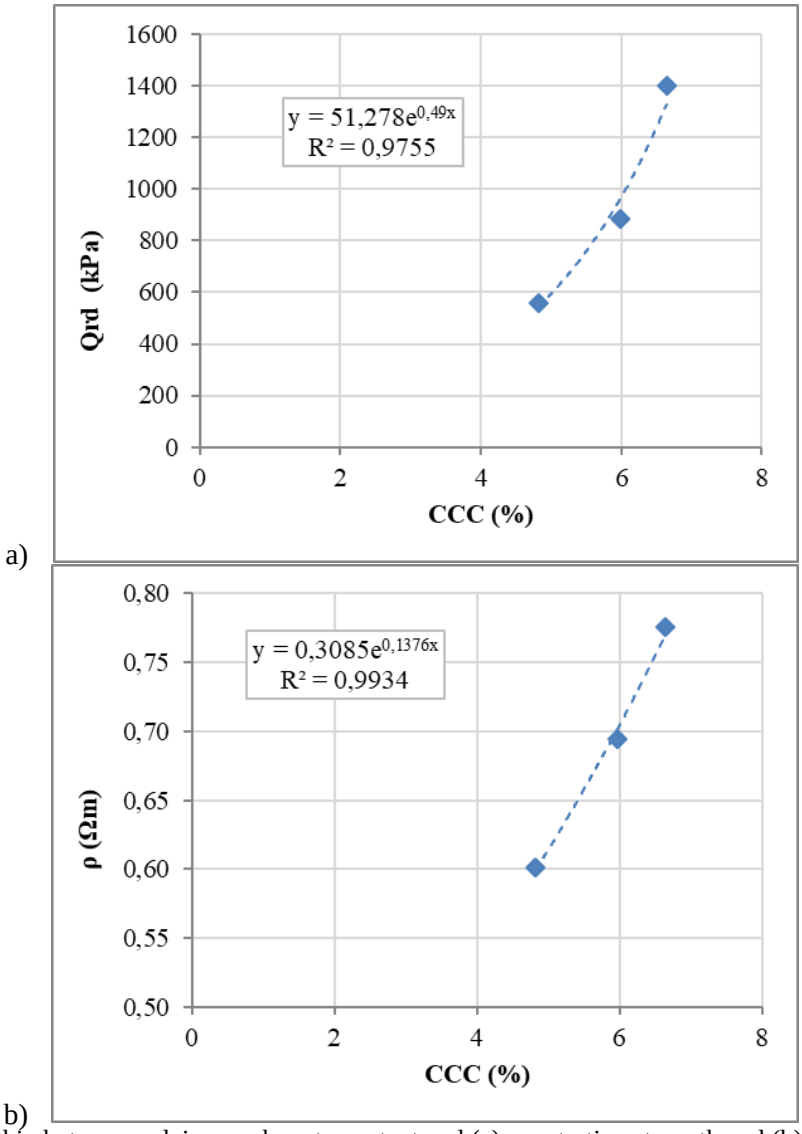

Fig. 3: Relationship between calcium carbonate content and (a) penetration strength and (b) electrical resistivity.

4. Conclusions

It was found out for the full saturated sand samples that the amount of precipitated calcium carbonate is directly dependent on the enzyme concentration added. For the concentrations tested, the percentage of precipitated calcium carbonate increases from 4.8 to 6.6% when the enzyme concentration is increased from 20 to 60 mg/mL.

The increase in calcium carbonate content translates into an improvement in the strength properties of the soil and an increase in its electrical resistivity, so these parameters could be used as an index measure to determine the effectiveness of a given treatment with regards to the amount of carbonate precipitated. By knowing the conditions of the treatment, being calcium carbonate dependent on the dosage of enzyme used, it will be possible to estimate the expected improvement as function of the amount of enzyme added to the soil. This is not an easy task, because the biological activity is affected by environmental conditions, minerals present in the soil and treatment sequence. In addition, soil's void ratio influences the precipitation of calcite and the distribution of the bonds, and therefore final porosity and strength. For this reason, further testing must be performed to establish reliable correlations between all these parameters, after fixing the most important variables affecting the treatment.

Acknowledgements

The authors would like to thank the Foundation for Science and Technology (FCT, I.P.) of Portugal for funding project BIOSOIL (Ref PTDC/ECI-EGC/32590/2017).

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