Soil Stabilization Against Water Erosion via Calcite Precipitation by Plant-Derived Urease

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Abstract. Stabilization is often necessary to improve the stiffness, strength and durability of both natural and compacted soils. Soils are typically stabilized via the addition of hydraulic binders, such as cement and lime, which present however relatively high carbon and energy footprints. Alternative stabilization techniques are therefore explored to minimize environmental impact while preserving good material properties. Among these techniques, enzyme induced calcite precipitation (EICP) has gained prominence in recent years. EICP exploits the action of the urease enzyme to catalyze the hydrolysis of urea and to produce carbonate ions, which then react with calcium ions inside the pore water to cause precipitation of calcium carbonate (i.e. calcite). The precipitated mineral bonds particles together, thus improving the hydro-mechanical characteristics of the soil. This paper presents a preliminary investigation on the use of plant-derived urease, instead of pure reagent-grade urease, to minimize environmental and financial costs. The urease enzyme is obtained from a liquid soybeans extract, inside which urea and calcium chloride are dissolved. This stabilizing solution is then mixed with a silty clay to produce stabilized samples, which are subjected to water erosion tests.

Keywords: Soil stabilization · Ground improvement · Bio-mineralization · Bio-cementation · Plant-derived urease · Enzyme induced calcite precipitation

1 Introduction

Ground improvement techniques are commonly employed by geotechnical engineers to enhance the mechanical properties or to reduce the permeability of natural and compacted soils. Current soil stabilization techniques are invasive (e.g. jet grouting, permeation grouting), present high energy costs (e.g. compaction, vibration, heating, freezing) and require chemical binders (e.g. cement or lime). Engineers have therefore explored alternative stabilization procedures that can enhance soil properties while

F. Calvetti et al. (Eds.): CNRIG 2019, LNCE 40, pp. 753–762, 2020.
https://doi.org/10.1007/978-3-030-21359-6_80

reducing the carbon/energy footprint of current practices. Some of these alternative stabilization procedures fall under the broad category of bio-mineralization or bio-cementation methods. Bio-cementation consists in the chemical alteration of the soil environment by means of biological activity, which eventually results in the precipitation of a binding mineral phase (Stocks-Fischer et al. 1999; Barkay and Schaefer 2001).

Until very recently, bio-cementation of coarse grained soils was mainly achieved by microbially induced calcite precipitation (MICP). MICP exploits the urease enzyme from the metabolic activity of microbes to catalyze the hydrolysis of urea (DeJong et al. 2006; Whiffin et al. 2007; De Muynck et al. 2010; Dilrukshi and Kawasaki 2016). This ureolytic reaction then induces the precipitation of calcium carbonate (e.g. calcite), and the consequent cementation of the soil, if enough calcium ions are present in the pore fluid.

In recent years, considerable progresses have been made on the development of alternative bio-cementation techniques that do not require the use of microbes (Yasuhara et al. 2012; Hamdan et al. 2013; Nam et al. 2015). Among these techniques, enzyme induced calcite precipitation (EICP) has shown promise for the stabilization of both coarse and fine grained materials. Like MICP, EICP relies on the precipitation of a cementing calcium carbonate phase as the result of the hydrolysis of urea inside the pore water. However, the main difference between EICP and MICP is that, in the former case, the urease enzyme is directly introduced in the soil without the need of any microbial organism. This is possible because the urease enzyme is not only produced by the metabolic activity of bacteria but it is also found in many common plants.

Another important difference is that MICP is only applicable to coarse grained materials because microbes have generally a size comprised between 300 and 5000 nm, which means that they cannot survive inside very small soil pores. Conversely, EICP is also applicable to fine grained soils thanks to the relatively small size of the urease enzyme molecule, which is around 12 nm (Blakeley and Zerner 1983).

The implementation of EICP requires three main ingredients, namely the urease enzyme, the urea substrate and a source of calcium ions such as, for example, a solution of calcium chloride. The ensuing bio-mineralization relies on relatively straightforward chemical reactions that induce the precipitation of large quantities of calcium carbonate in a relatively short period of time. The efficiency of cementation depends on a number of environmental factors such as, for example, the pH and temperature of the soil but also the concentration of urea and calcium ions in the pore water.

In this context, the present paper discusses the kinetics of the ureolytic reactions and explores how these reactions lead to the mineralization of soils with a consequent enhancement of hydro-mechanical characteristics. The paper also presents the results from a preliminary campaign of laboratory tests to demonstrate the enhancement of mechanical properties achieved by using plant-derived urease instead of expensive reagent-grade enzymes.

2 Calcite Precipitation via Hydrolysis of Urea

2.1 Background

Equation 1 indicates that the overall result of the hydrolysis of one mole of urea (ureolysis) is two moles of ammonium and one mole of carbonate ions. This reaction also produces hydroxide ions and therefore leads to an increase of alkalinity (Benkovic and Hammes-Schiffer 2003; Mitchell and Ferris 2005; Dilrukshi and Kawasaki 2016).

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

Equation 2 shows that, if calcium ions are present in solution, one mole of carbonate ions precipitates to form one mole of calcium carbonate (e.g. calcite).

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \tag{2}$$

Several factors may affect the kinetics of the above ureolytic reactions including pH, temperature, concentration of the urease enzyme and concentrations of urea and calcium chloride. Most calcite precipitation occurs under relatively alkaline conditions corresponding to pH levels from 8.7 to 9.5 (Stocks-Fischer et al. 1999; Dupraz et al. 2009). Similarly, the optimum temperature for the activity of the urease enzyme ranges from 20° to 37 °C (Mitchell and Ferris 2005; Okwadha and Li 2010). Benkovic and Hammes-Schiffer (2003) also investigated the importance of the concentration of calcium ions and found that the enzyme activity can increase up to tenfold in the presence of calcium ions compared to the case where calcium ions are absent.

2.2 Urease Enzyme and Chemical Reagents

The urease enzyme was the first nickel metalloenzyme ever purified and crystallised from jack beans (canavalia ensiformis). This important achievement yielded the Nobel Prize in Chemistry to James B. Summer in 1946. Since then, different types of nickel-dependent ureases have been isolated from bacteria, fungi and plants.

The urease enzyme acts on the urea substrate leading to the precipitation of calcium carbonate in aqueous solution. The occurrence of this reaction requires the presence of calcium ions, which typically originate from the dissolution of calcium containing salts.

Urea ($CO(NH_2)_2$) is a colourless and odourless organic material that serves an important role in the metabolism of nitrogen-containing compounds and is also the main nitrogen-containing substance of the urine of mammals. Urea 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 (Whiffin et al. 2007; Harkes et al. 2010).

In this work, the calcium chloride salt ($CaCl_2$) has been used as a source of calcium ions because of its relatively high hygroscopicity and solubility in water. The solubility of calcium chloride at room temperature is up to 100 times greater than other salts such as calcium hydroxide or calcium nitrate, which makes calcium chloride a very effective reagent to generate high concentrations of calcium ions. This is an important aspect to consider because a large concentration of calcium ions contributes to a high rate of calcite precipitation. Indeed, past experimental evidence has confirmed that calcium chloride produces higher rates of calcite precipitation compared to calcium hydroxide and calcium nitrate (Park et al. 2014).

Chemical suppliers commercialize pure reagent-grade urease enzyme, which is very effective for catalysing the hydrolysis of urea but is also very expensive. This research proposes instead an effective and economical procedure for obtaining the urease enzyme from the centrifugation of soybeans. The procedure consists in soaking the soybeans in water for 24 h, with 10 ml of distilled water for each gram of dry beans. The beans are then centrifuged in a blender together with the water they were soaked in. This yields a semi-transparent juice with a bright grey-yellow colour, which is collected and kept as a crude urease extract.

3 Experimental Program

3.1 Calcite Precipitation Tests

This section presents the results from preliminary tests that were carried out to confirm the occurrence of the ureolytic reaction and the precipitation of calcium carbonate inside standard laboratory beakers. As previously described, the urease enzyme was obtained from the centrifugation of wet soybeans, which yielded a juice with a pH of about 6.

The subsequent dissolution of two moles of urea in one liter of this crude soybeans extract resulted in an immediate rise in pH from 6 to about 7, which was followed by a further gradual increase to a value of 9 (Fig. 1). This marked increase of alkalinity indicates the occurrence of the hydrolysis of urea and confirms the activity of the enzyme in the extract. Note that the same increase of alkalinity is not observed if the urea is dissolved in distilled water.

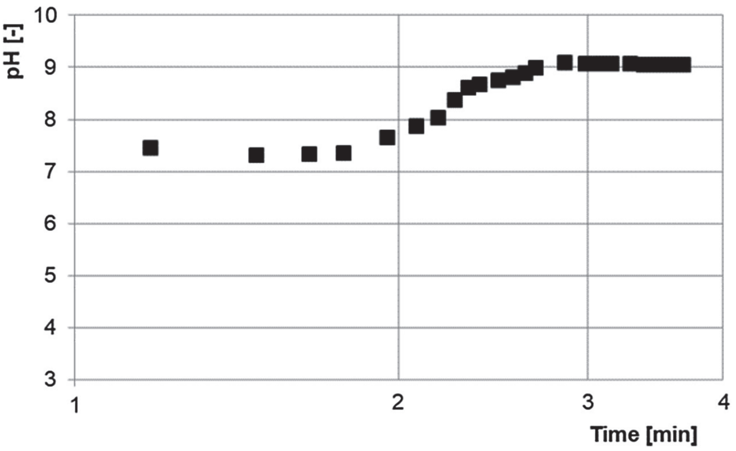

Fig. 1. Measurements of pH taken after dissolution of urea in the crude soybeans extract (urea concentration of 2 mol/L).

The alkaline environment is the result of the production of ammonia and the consequent release of carbon dioxide, which leads to calcite precipitation (Castanier et al. 1999). Note that the experimental values shown in Fig. 1 are the average of three tests, which were performed to confirm the repeatability of results. Inspection of Fig. 1 also indicates that the alkaline environment remains stable with a constant pH after dissolution of urea and the consequent occurrence of the ureolytic reaction.

Calcium chloride was then added to the solution with an equimolar concentration of 2 mol/L which produced a sudden drop of pH to about 6 as calcium carbonate precipitated. This happens because, during dissolution of calcium chloride, calcium hydroxide is formed and hydrogen ions are released making the solution more acidic. Figure 2(a) shows the precipitated material collected at the bottom of the beaker while Fig. 2(b) shows the precipitated material after oven-drying at a temperature of 105 °C.

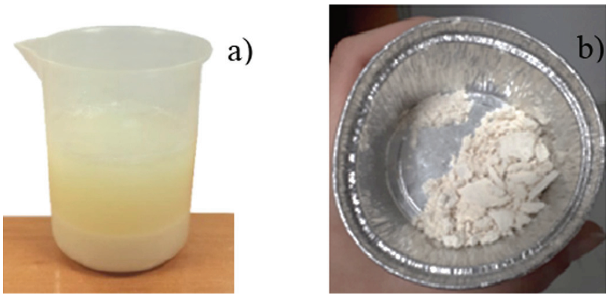

Fig. 2. Precipitation of calcium carbonate at the bottom of the beaker after addition of calcium chloride (a) and precipitated material after oven-drying (b).

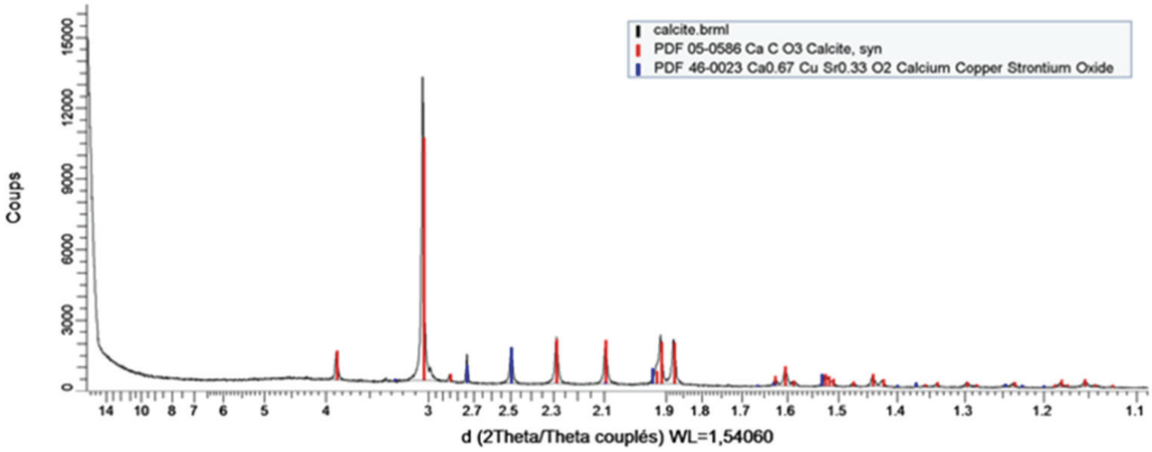

Fig. 3. XRD analysis of precipitated material.

The subsequent performance of XRD tests confirmed that the mineralogy of the precipitated material mainly comprises calcium carbonate (calcite), which provides evidence of the occurrence of the reactions described in Eqs. 1 and 2 (Fig. 3).

3.2 Properties of Crude Soybeans Extract

Samples of the crude soybeans extract were exposed to the laboratory atmosphere, at a temperature of 25 °C and a relative humidity of 40 ± 5%, for 72 h. Measurements taken over this period of time indicated that the extract becomes acidic after only few hours of exposure to the laboratory atmosphere attaining a pH of about 4.5. At this point, the pH stops reducing and the acidity level of the extract remains constant over time.

A thick foam developed at the top of the acidic soybeans extract during the time it was exposed to the laboratory atmosphere. The addition of 2 mol/L of urea did not produce any increase of pH in the extract after 72 h of exposure to the atmosphere. This suggests that, unlike previous experiments, no hydrolytic reaction took place in this case. The absence of hydrolytic reaction may be a consequence of the inhibition of the urease enzyme in an acidic environment (Stocks-Fischer et al. 1999).

Equally, the subsequent addition of calcium chloride with a concentration of 2 mol/L did not produce any precipitation of calcium carbonate. This was an expected result because the dissolution of calcium ions cannot cause the precipitation of calcite in the absence of the carbonate ions produced by the hydrolysis of urea.

The above experiment indicates that only a fresh soybeans extract can catalyze the hydrolysis of urea and hence the precipitation of calcite crystals. It is therefore important to use the extract as soon as possible after centrifugation to ensure that the pH does not decrease before addition of urea. Further investigation is being undertaken to assess whether the activity of the enzyme can be preserved for a longer time by sealing and/or freezing the extract prior to use.

3.3 Soil Stabilization

The proposed stabilization method was subsequently applied to a silty clay provided by the Bouisset brickwork factory in the south-west of France. This silty clay is used to manufacture conventional masonry bricks and is characterized by the index properties shown in Table 1.

Table 1. Main properties of Bouisset soil.

Index property			
Grain size distribution		*Atterberg limits*	
Gravel content (>2 mm, %)	0	Plastic limit (%)	18.7
Sand content (≤ 2 mm, %)	31	Liquid limit (%)	29.0
Silt content (≤ 63 μm, %)	35	Plasticity index (%)	10.3
Clay content (≤ 2 μm, %)	34	*Mineralogical composition*	
Specific gravity	2.65	Goethite, Muscovite, Orthose Kaolinite, Quartz	

The proposed stabilization method was assessed by comparing the durability to water erosion of stabilized and unstabilized soil samples. The stabilized sample was produced from a mix of 150 gm of dry soil and a cementing solution corresponding to 80% of the liquid limit of the soil. The cementing solution was prepared as previously discussed by dissolving equimolar concentrations (2 mol/L) of urea and calcium chloride into a freshly centrifuged soybeans extract. A relatively large amount of cementing solution was added to the dry soil to ensure a high rate of calcite precipitation and hence a good level of stabilization. The cementing solution was thoroughly mixed into the dry soil right after the addition of urea and calcium chloride, which resulted in the production of a slurry. To avoid the premature precipitations of calcite crystals, the slurry was immediately compacted by hand inside an oedometric ring to create a sample with a diameter of 60 mm and a height of 30 mm. A set of unstabilised samples was also prepared in a similar way by replacing the cementing solution with distilled water. All samples were equalized at the laboratory atmosphere, corresponding to a temperature of 25 °C and a relative humidity of 40 ± 5%, until the soil mass changed less than 0.1% over a period of at least one week. After equalization, the initial mass m_i of each sample was recorded and an immersion test was subsequently performed to evaluate the durability against water erosion (Tercruso 2013). The immersion test consisted in submerging the soil sample for ten minutes before draining it and equalizing it again to the laboratory atmosphere to attain the same moisture content as before immersion. The final sample mass m_f was then recorded and introduced, together with the initial mass m_i, in the following equation to calculate the percentage mass loss $\%\Delta m$ during immersion:

$$\%\Delta m = \frac{(m_i - m_f)}{m_i} \times 100 \tag{3}$$

Results from the above tests indicate that the unstabilized and stabilized samples lost about 7% and 2% of their respective initial masses. The mass loss is therefore reduced by a factor greater than three in the case of stabilized samples (Fig. 4). The visual examination of the tested samples also highlighted that, unlike the unstabilized material, the stabilized material did not experience any marked cracking and swelling after immersion. A similar result was also obtained if the samples were submerged in water until complete dissolution. In this case, the stabilized material resisted for almost 5 h whereas the unstabilized material was completely destroyed after only 3 h.

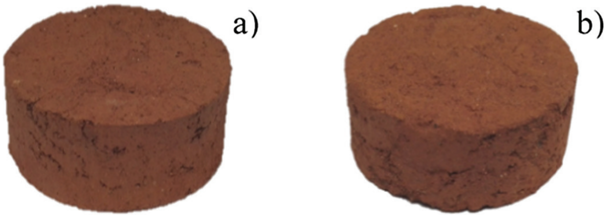

Fig. 4. Stabilized sample before (a) and after immersion (b) (mass loss of about 2%).

The durability of the unstabilized samples can be explained by the manufacture process of the wet clay, which results in a fabric orientation that seals the sample surface and reduces moisture infiltration (Maillard and Aubert 2014; Bruno et al. 2019). Furthermore, the initially high water content results in the development of a strong consolidation pressure during equalization due to the generation of elevated suctions inside the saturated material. The noticeable water resistance of the unstabilized samples is further enhanced by the proposed bio-cementation thanks to the formation of calcite bonds between soil particles and to the occlusion of pores with a consequent reduction of water permeability. More tests are currently under way to better understand the role played by the enzyme induced calcite precipitation in the improvement of material durability.

4 Conclusions

This paper explores the potential of bio-mineralization techniques for soil stabilization in geotechnical engineering. The stabilization method considered in the present work falls within the category of "enzyme induced calcite precipitation" (EICP) methods. It exploits the action of the urease enzyme to catalyze the hydrolysis of urea and therefore to produce carbonate ions, which then react with dissolved calcium ions resulting in the precipitation of calcium carbonate (i.e. calcite). The proposed technique employs the urease enzyme contained in a crude soybeans liquid extract as an alternative to the use of expensive reagent-grade enzymes from laboratory suppliers. The extract is obtained by centrifugation of soaked soybeans inside a common centrifugal juicer.

Results from preliminary tests in a laboratory beaker confirmed the ability of the enzyme inside the extract to catalyze the hydrolysis of urea. This is proven by the precipitation of calcium carbonate shortly after dissolution of urea and calcium chloride inside the extract. Moreover, XRD tests indicate that the precipitated material consists of calcium carbonate in the form of calcite.

The proposed technique was subsequently applied to the stabilization of a silty clay soil. The improvement of the durability of the soil to water erosion was assessed by means of standard immersion tests. A cementing solution consisting of crude soybeans extract (as a source of urease enzyme), urea (as a chemical substrate) and calcium chloride (as a source of calcium ions) was mixed with a dry silty clay in a proportion corresponding to 80% of the liquid limit. The mix was then compacted in the form of small cylindrical samples, which were subsequently submerged in water to evaluate erosion. Results from these tests indicate that the stabilized soil experiences a significantly lower mass loss during immersion compared to the unstabilized soil. This is due to the formation of calcite bonds between particles and, possibly, to the reduction of water permeability due to the occlusion of pores by the precipitated mineral. Furthermore, the stabilized samples did not experience swelling or cracking, which further confirms the effectiveness of the proposed stabilization method. Additional investigation is necessary to characterize the efficiency of the above bio-chemical reactions and, in particular, to optimize the key factors that affect the mineralization of calcite inside the soil.

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

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