**Proceedings of the XVII ECSMGE-2019**
Geotechnical Engineering foundation of the future
ISBN 978-9935-9436-1-3

doi: 10.32075/17ECSMGE-2019-0987

XVII European Conference on Soil Mechanics and Geotechnical Engineering
ECSMGE 2019 Reykjavik

# Advances in the enzymatic stabilisation of soils

## Avancées dans la stabilisation enzymatique des sols

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**ABSTRACT:** The demands of innovative, cost effective and sustainable construction practices requires the development of energy-efficient and environmental friendly building techniques. The achievement of this objective is helped by the exploitation of knowledge from different disciplines, including physics, biology and chemistry. In geotechnical engineering, Enzymatic Induced Calcite Precipitation (EICP) is a new stabilisation treatment that can improve the mechanical properties of soils without the use of hazardous or costly chemical binders. EICP employs the urease enzyme, a widely occurring hexameric protein of plants, to catalyse the hydrolysis of urea. This reaction creates a strongly alkaline environment, which in turn favours precipitation of calcium carbonate, thus binding soil grains together. This study investigates the key factors controlling the kinetics of urea hydrolysis with the objective of proposing an optimised EICP methodology. The proposed methodology is then applied to the stabilisation of a silty clay to assess the improvement of material durability in the presence of excess water.

**RÉSUMÉ:** La demande de pratiques de construction innovatrices, rentables et durables exige le développement de techniques de construction efficientes et respectueuses de l'environnement. La réalisation de cet objectif est facilitée par l'exploitation des connaissances de différentes disciplines, dont la physique, la biologie et la chimie. En géotechnique, l'EICP est un nouveau traitement de stabilisation qui permet d'améliorer les propriétés mécaniques des sols sans utiliser de liants chimiques dangereux ou coûteux. L'EICP utilise l'enzyme uréase, une protéine hexamérique largement répandue dans les plantes, pour catalyser l'hydrolyse de l'urée. Cette réaction crée un environnement fortement alcalin qui favorise la précipitation du carbonate de calcium, liant ainsi les grains du sol. Cette étude examine les facteurs clés contrôlant la cinétique de l'hydrolyse de l'urée dans le but de proposer une méthodologie EICP optimisée. La méthodologie proposée est ensuite appliquée à la stabilisation d'une argile limoneuse pour évaluer l'amélioration de la durabilité du matériau en présence d'eau.

**Keywords:** Ground improvement; Biocementation; EICP; Plant-derived urease

## 1 INTRODUCTION

Ground improvement techniques are generally invasive (e.g., jet grouting), energy intensive (e.g., compaction, vibration, heating) and require environmentally unfriendly or even hazardous binders (e.g., cement or lime). Alternative ground stabilisation methods have therefore been recently proposed to avoid the use of costly and carbon inefficient materials. Among them, bio-mediated stabilisation methods rely on the activity of microorganisms or proteins to enhance the engineering properties of soils (DeJong et al., 2010). These methods appear

very promising in terms of both financial and environmental efficiency.

Urea hydrolysis is a typical example of a bio-mediated process leading to the precipitation of calcium carbonate (i.e. calcite) which bonds particles together, thus stabilising the soil. Urea hydrolysis is catalysed by the urease enzyme, a hexameric protein that is found in higher order plants and is also produced by the metabolic activity of some microbes.

Past research has focused on the catalysis of urea hydrolysis by the urease enzyme produced via the metabolic activity of microbes that can be either exogenous or endogenous to the soil (DeJong et al., 2006; Whiffin et al., 2007; De Muynck et al., 2010; Dilrukshi et al., 2016). This technique, which is usually termed Microbially Induced Calcite Precipitation (MICP), presents however a number of disadvantages. One of the most important ones is the impossibility of being applied to fine-grained materials because of the size of bacterial cells (300-5000 nm) which tends to be larger than soil pores. The direct use of the urease enzyme has therefore been recently proposed as an alternative that avoids the need of cultivating bacteria inside the soil (Hamdan et al., 2013; Nam et al., 2015). This method is termed Enzyme Induced Calcite Precipitation (EICP) and is also applicable to fine-grained materials as the enzyme molecule has a size of only few nanometres. The urease enzyme can be obtained as crude extract from plants or synthetically produced in the laboratory.

Bio-stabilisation is affected by several factors such as the particle size and mineralogy of the soil but also the pH and chemical composition of the pore water (Dilrukshi et al., 2016). Information concerning the bio-treatment of compacted soils is very limited with only a very limited number of studies currently available (Morales et al., 2015).

The present paper provides an overview of the key factors that influence the kinetics of urea hydrolysis. The objective is to optimise the catalysing effect of the urease enzyme and therefore to maximise the precipitation of calcite. The optimised method is then applied to a silty clay for assessing the durability of the stabilised material when exposed to excess water.

## 2 BACKGROUND

Equation 1 shows that the hydrolysis of one mole of urea generates one mole of ammonia and one mole of carbamic acid (Dilrukshi et al., 2016). Hydrolysis of urea takes places spontaneously in water but at a very slow pace. The urease enzyme can however act as a catalyst and accelerate considerably the kinetics of the reaction. Equation 2 further indicates that one mole of carbamic acid is hydrolysed into another mole of ammonia plus one mole of carbonic acid.

Equations 3 and 4 then show that the two moles of ammonia and one mole of carbonic acid, obtained from the above reactions, equilibrate in water to form one mole of carbonate ions and two moles of ammonium ions. This also produces hydroxide ions and therefore leads to an increase of alkalinity.

Equation 5 summarizes the above four reactions (Equations 1-4) and indicates that the overall result of the hydrolysis of one mole of urea is the production of two moles of ammonium and one mole of carbonate ions.

Finally, Equation 6 shows that, in the presence of calcium ions, one mole of carbonate ions precipitates to form one mole of calcium carbonate (e.g., calcite) once supersaturation is attained.

$$CO\,(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \quad (1)$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad (2)$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^- \quad (3)$$

$$2OH^- + H_2CO_3 \rightarrow CO_3^{2-} + 2H_2O \quad (4)$$

$$CO\,(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad (5)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \quad (6)$$

The kinetics of urea hydrolysis is governed by different factors including pH, temperature, concentration of the urease enzyme, substrate and product inhibition. Past evidence has resulted in the definition of models that include a pH-dependent rate constant and a non-competitive product-inhibition term, which describe well the enzyme kinetics (Fidaleo et al., 2003). Furthermore, it has been demonstrated that urease activity grows with increasing temperature from 10°C to 40°C and is at a maximum when the pH ranges between 6.5 and 7.0.

## 3 MATERIAL AND METHODS

### *3.1 Urease extract*

The EICP method relies on the urease enzyme to catalyse the precipitation of calcium carbonate. Unlike the MICP method, the EICP method has the advantage of not requiring the inoculation and cultivation of microbial colonies inside the soil. Microbial colonies can continue to live inside the soil long after the stabilisation reactions have taken place, with potential negative impacts on the ecosystem. Conversely, the urease enzyme has a relatively short life span with an activity that naturally degrades with time (Pettit et al., 1976).

The urease enzyme is available from chemical suppliers as a synthesised product, which tends however to be very expensive. A cheaper alternative is to directly extract the enzyme from plants by means of centrifugation. Several families of plants are very rich in urease, including some varieties of beans (jack and soybeans) or seeds (melon, pumpkin and pineapple).

In this work, a soybean extract containing the urease enzyme was prepared according the following procedure: (i) soybeans were soaked in water for 24 hours with 10 ml of distilled water for each gram of dry beans; (ii) the soaked soybeans were centrifuged in a blender together with the water they were soaked in; (iii) the juice obtained after centrifugation was collected and kept as crude urease extract.

Soybean extract is semi-transparent and has a bright grey-yellow colour. The pH of the liquid suspension is about 6 and no change in colour is observed over time at room temperature.

### *3.2 Chemical reagents*

Urea ($CO(NH_2)_2$), which is also known as carbamide, is an organic compound that serves an important role in the metabolism of nitrogen-containing compounds and is also the main nitrogen-containing substance in the urine of mammals. It is a colourless and odourless solid, which is highly soluble in water and practically non-toxic. Importantly, the hydrolysis of urea exhibits a high calcite conversion rate compared to other precipitation processes (Harkes et al., 2010; Whiffin et al., 2007).

Calcium chloride ($CaCl_2$) is a ionic compound that is highly hygroscopic and dissolves in water via an exothermic reaction that increases the temperature of the solution. Past research has demonstrated that calcium chloride is a very effective reagent and generates high concentrations of calcium ions, which combine with carbonate ions to produce the precipitation of calcite. The solubility of calcium chloride at room temperature can be 100 times greater than other salts such as calcium hydroxide or calcium nitrate. This is also consistent with experimental observations that have indicated that calcium chloride results in higher rates of calcite precipitation compared to calcium hydroxide and calcium nitrate (Park et al., 2014).

## 4 EXPERIMENTAL PROGRAM

The precipitation of calcite is a relatively straightforward chemical process, which is controlled by four key factors: a) the concentration of reagents, b) the concentration of dissolved inorganic carbon, c) the pH of the solution and d) the availability of nucleation sites. The present

study focuses on the concentration of reagents and, in particular, on the definition of the optimal amounts of urea and calcium chloride to maximise calcite precipitation.

Samples of crude soybean extract containing different equimolar concentrations of urea and calcium chloride were prepared inside test tubes. The activity of the urease enzyme was then detected, for each concentration level, by measuring the conductivity and the pH of the solution by means of ion-selective electrodes and an acidity meter, respectively. The measurement of pH is useful because the release of ammonia during the hydrolysis of urea (Equations 1 and 2) produces an increase of pH. The differences of pH between distinct samples can be very small and a frequent calibration of the acidity meter is therefore required to minimize errors.

Finally, the amount of precipitated calcite was measured by weighing the solid material recovered after filtrating the content of test tubes. This in turn allowed the identification of the optimal equimolar concentration of urea and calcium chloride leading to the largest calcite precipitation. This optimal concentration of reagents was then employed for the stabilisation of the soil samples. The effectiveness of the treatment was finally evaluated by comparing the mass losses of both unstabilised and stabilised samples after prolonged immersion in water.

## *4.1 Test tube experiments*

Twelve test tubes with a capacity of 40ml were filled with crude soybean extract and mixed with the following twelve different equimolar concentrations of urea and calcium chloride: 0.010, 0.025, 0.050, 0.10, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50 and 4.00 mol/L. Urea was added to the soybean extract immediately after centrifugation while calcium chloride was added after 24 hours. Figure 1 show the values of electrical conductivity and pH recorded immediately after the addition of urea at different concentrations.

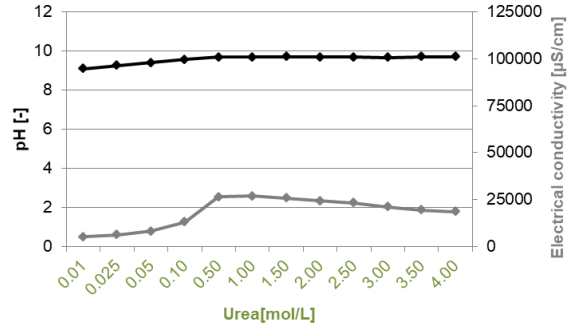


*Figure 1. Measurements of pH and electrical conductivity taken immediately after adding urea to the crude soybean extract (urea concentration varying from 0.010 to 4 mol/L).*

Inspection of Figure 1 indicates that, regardless of the concentration of added urea, the pH of the solution increases to about 10 from the initial value of 6 of the crude soybean extract. This marked increase confirms the occurrence of enzymatic hydrolysis. The alkaline environment generated by the production of ammonia and the consequent release of carbon dioxide are the key precursors of calcite precipitation (Castanier et al., 1999).

The measurements of electrical conductivity presented in Figure 1 demonstrate that the rate of hydrolysis increases with growing concentration of urea until a maximum is attained, after which the rate of hydrolysis decreases. This result may be explained by urease inhibition at high concentrations of reaction products (Nemati et al., 2003).

Figure 2 shows measurements taken 24 hours after the introduction of urea. Inspection of Figure 2 indicates a marked increase in electrical conductivity, which suggests an enduring activity of the enzyme over time, though the pH remains approximately unchanged. This means that urea is hydrolysed at a relatively slow pace and sufficient time must be allowed for the reaction to occur.

After 24 hours from the addition of urea, equimolar concentrations of calcium chloride were introduced in each test tube. Figure 3 shows the measurements of pH taken immedi-

ately after the addition of calcium chloride, which indicate a significant reduction of pH back to similar levels as those of the initial soybeans extract.

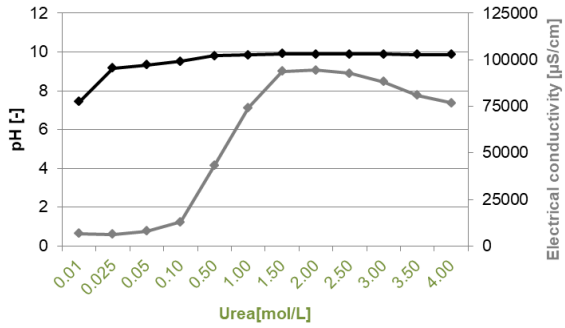


*Figure 2. Measurements of pH and electrical conductivity taken 24 hours after adding urea to the crude soybeans extract (urea concentration varying from 0.010 to 4 mol/L).*

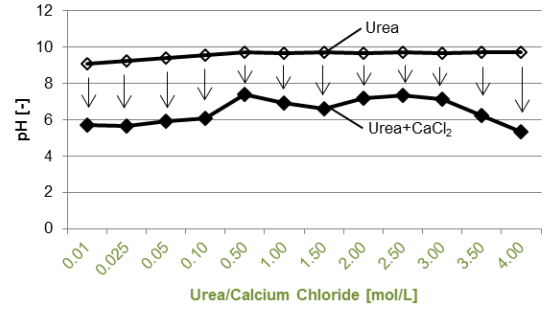


*Figure 3. Reduction of pH immediately after adding calcium chloride (equimolar urea and calcium chloride concentrations varying from 0.010 to 4 mol/L).*

Calcium chloride is a water-soluble ionic compound that releases heat as it dissolves. During dissolution, calcium hydroxide is formed and hydrogen ions are released causing the pH of the solution to reduce. Another way to think about it is that calcium chloride absorbs hydroxide and leaves free protons behind, making the solution more acidic.

Calcium carbonate precipitated at the bottom of the test-tubes and after 72 hours the level of precipitate did not change by visual inspection. The amount of precipitated material was then measured as follows: (i) the solution was passed through filter paper to collect the material deposited at the bottom of the test tube; (ii) the collected material was oven-dried at 40° C and subsequently weighed; (iii) the amount of precipitated calcium carbonate was obtained as the difference between the weight from step (ii) and the weight of the clean collecting pan. Each experiment was repeated three times to check the repeatability of results and values here reported are average values.

The precipitation ratio (*PR*) is defined as the ratio between the actual mass of precipitated calcite $M^a_{CaCO_3}$ measured from the above experiments and the theoretical mass of precipitated calcite $M^t_{CaCO_3}$ calculated from the chemical reactions of Equations 1-6. The precipitation ratio is therefore defined by the following expression (Carmona et al., 2016):

$$PR(\%) = \frac{M^t_{CaCO_3}}{M^a_{CaCO_3}} \times 100 = \frac{C \times V \times M}{M^a_{CaCO_3}} \times 100 \quad (7)$$

where $C$ is the concentration of the solution in mol/L, $V$ is the solution volume in liters, and $M$ is the molar mass of calcite (100.087 g/mol). Inspection of Figure 4 indicates that the precipitation ratio remains approximately equal to one for increasing equimolar concentrations of urea and calcium chloride up to about 2.50 mol/L. Beyond this level, the precipitation ratio reduces to below one as the actual mass of precipitated calcite stays approximately constant while the theoretical mass continues to increase. This is consistent with past research which has shown that ground improvement using this method becomes less effective if highly concentrated solutions of urea and calcium chloride are employed (e.g., Bull et al., 2014).

Inspection of Figure 4 therefore indicates an optimal concentration of urea and calcium chloride equal to about 2.50 mol/L. This concentration produces the largest precipitation of calcite with the least amount of reagents. Given that the amount of precipitated calcite is directly linked

to the degree of bonding between particles, the same concentration has been used in this work for the stabilisation of soil samples.

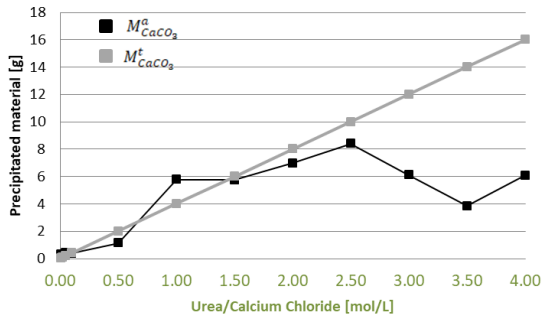


*Figure 4. Theoretical ( $M^t_{CaCO_3}$) and actual ($M^a_{CaCO_3}$) mass of precipitated calcite for equimolar concentrations of urea and calcium chloride from 0.010 to 4 mol/L (measured 72 hours after the addition of calcium chloride).*

## *4.2 Soil stabilisation experiments*

The EICP method has been used in this work to improve the durability of a silty clay soil when exposed to excess water. The soil was provided by a brickwork factory in south-west France and is the same material employed for the production of masonry bricks at the factory.

The cementing solution has been prepared by adding urea and calcium chloride to crude soybean extract. The concentrations of urea and calcium chloride are both equal to the optimum value of 2.5 mol/L as discussed earlier in the paper. Stabilised and unstabilised cylindrical samples of 50 mm diameter and 100 mm height were prepared by standard Proctor compaction. Samples were prepared with two repeats and results are the average values. Prior to compaction, the stabilised samples were mixed with an amount of cementing solution corresponding to 12% of the dry soil weight, which corresponds to the optimum water content of the material. Unstabilised samples were prepared in the same way but the cementing solution was replaced with an equal amount of distilled water.

After manufacturing, all samples were equalized at the laboratory atmosphere, corresponding to a temperature of 25 °C and a relative humidity of 40%±5%, until the sample mass changed less than 0.1% over at least one week. After equalisation, the initial masses $m_i$ of all samples were recorded and water immersion tests were performed (Tercruso, 2013). These tests consisted of submerging the samples in water for ten minutes. After this time, the samples were removed from the bath and equalised again to the laboratory atmosphere so that they could attain the same moisture content as before immersion. After equalisation, the final sample mass $m_f$ was recorded and introduced, together with the initial mass $m_i$, to calculate the percentage mass loss $\%\Delta m$ during immersion as

$$\%\Delta m = \frac{(m_i - m_f)}{m_i} \times 100. \quad (8)$$

Results from the tests indicate that unstabilised samples lost about 42% of their initial mass. The mass loss is however reduced by a factor greater than three, i.e. to about 13%, in the case of stabilised samples. Figure 5 shows photographs of unstabilised samples (Figure 5b) and stabilised samples (Figure 5c) after immersion in water together with a photograph of a reference sample before immersion.

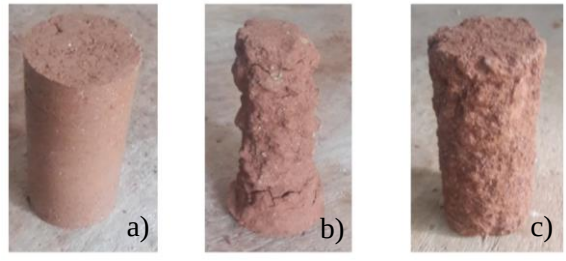


*Figure 5. a) Reference sample before immersion, b) unstabilised sample after immersion (mass loss of 42%), c) stabilised sample after immersion (mass loss of 13%).*

A significant improvement of water durability is therefore achieved by using the proposed enzymatic stabilisation treatment. The lower mass

loss experienced by the stabilised samples may be explained by the formation of calcite bonds between soil particles and by the occlusion of material voids with a consequent reduction of permeability.

This result, albeit promising, is however insufficient to justify the routine deployment of the proposed stabilisation technique at this point. Further refinements are necessary to reduce the mass loss during water immersion to a level that is acceptable for engineering applications. This could be achieved, for example, by the application of a greater compaction effort during the manufacture of soil samples or by an enhanced catalysis of calcite precipitation at higher reagents concentrations.

## 5 CONCLUSIONS

Growing interest is evident in recent years in the development of cost effective and environmental friendly ground stabilisation methods. To this end, the present paper has discussed the use of the urease enzyme to catalyse the hydrolysis of urea. This reaction leads to the production of carbonate ions, which react with dissolved calcium ions resulting in the precipitation calcium carbonate (e.g., calcite). Calcite precipitation cements soil particles together and occludes material pores, thus resulting in an improvement of the mechanical characteristics of the material. This soil stabilisation method is commonly referred to as Enzyme Induced Calcite Precipitation (EICP).

The use of commercially available urease enzyme is however unviable due to high financial costs. As a cheaper and ecological alternative, this work has proposed the use of plant-derived urease enzyme obtained as a crude extract via centrifugation of soaked soybeans. The soybean extract is then mixed with urea and calcium chloride (a source of calcium ions) to kick-start a series of reactions leading to calcite precipitation.

Test tube experiments have highlighted that the concentration of urea and calcium chloride plays an important role in the activity of the urease enzyme and on the amount of precipitated calcite. In particular, measurements of pH, electrical conductivity and precipitation ratio have indicated that the optimum concentration of urea and calcium carbonate, leading to the largest precipitation of calcite, is equal to 2.5 mol/L.

This optimal concentration has then been used for the manufacture of stabilised silty clayey samples, which have been subjected to water immersion tests. During immersion, the mass lost by the stabilised samples reduces to 13% compared to 43% for the unstabilised samples. This confirms the effectiveness of the proposed stabilisation method in enhancing the mechanical characteristics of the soil, though further improvements are necessary to make the proposed method competitive with conventional stabilisation techniques based on the use of cement and lime.

## 6 ACKNOWLEDGEMENTS

The authors wish to acknowledge the support of the European Commission via the Marie Skłodowska-Curie Innovative Training Networks (ITN-ETN) pro-ject TERRE 'Training Engineers and Researchers to Rethink geotechnical Engineering for a low carbon future' (H2020-MSCA-ITN-2015-675762).

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