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Effect of the soil type on the biocementation process by enzymatic way

Paulo J. Venda Oliveira[1,*], and *Luís* D. Freitas[1]

[1] Department of Civil Engineering, University of Coimbra, R. Luís Reis Santos, 3030-788 Coimbra, Portugal

Abstract. The effect of the enzymatic $CaCO_3$ precipitation on the behaviour of four soils (from a poorly graded sand to a fine and organic soil) is studied in this work. The analysis is based on the results of UCS tests, where the results from the non-stabilised specimens are compared with specimens stabilised with a urease concentration of 8 kU/L and an equimolar solution of urea-$CaCl_2$ of 0.5 mol/L. Additionally, pH and scanning electron microscopy (SEM) tests with energy dispersive X-ray (EDX) analyses are performed to analyse the microstructure and the local chemical composition. The results of the UCS tests show that, in the case of the sandy and silty soils, the process of enzymatic $CaCO_3$ precipitation potentiates the strengthening of the soils while, in the organic soil, a detrimental effect is observed. The SEM tests show the existence of vestiges of calcium in the biostabilised soils studied.

1 Introduction

Nowadays, biocementation has emerged as an alternative method to improve the behaviour of natural soils [1-3]. In general, this methodology uses bacteria to bio-catalyse the urea hydrolysis via the urease enzyme inducing the precipitation of calcium carbonate ($CaCO_3$) (MICP) [3-6].

As the bacteria's cultivation and storage requires special environmental conditions (temperature, pH, etc.), some alternative methods to promote biocementation in a porous medium have been studied, one of which is enzymatic $CaCO_3$ precipitation, which is performed by mixing the soil, urea, calcium chloride ($CaCl_2$) and the urease enzyme [7-12].

The few works about the use of enzymatic $CaCO_3$ precipitation in soils show that this process improves the strength, the stiffness [8-9, 11] and decreases the permeability and the porosity of the porous media [7, 8, 10]. Although with high scattering, the results also show that the level of improvement increases with the amount of $CaCO_3$ precipitated [8, 11, 13].

Considering the lack of research concerning this methodology, it is very pertinent to study the effect of enzymatic $CaCO_3$ precipitation on the process of the strengthening of biostabilised soils by examining four types of soils, from a poorly graded sand to a fine and organic soil. The analysis is mainly based on the results of UCS tests, where the results of the non-stabilised specimens are compared with specimens biostabilised with the use of enzymes. Additionally, pH and scanning electron microscopy (SEM) tests with energy dispersive X-ray (EDX) analyses are performed to study the microstructure and the local chemical composition.

2 Precipitation of $CaCO_3$

The precipitation of $CaCO_3$ using the enzyme urease to promote urea hydrolysis is described by the equation (1) [6, 14]:

$$\underset{\text{Urea}}{CO(NH_2)_2}\,(s) + H_2O\,(l) \xrightarrow{\text{Urease}} 2NH_4^+(aq) + CO_3^{2-}(aq) \quad (1)$$

At a pH of 7.0 and 38ºC, the urease promotes the hydrolysis of the urea 1,014 times faster than spontaneous hydrolysis [15]. Thus, in an environment with a high pH value and rich in calcium ions (Ca^{2+}) the carbonate ion (CO_3^{2-}) reacts spontaneously with Ca^{2+} producing calcium carbonate ($CaCO_3$), as described in equation (2):

$$Ca^{2+}(aq) + CO_3^{2-}(aq) \leftrightarrow CaCO_3(s) \quad (2)$$

3 Materials

The main characteristics of the soils studied (A, B, C and D) are shown in Table 1. Soil A is a poorly graded sand with silt (SP-SM), soil B is a silty sand (SM), soil C is a silt with sand (ML) and soil D is an organic silt with sand (OL). Soil D is plastic (w_L = 48.5%; w_P = 38.4%) and presents a high organic matter content (11.0%). Soils A and B show a pH value of about 8.4, while the pH of soil C is slightly lower (7.8) and soil D presents a much lower pH (4.3). The standard Proctor test [16] was used to evaluate the maximum dry unit weight (γ_{dmax}) and the optimum water content (w_{opt}), which are used to prepare the specimens tested.

* Corresponding author: pjvo@dec.uc.pt

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The grout to promote the biocementation is composed by the urease enzyme, urea (CO(NH2)2) and calcium chloride ($CaCl_2$), with purity levels of 99.5% and 95%, respectively. The urease enzyme is obtained from *Canavalia ensiformis* (jack beans) and has an activity of 34,310 U/g (1U corresponds to the amount of enzyme which hydrolyses 1 μ_{mol} of urea per minute at pH 7.0 and 25ºC).

Table 1. Main characteristics of the soils (based on [12]).

Property	Soil A	Soil B	Soil C	Soil D
Grain size distribution:				
Clay (%)	2.7	2.0	4.7	21.5
Silt (%)	4.2	14.8	72.9	57.9
Sand (%)	93.1	83.2	22.4	20.6
Liquid limit, w_L (%)	--	NP(**)	NP(**)	48.5
Plastic limit, w_P (%)	--	NP(**)	NP(**)	38.4
Plasticity index, PI (%)	--	NP(**)	NP(**)	10.1
Org. matter content (%)	0.3	0.0	0.0	11.0
Standard Proctor test [ASTM D698]:				
Maximum dry unit weight, γ_{dmax} (kN/m^3)	17.3	19.2	17.1	13.3
Optimum water content, w_{opt} (%)	12.0	8.93	14.9	32.5
pH	8.40	8.44	7.75	4.32
Soil classification(*)	SP-SM	SM	ML	OL

(*) Unified Soil Classification System [17]; (**) Non plastic.

4 Testing methodology

The specimens of the soil used in the tests (Table 1) were prepared as follows: (i) the paste composed by the soil, the equimolar solution of urea-$CaCl_2$ of 0.5 mol/L and urease (8 kU/L), was mixed for the optimum water content (obtained from the standard Proctor test) in order to obtain a homogeneous paste; (ii) the paste was compacted directly into the PVC mold (37 mm in diameter, 76 mm in height) in 8 layers; (iii) each layer was lightly tapped by hand and compacted with standard Proctor test's energy; (iv) the surface of each layer was lightly scarified and another layer was introduced; (v) after preparation, the specimens were put inside a plastic bag and cured for 14 days inside a room equipped with a automatic system to control the humidity (95±5%) and the temperature (20±2ºC); (vi) after the curing time, each sample was removed from the PVC mould and placed on the pedestal of the equipment used to perform the UCS test; (vii) the load cell and strain gauge transducer were set up and adjusted; (viii) finally, unconfined compression strength (UCS) tests [18] were performed under a constant strain rate of 1%/min. All the UCS tests were repeated twice (soil A) or three times (soils B, C and D). The amount of *urease* used (8 kU/L) were based on the results obtained by Carmona et al. [13] for soil A.

Finally, the water content, the pH value (evaluated by a digital sensor) and SEM/EDX tests were performed.

5 Analysis of the experimental results

The stress-strain behaviour of the specimens tested, illustrated in Figure 1, is described by an initial trend with a quasi-linear elastic behaviour followed by a significant decrease in the strength after peak strength. An increase in the unconfined compressive strength is obtained with biocementation for soils A-C, while in soil D a detrimental impact on the strength is observed after biocementation.

Figure 2 shows the effect of the change of soil on the maximum unconfined compressive strength (q_u). As expected, these results present some scattering, due to the non-homogeneity inherent to the biocementation process. This figure also highlights the negative effect of the biocementation on the organic soil (soil D) with a loss of strength higher than 45%. For the soils A, B and C, the biocementation has a positive impact on q_u, with a gain from 42,9% (soil C) to 106.2 % (soil B).

The behaviour observed for soils B, C and D is in line with some results obtained from MICP experiments [5 19], which show the increase in the precipitation of $CaCO_3$ when using well-graded sands and silts. In its turn, soil D shows a high organic matter content (11.0%) and a high clay content (21.5%), which hinders the establishment of effective bonds between the soil particles and $CaCO_3$ crystals. Additionally, the positive effect obtained with a poorly graded sand (soil A) does not match with the MICP experiments of Mortensen et al. [5] and Rebata-Landa [19], which obtained a detrimental effect. In fact, the results obtained in the present work, indicate that enzymatic $CaCO_3$ precipitation could be applied to a wider range of soils than previous MICP experiments suggested, although its use in organic clayed soils is not effective.

The variation of the pH value is illustrated in Figure 3. The results show a slight decrease in the pH value after biostabilisation for all soil types. The pH value of soil D (without stabilisation) is 4.32, which is not suitable to potentiate $CaCO_3$ precipitation [3, 20]; naturally, this is a key factor in the inefficiency of the biostabilisation process for this soil.

Figure 4 depicts the results of SEM/EDX tests carried out on the samples of the biostabilised soils C and D. The local chemical composition of the small particles of all the soils, obtained from the EDX tests, displays vestiges of calcium (Ca) (soil C: 17.0%; soil D: 1.0%) suggesting the existence of $CaCO_3$ crystals. Soil C (Fig. 4a) has the coarser and better defined particles, while the organic matter present in soil D (Fig. 4b) appears to coat the soil particles, which seems to hinder the creation of bonds between the soil particles and the crystals of $CaCO_3$.

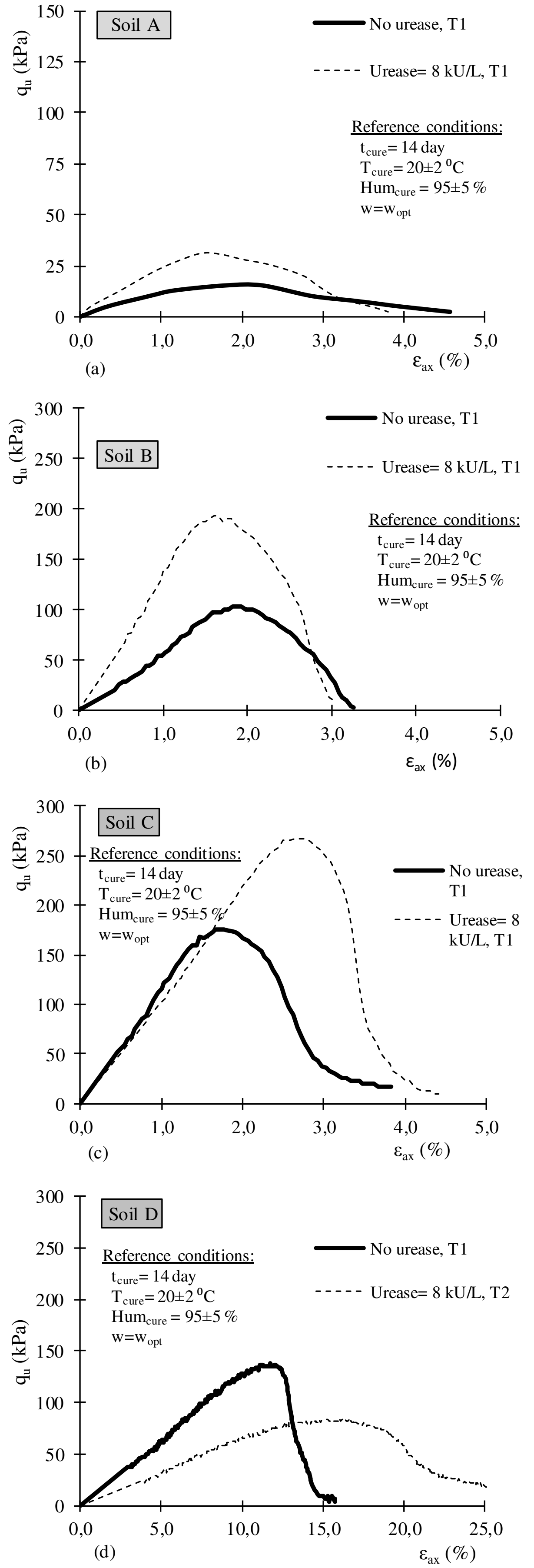

Fig. 1. Stress-strain curves (UCS tests). (a) soil A; (b) soil B; (c) soil C; (d) soil D (based on [12]).

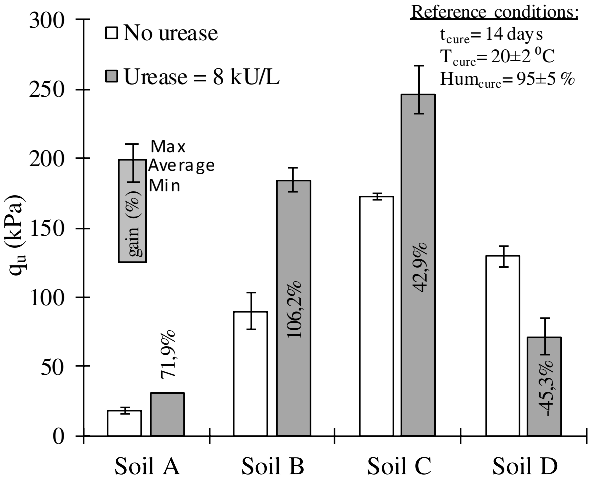

Fig. 2. UCS tests. Effect of soil type on the q_u (based on [12]).

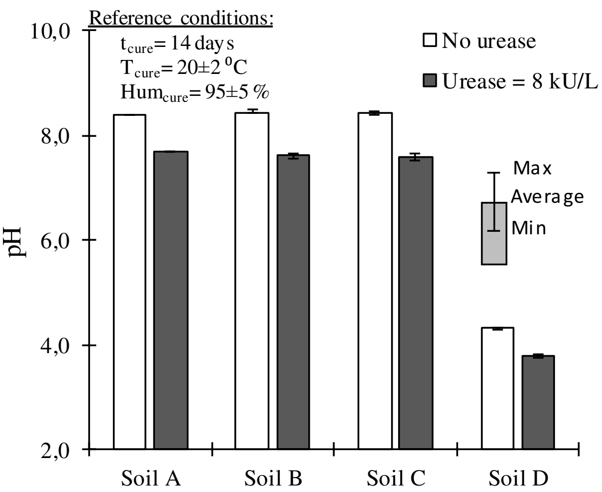

Fig. 3. Effect of the soil type on the pH value (based on [12]).

The low pH value of the organic soil combined with the coating of the soil particles by the organic matter, are probably the key factors responsible for the inefficiency of enzymatic $CaCO_3$ precipitation in soil D. Further, the use of biostabilisation is not only ineffective but it also induces a decrease in the strength, which may possibly be explained by the fact that the $CaCO_3$ crystals seem not to link the soil particles as might be expected, but even to break some of the bonds between the particles of silt and clay, which promotes the slippage of the soil particles inducing the decreases in the strength of the biostabilised material in relation to the unstabilised soil. Further experiments are needed to prove this theory.

6 Conclusions

Considering the results of the UCS, SEM and pH tests performed to study the effect of soil type on the efficiency of the process of enzymatic $CaCO_3$ precipitation, the following observations and conclusions can be stated: *(i)* the process of enzymatic $CaCO_3$

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precipitation is potentiated in sandy and silty soils, with a range of strength gain from 43% to 106%; *(ii)* the process of enzymatic CaCO3 precipitation has a detrimental effect on the organic soil, with decreases in strength; *(iii)* SEM/EDX tests carried out with the biostabilised specimens, show vestiges of calcium in the soils tested, which, combined with the results of pH, demonstrate the existence of $CaCO_3$ precipitation; *(iv)* the results suggest that the inefficiency of this process in organic soils is due to the combination of two key factors, their low pH value, which is not suitable to potentiate $CaCO_3$ precipitation and the organic matter that coats the soil particles and hinders the creation of bonds between the soil particles and the crystals of CaCO3.

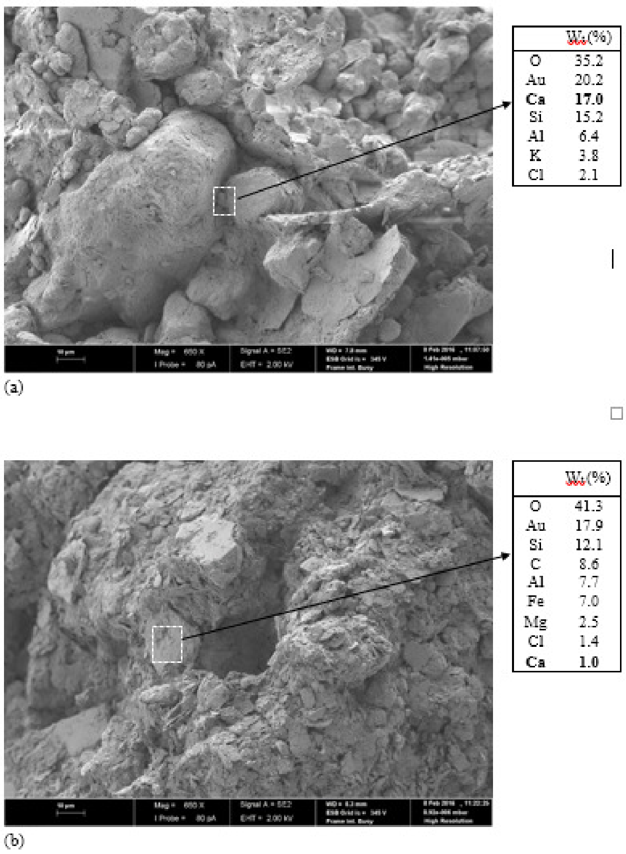

Fig. 4. SEM tests with EDX analyses of the biostabilised soils. (a) Soil C; (b) Soil D (based on [12]).

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