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# Biostabilization of a Sandy Soil Using Enzymatic Calcium Carbonate Precipitation

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**Abstract**
The use of soils with poor mechanical properties frequently requires the improvement of their characteristics, mainly the strength and stiffness. One possible technique utilizes precipitated calcium carbonate ($CaCO_3$). However this methodology usually requires a complex and sensitive process of cultivation and storage of the bacteria, which may make field applications difficult. In order to avoid this, an alternative process to promote $CaCO_3$ precipitation is the enzymatic $CaCO_3$ precipitation which is performed by mixing the soil with urea, calcium chloride and the urease enzyme. Thus, the aim of this work is to analyze the effect of the amount of the urea and calcium chloride on the biocalcification process. Initially, the methodology is studied in test-tube experiments, through the evaluation of the amount of $CaCO_3$ precipitated and XRD tests to verify the existence of $CaCO_3$. After these tests, the methodology is tested with a sandy soil to examine its strengthening effects, using the results of unconfined compressive strength tests. The results obtained confirm the precipitation of $CaCO_3$ and the effectiveness of this methodology in improving the characteristics of a sandy soil, they also show that the increase of the urea-$CaCl_2$ concentration may inhibit the activity of urease.

*Keywords:* Calcium carbonate precipitation, biostabilization, sandy soil, enzymatic precipitation, urease enzyme.

# 1 Introduction

The use of soils with poor geotechnical properties for construction usually requires the improvement of their mechanical properties. One of the most promising techniques to improve the characteristics of natural soils is microbially induced calcium carbonate precipitation ($CaCO_3$), also known as biocalcification or biostabilization (Dejong et al., 2010; Chou et al., 2011).

Research concerning microbially induced $CaCO_3$ precipitation in a porous soil usually consists in introducing the exogenous bacterium *Sporosarcina pasteurii* (*S. pasteurii*) in the soil to promote urea hydrolysis via the enzyme urease (Whiffin et al., 2007; Chou et al., 2011), which is described by the following equations (Hammes and Verstraete, 2002):

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$$\underbrace{CO(NH_2)_2(s)}_{\text{Urea}} + H_2O(l) \xrightarrow{\text{Urease enzyme}} 2\,\underbrace{NH_3}_{\text{Ammonia}}(aq) + CO_2(g) \quad [pH\uparrow] \tag{1}$$

$$2\,\underbrace{NH_3}_{\text{Ammonia}}(aq) + CO_2(g) + H_2O(l) \rightarrow 2NH_4^+(aq) + CO_3^{2-}(aq) \tag{2}$$

With an appropriate amount of $Ca^{2+}$, calcium carbonate is produced spontaneously (Chou et al., 2011; Burbank et al., 2013) by the reaction (3):

$$Ca^{2+}(aq) + CO_3^{2-}(aq) \leftrightarrow CaCO_3(s) \tag{3}$$

In general, experimental results show that the production of $CaCO_3$ due to biocalcification, promotes links/bonds between the soil particles (Chou et al., 2011; Chu et al., 2012) resulting in increases in the strength (Dejong et al., 2010; Whiffin et al., 2007; Al Qabany and Soga 2013; Venda Oliveira et al., 2015) and stiffness of soils (van Paassen et al., 2010a; Mortensen et al., 2011; Venda Oliveira et al., 2015). Naturally, the deposition of $CaCO_3$ in the void spaces and/or around the surfaces of the soil particles contributes to the clogging of the porous medium, which reduces the porosity (Whiffin et al., 2007) and the hydraulic conductivity (Chou et al., 2011; Al Qabany and Soga 2013). Biocalcification also changes the behavior of the loose specimens treated; thus, volumetric expansion during direct shear tests was observed (Chou et al., 2011) as well as a non-collapsible behavior (Dejong et al., 2006). These facts, together with the strengthening of the soil, contribute to mitigating the soil's liquefaction potential (Dejong et al., 2010; Montoya et al., 2012; Burbank et al., 2011, 2013; Cheng et al., 2013).

Recent research performed at the University of Coimbra (Taborda et al., 2009; Costa 2012; Venda Oliveira et al., 2015) used the bacteria *Idiomarina insulisalsae* (*I. insuliasalsae*) to improve the properties of a sandy soil and showed that this bacteria is more efficient than *S. pasteuri* in strengthening the soil and an increase in the concentration of *I. insuliasalsae* induce better geomechanical properties, i.e., an increase in the stiffness, compressive and tensile strength.

In order to avoid the bacteria's complex cultivation and storage processes, which frequently require special environments, some alternative methods to promote the $CaCO_3$ precipitation in a porous medium have been studied and applied, such as: (i) flushing with a mixture of chemical solutions inducing the precipitation of calcite, due to the reaction of the solution's ingredients (Ismail et al., 2002); (ii) enzymatic $CaCO_3$ precipitation, which is performed by mixing the soil with urea, calcium chloride and the urease enzyme (Nemati and Voordouw, 2003; Yasuhara et al., 2012; Neupane et al., 2013, 2015); (iii) microbial denitrification of calcium nitrate, using the calcium salts of fatty acids as an electron donor and source of carbon (van Paassen et al., 2010b).

The aim of this work is to analyze the effect that the amount of urea and calcium chloride has on the behavior of the enzymatic calcium carbonate precipitation process. The first set of tests uses test-tube experiments to evaluate the amount of $CaCO_3$ precipitated and XRD tests to confirm the existence of $CaCO_3$. At the end of the study, the results of unconfined compressive strength tests are used to evaluate the strengthening of a sandy soil stabilized with the methodology studied.

# 2 Characteristics of the Materials

The soil used in this study is an inorganic soil, with a uniformly graded grain size distribution and is predominantly sandy (gravel = 15.5%, sand = 78.7%; silt = 3.5%; clay = 2.3%), it is therefore classified as poorly graded sand (ASTM D2487 2000). The specific gravity is 2.71 and the pH value is equal to 8.8 (Costa, 2012). The compaction of the soil was characterized by a standard Proctor test (ASTM D698

2003), which provides a maximum dry unit weight ($\gamma d_{max}$) of 17.3 kN/m$^3$ and an optimum water content ($w_{opt}$) of 12%.

The products used to produce the grout are the urease enzyme, urea [$CO(NH_2)_2$] and calcium chloride ($CaCl_2$), with purity levels of 99.5% and 95%, respectively. The urease used is produced by Sigma-Aldrich Company Ltd. from Canavalia ensiformis (jack bean) in powder and presents an activity of 34,310 U/g (1U corresponds to the amount of enzyme which hydrolyzes 1 μmol urea per minute at pH 7.0 and 25ºC).

# 3 Description of the Tests

The program of tests carried out in this work is composed by two sets of tests; the first in test-tube experiments to examine the process of calcium carbonate precipitation, while the second uses this methodology to stabilize a sandy soil.

## 3.1 Test-tube Experiments

This set of tests examines the rate of urea hydrolysis that is bio-catalyzed by the urease enzyme. For this purpose, the quantity of precipitated calcium carbonate in test-tubes (Figure 1) for an equimolar solution of urea-$CaCl_2$, with concentrations of 0.25, 0.5, 0.75, 1.0 and 1.25 mol/L is evaluated. The amount of urease used in all the tests is constant and equal to 4 kU/L. The various solutions of urea-$CaCl_2$ were thoroughly mixed with water in order to guarantee that the chemical products are completely dissolved. The urease was thoroughly mixed in water for 5 minutes and filtered using filter papers to remove the undissolved particles of urease, in accordance with the Neupane et al. (2013) procedure. 10 mL of urease solution and 10 mL of urea-$CaCl_2$ solution were mixed in the test tubes, resulting in a total solution volume a 20 mL. As seen in Figure 1, the precipitated $CaCO_3$ settles at the bottom of the test tubes. After 14 days, the amount of material precipitated was evaluated as follows: (i) the solution was filtered through the filter paper; (ii) the paper filter and the test-tube were dried and the amount of the particles deposited was evaluated; (iii) the total amount of $CaCO_3$ is the addition of the material deposited on the filter paper and in the test tube. Each type of test was repeated three times to check the reproducibility of the procedure.

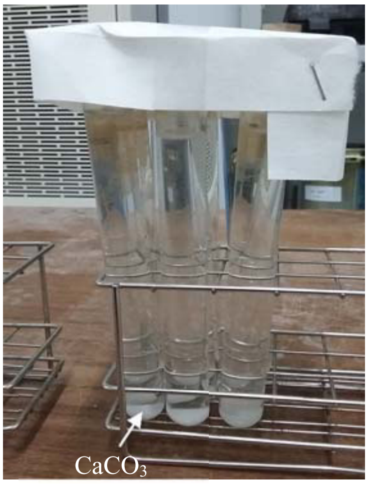



**Figure 1:** Test-tube experiments.

The precipitation ratio (PR) is expressed by the equation:

$$PR\ (\%) = \frac{\mathrm{M}^{\mathrm{t}}_{\mathrm{CaCO_3}}}{\mathrm{M}^{\mathrm{a}}_{\mathrm{CaCO_3}}} \times 100 = \frac{\mathrm{C} \times \mathrm{V} \times \mathrm{M}}{\mathrm{M}^{\mathrm{a}}_{\mathrm{CaCO_3}}} \times 100 \tag{4}$$

where $M^{a}_{CaCO_3}$ represents the actual mass of $CaCO_3$ and the $M^{t}_{CaCO_3}$ is the theoretical mass of $CaCO_3$ evaluated by C×V×M, where C is the concentration of the solution in mol/L, V is the solution volume in liters, and M corresponds to the molar mass of $CaCO_3$ (100.087 g/mol).

X-ray powder diffraction (XRD) tests were conducted to analyze the mineralogical composition of the precipitated material.

## 3.2 Stabilized Soil Specimens

The second set of tests uses biostabilized soil specimens, which were prepared as follows: (i) the natural soil was passed through a 2.0 mm mesh sieve to remove the major particles; (ii) the soil and the final aqueous solution with a water content of 12% ($w_{opt}$ of the standard Proctor test) were mixed to obtain a homogeneous paste; (iii) compaction of the paste directly into the PVC mold (37 mm in diameter, 76 mm in height) in 8 layers; (iv) each layer was lightly tapped by hand and compacted with an energy of compaction corresponding to the standard Proctor test; (v) the surface of each layer was lightly scarified and another layer was introduced; (vi) after preparation, the specimens were cured for 14 days inside a room with controlled humidity (60±5%) and temperature (20±2ºC); (viii) after 14 days of curing time, each sample was removed from the mold for use in the experiments; (ix) the electronic devices (load cell and/or strain gauge transducer) were set up and adjusted; (x) finally, the tests could start and the data were recorded by automatic data acquisition; (xi) unconfined compression strength (UCS) tests (ASTM D2166 2005) were performed under a constant strain rate of 1%/min. The tests were repeated three times to guarantee reliability.

# 4 Presentation and Analysis of the Results

Figure 2 illustrates the effect of the equimolar concentration of the urea-$CaCl_2$ solution on the PR, based on the results of test-tube experiments. The results show that the PR is about 95% for the lowest concentration of urea-$CaCl_2$ (0.25 mol/L), which means that for this concentration the amount of urease existent in the solution is able to hydrolyze almost all the urea.

Unexpectedly, the results also show that with the increase in the urea-$CaCl_2$ concentration induces a reduction of the efficiency of the process (i.e., PR decreases), which means that part of the urea existent in the solution is not hydrolyzed by the urease. This behavior may be due to an insufficient quantity of the urease enzyme and/or due to a negative effect of the high urea-$CaCl_2$ concentration, which tends to inhibit the action of urease, consequently the bio-catalyzation process is less effective. In fact, the negative impact of the increases of the urea-$CaCl_2$ concentration was also reported by Yasuhara et al. (2012) in specimens prepared in a similar way.

The results of XRD obtained with the material precipitated from the mixture with 0.5 mol/L of urea-$CaCl_2$ (Figure 3) show that the mineralogical composition of the precipitated material is fundamentally composed by calcite (i.e., $CaCO_3$), which confirms that the theoretical reactions (equations 1-3) actually occur.

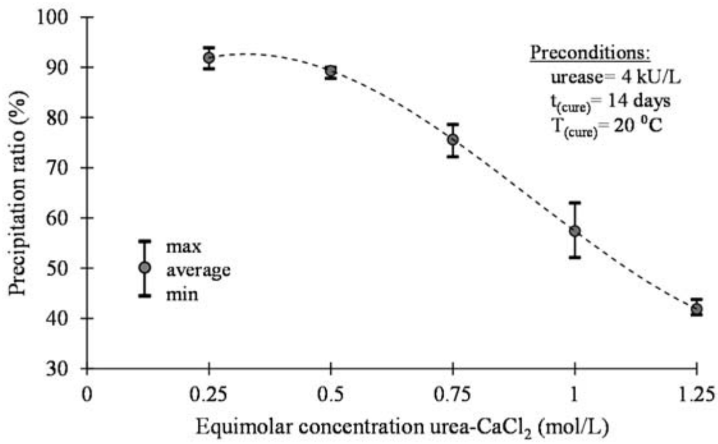



**Figure 2:** Test-tube experiments. Relation between urea-$CaCl_2$ concentration and $CaCO_3$ precipitation ratio.

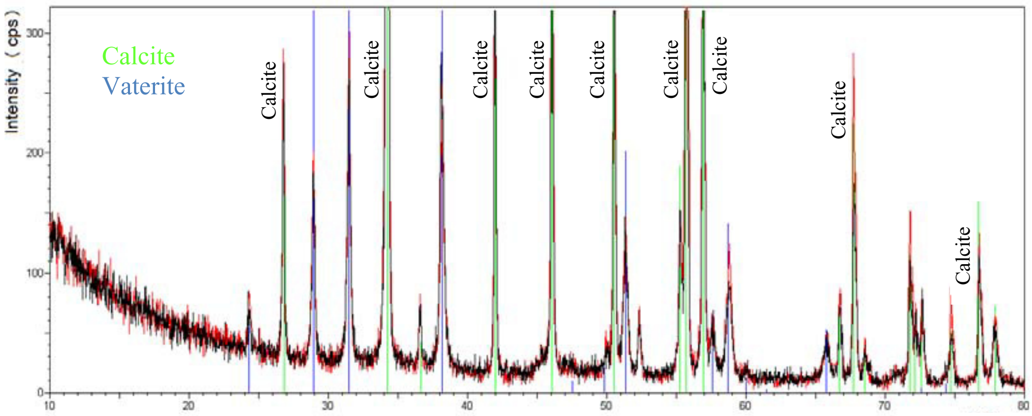
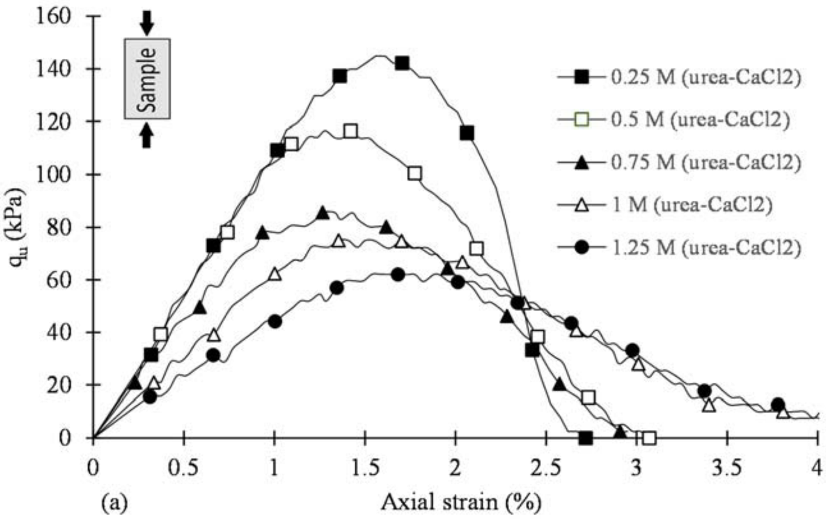



**Figure 3:** Tube-test experiments. XRD results with 0.5 mol/L of urea-$CaCl_2$.

Figure 4 depicts the UCS test results with specimens of soil biostabilized by enzymatic calcium carbonate precipitation for a constant urease concentration (4 kU/L) and various urea-$CaCl_2$ concentrations (0.25 – 1.25 mol/L). The stress-strain curves obtained (Figure 4a) reveals a greater effectiveness of the biocalcification process, expressed by the upper stress-strain curves, leading to the increases in the unconfined compressive stress ($q_u$) up to a maximum value of about 140 kPa, while the non-stabilized specimens show a negligible strength. The stress-strain curves also show that the specimens with higher strength also exhibit greater stiffness, which reflects a stabilized soil matrix with more calcite minerals in the void spaces and/or around the surfaces of the soil particles, thus increasing the links/bonds between them.

Concerning the variation of $q_{u\ max}$ with the urea-$CaCl_2$ concentration (Figure 4b), some scattering of the results, which tend to be higher for concentrations of urea-$CaCl_2$ of 0.25 and 1.25 mol/L, is observed. The results also show a clear and quasi-linear decrease of $q_u$ with the increment of the urea-$CaCl_2$ concentration from about 140 kPa to 50 kPa, for a variation of urea-$CaCl_2$ concentration from 0.25 to 1.25 mol/L. In fact, this variation is coherent with the results obtained from the test-tube experiments; indeed the decreases of the $CaCO_3$ precipitated with the increase of the urea-$CaCl_2$ concentration is related to the reduction of the links/bonds between the soil particles, which induce a weaker stabilized soil matrix.

Thus, the results clearly show that the best urea-$CaCl_2$ concentration for a concentration of urease of 4 kU/L is 0.25 mol/L. Higher concentrations of urea-$CaCl_2$ inhibit the activity of the urease enzyme, leading to a decrease in precipitation material and a weaker biostabilized soil.



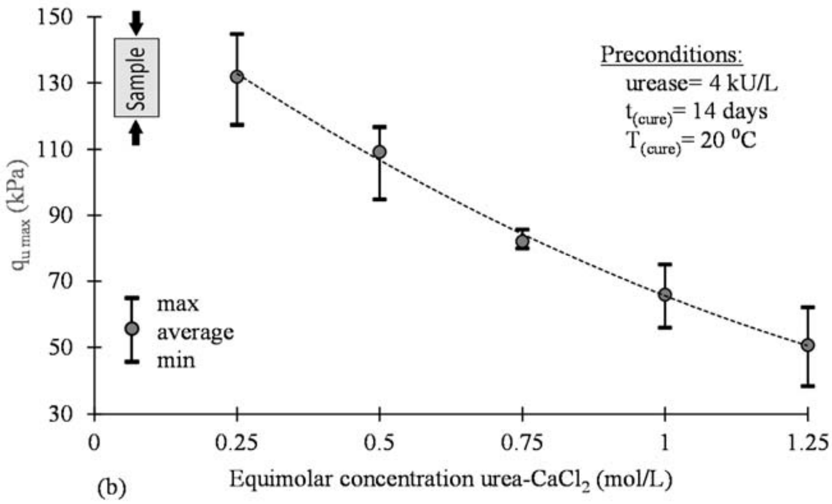




**Figure 4:** UCS with specimens of bio-stabilized soil for various urea-$CaCl_2$ concentrations. a) Stress-strain curves. b) Variation of $q_u$ with urea-$CaCl_2$ concentration.

# 5 Conclusions

Considering the effect of urea-$CaCl_2$ concentration on the biostabilization process and based on the results of test-tube experiments and UCS tests, the following observations and conclusions can be drawn:

i) The use of the urease enzyme promotes the hydrolysis of urea and the precipitation of calcium carbonate, which has a positive impact on the strengthening of the stabilized soil.

ii) For a constant concentration of urease of 4 kU/L, the urea-$CaCl_2$ concentration of 0.25 mol/L induces a precipitation ratio of about 90%, consequently the unconfined compressive strength increases.

iii) This methodology originates some scattering of the results of the UCS tests.

iv) The increases of the urea-$CaCl_2$ concentration reduces the precipitation ratio and consequently the strength of the stabilized soil, which may be due to an insufficient amount of urease and/or the inhibition of the urease activity for higher urea-$CaCl_2$ concentrations.

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