

# Earth stabilisation via carbonate precipitation by plant-derived urease for building applications

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**ABSTRACT:** Raw (unfired) earth represents a sustainable and efficient alternative to traditional construction materials but its dissemination into building practice has been hindered by a relatively high vulnerability to water erosion. Enzyme induced carbonate precipitation (EICP) can improve the durability of earth materials without using traditional chemical binders such as cement and lime. EICP utilises the urease enzyme to catalyse the hydrolysis of urea, which produces carbonate ions that react with the calcium ions dissolved in the pore water, thus resulting in the precipitation of calcium carbonate. The calcium carbonate fills the soil voids and binds particles together, which reduces water permeability and increases material strength. The urease enzyme is a hexameric protein that is found in the tissues of many common plants. This work proposes a low-cost and simple stabilisation technology that makes use of crude urease enzyme extracted from soybeans. This technology is applied to the stabilisation of compacted earth, whose properties are then assessed via unconfined compression, moisture buffering and durability tests. The findings suggest a noticeable improvement of material strength and durability, though further investigation is necessary to increase the competitiveness of EICP stabilisation against standard techniques using cement and lime.

**Keywords:** Soil stabilisation; Biocementation; Enzyme induced carbonate precipitation (EICP); Plant-derived urease; Earth construction

## 1. INTRODUCTION

### 1.1. Context

Raw earth is a construction material consisting of a compacted mix of soil and water which is put in place with the least possible transformation.<sup>1</sup> Archaeological evidence from clay-rich regions of ancient Mesopotamia (between the Euphrates and Tigris rivers) and Afghanistan indicates that raw earth was used for the construction of buildings since 10000 BC. Earth is harmless to humans, can be locally sourced and is easily transported to the construction site. Earth is also recyclable, inexhaustible and, when properly manufactured, offers high strength, excellent hygrothermal properties and small embodied energy at very low costs. Therefore, raw earth materials can dramatically reduce exploitation of natural resources not only during construction of buildings but also during the service life by cutting heating/air conditioning needs and limiting demolition waste.<sup>2</sup>

Despite these advantages, the dissemination of earth materials into routine building has been limited mainly because of their high vulnerability to water erosion. Earth rapidly absorb any free water that it meets,<sup>3,4</sup> generally exhibiting a linear increase of moisture content with the square root of time.<sup>5,6</sup> Moisture ingress reduces the strength and stiffness of the earth while producing structural damages caused by swelling of the clay fraction. Erosion depths between 5 mm and 10 mm have been reported on the surface of 400 mm thick unstabilised earth wallets exposed to a wet continental climate for twenty years.<sup>7</sup> Therefore, well-preserved earth buildings, dating back hundreds of years, are relatively common in dry climates but are unusual in wet climates.

One of the main challenges lies in the improvement of earth durability by means of stabilisation methods that avoid energy intensive processes or environmentally unfriendly binders such as cement or lime. Stabilisation by cement or lime undermines the hygroscopic properties of raw earth, thus reducing the hygro-thermal inertia of building walls with a consequent increase of operational energy.<sup>8,9</sup> Alternative

48 chemical binders, such polymers or alkoxysilanes, have also been used to stabilise the superficial layer  
49 of earth walls exposed to environmental actions. These techniques have, however, encountered limited  
50 success as they do not counteract the swelling/shrinkage of clay minerals but only diminish the  
51 symptoms of the wearing process, thus being ineffective in the long-term.<sup>10</sup>

## 52 1.2. Research objectives

53 This paper describes a biomediated earth stabilisation method that relies on the hydrolysis of urea  
54 (ureolysis) to induce the precipitation of calcium carbonate and the consequent enhancement of material  
55 properties.<sup>11-16</sup> The application of microbial processes to the catalysis of ureolytic reactions inside soils  
56 has captured the attention of many scientists across the world.<sup>13,17-21</sup> Past research has shown that  
57 ureolytic reactions are catalysed by the urease enzyme, which is produced by the metabolic activity of  
58 microbes, either exogenous or endogenous to the soil.<sup>13-15,18,22-31</sup> Based on these findings, researchers  
59 have developed microbially induced carbonate precipitation (MICP) techniques, which have proven  
60 generally successful in stabilising earth materials but have also displayed several shortcomings. The  
61 main shortcoming is perhaps the unsuitability of MICP to the stabilisation of compacted fine-grained  
62 materials (i.e., clays and silts) due to the impossibility of microorganisms to migrate through very small  
63 pores.<sup>32</sup> For example, the *Sporosarcina pasteurii* bacteria have a size of the order of microns, which is  
64 larger than most pores inside fine-grained materials. Furthermore, microbial colonies remain alive inside  
65 the soil long after the stabilisation reactions have occurred with potential negative impacts on the  
66 surrounding ecosystem.

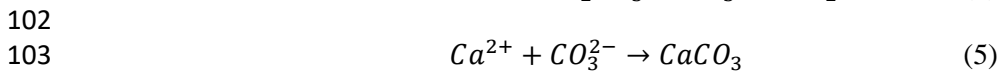
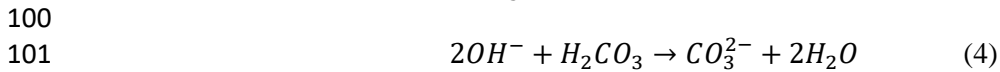
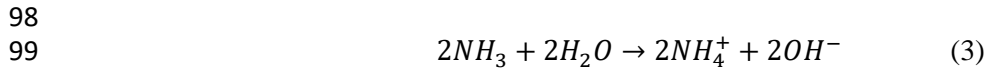
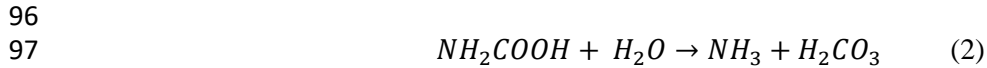
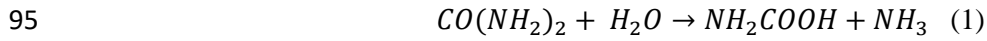
67 Because of the above limitations, some authors have recently proposed the direct use of the urease  
68 enzyme as an alternative to the cultivation of microbes inside the soil, which has led to a new category  
69 of enzyme induced carbonate precipitation (EICP) techniques for the stabilisation of earth materials.<sup>16,32-  
70 40</sup> The molecule of the urease enzyme has a size of around 12 nm and can fit in very small pores, thus  
71 overcoming the main disadvantage of MICP. Furthermore, unlike microbial colonies, the urease enzyme  
72 has a relatively short life span with an activity that naturally degrades with time, thus avoiding any  
73 significant impact on the surrounding ecosystem.<sup>41</sup> Traditional EICP techniques make use of pure  
74 reagent-grade urease enzyme that is purchased from chemical suppliers at relatively high cost.  
75 Conversely, the present work promotes the use of crude plant-derived urease enzyme via simple and  
76 cost-effective processes that can be easily adopted by building practitioners.<sup>36,42-45</sup>

77 The paper starts with an overview of the key aspects that govern the kinetics of urea hydrolysis. This  
78 provides the basis for optimising the catalyst action of the urease enzyme and, therefore, maximising  
79 calcium carbonate precipitation. A larger precipitation of calcium carbonate is here assumed to produce  
80 a greater improvement of the mechanical characteristics of the earth material. An optimised EICP  
81 method is devised and applied to the stabilisation of compacted silty clay samples, which are subjected  
82 to unconfined compression, moisture buffering and durability tests to assess the improvement of hygro-  
83 mechanical properties.

## 84 2. ENZYME INDUCED CARBONATE PRECIPITATION (EICP)

### 85 2.1. Chemical reactions leading to calcium carbonate precipitation

86 Eqs. (1)-(5) illustrate the EICP reactions that are catalysed by the free urease enzyme in an aqueous  
87 solution of urea and calcium chloride. The urease enzyme hydrolyses one mole of urea ( $\text{CO}(\text{NH}_2)_2$ ) into  
88 one mole of ammonia ( $\text{NH}_3$ ) and one mole of carbamic acid ( $\text{NH}_2\text{COOH}$ ) as shown in Eq. (1).<sup>16</sup> One  
89 mole of  $\text{NH}_2\text{COOH}$  is then hydrolysed into another mole of  $\text{NH}_3$  plus one mole of carbonic acid ( $\text{H}_2\text{CO}_3$ )  
90 as shown in Eq. (2). Carbonate ions ( $\text{CO}_3^{2-}$ ), ammonium ions ( $\text{NH}_4^+$ ) and hydroxide ions ( $\text{OH}^-$ )  
91 equilibrate in water as the result of the above reactions (Eqs. (3) and (4)). Hydroxide ions ( $\text{OH}^-$ ), in  
92 particular, lead to an increase of the alkalinity of the solution. In the presence of calcium ions ( $\text{Ca}^{2+}$ ),  
93 under supersaturated conditions, one mole of  $\text{CO}_3^{2-}$  then precipitates into one mole of calcium carbonate  
94 ( $\text{CaCO}_3$ ) Eq. (5), which is the ultimate objective of the proposed stabilisation method.



104 The above reactions indicate that precipitation of calcium carbonate is a rather straightforward chemical  
105 process that is governed by the following factors: the source of calcium ions, the concentration of urea  
106 and calcium ions<sup>21,46</sup> and, finally, the pH and temperature of the solution.<sup>47,48</sup> The next sections briefly  
107 describe the influence of each one of these factors on the precipitation of calcium carbonate.

## 108 2.2 Source of urease enzyme

109 The urease enzyme was the first nickel metalloenzyme ever purified and crystallised from jack beans  
110 (*Canavalia ensiformis*), an achievement that yielded the Nobel Prize in Chemistry to James B. Sumner  
111 in 1946. Since then, different types of nickel dependent ureases have been isolated from bacteria, fungi  
112 and plants. Chemical suppliers commercialise pure reagent-grade urease enzyme, which is effective for  
113 catalysing the hydrolysis of urea, but it is also very expensive and, therefore, not suitable for earth  
114 construction. A few studies have therefore explored the use of urease extracted from plants by means of  
115 simple and cost-effective techniques, referred to herein as crude extracts.<sup>36,42-45</sup> Among these studies,  
116 those making use of crude jack bean extract are the majority.

117 Larsen et al.<sup>42</sup> used jack bean meal as a source of urease (with an activity of 1,700 Units/g) for plugging  
118 pervious strata in oil wells. The use of jack bean meal resulted in increasing the amount of precipitation  
119 compared to the use of high-purity commercially available urease (with an activity of 150,000 Units/g).  
120 The authors of the study concluded that the presence of other proteins in crude extracts prevents the  
121 coprecipitation of the urease protein with  $CaCO_3$  by exercising a protective effect on the urease enzyme  
122 and consequently leading to greater  $CaCO_3$  precipitation. The study reported a tenfold increase in  $CaCO_3$   
123 precipitation (from 20 to 200 grams per litre) when high-purity commercial enzyme was replaced with  
124 jack bean meal. Similar results were obtained from Tirkolaei et al.<sup>45</sup> who studied the cementation of a  
125 granular soil using crude extracts from different plants (i.e., jack bean, soybean and watermelon seeds)  
126 and commercially available enzymes. Test-tubes experiments showed that, among the plants tested, the  
127 crude jack bean provided the highest unit yield defined as urease content per initial mass of source  
128 material. Unconfined compression tests on the cemented soil demonstrated that the crude extract is also  
129 more effective at enhancing soil strength compared to commercially available highly purified urease. In  
130 line with Larsen et al.<sup>42</sup>, Tirkolaei et al.<sup>45</sup> suggested that the presence of organic impurities and  
131 complementary proteins enhances the effectiveness of EICP. Park et al.<sup>36</sup> treated a sandy soil with  
132 various amounts of jack bean extract, urea, and different calcium sources (i.e., calcium chloride, calcium  
133 hydroxide and calcium nitrate). They found that, as the amount of urea increased, the unconfined  
134 compression strength of the treated soil increased up to 317 kPa. This level of measured strength was  
135 tenfold greater than that of the untreated soil and similar to that of the soil stabilised with 4% of high-  
136 early strength Portland cement. Furthermore, the use of calcium chloride yielded the highest strength  
137 levels compared to the other two calcium sources. Other studies<sup>43,44</sup> explored extraction of urease  
138 enzyme from soybeans and watermelon seeds with similar conclusions.

## 139 2.3 Source of $Ca^{2+}$ ions

140 Mixing concentrated solutions of  $Ca^{2+}$  and  $CO_3^{2-}$  ions leads to the precipitation of calcium carbonate in  
141 three consecutive steps: a) formation of amorphous calcium carbonate characterised by low stability and  
142 high solubility, b) transformation of amorphous calcium carbonate into vaterite and c) transformation of  
143 thermodynamically unstable vaterite into stable calcite.<sup>49-51</sup> Furthermore, different sources of  $Ca^{2+}$  ions

144 induce precipitation of calcium carbonate crystals with different shapes as revealed by electron  
145 microscopy. The rhombohedral shape, which is induced by calcium chloride ( $\text{CaCl}_2$ ), is characteristic  
146 of calcite, i.e. the most stable form of calcium carbonate.<sup>52-54</sup> Instead, calcium acetate induces a lettuce  
147 like or lamellar shape that is characteristic of vaterite, a metastable form of calcium carbonate. Finally,  
148 calcium lactate and calcium gluconate induce a more complex growth of vaterite crystals with spherical  
149 shapes.<sup>55</sup> Overall, calcium chloride appears the best option to enhance urease activity and maximise the  
150 production of a stable form of calcium carbonate.<sup>56</sup>

## 151 **2.4 Concentration of chemical reagents (urea and $\text{Ca}^{2+}$ ions)**

152 Nemati and Voordouw<sup>33</sup> conducted one of the earliest investigations into the parameters that affect the  
153 efficiency of EICP. Their objective was to enhance fossil fuels recovery by consolidating sand in oil  
154 reservoirs and to trace contaminants in ground water systems. Nemati and Voordouw<sup>33</sup> conducted test-  
155 tubes experiments to evaluate the influence of the amount of urease enzyme and the concentration of  
156 reagents (i.e. urea, calcium chloride and skim milk powder as stabiliser) on the rate and quantity of  
157 calcium carbonate precipitation. They concluded that, for a constant concentration of reagents, an  
158 increase in the amount of urease enzyme produced a faster reaction. Conversely, a proportional increase  
159 of reagents concentration resulted in a slower reaction, though the final quantity of precipitate increased  
160 if sufficient time was allowed. Nemati and Voordouw<sup>33</sup> concluded that, as the concentration of reagents  
161 increases, longer curing times are needed for completely converting reactants into products. Other  
162 studies<sup>34,35,39,56-59</sup> confirmed that, for a fixed amount of urease enzyme, higher concentrations of urea and  
163 calcium chloride yield greater quantities of precipitate if enough time is allowed. Nevertheless, if the  
164 amount of urease enzyme and curing time are both fixed, there is an optimal concentration of the  
165 reagents leading to the highest precipitation of calcium carbonate.

166 Urease activity also plays a key role in determining the optimal amount of enzyme, i.e. the amount that  
167 maximises reaction rate while avoiding inhibition of catalysis at high reagents concentrations. Nemati  
168 and Voordouw<sup>33</sup> used purified urease with a very high activity of 26,100 Units/g, which explains why  
169 only a very little amount of enzyme was necessary (0.01-0.1 g/L) to initiate the ureolytic reactions  
170 without any inhibition of catalysis at all reagents concentration. Other studies, which employed enzymes  
171 of different nature and activity, observed instead inhibition of catalysis at large reagents concentrations  
172 with a consequent reduction of calcium carbonate precipitation.

## 173 **2.5 pH**

174 The pH of the reaction medium is a crucial abiotic factor that influences the activity of the urease enzyme  
175 during hydrolysis of urea.<sup>60</sup> Rohy et al.<sup>61</sup> observed that an acidic reaction medium delays the EICP  
176 reaction rate, which is also consistent with the results from Cheng et al.<sup>62</sup> about delayed MICP  
177 stabilisation. Rohy et al.<sup>61</sup> investigated the compressive strength of stabilised soil samples with 100 mm  
178 height and 50 mm diameter where, for each increment in molarity of the reagents (urea- $\text{CaCl}_2$ ), a greater  
179 quantity of purified urease was used. Measurements of pH showed that acidity tended to increase with  
180 the increase in molarity of the reagents with values of pH equal to 5.69 and 4.46 for 1 mol/L and 3 mol/L  
181 solutions, respectively. This decrease of pH was shown to be due to the increase in urea concentration,  
182 which led to a slower rate of carbonate precipitation upon the addition of free urease. Samples prepared  
183 with a 3 mol/L solution showed a compressive strength of 3 MPa when cured for 14 days, which is an  
184 increase of 500% compared to the strength of the same samples cured for only 3 days. This confirmed  
185 that a curing period of 3 days was not enough for full stabilisation to take place because of the slower  
186 rate of carbonate precipitation at lower levels of pH.

## 187 **2.6 Temperature**

188 Neupane et al.<sup>63</sup> investigated the effect of temperature on both EICP reaction rate and amount of  
189 precipitate inside coarse sand samples of 5 cm diameter and 100 cm height. Their objective was to  
190 reduce the reaction rate for achieving an uniform distribution of precipitate over large soil areas. The  
191 sample columns were injected with two cementing solutions, containing fixed amounts of urea, calcium

192 chloride and purified urease enzyme at mixing temperatures of 5 °C and 23.5 °C, respectively. The  
 193 solution at temperature of 23.5 °C generated very large amounts of calcium carbonate near the injection  
 194 point. Precipitation progressively reduced to a depth of 60 cm, beyond which no significant amount of  
 195 precipitate was detected. On the contrary, the columns injected with cementing solution at temperature  
 196 of 5 °C, exhibited a smaller but much more uniform precipitation of calcium carbonate. This indicates  
 197 that a decrease of temperature from 23.5 °C to 5 °C reduces the reaction rate and the amount of  
 198 precipitation. These results are in agreement with those of Nemati and Voordouw,<sup>33</sup> who found that the  
 199 reactants conversion rate was 100% after 120 hours at temperatures between 30°C and 50°C but it  
 200 dropped to 70% after 300 hours at a temperature of 20 °C. Similarly, van Paassen<sup>64</sup> found that the rate  
 201 of ureolysis doubled approximately every 8 °C as temperature increased from 5 °C to 70 °C.

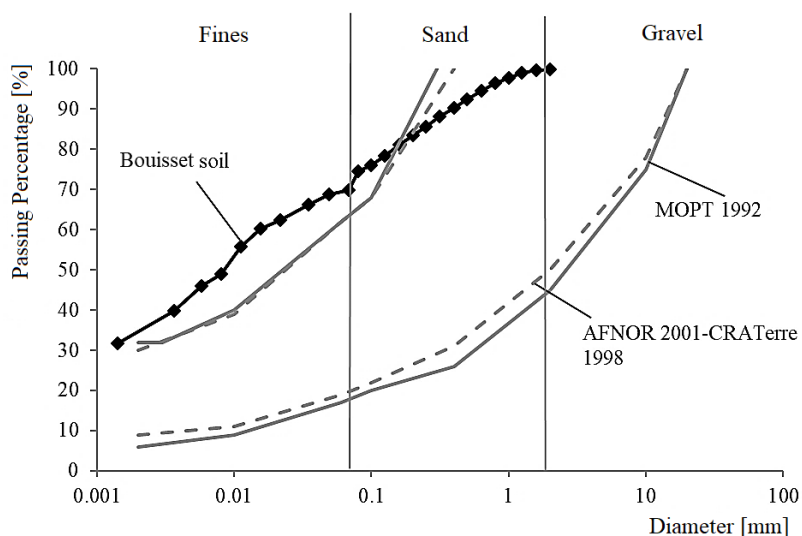
202 Nevertheless, increasing temperature to very high levels may lead to a progressive denaturation of the  
 203 urease enzyme. For example, Illeová et al.<sup>65</sup> demonstrated that enzyme activity was completely lost after  
 204 40 min of exposure to a temperature of 87.5 °C. Dhami et al.<sup>66</sup> found that the urease enzyme was stable  
 205 at 35 °C but its activity decreased by almost 47% when temperature increased to 55 °C. Wu et al.<sup>67</sup>  
 206 investigated urea hydrolysis in the absence of a calcium source and observed decreasing rates of  
 207 ammonium production at temperatures above 30 °C with no ammonium production at 50 °C.

### 208 3. MATERIALS AND METHODS

#### 209 3.1. Bouisset soil

211 The soil used in the present work was provided by the brickwork factory Bouisset from the region of  
 212 Toulouse (France) and is one of the five soils from different brickwork factories tested in the project  
 213 TERCRUSO.<sup>68</sup> The project TERCRUSO<sup>68</sup> showed that the Bouisset soil possesses similar hygro-  
 214 mechanical characteristics and better water durability properties than the other four soils, which is the  
 215 reason why it was selected in the present study.

216 Fig. 1 shows the grain size distribution of the Bouisset soil, which was determined as the average of  
 217 three independent measurements by means of wet sieving and sedimentation tests in compliance with  
 218 the norms XP P94-041<sup>69</sup> and NF P 94-057.<sup>70</sup> Fig. 1 also presents the lower and upper limits of the  
 219 recommended grading ranges for the manufacture of compressed earth bricks according to AFNOR<sup>71</sup>;  
 220 CRATerre-EAG<sup>72</sup> and MOPT.<sup>73</sup>



221  
 222 Fig. 1: Grain size distribution of the Bouisset soil in relation to existing recommendations for the manufacture of  
 223 compressed earth bricks by AFNOR<sup>71</sup>; CRATerre-EAG<sup>72</sup> and MOPT.<sup>73</sup>

224 Inspection of Fig. 1 indicates a well graded grain size distribution that is slightly finer than the limit  
 225 prescribed by current recommendations for compressed earth bricks. The specific gravity of the solid

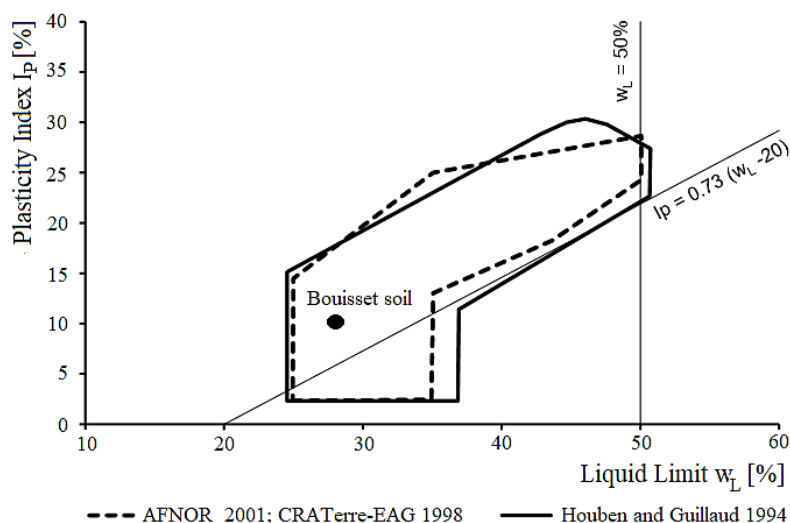
226 particles,  $G_s$  is reported in Table 1 and was measured as the average of four pycnometer tests performed  
 227 according to the norm NF P 94-054.<sup>74</sup> The plasticity properties of the fine fraction (i.e. the fraction  
 228 smaller than 0.400 mm) were measured according to the norm NF P94-051.<sup>75</sup> The Atterberg limits, i.e.  
 229 the liquid limit  $w_L$ , plastic limit  $w_P$  and plasticity index  $I_P$ , were all determined as the average of four  
 230 independent measurements and are given in Table 1.

231 Fig. 2 shows the plasticity characteristics of the Bouisset soil with reference to the Casagrande chart,  
 232 which indicates that the material can be classified as a low plasticity clay. Note also that the Bouisset  
 233 soil falls inside the recommended plasticity region for the manufacture of compressed bricks according  
 234 to AFNOR<sup>71</sup>; CRATerre-EAG<sup>72</sup> and Houben and Guillaud.<sup>76</sup>

235 Previous studies of the Bouisset soil<sup>68</sup> have indicated a predominantly kaolinitic clay fraction, which  
 236 indicates a small tendency to swell/shrink upon wetting/drying and, therefore, makes this soil suitable  
 237 for earth construction. Another measure of the soil sensitivity to water is the clay activity,  $A$ , which is  
 238 defined as the ratio between the plasticity index and the percentage of clay fraction (i.e. the percentage  
 239 of the soil that is smaller than 0.002 mm). For the Bouisset soil, the activity has been established to be  
 240 0.30, which classifies this material as low active.<sup>77</sup>

241 Table 1: Main properties of Bouisset soil (after Cuccurullo et al.<sup>78</sup>).

<b>Grain size distribution</b>	
Gravel content (> 2 mm, %)	0
Sand content ( $\leq 2$ mm, %)	31
Silt content ( $\leq 63$ $\mu\text{m}$ , %)	35
Clay content ( $\leq 2$ $\mu\text{m}$ , %)	34
<b>Specific gravity, <math>G_s</math></b>	2.65
<b>Atterberg limits</b>	
Plastic limit, $w_P$ (%)	18.7
Liquid limit, $w_L$ (%)	29.0
Plasticity index, $I_P$ (%)	10.3
<b>Clay activity, <math>A</math></b>	0.30



242  
 243 Fig. 2: Plasticity properties of the Bouisset soil in relation to existing recommendations for the manufacture of  
 244 compressed earth bricks by Houben and Guillaud<sup>76</sup> and AFNOR<sup>71</sup>; CRATerre-EAG<sup>72</sup> (after Bruno<sup>9</sup>).

245 **3.2. Plant-derived urease enzyme**

246 The present work has explored a new cost-effective EICP stabilisation method that is based on the use  
247 of crude urease enzyme extracted from soybeans. Soybeans have been either centrifuged to extract a  
248 liquid, i.e. water-diluted, juice or crushed into a dry fine powder resulting in two alternative enzyme  
249 sources, which were both tested to assess their respective effectiveness.

### 250 3.2.1. Soybeans liquid extract

251 The procedure for obtaining the liquid urease extract consisted in soaking dry soybeans into distilled  
252 water (1g of dry soybeans to 10 ml of distilled water) for 24 hours before centrifuging both beans and  
253 water in a 700-Watt blender for 1 minute (Fig. 3). This resulted in the extraction of a semi-transparent  
254 grey-yellow juice, which was collected in a glass while the crushed beans were retained in the blender.  
255 To increase the enzyme concentration, the crushed beans were recovered from the blender and  
256 centrifuged two more times to extract additional liquid that was mixed with the previously collected  
257 juice.



258  
259 Fig. 3: Procedure for obtaining soybeans liquid extract: soybeans are soaked in water (a), soaked soybeans are  
260 centrifuged in a blender (b), soybeans juice is collected (c).

### 261 3.2.2. Fine soybeans powder

262 An alternative procedure was also followed to obtain the urease enzyme in the form of fine soybeans  
263 powder. Soybeans were crushed by using an electrical grinder and passed through a sieve to collect the  
264 finest particles with dimensions smaller than 0.400 mm (Fig. 4). This cut-off size was chosen to boost  
265 the fine fraction of the earth mix and to avoid that material imperfections, caused by the presence of  
266 large inclusions, might increase vulnerability to water erosion.



267  
268 Fig. 4: Sieving of the fine fraction (smaller than 0.400 mm) of crushed soybeans.

### 269 3.3. Chemical reagents

270 The chemical reagents were urea and calcium chloride supplied by Labbox (Fig. 5), whose main  
271 properties are synthetically described in Table 2. Urea is a colourless and odourless organic material,  
272 which serves an important role in the metabolism of nitrogen compound and is the main nitrogen  
273 containing substance of the urine of mammals. It is also highly soluble in water and practically non-  
274 toxic. The hydrolysis of urea exhibits a high carbonate conversion rate compared to other calcium  
275 carbonate precipitation processes.<sup>14,79</sup> Calcium chloride is instead a salt that provides an excellent source  
276 of calcium ions because of its high hygroscopicity and solubility in water. The water solubility of  
277 calcium chloride at room temperature is up to 100 times greater than that of other calcium salts such as

278 calcium hydroxide or calcium nitrate, which makes calcium chloride a very effective reagent to generate  
279 high concentrations of calcium ions. This is an important aspect to consider during EICP stabilisation  
280 because larger concentrations of calcium ions correspond to greater calcium carbonate precipitation.<sup>36</sup>  
281



282

283

Fig. 5: Urea ( $\text{CO}(\text{NH}_2)_2$ ) powder and calcium chloride ( $\text{CaCl}_2$ ) flakes.

284

Table 2: Properties of the urea ( $\text{CO}(\text{NH}_2)_2$ ) and calcium chloride ( $\text{CaCl}_2$ ) used in the present work.

	<b>Chemical formula</b>	<b>Labbox reference</b>	<b>MW (g/mol)</b>	<b>Appearance</b>	<b>Assay (%)</b>
<b>Urea</b>	$\text{CO}(\text{NH}_2)_2$	UREA-00P-1K0	60.06	White crystals, powder	>99.6 %
<b>Calcium chloride</b>	$\text{CaCl}_2$	CACH-A0P-1K0	110.98	White pearls	>94 %

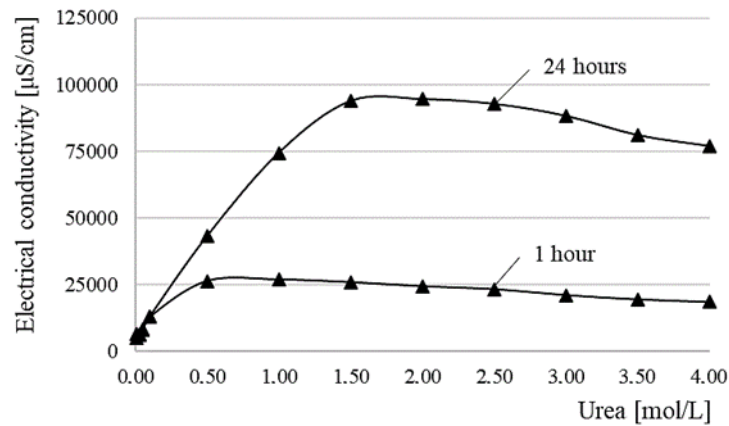
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### 286 3.4. Preliminary test-tubes experiments

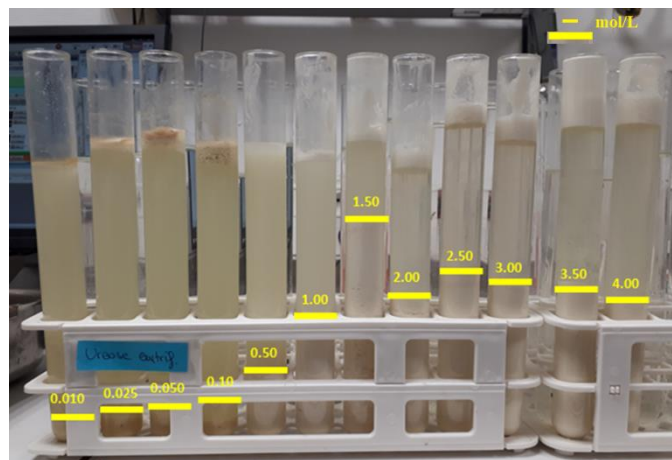
#### 287 3.4.1. Liquid soybeans extract

288 This section briefly recalls past research by the same authors<sup>80</sup> about the influence of reagents  
289 concentration on the efficiency of the EICP reactions inside test-tubes containing liquid soybeans  
290 extract. Twelve test-tubes with a capacity of 40 ml were filled with fresh liquid soybeans extract  
291 produced as described in Section 3.2.1. The extract in each test-tube was mixed with twelve different  
292 equimolar concentrations of urea and calcium chloride, namely 0.010 mol/L, 0.025 mol/L, 0.050 mol/L,  
293 0.10 mol/L, 0.50 mol/L, 1.00 mol/L, 1.50 mol/L, 2.00 mol/L, 2.50 mol/L, 3.00 mol/L, 3.50 mol/L and  
294 4.00 mol/L. The urea was added to the fresh liquid extract shortly after centrifugation of the soybeans,  
295 while the calcium chloride was added after 24 hours. Electrical conductivity was measured at 1 and 24  
296 hours after the addition of urea. Fig. 6 compares the values of electrical conductivity measured at these  
297 two times inside the test-tubes containing different concentrations of urea. The peak value of electrical  
298 conductivity changes from about 27000  $\mu\text{S}/\text{cm}$  for an urea concentration of 0.50 mol/L after 1 hour to  
299 about 100000  $\mu\text{S}/\text{cm}$  for an urea concentration of 2.00 mol/L after 24 hours. After 24 hours, equimolar  
300 concentrations of calcium chloride were introduced in each test-tube, which induced the rapid  
301 precipitation of calcium carbonate as visually observed. An equilibrium condition was reached after  
302 about 72 hours from the addition calcium chloride, with no more material being deposited at the bottom  
303 of the tubes (Fig. 7). The material inside each test-tube was then filtered and weighted, which showed  
304 that the amount of precipitate was largest for a concentration of reagents between 1.50 mol/L and 2.50  
305 mol/L. This concentration level is also consistent with the electrical conductivity values measured in  
306 Fig. 6 and with the visual observation of precipitate inside test-tubes in Fig. 7.





307  
 308 Fig. 6: Measurements of electrical conductivity 1 and 24 hours after adding urea to the liquid soybeans extract  
 309 (urea concentration varying from 0.010 mol/L to 4 mol/L) (after Cuccurullo et al.<sup>80</sup>).



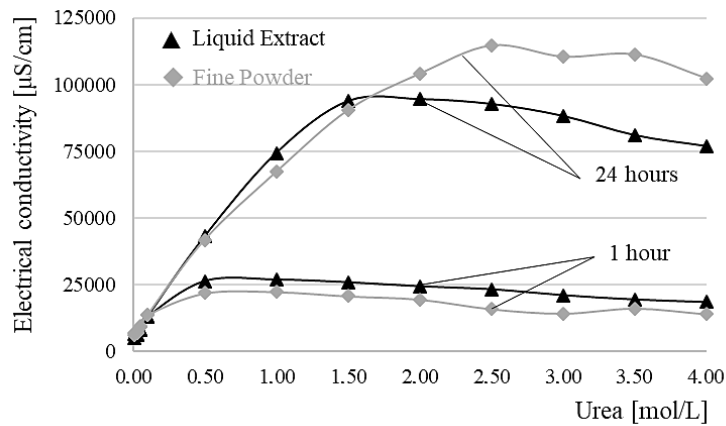
310  
 311 Fig. 7: Visual inspection of test-tubes 72 hours after adding calcium chloride (equimolar urea and calcium  
 312 chloride concentrations varying from 0.010 mol/L to 4 mol/L).

### 3.4.2. Fine soybeans powder

314 Past tests on the liquid soybeans extract were repeated, during the present work, on the fine soybeans  
 315 powder. As before, the objective was to find the optimum equimolar concentrations of urea and calcium  
 316 chloride that maximise precipitation of calcium carbonate. Twelve test-tubes were filled with 40 ml of  
 317 distilled water which was mixed with 4 g of the fine soybeans powder produced as described in Section  
 318 3.2.2. This amount of soybeans powder was chosen for consistency with the liquid extract, which was  
 319 produced by centrifuging dry soybeans and distilled water in the proportion of 1g:10 ml (see Section  
 320 3.2.1).

321 Similar to the previous tests, urea was added to each test-tube in twelve different concentrations of 0.010  
 322 mol/L, 0.025 mol/L, 0.050 mol/L, 0.10 mol/L, 0.50 mol/L, 1.00 mol/L, 1.50 mol/L, 2.00 mol/L, 2.50  
 323 mol/L, 3.00 mol/L, 3.50 mol/L and 4.00 mol/L. Fig. 8 shows the measurements of electrical conductivity  
 324 1 and 24 hours after the addition of urea to the test-tubes. As before, the electrical conductivity appears  
 325 to increase significantly over time with a maximum value of about 125000 µS/cm for a concentration  
 326 of 2.50 mol/L after 24 hours. Fig. 8 also includes the previous measurements of electric conductivity on  
 327 the liquid soybeans extract and indicates that the fine powder leads to higher levels of electrical  
 328 conductivity for higher concentrations of urea after 24 hours while a negligible difference between the  
 329 two cases is observed after 1 hour.

330



331  
 332 Fig. 8: Comparison of electrical conductivity measurements 1 and 24 hours after adding urea to test-tubes (urea  
 333 concentration varying from 0.010 mol/L to 4 mol/L).

334 The subsequent addition of equimolar concentrations of calcium chloride to the test-tubes led to the  
 335 rapid formation of a sticky yellow semi-liquid paste (Fig. 9). Unlike the case of the liquid soybeans  
 336 extract, the consistency of the paste did not allow collection of the precipitated calcium carbonate by  
 337 filtration (see Section 3.4.1.).



338  
 339 Fig. 9: Formation of a semi-liquid paste after addition of calcium chloride to test-tubes containing distilled water,  
 340 soybeans fine powder and urea (urea/calcium chloride concentration of 2.50 mol/L).

#### 341 4. EXPERIMENTAL PROGRAM ON STABILISED EARTH

##### 342 4.1. Samples manufacture

343 Three different stabilisation methods were selected for the campaign of laboratory tests on earth  
 344 samples. For two of these methods, the source of urease enzyme was provided by either the liquid  
 345 soybeans extract and a suspension of fine soybeans powder in distilled water. In the third method, a  
 346 suspension of fine soybeans powder in liquid soybeans extract was instead employed. In all cases, the  
 347 cementing solution was completed by adding urea and calcium chloride in equimolar concentrations of  
 348 2.50 mol/L. This concentration was chosen based on the previous test-tube experiments (see section  
 349 3.4), which suggested a maximum carbonate precipitation for this amount of reagents.

350 The three different stabilisation methods are summarised in Table 3 using the following codes:

- 351 - stabilisation using liquid soybeans extract is referred as SC;
- 352 - stabilisation using fine soybeans powder suspended in distilled water is referred as SP;
- 353 - stabilisation using fine soybeans powder suspended in liquid soybeans extract is referred as SCP.

354  
 355  
 356  
 357  
 358

Table 3: Composition of cementing solution for SC, SP and SCP stabilisation methods.

Stabilisation method ID	Liquid soybeans extract	Fine soybeans powder	Distilled water	Reagents (2.50 mol/L urea/calcium chloride)
SC	×	-	-	×
SP	-	×	×	×
SCP	×	×	-	×

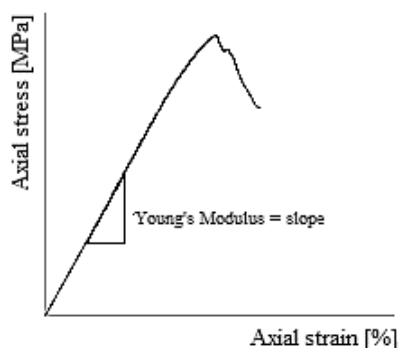
360 For the SP and SCP stabilisation methods, the amount of soybeans powder was equal to 1/12 of the dry  
 361 sample mass. This relatively little amount of soybeans powder was chosen to limit the growth of mould  
 362 on the sample surface during prolonged exposure to water. Finally, as a term of comparison, a set of  
 363 control (unstabilised) samples made of dry earth and distilled water were prepared according to the same  
 364 manufacturing procedure, as described below.

365 Cylindrical earth samples with a diameter of 50 mm and a height of 100 mm were manufactured as  
 366 follows: (i) the dry earth was passed through a 2 mm sieve to remove the largest particles; (ii) the sieved  
 367 material was carefully mixed with an amount of cementing solution equal to the Proctor optimum water  
 368 content of the Bouisset soil, that is 12.4%; (iii) the moist soil was statically compacted in 10 layers inside  
 369 a cylindrical mould to attain the Proctor optimum dry density of the Bouisset soil, that is 1.97 g/cm<sup>3</sup>  
 370 (note that the earth was compacted immediately after mixing it with the cementing solution to prevent  
 371 the premature precipitation of calcium carbonate crystals); (iv) the surface of each compacted earth layer  
 372 was lightly scarified before applying the subsequent layer; (v) the final samples were equalised under  
 373 controlled humidity ( $40 \pm 5$  %) and temperature ( $20 \pm 5$  °C) until the mass changed less than 0.1 % over  
 374 at least one week.

375

## 376 4.2. Mechanical properties

377 After equalisation, the cylindrical samples were subjected to unconfined compression tests with a  
 378 constant axial displacement rate of 0.001 mm/s. Three samples were tested for each stabilisation method  
 379 to confirm the repeatability of measurements. The peak compressive strength and Young's modulus  
 380 were calculated as the average of these three tests. The Young's modulus was simply measured as the  
 381 slope of the tangent to the stress-strain curve over the initial stress range where the material response is  
 382 reasonably linear (Fig. 10).

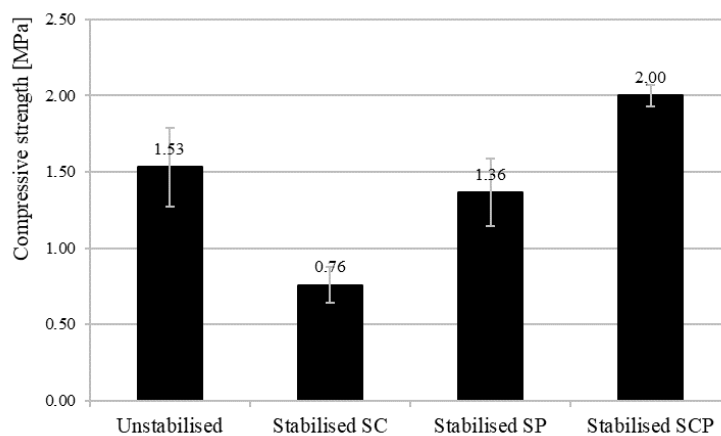


383

384 Fig. 10: Stress-strain curve from unconfined compression tests and measurement of the Young's modulus as the  
 385 slope of the initial tangent to the stress-strain curve.

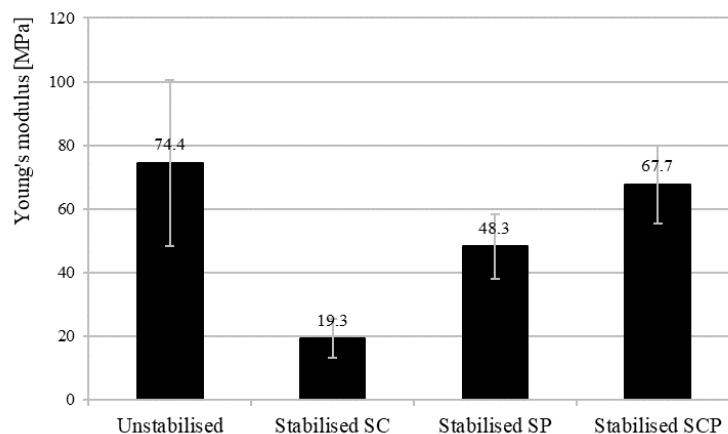
386 Fig. 11 shows the average peak compressive strength of all stabilised and unstabilised samples together  
 387 with the corresponding standard deviation bars. Inspection of Fig. 11 indicates that compressive strength  
 388 varies depending on the chosen stabilisation method with the lowest, intermediate and highest levels  
 389 recorded for the SC, SP and SCP stabilised samples, respectively. Interestingly, only SCP stabilisation,  
 390 which is a combination of the SC and SP methods, generates a level of strength which is higher than  
 391 that of the unstabilised samples. Instead, the use of the liquid soybeans extract as a source of urease  
 392 enzyme (SC stabilisation) markedly undermines the mechanical properties of the material resulting in a  
 393 compressive strength that is twofold lower than that of the unstabilised samples. This result is in apparent  
 394 contradictions with the observations made in test-tube experiments (Cuccurullo et al.<sup>80</sup>), where  
 395 precipitation of carbonate crystals was observed in a solution of liquid soybeans extract, urea and  
 396 calcium chloride. An explanation might be given by the presence of proteins, other than the urease  
 397 enzyme, which may affect the mechanical performance of the earth material. This is an important aspect  
 398 that should be investigated in detail during future research. The visual examination of SP and SCP  
 399 stabilised samples also highlighted the formation of a crystallised white covering on the external material  
 400 surface. This observation may provide further evidence of the greater efficiency of the fine soybeans  
 401 powder as a source of urease enzyme, either alone or in combination with the liquid soybeans extract.

402 Fig. 12 shows the average measurements of Young's modulus of all stabilised and unstabilised samples  
 403 together with the corresponding standard deviation bars. Like compressive strength, the Young's  
 404 modulus varies depending on the chosen stabilisation method with the lowest, intermediate and highest  
 405 levels of stiffness recorded for the SCP, SP and SC stabilised samples, respectively. In this case, all  
 406 three stabilisation methods generate lower values of Young's modulus compared to the unstabilised  
 407 material.



408

409 Fig. 11: Compressive strength measured from unconfined compression tests on unstabilised, SC stabilised, SP  
 410 stabilised and SCP stabilised samples.



411

412 Fig. 12: Young's modulus measured from unconfined compression tests on unstabilised, SC stabilised, SP  
413 stabilised and SCP stabilised samples.

#### 414 4.3. Hygroscopic properties

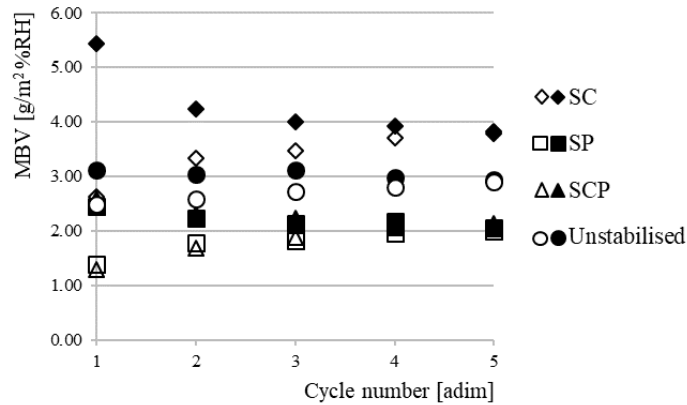
415 The hygroscopic behaviour of the stabilised earth was investigated via the measurement of the moisture  
416 buffering value (MBV) corresponding to cyclic daily variations of ambient humidity. Samples were  
417 exposed to step cycles of relative humidity, between 53% and 75%, under a constant temperature of 23  
418 °C inside a climatic chamber (CLIMATS Type EX2221HA). Each of these two humidity levels was  
419 maintained for twelve hours while the sample mass was recorded every two hours as prescribed by the  
420 norm ISO 24353<sup>81</sup>. Each sample was placed in the upright position inside an aluminium foil pan so that  
421 only the top and lateral surfaces were directly exposed to the atmosphere inside the climatic chamber.  
422 The total area of the exposed surface was, therefore, higher than the minimum value of 0.010 m<sup>2</sup> required  
423 by the norm ISO 24353.<sup>81</sup>

424 Before the start of the tests, all samples were equalised at a temperature of 23 °C and a relative humidity  
425 of 53%. Equalisation was assumed to be complete when the mass of the sample changed less than 0.1%  
426 over at least one week, which took generally a period of two weeks. After equalisation, the samples were  
427 exposed to the cyclic changes of relative humidity, as previously described. Distinct MBVs were  
428 calculated during the moisture uptake and moisture release stages of each cycle according to the  
429 following equation:

$$430 \quad MBV = \frac{\Delta m}{S \Delta \%RH} \quad (6)$$

431 where  $\Delta m$  is the absolute value of the sample mass variation (in grams) at end of each relative humidity  
432 stage,  $S$  is the exposed surface (in square meters) and  $\Delta \%RH$  is the imposed change of relative humidity  
433 (in percentage). The values of  $\Delta m$  measured at the end of a moisture uptake stage (i.e. at the end of the  
434 exposure to the high humidity level) provide the “MBV uptake” while the values of  $\Delta m$  measured at the  
435 end of a moisture release stage (i.e. at the end of the exposure to the low humidity level) provide the  
436 “MBV release”. To take into account the change of sample dimensions caused by swelling, the exposed  
437 surface was calculated from the average of three height measurements and three diameter measurements  
438 taken at the beginning of the test (i.e. at  $T = 23$  °C and  $RH = 53\%$ ) and at the end of each moisture  
439 uptake stage (i.e. at  $T = 23$  °C and  $RH = 75\%$ ). This assures that small variations of sample dimensions,  
440 due to swelling upon wetting at high humidity, are duly considered. To confirm the repeatability of  
441 measurements, three replicate samples were tested, with the relevant MBVs calculated as the average of  
442 these three measurements.

443 Fig. 13 shows the variation of both MBV uptake and MBV release over the consecutive humidity cycles  
444 for all stabilised and unstabilised samples (solid markers indicate the MBV uptake while hollow markers  
445 indicate the MBV release). As expected, the MBVs of the uptake and release stages tend to converge  
446 towards the same steady state value as the number of cycles increases. In general, five cycles were  
447 sufficient to attain steady state conditions for all materials.



448

449 Fig. 13: MBVs measured during the uptake and release stages of subsequent humidity cycles on unstabilised, SC  
 450 stabilised, SP stabilised and SCP stabilised samples. Solid markers indicate the MBV uptake while hollow  
 451 markers indicate the MBV release.

452 The final MBVs of the different materials were calculated as the averages of the uptake and release  
 453 values of the last three stable cycles (Table 4). A stable cycle is defined as a humidity cycle where the  
 454 uptake and release values are approximately equal, thus indicating the achievement of steady state  
 455 conditions. Inspection of Table 4 indicates that the unstabilised earth exhibits a moisture buffering  
 456 capacity that is greater than that of the SP and SCP stabilised earth, which incorporates the fine soybeans  
 457 powder as a source of urease enzyme. An explanation of this result is proposed later when the results of  
 458 water immersion tests are presented. Yet, even for the SP and SCP stabilised earth, the MBV value  
 459 remains larger than 2, which indicates good hygroscopic properties.

460 Interestingly, the highest moisture buffering capacity is recorded for the SC stabilised earth, which  
 461 incorporates the liquid soybeans extract as a source of urease enzyme. In this case, a very high MBV  
 462 close to 4 is attained, which is even greater than that of the unstabilised earth. A possible explanation of  
 463 this result might be found in the modification of the pore size distribution of the earth due to the  
 464 precipitation of calcium carbonate inside the small voids that have been penetrated by the liquid  
 465 soybeans extract. This may increase the fraction of nanopores compared to the unstabilised earth, thus  
 466 increasing the material capacity of absorbing/releasing vapour under changing ambient humidity.  
 467 Therefore, SC stabilisation might not be very effective in increasing the stiffness and strength of the  
 468 earth but offers a way to improve the material hygroscopic properties.

469 Table 4: MBVs of unstabilised, SC stabilised, SP stabilised and SCP stabilised samples.

Sample ID	MBV [g/m²%RH]
Unstabilised soil	2.91
SC	3.79
SP	2.03
SCP	2.07

470

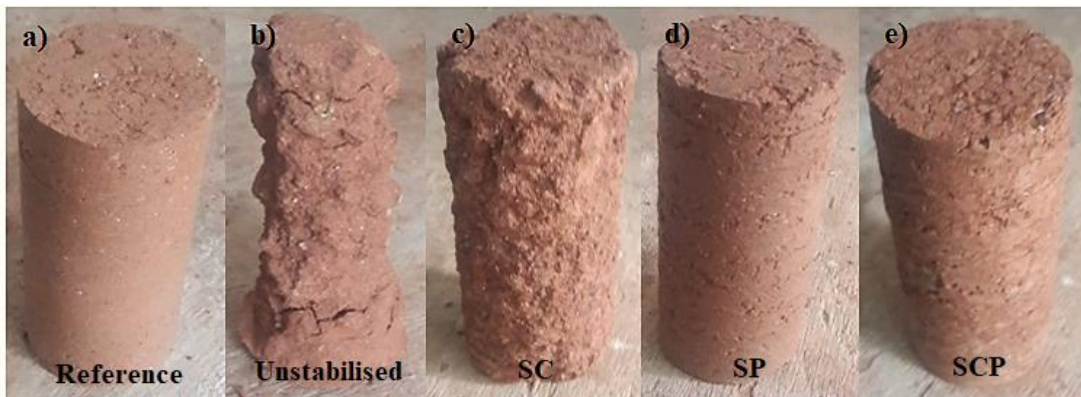
#### 471 4.4. Durability properties

472 Durability against water erosion was assessed by means of immersion tests according to the norm DIN  
 473 18945.<sup>82</sup> After equalisation to the laboratory atmosphere (corresponding to a temperature of  $20 \pm 5$  °C  
 474 and a relative humidity of  $40 \pm 5\%$ ), the samples were weighed to record their initial mass  $m_i$  and then  
 475 immersed in water for ten minutes. They were subsequently removed from water and equalised again to

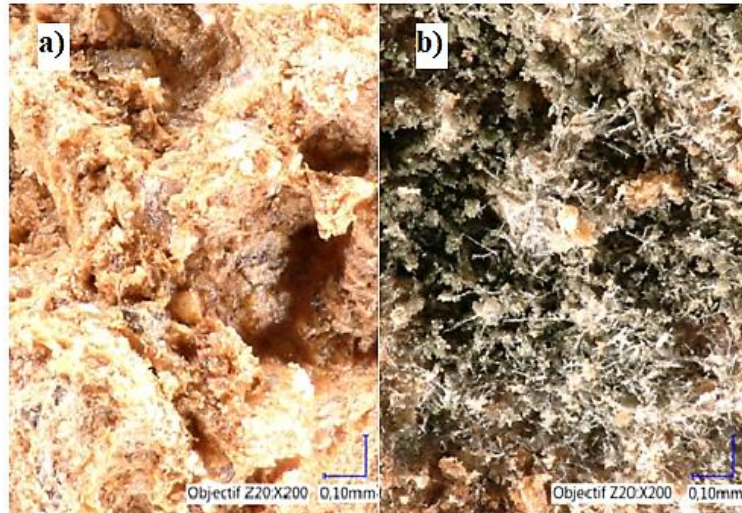
476 the laboratory atmosphere to attain the same moisture content as before immersion. After equalisation,  
477 the final mass  $m_f$  was recorded and introduced, together with the initial mass  $m_i$ , in the following  
478 equation to calculate the percentage mass loss  $\% \Delta m$  :

479 
$$\% \Delta m = \frac{m_i - m_f}{m_i} \times 100 \quad (7)$$

480 Each test was performed in three replicates to confirm the repeatability of the experimental procedure.  
481 The mass loss was about 42% for the unstabilised samples but reduced to 13% for the SC stabilised  
482 samples. An even better result was obtained by SP and SCP stabilisation, when only 1% of the initial  
483 mass was lost. Fig. 14 compares the pictures of the different samples before and after immersion  
484 confirming that a significant improvement of durability is achieved by means of enzymatic stabilisation,  
485 especially when the fine soybeans powder is used as a source of urease enzyme. Interestingly, the  
486 introduction of soybeans powder as a source of urease enzyme improves the erosion resistance of the  
487 material to levels that are acceptable for routine building applications. The lower mass loss experienced  
488 by the stabilised samples may be explained by the formation of calcium carbonate bonds between earth  
489 particles and by the partial occlusion of voids with a consequent reduction of permeability to water. Fig.  
490 15 compares the images captured by a 4K digital KEYENCE microscope on the surface of SC and SP  
491 stabilised samples. The growth of organic ramifications on the surface of SP stabilised earth may be  
492 responsible for the occlusion of voids, which explains the hydrophobic behaviour and relatively low  
493 moisture buffering capacity of this material (see Section 4.3).



495 Fig. 14: Reference sample before immersion (a); unstabilised sample after immersion exhibiting a mass loss of  
496 42% (b); SC stabilised sample after immersion exhibiting a mass loss of 13% (c); SP stabilised sample after  
497 immersion exhibiting a mass loss of 1% (d); SCP stabilised sample after immersion exhibiting a mass loss of 1%  
498 (e).



499

500 Fig. 15: Images captured by a 4K digital KEYENCE microscope on the surface of SC stabilised samples (a) and  
501 SP stabilised samples (b). Note the organic ramifications on the surface of the SP stabilised sample.

#### 502 4.5. X-Ray Diffraction (XRD) analysis

503 Five grams of the unstabilised, SC stabilised and SP stabilised material were ground into a fine powder  
504 and air dried to the laboratory environment before performing a X-Ray Diffraction (XRD) analysis.  
505 Results from these XRD tests revealed the presence of kaolinite, microcline intermediate, muscovite,  
506 quartz and calcium carbonate in the form of calcite (Figs. 16-18). Calcite was identified in all samples,  
507 both unstabilised and stabilised. An approximate XRD quantitative analysis was performed based on the  
508 construction of diffraction peak mathematic function model by means of polynomial whole pattern  
509 fitting to the spectral lines using computer software (Rietveld refinement). This indicated an amount of  
510 calcite of about 2% for the unstabilised samples compared to about 8% for the SP stabilised samples.



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512

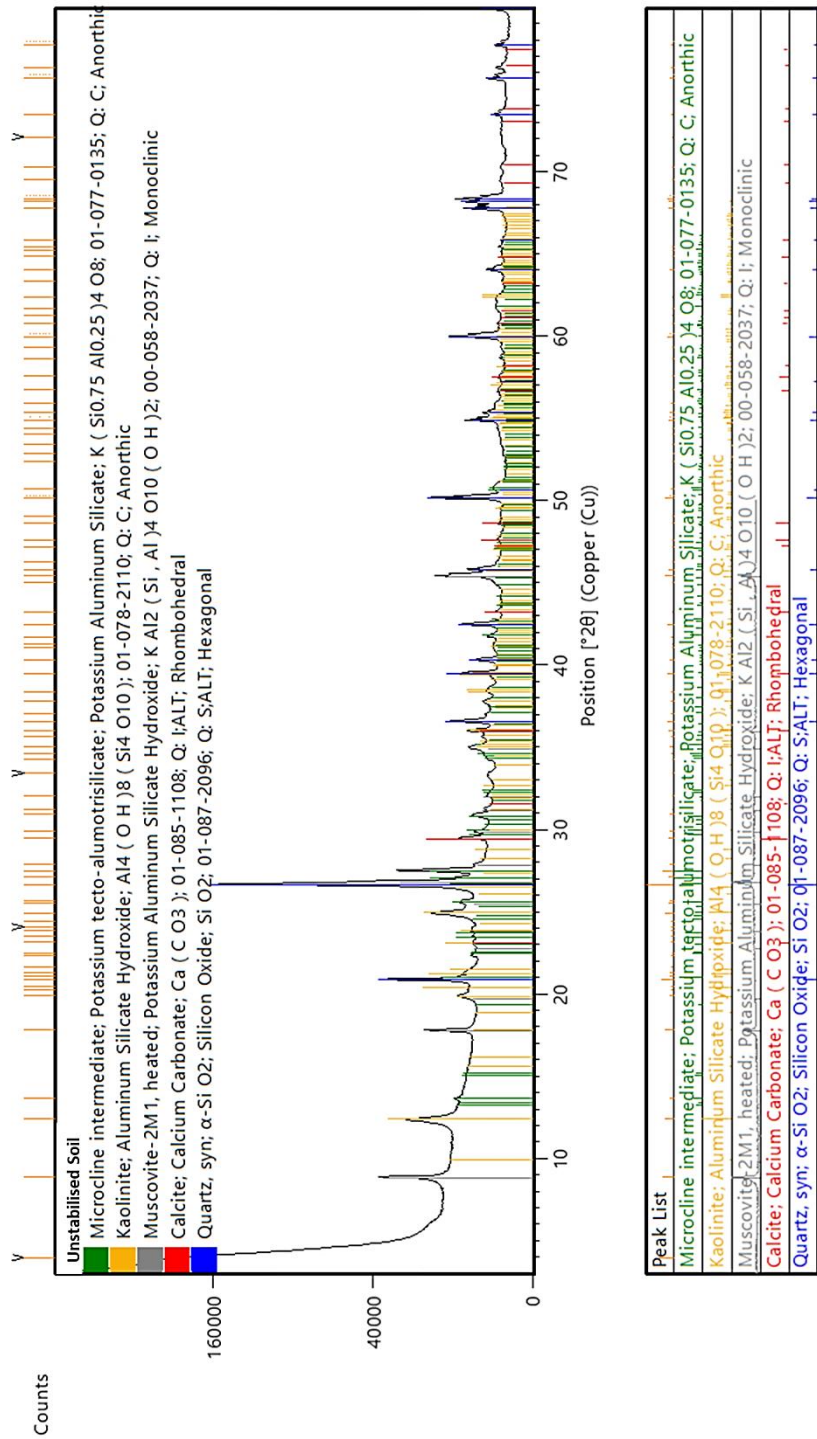


Fig. 16: XRD analysis on unstabilised sample.

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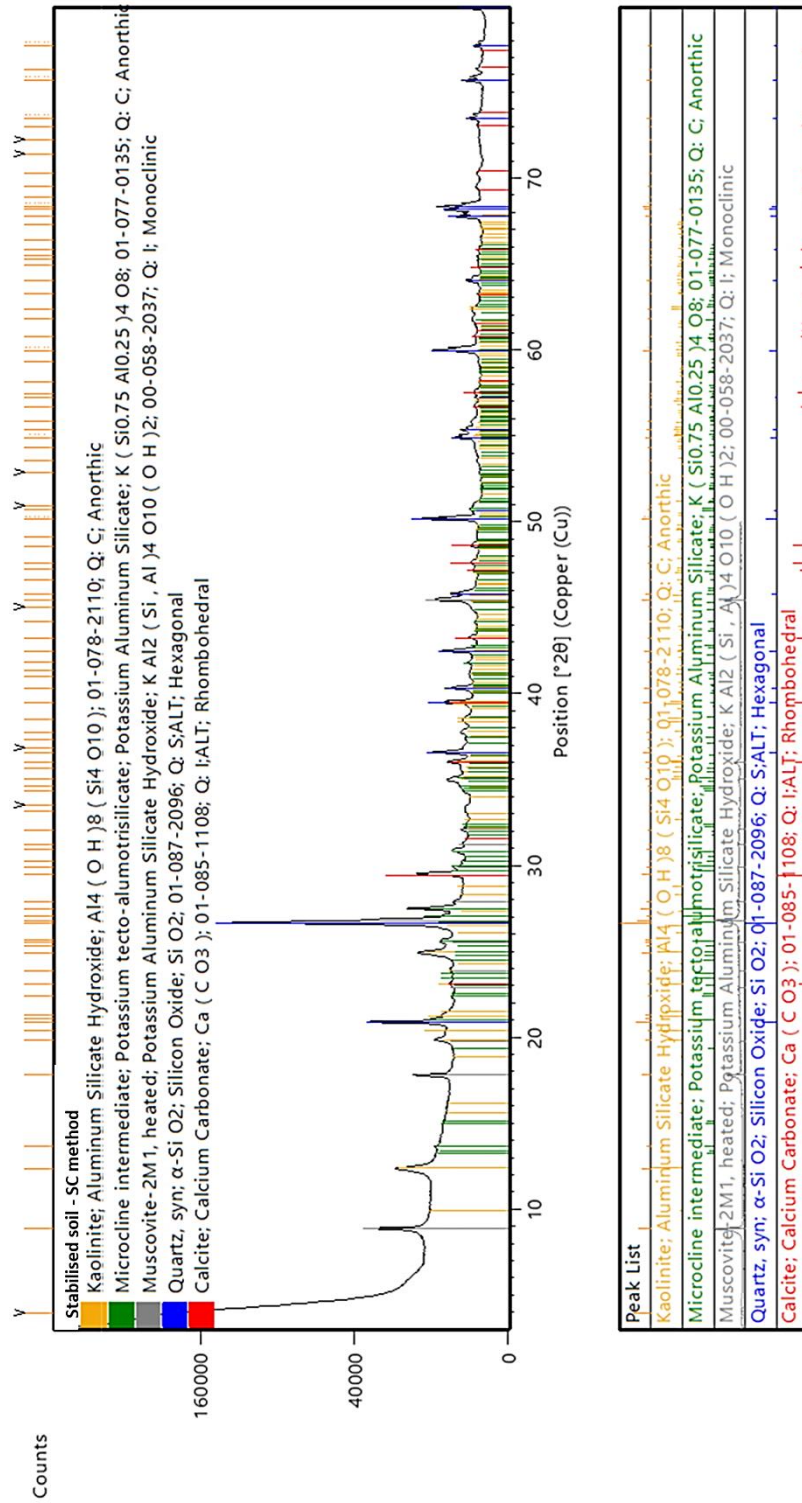
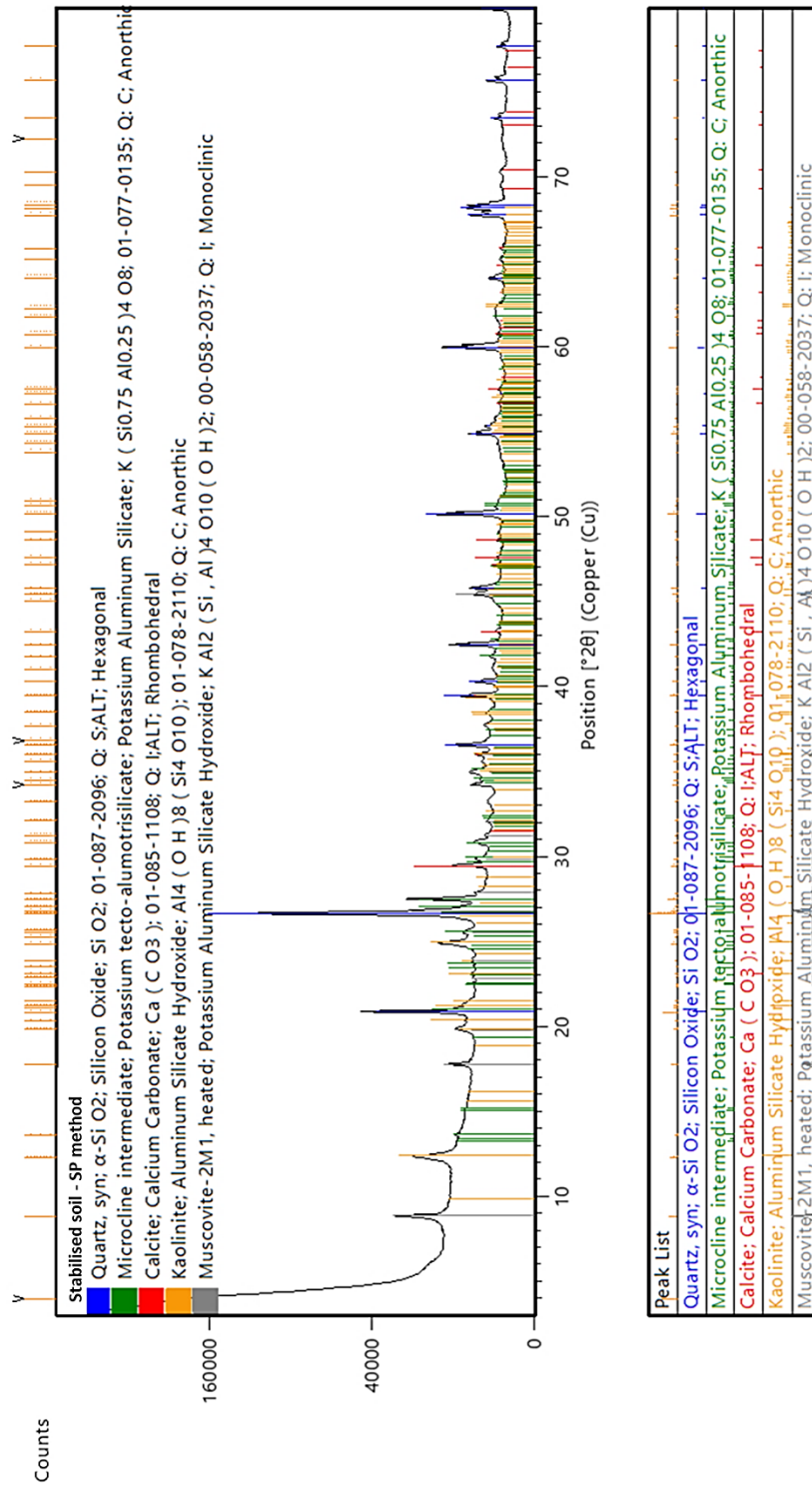


Fig. 17: XRD analysis on SC stabilised sample.



515  
516

Fig. 18: XRD analysis on SP stabilised sample.

517 **5. CONCLUSIONS**

518 This paper has presented an experimental study of enzyme induced carbonate precipitation (EICP) as a  
519 technique for improving the hygro-mechanical and durability properties of compacted earth in building  
520 applications. The main outcomes of this work are summarised as follows:

- 521 - Earth materials have been stabilised by exploiting the urease enzyme that is present in soybeans  
522 to catalyse hydrolysis of urea inside the pore water which leads to the production of carbonate

- 523 ions and the precipitation of calcium carbonate. Both a liquid soybeans and a fine soybeans  
524 powder provide a good source of the urease enzyme as a catalyst of the hydrolysis of urea.
- 525 - Results of unconfined compression tests on stabilised earth samples indicate that material  
526 strength changes depending on the chosen stabilisation method with the highest levels of  
527 strength obtained when the fine soybeans powder and liquid soybeans extract are used together  
528 as a source of the urease enzyme. Interestingly, if the fine soybeans powder and liquid soybeans  
529 extract are used alone, strength levels drop to values that are lower than those of the unstabilised  
530 material.
- 531 - Unstabilised earth has an excellent capacity to buffer moisture and hence exhibits a high hygro-  
532 thermal inertia. This capacity is not significantly changed by the stabilisation methods tested in  
533 the present work. The moisture buffering capacity of the material reduces slightly when the fine  
534 soybeans powder is employed while it increases when the liquid soybeans extract is used.
- 535 - The water durability of both unstabilised and stabilised earth has been assessed by immersion  
536 tests according to the norm DIN 18945<sup>82</sup>. During water immersion, the unstabilised samples lost  
537 about 42% of their initial mass while the samples stabilised with a combination of liquid  
538 soybeans extract and fine soybeans powder exhibited a mass loss of only 1%. This negligible  
539 mass loss makes the stabilised material adequate for exposure to natural weathering according  
540 to the norm DIN 18945.<sup>82</sup> Digital microscope images have showed the presence of organic  
541 ramifications on the surface of the earth stabilised with the fine soybeans powder. This may  
542 explain the hydrophobic behaviour and smaller moisture buffering capacity of this material  
543 compared to the unstabilised one.
- 544 - Results from X-Ray Diffraction (XRD) analyses of both stabilised and unstabilised earth have  
545 revealed the presence of minerals like kaolinite, microcline intermediate, muscovite, quartz and  
546 calcium carbonate in the form of calcite. An approximate quantitative interpretation of these  
547 results detected an amount of calcite of about 2% in the unstabilised samples, which increased  
548 to about 8% in the samples stabilised with the fine soybeans powder.

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