

## Optimum Condition for the Application of Enzyme-Mediated Calcite Precipitation Technique as Soil Improvement Method

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**Abstract**— The optimum condition of enzyme-mediated calcite precipitation has been evaluated for its possible application as a soil improvement technique. Magnesium chloride ( $MgCl_2$ ) and magnesium sulfate ( $MgSO_4$ ) were substituted to the grouting solution composed of urease, urea, and calcium chloride ( $CaCl_2$ ), and its effects on the precipitation process, amount, and the mineralogical substances of the precipitated materials were investigated. The evolution of the strength of treated sand was also evaluated through unconfined compressive strength (UCS) tests. The substitution of magnesium compounds was found to be able to augment the precipitated amount and reduce the hydrolysis rate of urea. The mineralogical analysis indicated that the addition of magnesium to the grouting solution was a potential method for promoting the formation of aragonite. Furthermore, the formation of gypsum was also promoted when magnesium sulfate was substituted. The mechanical analysis showed that the producing of the high precipitated amount resulted in the significant improvement in the strength of the treated sand. The relation between the UCS of the treated soil and the precipitated mass indicated that the strength could be controlled by the precipitated mass within the soil.

**Keywords**— EMCP; calcite; magnesium; aragonite; hydrolysis rate; soil improvement

### I. INTRODUCTION

Several methods have been developed for their various possible applications as soil improvement technique, such as the application of natural and synthetic materials (i.e., recycled glass fiber, tires, geosynthetics) [1]–[5], injection of grouting material or deep mixing [6], [7] and application of sand column or stone column [6], [8]. The majority of the present techniques are dependent on the mechanical or man-made material, which requires the substantial energy for production and installation [9]. The application of grouting technique using the chemical material is the most commonly used in soil improvement method [10]. Chemical grouting can be achieved with a variety of additives material including cement, lime, asphalt, sodium silicate, depends upon the objective of the improvement [3], [11], [12].

In this technique, chemical grouting is injected into soil. Thus, the chemicals harden in the voids, binding the soil particles, and increasing the strength of soil and reducing the permeability of the soil. The grouting technique can use to improve the engineering properties of soil below the existing structure. However, the chemical material often increases the pH of soils to highly alkaline levels and thus may contaminate the soil and cause the serious environmental

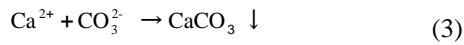
problems [9], [10], [13]. The environmental concern for the chemical grouting should be considered. Furthermore, the high viscosity and fast hardening rate of the chemical cause the abruptly reduce the permeability of the soil, hence the treatment area is limited.

The above-mentioned limitations in the current practice of soil improvement necessitate exploration of the alternative of soil improvement technique that should be environmentally friendly, sustainable, and able to fulfill the increasing demands for soil improvement, especially for civil engineering infrastructure developments. Hence, the promising techniques for utilization of biological process have been recently proposed, which have been made possible through interdisciplinary research at the confluence of microbiology, geochemistry and geotechnical engineering.

One of the emerging and promising biochemical soil improvement methods is biocementation which is called calcite precipitation method (CPM) [14]–[18]. Enzyme-mediated calcite precipitation (EMCP) may be one of the promising methods [16], [19]. The increasing in unconfined compressive strength ranges from 200 kPa to 1.6 MPa depending upon the amount of the precipitated calcite [16], [18]–[20].

This technique employs the enzyme of urease to dissociate urea into ammonium ( $NH_4^+$ ) and carbonate ( $CO_3^{2-}$ )

ions. The carbonate ions are precipitated as calcite crystal in the presence of calcium ions ( $\text{Ca}^{2+}$ ). The reaction of the urea hydrolysis and the calcite formation are expressed in Equations (1)-(3).



An enzyme-reagents mixed solution (i.e., purified urease and  $\text{CaCl}_2$ -urea), which produces the precipitated calcite, is injected into the soil. The calcite may provide bridges between the grains of sand, restricting their movement, and hence, improving the engineering properties of the sand [21]. A schematic of the precipitation process on EMCP technique and the improving mechanism expected are illustrated in Fig. 1.

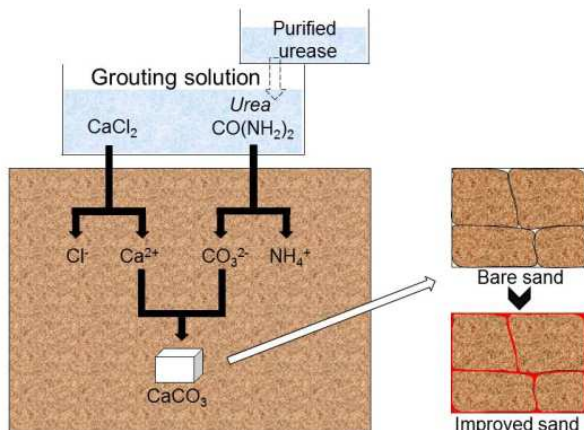


Fig. 1 Schematic of calcite precipitation process on EMCP technique [19]

Calcite precipitation methods require many injection wells to obtain a sufficient amount of the calcite to improve the strength of porous media [22]. Whiffin et al. [15] reported that precipitated calcite comprising approximately 4% of soil mass should be precipitated in the treated sand in order to obtain the strength of 0.3 MPa. However, the highly concentrated reagents (urea and  $\text{CaCl}_2 > 0.5$  mol/L) decrease the efficiency of calcite precipitation [16], [23]. Reagents with concentrations of 0.05–0.50 mol/L, were reported to be the most efficient in the application of calcite precipitation techniques [16], [23], [24].

Upscale of the application of calcite precipitation method, from the laboratory scale to the real field, and the uniform distributions of precipitated minerals within the soil are the main challenges [20], [25]. Several methods have been developed to enhance the uniformity of the precipitated distribution, such as the one controlling the reaction rate and modifying the injection pattern [7], [14], [25].

The addition of magnesium in the carbonation process was reported as a potential method to control the precipitation rate [26]–[28]. The presence of magnesium ions in the calcination process, which has  $\text{pH} < 11$ , was also possible to promote the formation of aragonite in addition to calcite [29]. Magnesium ions are found to be the most effective ions for the aragonite formation [30]. Aragonite also has higher Mohs hardness than calcite, namely, 3.25–4.00 and 3.00, respectively [31], [32].

In the present work, magnesium chloride ( $\text{MgCl}_2$ ) and magnesium sulfate ( $\text{MgSO}_4$ ) were substituted to the grouting solution composed of urease, urea, and calcium chloride ( $\text{CaCl}_2$ ), and its effects on the precipitation process, amount, and the mineralogical substances of the precipitated materials were explicitly investigated. Magnesium compounds were added to optimize the applicability of EMCP technique. The evolution of mineralogical substances of precipitated minerals and the improvement in the engineering properties of soil were also investigated by X-ray diffraction (XRD) and unconfined compressive strength (UCS), respectively.

## II. MATERIAL AND METHOD

### A. Material

The mixed solutions of reagents, i.e.,  $\text{CaCl}_2$ ,  $\text{MgCl}_2$ ,  $\text{MgSO}_4$ , urea, and purified urease, were used as the grouting material in this study.  $\text{CaCl}_2$ ,  $\text{MgCl}_2$ ,  $\text{MgSO}_4$ , and urea, with purity levels greater than 95%, were obtained from Kanto Chemicals Co., Inc., Tokyo, Japan. As the biocatalytic dissociation of urea, the purified enzyme of urease from jack bean meal was obtained from the Kishida Chemical Co., Inc., Japan. The poorly graded silica sand with  $e_{\text{max}}$ ,  $e_{\text{min}}$ , the coefficient of uniformity (CU), and the specific gravities (Gs) of 0.899, 0.549, 1.550, and 2.653, respectively, was used as the soil sample. The curve of grain size distribution of silica sand is shown in Fig. 2.

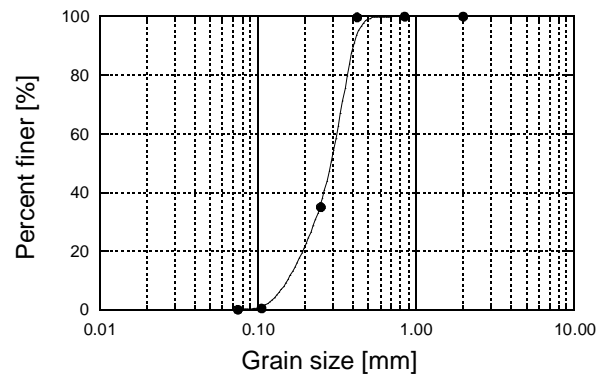


Fig. 2 Grain size distribution of silica sand [33], [34]

### B. Test-tube Experiment

The precipitation of calcite is evaluated directly in the transparent polypropylene (PP) tubes. The experimental procedures developed by Neupane et al. [16] were adopted in this study. Magnesium compounds (i.e.,  $\text{MgCl}_2$  and  $\text{MgSO}_4$ ) were newly substituted for the injecting solution composed of urea and  $\text{CaCl}_2$ . Magnesium chloride with the concentrations of 0.10 and 0.20 mol/L and magnesium sulfate with the concentration of 0.04 and 0.10 were added to obtain the total concentrations of  $\text{CaCl}_2$ -Mg of 0.50 and 1.0 mol/L, respectively. Purified urease with the concentration 1.0 and 2.0 g/L were utilized to dissociate 0.50 and 1.00 mol/L of urea, respectively. The enzyme of urease was mixed with distilled water for 2 mins and was filtered using filter paper (pore size of 11  $\mu\text{m}$ ) to remove the undissolved particle of urease. The filtered urease and the reagents solution (i.e., urea,  $\text{CaCl}_2$ ,  $\text{MgCl}_2$ ,  $\text{MgSO}_4$ ) were mixed thoroughly in the PP tubes, in a total solution volume of 30

mL and allowed to react for 3-day curing time. The procedures and experimental conditions for test-tube

experiments are shown in Fig. 3 and Table 1, respectively.

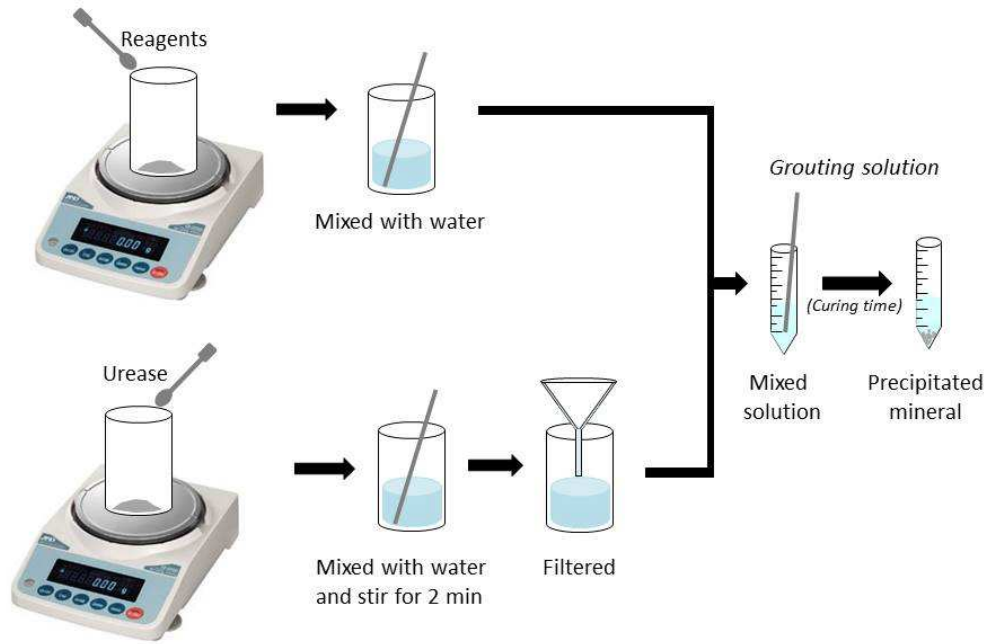


Fig. 3 Procedure of test-tube experiment [16]

TABLE I  
EXPERIMENTAL CONDITIONS FOR TEST-TUBE EXPERIMENTS

Case	Conc. of urease	Reagent concentration		Conc. of MgCl <sub>2</sub>	Conc. of MgSO <sub>4</sub>
		Urea	CaCl <sub>2</sub>		
	(g/L)	(mol/L)	(mol/L)	(mol/L)	(mol/L)
A1	1.00	0.50	0.50	-	-
A2	2.00	1.00	1.00	-	-
C1	1.00	0.50	0.45	0.05	-
C2	1.00	0.50	0.40	0.10	-
S1	2.00	1.00	0.96	-	0.04
S2	2.00	1.00	0.90	-	0.10

The hydrolysis rate was evaluated directly after the mixing. The experimental procedures developed by Whiffin et al. [35] were adopted. The evolution of the resistance with time was measured using LCR meter KC-555 KDK from Koyo Electronics Industries Co., Ltd, Tokyo, Japan, and the conductivity change was determined. A standard curve was provided by determining the conductivity resulting from the complete hydrolysis of several concentrations of urea. The hydrolysis rate is determined by calculating the gradient slope of the conductivity changes versus time, which is expressed in Equations (4)-(5).

$$\text{Conductance, (mS)} = \frac{1}{R} \quad (4)$$

$$\text{Hydrolysis rate, (}\frac{\mu\text{S}}{\text{g}}\text{)} = \frac{\theta_{ms}}{\theta_{sc}} \cdot v \cdot N \quad (5)$$

$R$  is measured resistance ( $\Omega$ ),  $\theta_{ms}$  is the gradient slope of measured sample,  $\theta_{sc}$  is the gradient slope of the standard curve,  $v$  is the volume of sample (L), and  $N$  is a final concentration of ammonia (mMol/L).

Test-tubes were kept without shaking at a room temperature of 20 °C. In order to evaluate the precipitated

amount, the grouting solution was filtered through filter paper (pore size of 11  $\mu\text{m}$ ). The undissolved particles on the filter paper and the particles remaining in the tubes are dried at 60 °C for 24 hrs, and then the total mass of the precipitated amount is examined [16], [36]. The morphologies substances of the dried precipitated materials were also characterized by X-ray diffraction (XRD). Finally, by comparing the hydrolysis rate, precipitated amount and XRD patterns within the grouting solution composed CaCl<sub>2</sub>-Urea, CaCl<sub>2</sub>-urea-MgCl<sub>2</sub> and CaCl<sub>2</sub>-urea-MgSO<sub>4</sub>, the effects of the substitution of magnesium compound are explicitly investigated.

### C. Unconfined Compressive Strength (UCS) Test

UCS tests were conducted to evaluate the effect of the substitution of magnesium compounds on the engineering properties of the soil. The experimental procedures developed by Putra et al. [36] were followed in this study. Soil specimens were prepared in PVC cylinders and treated with concentration-controlled solutions composed of reagents-enzyme. Polyvinyl chloride cylinders (5 cm in diameter and 10 cm in height) were used to prepare the sand samples. The fixed volume of the solution was injected into

the prepared sand specimens. The injected volume is controlled by the number of pore volumes (PV), and the amount of one PV is ~75 mL. The sand samples with a

relative density 50% were treated by 1-3 PV with a 3-day curing time. The procedure of sample preparation for UCS test is illustrated in Fig. 4.

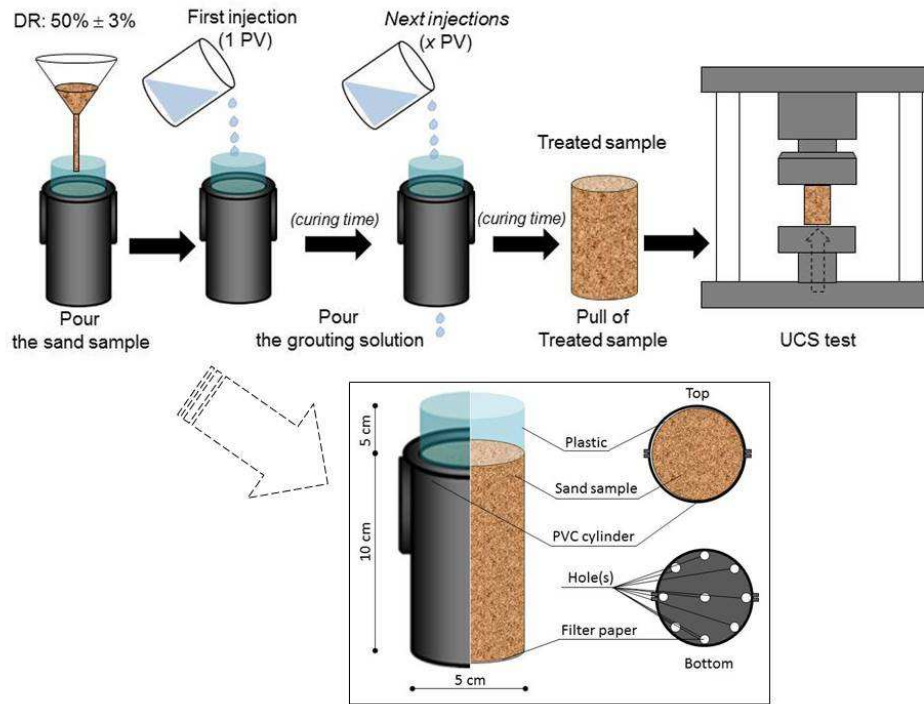


Fig. 4 Schematic of UCS tests [36]

The acid leaching method was used to evaluate the amount of precipitated calcite within the sand samples [16], [19], [21], [34]. Finally, by comparing the relationship between the precipitated amount and the UCS, the optimum conditions for the application of EMCP technique were explicitly evaluated.

### III. RESULTS AND DISCUSSION

The evolution of precipitation mass as the effect of the substitution of the magnesium compound is depicted in Figure 5. As is apparent, the precipitated amount increased as the magnesium compounds were added. In order to evaluate the effect of the substitution of magnesium on the efficiency of precipitation calcite, the precipitated amount is also shown as the precipitation ratio, namely, the ratio of the mass of the precipitated minerals to the maximum theoretical mass of precipitated  $\text{CaCO}_3$ . Fig. 5 reveals that the efficiency of precipitation calcite decreased in a high concentration of reagent. The reduction of the precipitation ratio of 13% was obtained when the reagents concentration were increased from 0.50 to 1.00 mol/L (case A1 and A2). The substitution of magnesium significantly enhanced the efficiency of precipitation calcite. The increase of precipitation ratio of 20% and 10% were achieved by the substitution of  $\text{MgCl}_2$  of 0.10 mol/L and of  $\text{MgSO}_4$  of 0.04 mol/L, respectively. A precipitation ratio of more than 100% was also obtained in the case of S2. The result indicated that the minerals other than calcite were also formed during the precipitation process. Hence, the XRD analyses were needed

to determine the mineralogical substances of the precipitated materials.

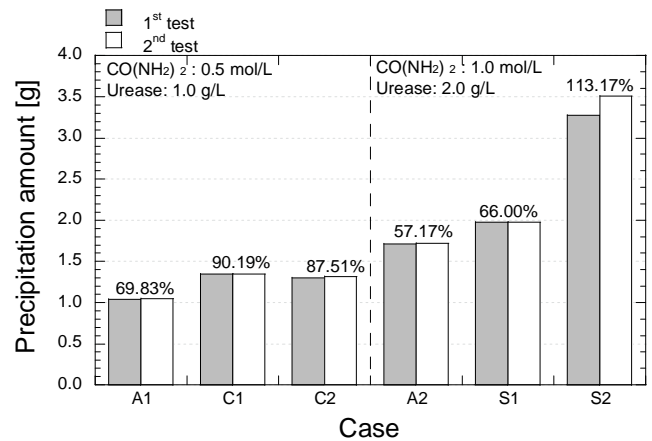


Fig. 5 Evolution of the precipitation amount [34], [36]

The effect of the substitution of magnesium compounds on the hydrolysis rate was also evaluated. The changes of conductance with time are shown in Fig. 6. As is apparent in Fig. 6 (a), the higher gradient slope of conductivity versus time was obtained from the larger concentration urea-urease. The result confirmed that the high concentration of urea-urease solution produced a high amount of ammonia and carbonate ions. Fig. 6(b) and 6(c) show the effect of the substitution of magnesium chloride and magnesium sulfate on hydrolysis rate of urea, respectively. The delay of hydrolysis rate of urea was obtained by the addition of magnesium chloride. The hydrolysis occurred after 30 min

and 150 min in the cases C1 and C2, respectively. The reduction of gradient slopes as the effect of the substitution

of magnesium indicated that the presence of magnesium compounds could delay the reaction.

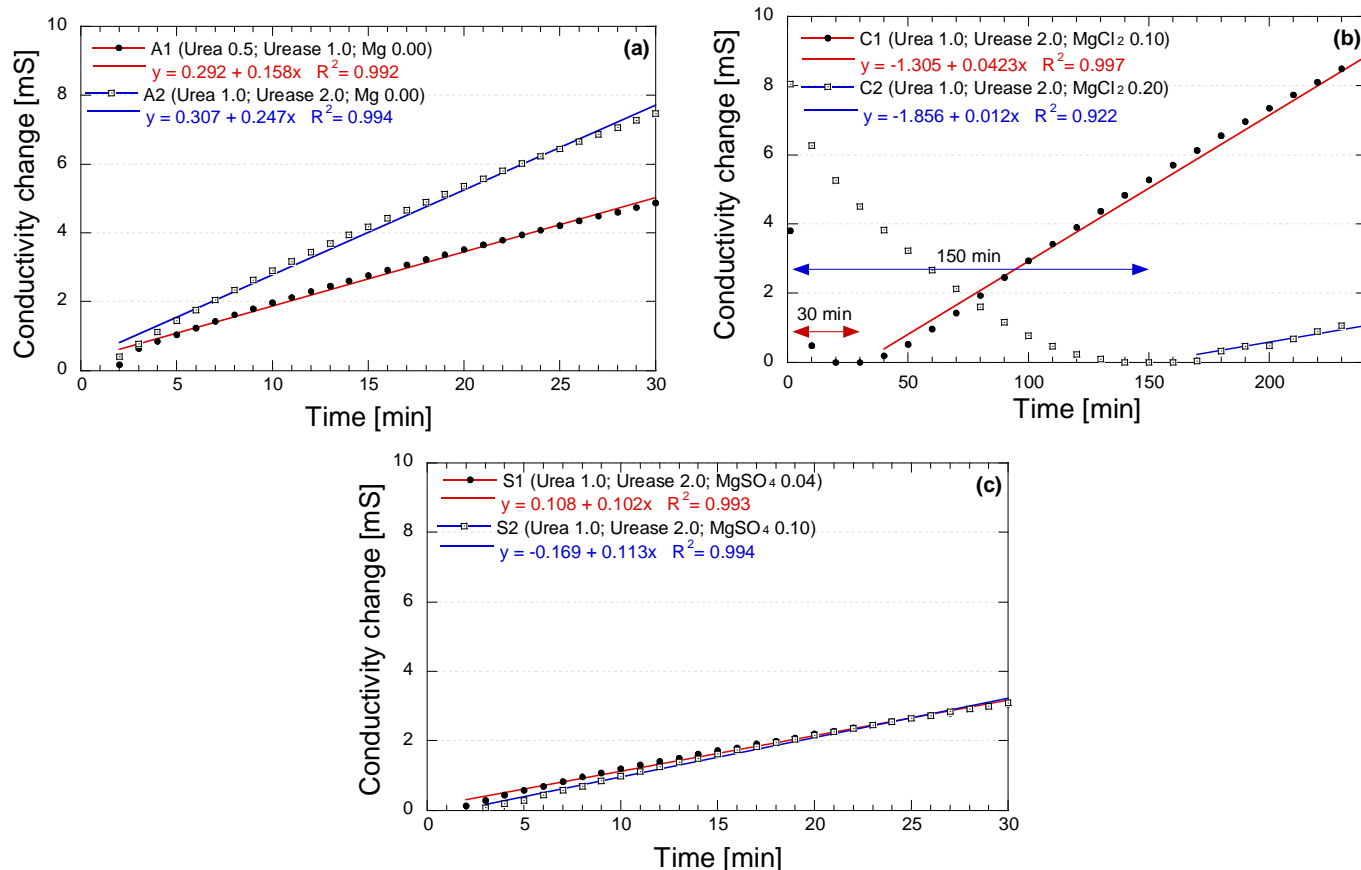


Fig. 6 Evolution of the conductivity with time; (a) Urea-urease; (b) Effect of  $MgCl_2$ ; (c) Effect of  $MgSO_4$

In order to determine the hydrolysis rate of urea, a standard curve was provided by determining the conductivity resulting from the complete hydrolysis of several concentrations of urea. The standard curve is depicted in Fig. 7(a). By using Equation (5), the hydrolysis rate of urea was determined and was shown in Fig. 7(b). As

is apparent, the substitution of magnesium chloride has a significant effect on the hydrolysis rate. Magnesium sulfate was also found to be able to reduce the hydrolysis rate by more than 50%. The results confirmed that the substitution of magnesium compounds as the delaying agent in EMCP technique might be a potential method.

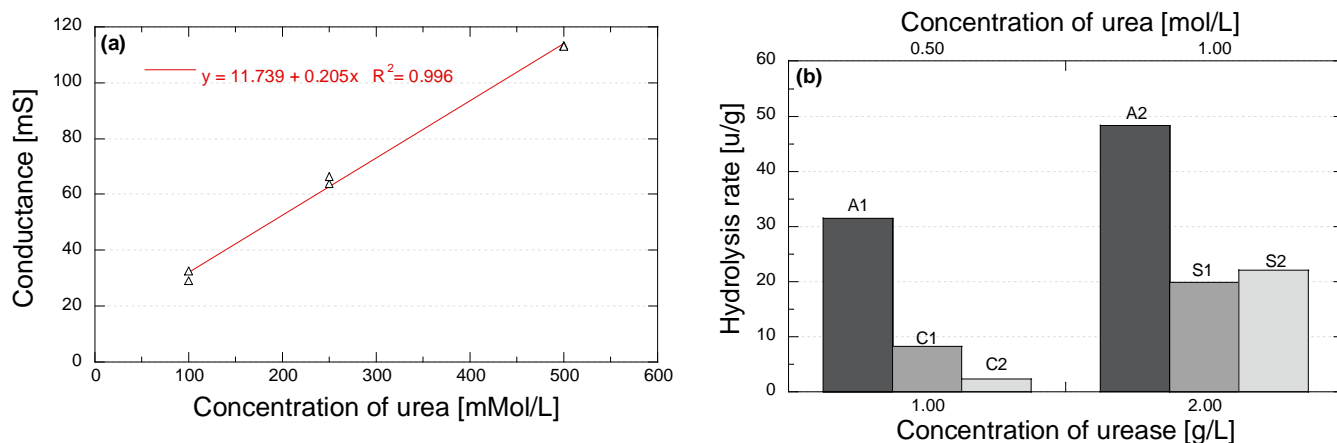


Fig. 7 (a) Standard curve for hydrolysis rate analysis; (b) Hydrolysis rate

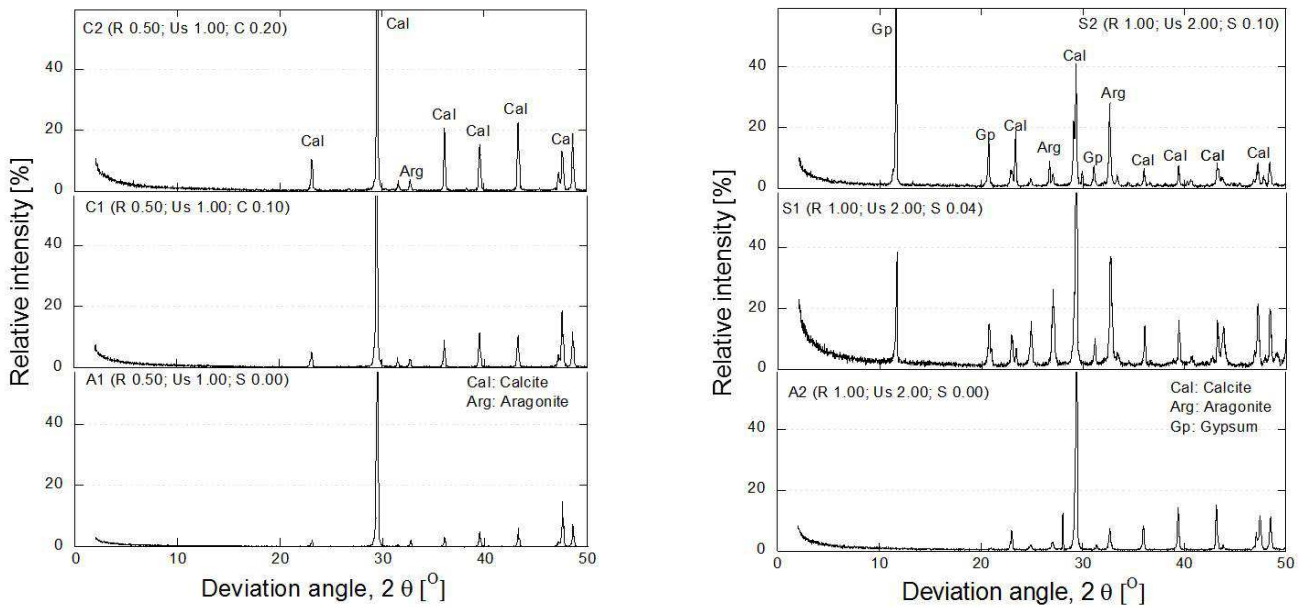


Fig. 8 X-ray diffraction results of precipitated minerals [34], [36]

UCS tests were performed to evaluate the improvement of strength with the precipitated amount within the sand samples. The results of the UCS tests are depicted in Fig. 9. The precipitated amount varying in the range of 1-10% of the soil mass, which corresponds to the strength of 20-555 kPa, was obtained by 1-3 PV injections. UCS improved as the increase of the precipitated amount. In the cases of C2, with a total reagent concentration of 0.5 mol/L, the substitution of magnesium chloride of 0.20 mol/L, was found to be able to improve the strength in the same precipitated amount.

In the case of S1 and S2, the substitution of magnesium sulfate has the great improvement on the precipitated amount and the strength. In the case of S2, the precipitated mass is lower than that of S1. The presence of a high concentration of sulfate in the grouting solution enhanced the reaction rate. The reaction between  $\text{SO}_4^-$  and  $\text{Ca}^{2+}$  quickly occurred after the mixing, and the precipitated materials of gypsum might have been formed before all the solution had been passed through the soil specimens. The results indicated that the substitution of a low concentration of magnesium sulfate into the grouting solution has the potential to optimize the application of EMCP technique.

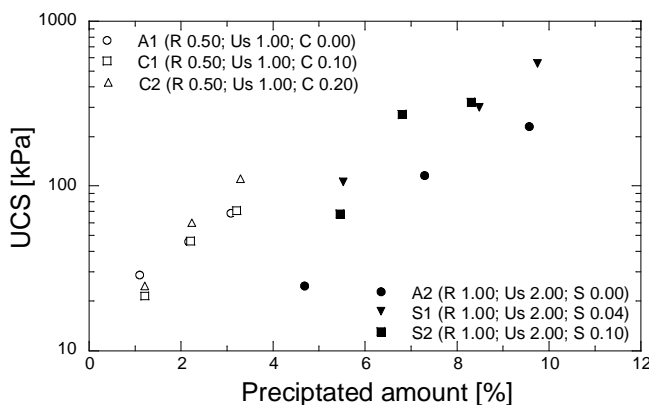


Fig. 9 The evolution of strength with precipitated amount [34], [36]

#### IV. CONCLUSIONS

Enzyme-mediated calcite precipitation (EMCP) as a soil improvement technique has been evaluated as an alternative method for improving engineering properties of soil. Magnesium chloride and magnesium sulfate were added to the injected solution composed of urea and calcium chloride to optimize the precipitated amount and control the precipitation process. The effect of the addition of magnesium to the injected solution on the mineralogical substance of the precipitated minerals was also evaluated. The evolution of the engineering properties of the treated sand was evaluated through UCS tests.

The precipitated mass increased as the magnesium compounds were added. The substitution of magnesium compounds was also found to be able to delay the hydrolysis rate of urea. The XRD analysis revealed that the presence of magnesium ion was potential to promote the formation of aragonite. In addition, gypsum was also formed when magnesium sulfate was substituted. The UCS test results showed that the substitution of magnesium led to a greater improvement in the strength of the soil than the sand treated by  $\text{CaCl}_2$ -urea only. A maximum strength of 555 kPa was obtained from the improved samples in the presence of a mineral mass of 10 %. The results of this study show that the application of magnesium compound in EMCP technique might be able to optimize the applicability of EMCP technique as a soil improvement method.

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