Enzyme-induced carbonate precipitation utilizing synthetic Ca²⁺-zeolite for low ammonium

S. Lee, J. Kim ✉

Department of Civil and Environmental Engineering, Sejong University, (Seoul, Korea)
✉: jongmin@sejong.ac.kr

ABSTRACT: In this study, a low-ammonium enzyme-induced carbonate precipitation (LA-EICP) technique is proposed that utilizes the cation exchange capability of zeolite to remove ammonium, an environmentally harmful by-product of urea hydrolysis. The LA-EICP process is a modified enzyme-induced carbonate precipitation (EICP) suitable for soil stabilization, by mixing zeolite and resulting solution of urea hydrolysis. The amounts of calcium carbonate precipitated and ammonium ions removed by the synthetic calcium-modified zeolite were analyzed through tube precipitation tests. In addition, the unconfined compressive strengths of the soil specimens were measured and compared to investigate the reinforcing effect of LA-EICP. The precipitation of calcium carbonate within the soil specimen was also confirmed by scanning electron microscope and energy dispersive spectrometry analyses. The results showed LA-EICP can precipitate the same amount of calcium carbonate as conventional EICP, while removing almost all ammonium ions. In addition, the LA-EICP-treated specimen showed a higher strength improvement than the conventional EICP-treated specimen, due to the combined effect of the calcium carbonate and the zeolite.

KEY WORDS: Urea hydrolysis; Ammonium; Ion exchange material; Calcium-modified zeolite; EICP; Soil stabilization.

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RESUMEN: *Precipitación de carbonatos inducia por enzimas empleando Ca²⁺-zeolitas sintéticas de bajo amonio.* En este estudio, se propone una técnica de precipitación de carbonato inducida por enzimas con bajo contenido de amonio (LA-EICP) que utiliza la capacidad de intercambio catiónico de la zeolita para eliminar el amonio, un subproducto de la hidrólisis de la urea perjudicial para el medio ambiente. El proceso LA-EICP es una precipitación de carbonato inducida por enzimas modificadas (EICP) adecuada para la estabilización de suelos, mediante la mezcla de zeolita y la solución resultante de hidrólisis de urea. Las cantidades de carbonato de calcio precipitado y los iones de amonio eliminados por la zeolita sintética modificada con calcio se analizaron a través de pruebas de precipitación en tubos. Además, se midieron y compararon las resistencias a la compresión no confinada de las muestras de suelo para investigar el efecto de refuerzo de LA-EICP. La precipitación de carbonato de calcio dentro de la muestra de suelo también se confirmó mediante análisis de microscopía electrónica de barrido y espectrometría de dispersión de energía. Los resultados mostraron que LA-EICP puede precipitar la misma cantidad de carbonato de calcio que el EICP convencional, mientras elimina casi todos los iones de amonio. Además, la muestra tratada con LA-EICP mostró un mayor aumento de la resistencia que la muestra tratada con EICP convencional, debido al efecto combinado del carbonato de calcio y la zeolita.

PALABRAS CLAVE: Hidrólisis de urea; Amonio; Material de intercambio catiónico; Zeolita de calcio modificada; EICP; Estabilización de suelos.

1. INTRODUCTION

Cement is the most popularly used material for soil stabilization, applied as grouting. However, the generation of carbon dioxide during the cement manufacturing process accelerates global warming. In recent years, enzyme-induced carbonate precipitation (EICP) has received attention as an alternative to the cement-based soil stabilization technique (1-11). In EICP, urease hydrolyzes urea ($CO(NH_2)_2$) to the ammonium (NH_4^+) and carbonate (CO_3^{2-}) ions. When a calcium ion (Ca^{2+}) is provided under the appropriate pH conditions, the calcium carbonate ($CaCO_3$) is precipitated by the chemical reaction between CO_3^{2-} and Ca^{2+}. The $CaCO_3$ precipitate binds the soil particles and stabilizes the ground.

In the past, purified urease was generally used as a catalyst for hydrolysis. However, recent studies have investigated the application of nature-driven urease to EICP because of the high cost of purified urease (11-15). Yellow soybean is a good source of urease, and is a good alternative to purified urease since it is urease-abundant, inexpensive, and readily available (11). However, NH_4^+, which is a byproduct of the urea hydrolysis, may cause environmental concerns such as groundwater contamination (16). In order to solve these concerns, ammonium-free EICP techniques have been proposed by some researchers. The studies by Putra et al. (17) and Keykha et al. (18) utilized zeolite to make EICP ammonium-free. It is known that zeolite can capture ammonium ions in an aqueous solution by taking advantage of its cation exchange ability (19-20). In their studies, the ammonium ions in the resulting solution of urea hydrolysis were exchanged by the cations of zeolite. An ammonium-free solution was thus prepared and injected into the soil specimens with a solution containing Ca^{2+} in order to precipitate calcium carbonate without producing ammonium. However, since the hydrolysis of urea is completed during preparation of the solution, an immediate reaction between the CO_3^{2-} and Ca^{2+} is expected. This results in the immediate precipitation of $CaCO_3$, which may cause clogging and non-uniform distribution of $CaCO_3$ within the soil. In addition, it is known that the immediate precipitation of $CaCO_3$ is unfavorable in terms of strength gaining, because the immediate precipitation results in small crystals, mostly distributed over just the surface of the soil particles, while the slow precipitation of $CaCO_3$ results in relatively large $CaCO_3$ crystals growing at inter-particle contacts, which bind the particles (21). Therefore, the use of an ammonium-free solution prepared beforehand seems unsuitable for the EICP technique.

As an ammonium-free microbial-induced carbonate precipitation (MICP) technique for soil stabilization, Mohsensadeh et al. (22) proposed a similar 2-stage treatment process, which was composed of rinsing of ammonium followed by a chemical recovery as a struvite. However, the forementioned methods require two independent processes, which is complicated and impractical.

In this study, the authors examined a mixing-based EICP technique using zeolite to remove the ammonium while maintaining a simple and efficient process for strength improvement. By mixing zeolite and EICP solution simultaneously in the soil, the hydrolysis of urea, the cation exchange, and the precipitation of $CaCO_3$ occur sequentially in the soil pores, so that both the production of ammonium and the immediate precipitation can be minimized.

Regarding the zeolite, it is well known that certain types of synthetic zeolite such as Na-P1, X and A-type have excellent cation exchange capacity, thus high efficiency in ammonium removal (23, 24). Therefore, a synthetic calcium-modified zeolite (Ca^{2+}-zeolite) was adopted in this study to provide Ca^{2+} for the precipitation of the carbonate and to capture NH_4^+ by cation exchange in the aqueous solution.

Regarding environmental concerns, the ammonium ions in the pore water of the soil are captured by zeolite in a solid form of NH_4^+-zeolite once treated with the EICP technique using zeolite. NH_4^+-zeolite is insoluble, stable, and will continuously capture ammonium ions unless an exceptionally high concentration of ionized water flows into the reinforced soil. The inflow of high concentrations of ionized water is not only exceptional in practice but also a pollutant by itself. Therefore, the negative effect of ammonium after LA-EICP treatment is expected to be negligible in practice.

Regarding the reinforcing effect, a greater improvement in strength is also expected, compared with EICP utilizing zeolite reported in previous studies, because the immediate precipitation of $CaCO_3$ can be prevented due to the delayed reaction between CO_3^{2-} and Ca^{2+} after the hydrolysis, and the removal of the ammonium. Densification also occurs, as pores are filled with NH_4^+-zeolite as a result of the cation exchange, and is another contributor to improved strength. In summary, the low-ammonium enzyme-induced carbonate precipitation (LA-EICP) proposed in this study can bond soil particles and improve the strength of the ground without environmental concerns. The entire LA-EICP process is shown in Figure 1.

The organization of this study is as follows. The amounts of $CaCO_3$ precipitated and the NH_4^+ removed by the LA-EICP were measured through a series of tube precipitation tests. In addition, a comparison between natural and synthetic zeolite was made in terms of $CaCO_3$ precipitation and NH_4^+ removal. The LA-EICP-treated soil specimens were prepared in accordance with the results of the tube precipitation tests for the unconfined compression strength (UCS) test, in order to verify the reinforcing effect of the LA-EICP. Finally, the precipitation
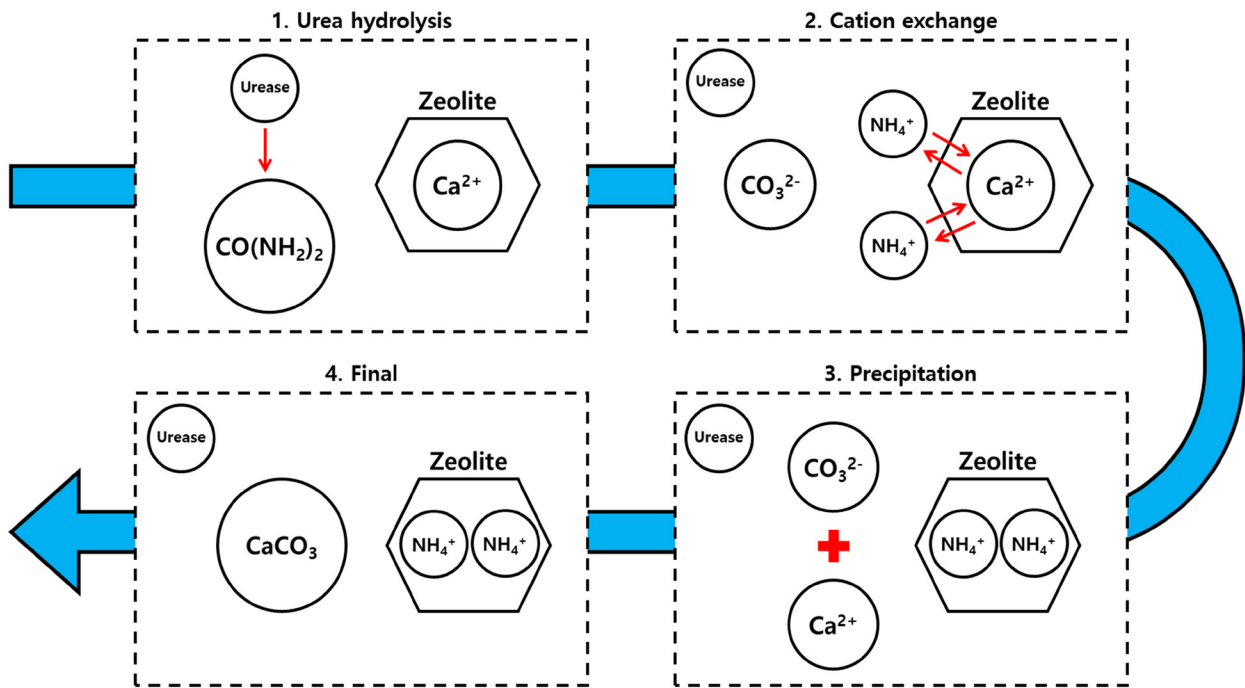
FIGURE 1. The low-ammonium EICP (LA-EICP) process.

of $CaCO_3$ within the specimen was confirmed by Scanning Electron Microscope (SEM) and Energy Dispersive Spectrometry (EDS) analyses.

2. MATERIALS AND METHODOLOGY

2.1. Materials

2.1.1. Urease solution

Yellow soybean powders were selected as the source of the urease solution. The urease activity of yellow soybean powders used in this study was estimated as 6.535×10^{-2} U/mg (11). 1L(liter) of the urease solution was prepared by mixing 75 g of the yellow soybean powders in distilled water, and the mixed solution was then centrifuged at 3,000 rpm and 4°C for 20 min. The centrifuged supernatant is the urease solution.

2.1.2. Cementation solution

In order to compare the conventional EICP and LA-EICP, two types of cementation solutions were prepared. For the conventional EICP, a cementation solution was prepared by mixing 1M of urea and 1M of calcium chloride dehydrate. For the LA-EICP, the cementation solutions were prepared by mixing 1M of urea and various concentrations, ranged from 0 to 900 g/L, of Ca^{2+}-zeolite. An A5-type synthetic zeolite was used as the calcium source for the LA-EICP due to its calcium richness and high cation exchange capacity (CEC) values (23, 24). The range of particle sizes was 2.5~4.5μm, and the CEC of the A5-type synthetic zeolite was 7 meq/g. In addition, the efficiency of natural zeolite (~10μm) for LA-EICP was also investigated to verify the suitability of the A5-type synthetic zeolite for LA-EICP. In order for the natural zeolite to be used in EICP, its inherent cations must be exchanged by calcium

TABLE 1. The components of the synthetic zeolite A5 and natural zeolite.

Components	Al_2O_3	SiO_2	CaO	Na_2O	Fe_2O_3	MgO	LOI
Synthetic zeolite A5	36.82	42.95	14.46	5.44	-	-	-
Natural zeolite	11.65	70.25	1.42	-	2.32	1.30	6.36

ions to make natural Ca^{2+}-zeolite first. This can be achieved by immersing natural zeolite into calcium-rich solution such as calcium chloride solution. In this study, the two types of natural Ca^{2+}-zeolite were prepared by immersing the natural zeolite with 3M and 4M of calcium chloride solution at room temperature for 24 hours to enrich calcium through cation exchange. After extraction and drying process, natural Ca^{2+}-zeolite was used to compare with the synthetic Ca^{2+}-zeolite in the LA-EICP. The components of the A5-type synthetic zeolite and natural zeolite are summarized in Table 1.

2.1.3. Soil

In this study, weathered granite soil, the most widely distributed soil type in Korea, was used to prepare specimens for the UCS test. The soil is classified as SW according to the unified soil classification system (25). The grain size distribution curve is illustrated in Figure 2, and the basic properties of the weathered granite soil are summarized in Table 2.
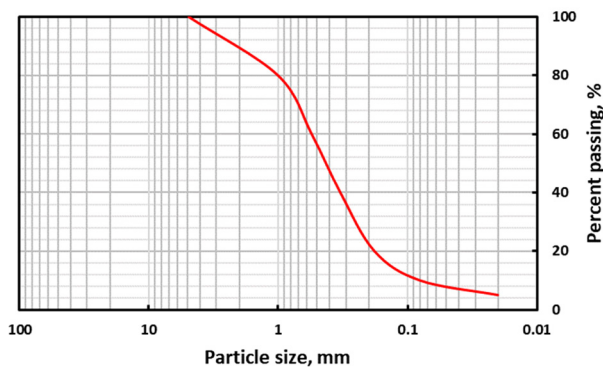

FIGURE 2. The grain size distribution curve.

TABLE 2. The properties of the weathered granite soil.

Property	G_s	C_c	C_u	e_{max}	e_{min}	PI	LL
Value	2.66	1.54	6.88	0.96	0.61	11.64	17.42

2.2. Methodology

2.2.1. Tube precipitation test

A series of tube precipitation tests were conducted to investigate the amount of $CaCO_3$ precipitated and NH_4^+ removed with respect to the concentration of synthetic Ca^{2+}-zeolite. The procedure for the tube precipitation test was as follows: (a) 10 ml of urease solution was mixed with 10 ml of the cementation solution in a 50 ml-sized conical tube. (b) The mixed solution was kept in a shaking incubator at 200 rpm and 25°C for a day. (c) Each conical tube was centrifuged at 3,000 rpm and 25°C for 20 minutes. (d) The NH_4^+ concentration in the supernatant was measured after centrifugation. (e) The precipitate was mixed again with distilled water, centrifuged, extracted, and dried at 100°C in order to eliminate any impurities, followed by measuring the amount of precipitated $CaCO_3$. Figure 3 shows the separation of the supernatant and the precipitate in the conical tube after centrifugation.

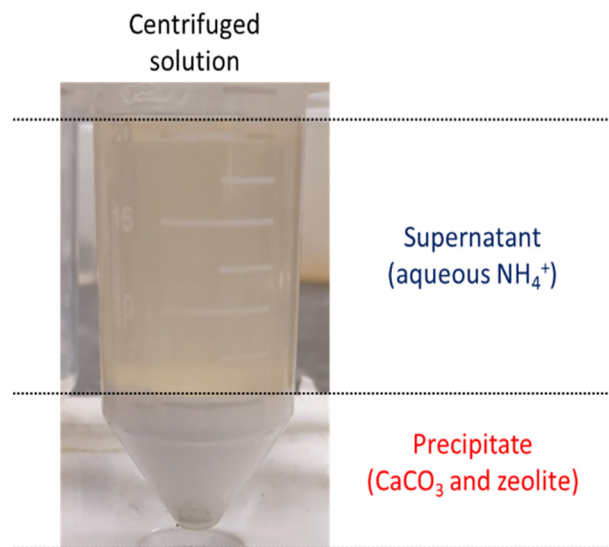**FIGURE 3.** The solution centrifuged in a conical tube.

2.2.2. Estimating the amount of NH_4^+

The amount of NH_4^+ in the supernatant solution can be calculated by the product of the volume of the solution and the concentration of NH_4^+, which was estimated by the indophenol method using an assay kit (Machery-Nagel NANOCOLOR® ammonium 2000, Machery-Nagel, Düren, Germany) and a spectrophotometer. In this study, several ammonium solutions with different concentrations in the measurement range of the assay kit were prepared, and the optical density of each solution at a 585 nm wavelength (OD_{585}) were measured using a spectrophotometer. A linear relationship between OD_{585} and the concentration of NH_4^+ was then obtained as in Equation [1]:

$$NH_4^+ \text{ (mg/L)} = 1566.8 \times OD_{585} + 400, \ (R^2 = 0.9977) \ [1]$$

In order to apply Equation [1], the supernatant from the tube precipitation test needs to be diluted to make the NH_4^+ concentration fit into the measurement range of the assay kit, which Equation [1] is based on. Therefore, the actual concentration from

the supernatant must be adjusted by multiplying the concentration of NH_4^+, estimated in Equation [1], to the inverse of the dilution ratio. Finally, the amount of NH_4^+ in the unit of mmol was calculated by multiplying the volume of the supernatant solution, the actual concentration of NH_4^+, and the inverse of its molar mass (0.055 mmol/mg).

2.2.3. Estimating the amount of CaCO₃

In this study, the amount of precipitated $CaCO_3$ was estimated by measuring the vapor pressure of the carbon dioxide (CO_2 pressure) generated when $CaCO_3$ reacts with hydrochloric acid. The magnitude of CO_2 pressure is proportional to the amount of $CaCO_3$. The method to establish the relationship between the amount of $CaCO_3$ and CO_2 pressure is as follows. (a) Various amounts, ranging from 0 to 1 g, of pure $CaCO_3$ powder were mixed with 20 g of Jumunjin sand in a 50 ml conical tube. The role of sand is to confine the $CaCO_3$ powder temporarily to delay the reaction with hydrochloric acid and prevent the generation of CO_2 before measurement. (b) The conical tube was placed in an acrylic mold containing 1M of hydrochloric acid. It should be noted that the conical tube floats in hydrochloric acid until reaction in order to prevent the generation of CO_2 gas before measurement. (c) After completely sealing the acrylic mold, it was shaken long enough to fully generate CO_2 by mixing the $CaCO_3$ and hydrochloric acid together. The CO_2 pressure was measured with a pressure transmitter connected to the top of the acrylic mold. Figure 4 shows a schematic of the CO_2 pressure measurement. By measuring CO_2 pressures for various amount of $CaCO_3$ powder, a linear relationship between the amount of $CaCO_3$ and CO_2 pressure was obtained as in Equation [2]:

$$CaCO_3 \text{ (mg)} = 20.1 \times CO_2 \text{ pressure (kPa), } (R^2 = 1) \quad [2]$$

As a result, the amount of precipitated $CaCO_3$ can be estimated by substituting the CO_2 pressure, measured as above, into Equation [2].

2.2.4. Unconfined compressive strength

In order to investigate the reinforcing effect of LA-EICP, the unconfined compression strengths (UCS) of the soil specimens were investigated under various conditions. The conditions of the specimens in this study are summarized in Table 3. Distilled water was used for the saturation of the non-treated and zeolite-treated specimens. The cylindrical specimens (5cm-diameter and 10cm-height) were prepared at a relative density of 80%, equivalent to void ratio of 0.68, to ensure the definite development of bonding between the soil particles. This required 310.89 g of weathered granite soil and resulted in 78 ml of pore volume for a 5 cm x 10 cm cylindrical mold. To fully saturate the specimens, 39 ml of the urease and cementation solutions each were prepared as in Table 3. The mixture of soil and the solutions, similar to liquid mortar before it hardens, was poured into the mold and slightly tamped to remove air and cured for 24 hours to precipitate all of the calcium carbonate at 25°C before extraction from the mold. To prevent cracking of the specimen, the extracted specimen was slowly dried in an oven
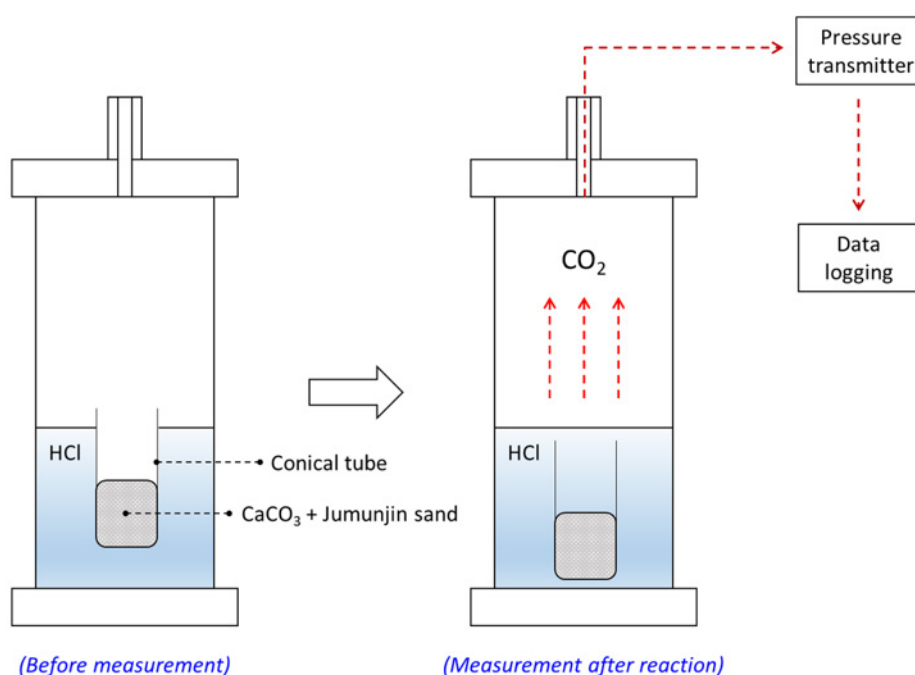

FIGURE 4. Schematic of the CO_2 pressure measurement.

TABLE 3. Concentration of solutions according to the specimen type.

Specimen	Urease solution		Cementation solution	
	Yellow soybean powder	Urea	Calcium chloride dehydrate	synthetic Ca^{2+}-zeolite
Non-treated	-	-	-	-
Zeolite-treated	-	-	-	900 g/L
EICP-treated	75 g/L	60.06 g/L (1M)	147.01 g/L (1M)	-
LA-EICP-treated	75 g/L	60.06 g/L (1M)	-	900 g/L

at a medium temperature of 40°C (21). The degree of dryness was confirmed by checking the change in the weight of specimen during the drying process. Finally, the dried specimen was subjected to a UCS test at a constant strain rate of 1 mm/min in accordance with ASTM D2166 (26).

2.2.5. Estimation of the $CaCO_3$ content in the specimen

After each UCS test, 3 chunks of about 20 g per each were taken from a specimen to estimate the amount of precipitated $CaCO_3$ by measuring CO_2 pressure, as described in Section 2.2.3. The $CaCO_3$ content was then calculated as defined in Equation [3]:

$$CaCO_3 \text{ content } (\%) = \frac{W_{cal}}{W_s} \times 100 \qquad [3]$$

where, W_{cal} = weight of $CaCO_3$ (g) and W_s = weight of soil particles (g).

In addition, the precipitation of $CaCO_3$ in the specimen was confirmed by Scanning Electron Microscopy (SEM) and Energy Dispersive Spectrometry (EDS) analyses. The equipment for the SEM and EDS analyses in this study were a Hitachi SU-8010 (Hitachi, Japan) and a Horiba EMAX X-ray detector (Horiba, Japan), respectively.

3. RESULTS AND DISCUSSION

3.1. Precipitation of $CaCO_3$ and removal of NH_4^+ by LA-EICP

In this section, the results of the tube precipitation tests are provided, to assess the amounts of $CaCO_3$ precipitated and NH_4^+ removed, in order to investigate the efficiency of the LA-EICP. Figure 5 shows the results of the tube precipitation tests using conventional EICP and LA-EICP with various concentrations of synthetic Ca^{2+}-zeolite for the same conditions of specimens. The theoretical maximum amounts of $CaCO_3$ and NH_4^+ as a result of the hydro-

lysis of 1M (mol/L) of urea are also presented, as a red-dotted line in Figure 5(a) and (b), respectively.

The amount of $CaCO_3$ precipitated by the conventional EICP was 984.90 mg, which is close to its theoretical maximum. This result shows that 75 g/L of soybean extract can sufficiently hydrolyze 1 M of urea for one day. On the other hand, the amount of NH_4^+ released was measured to be 17.42 mmol, which is about 85% of the theoretical maximum. The shortfall of 15% is believed to be released into the atmosphere as ammonia (NH_3). In regards to the LA-EICP, both the amounts of $CaCO_3$ precipitated and NH_4^+ removed increases with increasing concentrations of synthetic Ca^{2+}-zeolite. This shows that the synthetic Ca^{2+}-zeolite efficiently removes NH_4^+ by cation exchange without interrupting the hydrolysis of the urea. Figure 5 shows that 900 g/L of synthetic Ca^{2+}-zeolite removes almost all of the NH_4^+ while precipitating 990.93mg of $CaCO_3$, which is even closer to the theoretical maximum than the conventional EICP. Therefore, 900 g/L of synthetic Ca^{2+}-zeolite was used to prepare the LA-EICP-treated soil specimens for the UCS tests in the following section.

3.2. Comparison between natural zeolite and synthetic Ca^{2+}-zeolite for LA-EICP

To verify the suitability of synthetic Ca^{2+}-zeolite for LA-EICP, the amounts of $CaCO_3$ precipitated and NH_4^+ removed by LA-EICP using the natural Ca^{2+}-zeolite were measured via tube precipitation tests. The concentration of natural Ca^{2+}-zeolite was 900 g/L, the same as that of synthetic Ca^{2+}-zeolite. The results are shown in Figure 6.

It is observed from the figure that for the natural Ca^{2+}-zeolite, there was little difference in the amounts of $CaCO_3$ precipitated and NH_4^+ removed regardless of Ca^{2+} in calcium chloride solution. This shows that the concentrations (3M and 4M) of calcium chloride solution for the preparation of natural Ca^{2+}-zeolite are beyond the cation exchange capacity of natural zeolite used in this study. Thus, the

(a) Precipitated CaCO$_3$ (mg)

$$Theoretical\ CaCO_3\ amount\ (mg) = Mm \times M \times V \times 1000 = 1000.09\ mg$$

Where, Mm = molar mass of CaCO$_3$ (100.09 g/mol);
M = molarity of CO$_3^{2-}$ (1 mol/L);
V = volume of cementation solution (0.01 L)
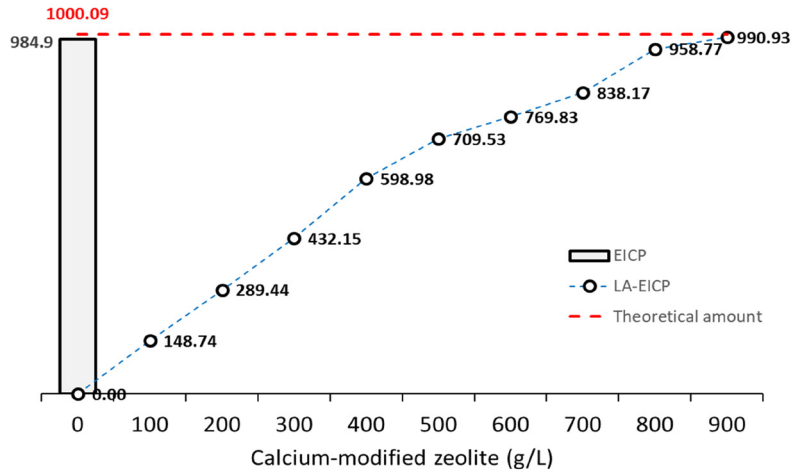
(b) Aqueous NH$_4^+$ (mmol)

$$Theoretical\ NH_4^+\ amount\ (mmol) = M \times V \times 1000 = 20\ mmol$$

Where, M = molarity of NH$_4^+$ (2 mol/L);
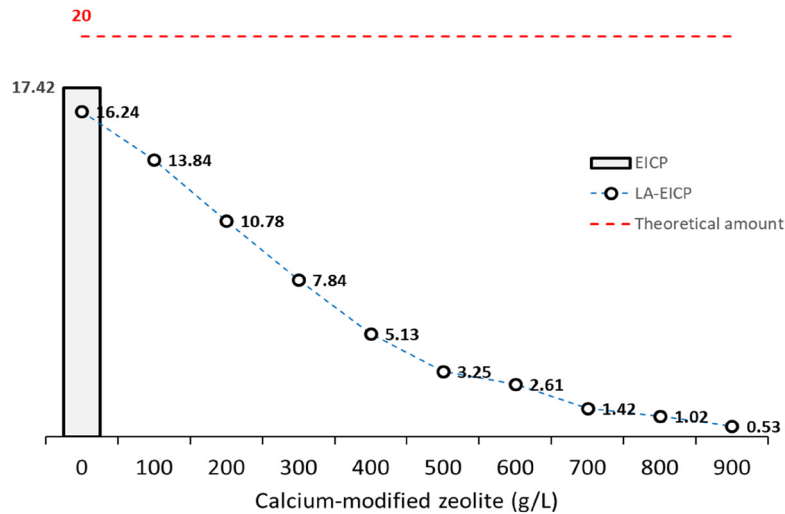*V = volume of cementation solution (0.01 L)*

Figure 5. The results of the tube precipitation tests using the EICP and the LA-EICP: (a) amount of CaCO$_3$ precipitated and (b) amount of NH$_4^+$ remaining.

amounts of CaCO$_3$ precipitated and NH4$^+$ removed by the natural Ca^{2+}-zeolite in Figure 6 are the maxima. When compared to synthetic Ca^{2+}-zeolite, LA-EICP using natural Ca^{2+}-zeolite decreases both amounts of CaCO$_3$ precipitated and the amount of NH$_4^+$ removed by 299.46 mg and 2.91 mmol, respectively. This means that the CEC of natural Ca^{2+}-zeolite is significantly smaller than that of synthetic Ca^{2+}-zeolite. In addition to the time required for the preparation of Ca^{2+}-zeolite, the application of natural Ca^{2+}-zeolite to LA-EICP is uneconomical due to the cost of calcium chloride with high concentration. Therefore, synthetic Ca^{2+}-zeolite is more suitable for LA-EICP than natural Ca^{2+}-zeolite, in terms of effectiveness of CaCO$_3$-precipitation, NH$_4^+$-removal, simplicity, and cost.
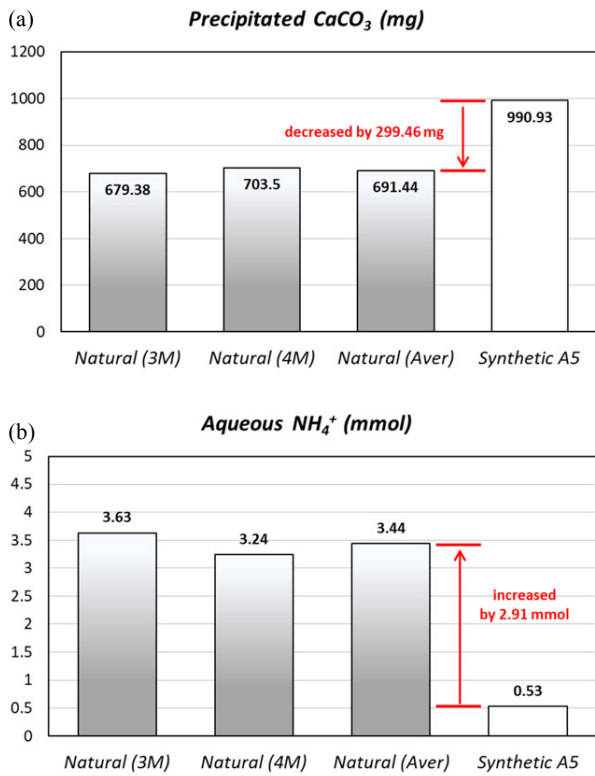
FIGURE 6. The results of the tube precipitation tests comparing natural and synthetic zeolite: (a) amount of CaCO$_3$ precipitated and (b) amount of NH$_4^+$ remaining.

3.3. Identification of CaCO$_3$ precipitation in various soil specimens

Table 4 summarizes the CaCO$_3$ content, a weight ratio of CaCO$_3$ to soil grains, for different specimen conditions. For the last two specimens in Table 4, the amount of CaCO$_3$ in the 3 chunks from a specimen were measured and used to estimate the CaCO$_3$ content. The average CaCO$_3$ contents in the conventional EICP and the LA-EICP were 0.73% and 0.76%, respectively.

In addition to the quantitative estimation, the precipitation of CaCO$_3$ was also confirmed by SEM and

EDS analyses. Figure 7 shows an SEM image and EDS mapping of the (a) Non-treated, (b) Conventional EICP-treated, and (c) LA-EICP-treated specimens. For the EDS mapping, the silicon (Si) from silicon dioxide (SiO$_2$) and the calcium (Ca) from calcium carbonate (CaCO$_3$) are represented by yellow dots and red dots, respectively. In addition, their proportion in weight (W$_t$) determined by EMAXevo software (Horiba, Japan) of the EDS equipment is also presented. The precipitation of CaCO$_3$ is clearly observed in the conventional EICP-treated and the LA-EICP-treated specimens from the EDS mappings in Figure 7(b) and 7(c). In Figure 7(c), the red dots for CaCO$_3$ are distributed around the hexahedral synthetic Ca^{2+}-zeolite. The precipitation of CaCO$_3$ can be also confirmed by the variation in the W$_t$ of Ca, which increases from 0% for the non-treated specimens to 14.35% for the conventional EICP-treated specimens and 17.53% for the LA-EICP-treated specimens. The reason the W$_t$ of Ca for LA-EICP is higher than the conventional EICP is due to the existence of CaO inherent in the synthetic Ca^{2+}-zeolite, although the difference seems small. Therefore, the applicability of LA-EICP to the soil specimen was confirmed in terms of the precipitation of CaCO$_3$ within it.

3.4. Reinforcing effects of the LA-EICP

The results of the UCS tests are provided in this section, to investigate the reinforcing effects of the LA-EICP. The results are shown in Table 5 and Figure 8, where the UCS of the non-treated, zeolite-treated, conventional EICP-treated, and LA-EICP-treated soil specimens are presented.

The average UCS of the zeolite-treated specimen was 449.34 kPa, which is 3.09 times higher than that of the non-treated specimen. This improvement in strength of the zeolite-treated specimen is attributed to the densification of the specimen, by zeolite filling pores. For the EICP-treated specimen, the average UCS was 1201.97 kPa, which is 8.28 times higher

TABLE 4. CaCO$_3$ contents in various soil specimens.

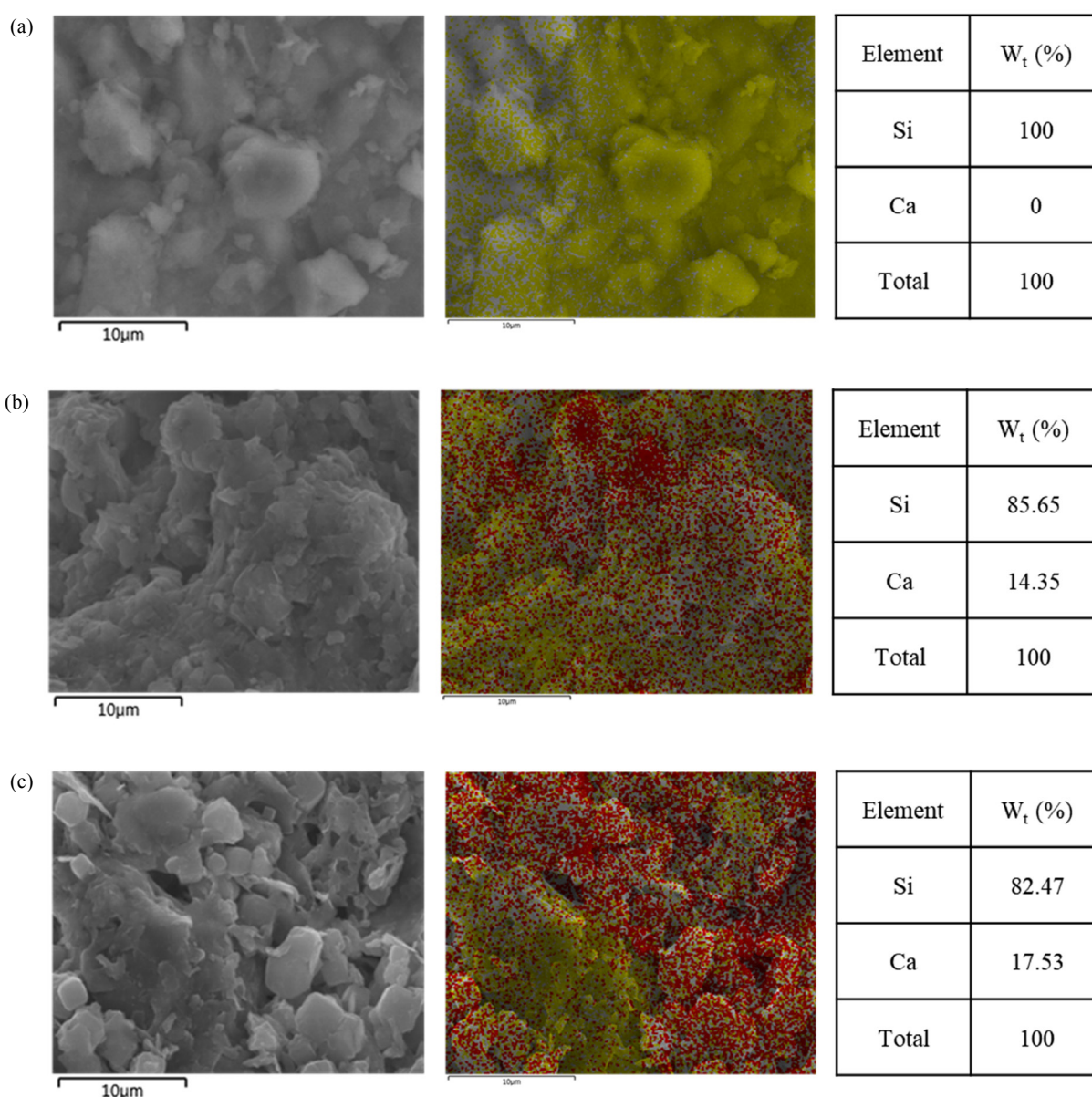| Specimen type		CaCO$_3$ content (%)	
Non-treated		0	
Zeolite-treated		0	
EICP-treated	chunk 1	0.79	
	chunk 2	0.69	Average = 0.73
	chunk 3	0.71	
LA-EICP-treated	chunk 1	0.85	
	chunk 2	0.67	Average = 0.76
	chunk 3	0.76	

FIGURE 7. SEM and EDS results: (a) Non-treated, (b) EICP-treated, and (c) LA-EICP-treated.

than that of the non-treated specimen. When the low $CaCO_3$ content of 0.73% is considered, even a small amount of $CaCO_3$ can form bridges that sufficiently bond the soil particles and contribute to strength improvement.

For the LA-EICP-treated specimen, the average UCS was 1730.91 kPa, which is 11.92 times higher than that of the non-treated specimen and actually the highest among all the specimens. Despite the $CaCO_3$ content being similar to that of the conventional EICP, LA-EICP showed more strength improvement, which is attributed to the zeolite.

To further investigate the role of zeolite in the LA-EICP process other than densification, the UCS of the LA-EICP-treated specimen was compared with a simple sum of UCS of zeolite-treated and conventional EICP-treated specimens and is presented as 'Combined UCS' in Figure 8. It can be seen from the figure that the UCS of the LA-EICP-treated specimen is 1.15 times higher than the combined UCS under same conditions. In the authors' opinion, this additional reinforcing effect of LA-EICP shows the role of zeolite in the EICP process, i.e., in addition to its densification effect, $CaCO_3$ is precipitated and accumulated on the surface of zeolite particles to form more bridges to bind the soil particles.

The results of the UCS tests confirmed that the reinforcing effect of LA-EICP is a result of bridging soil particles by $CaCO_3$ precipitated combined with densification and enhancement of bridging by zeolite.

TABLE 5. Unconfined compressive strength of all soil specimens.

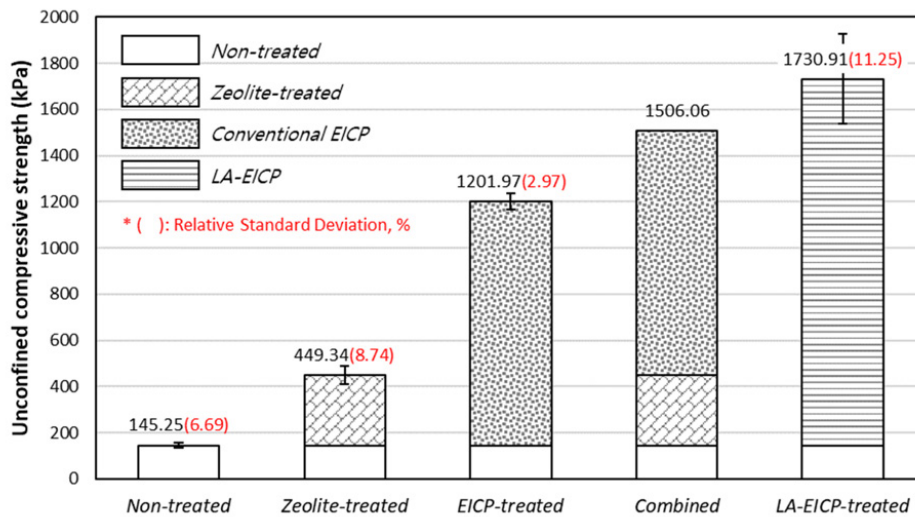| Specimen type	Unconfined compressive strength (kPa)		
Non-treated	Specimen 1	138.88	
	Specimen 2	156.44	Average = 145.25
	Specimen 3	140.44	
Zeolite-treated	Specimen 1	492.43	
	Specimen 2	440.02	Average = 449.34
	Specimen 3	415.58	
EICP-treated	Specimen 1	1186.70	
	Specimen 2	1176.42	Average = 1201.97
	Specimen 3	1242.80	
LA-EICP-treated	Specimen 1	1928.60	
	Specimen 2	1539.29	Average = 1730.91
	Specimen 3	1724.85	

FIGURE 8. Average unconfined compressive strength according to the type of soil specimen.

4. CONCLUSIONS

In this study, a modified EICP technique (LA-EICP) using zeolite to remove ammonium was proposed and investigated by laboratory tests. The amounts of $CaCO_3$ and NH_4^+ produced during the LA-EICP process were measured using the tube precipitation test with varying concentrations of synthetic Ca^{2+}-zeolite, and compared with the amounts obtained from conventional EICP. A comparative analysis of natural and synthetic zeolite was also conducted to investigate the suitability to LA-EICP. In addition, the UCS of the LA-EICP-treated specimen was investigated verify its reinforcing effect. Finally, the precipitation of $CaCO_3$ in the soil specimens was confirmed by measuring CO_2 pressure,

and by SEM/EDS analyses. The conclusions drawn from the study are provided below.

From a series of the tube precipitation tests for LA-EICP utilizing synthetic Ca^{2+}-zeolite, it was observed that most of the NH_4^+ was removed while almost the same amount of $CaCO_3$ as the conventional EICP was precipitated, when 900 g/L of synthetic Ca^{2+}-zeolite was mixed with 1M of urea for a cementation solution, and 75 g/L of yellow soybean powder was used for hydrolysis of urea.

The suitability of synthetic Ca^{2+}-zeolite over natural zeolite for LA-EICP was verified by a comparative study on both in terms of $CaCO_3$ precipitation and NH_4^+ removal. For LA-EICP using natural zeolite, the amounts of $CaCO_3$ precipitated and NH_4^+ removed were reduced by 299.46 mg and 2.91 mmol

respectively when compared to LA-EICP using synthetic Ca²⁺-zeolite with same concentration.

From a series of precipitation tests on soil specimens, the efficiency of conventional and LA-EICP were estimated and compared in terms of $CaCO_3$ content. The amount of $CaCO_3$ was estimated by measuring CO_2 pressure and conducting SEM/EDS analyses in the soil specimen precipitation tests. The difference in $CaCO_3$ content between the conventional and LA-EICP was negligible for the same soil conditions. It was also confirmed by SEM image and EDS mapping analysis. This result shows the potential of the LA-EICP technique as an eco-friendly alternative to the conventional EICP technique, which generates NH_4^+ causing environmental concerns.

The LA-EICP-treated specimen showed the highest UCS among the specimens of untreated, zeolite-treated, conventional EICP-treated and LA-EICP-treated soil under the same conditions. The highest strength improvement by the LA-EICP was due to the combined effects of cementation by precipitated $CaCO_3$ and the densification and bridging of soil particles via $CaCO_3$ by zeolite.

Accordingly, the proposed LA-EICP technique can resolve environmental concerns and provide additional strength improvement compared to the conventional EICP technique. It should be noted that, as a source of Ca²⁺, the synthetic zeolite for LA-EICP is generally more expensive than calcium chloride required for conventional EICP. However, when a low-cost natural zeolite is used for LA-EICP, both the amount of NH_4^+ removed and $CaCO_3$ precipitated decrease, due to its low cation exchange capacity. In addition, it takes additional time and cost to produce natural Ca²⁺-zeolite. Therefore, further studies on the application of lower cost Ca²⁺-zeolite with higher cation exchange capacity are required to develop a more practical LA-EICP technique as an eco-friendly soil stabilization method.

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AUTHOR CONTRIBUTIONS:

Conceptualization: S. Lee, J. Kim. Data curation: S. Lee, J. Kim. Formal analysis: S. Lee, J. Kim. Investigation: S. Lee. Methodology: S. Lee. Project administration: J. Kim. Resources: J. Kim. Supervision: J. Kim. Validation: S. Lee, J. Kim. Visualization: S. Lee. Writing – original draft: S. Lee, J. Kim. Writing – review & editing: S. Lee, J. Kim.

REFERENCES

1. Namati, M.; Voordouw, G. (2003) Modification of porous media permeability using calcium carbonate produced enzymatically in situ. *Microb. Technol.* 33 [5], 635-642. https://doi.org/10.1016/S0141-0229(03)00191-1.

2. Park, S.S.; Choi, S.G.; Nam, I.H. (2012) Development of soil binder using plant extracts. *Journal of the Korean Geotechnical Society.* 28 [3], 67-75. https://doi.org/10.7843/kgs.2012.28.3.67.

3. Yasuhara, H.; Neupane, D.; Hayashi, K.; Okamura, M. (2012) Experiments and predictions of physical properties of sand cemented by enzymatically-induced carbonate precipitation. *Soils. Found.* 52 [3], 539-549. https://doi.org/10.1016/j.sandf.2012.05.011.

4. Neupane, D.; Yasuhara, H.; Kinoshita, N.; Unno, T. (2013) Applicability of enzymatic calcium carbonate precipitation as a soil-strengthening technique. *J. Geotech. Geoenviron. Eng.* 139 [12], 2201-2211. https://doi.org/10.1061/(ASCE)GT.1943-5606.0000959.

5. Kavazanjian, E.; Hamdan, N. (2015) Enzyme Induced Carbonate Precipitation (EICP) columns for ground improvement. In: IFCEE 2015, American Society of Civil Engineers, Reston, VA, USA, pp. 2252-2261. https://doi.org/10.1061/9780784479087.209.

6. Neupane, D.; Yasuhara, H.; Kinoshita, N.; Ando, Y. (2015) Distribution of mineralized carbonate and its quantification method in enzyme mediated calcite precipitation technique. *Soils. Found.* 55 [2], 447-457. https://doi.org/10.1016/j.sandf.2015.02.018.

7. Neupane, D.; Yasuhara, H.; Kinoshita, N.; Putra, H. (2015b) Distribution of grout material within 1-m sand column in in situ calcite precipitation technique. *Soils. Found.* 55 [6], 1512-1518. https://doi.org/10.1016/j.sandf.2015.10.015.

8. Oliveira, P.J.V.; Freitas, L.D.; Carmona, J.P. (2016) Effect of soil type on the enzymatic calcium carbonate precipitation process used for soil improvement. *J. Mater. Civ. Eng.* 29 [4], 04016263. https://doi.org/10.1061/(ASCE)MT.1943-5533.0001804.

9. Almajed, A.; Tirkolaei, H.K.; Kavazanjian, E. (2018) Baseline investigation on enzyme-induced calcium carbonate precipitation. *J. Geotech. Geoenviron. Eng.* 144 [11], 04018081. https://doi.org/10.1061/(ASCE)GT.1943-5606.0001973.

10. Song, J.Y.; Ha, S.J.; Jang, J.W.; Yun, T.S. (2020) Analysis of improved shear stiffness and strength for sandy soils treated by EICP. *Journal of the Korean Geotechnical Society.* 36 [1], 17-28. https://doi.org/10.7843/kgs.2020.36.1.17.

11. Lee, S.; Kim, J. (2020) An experimental study on enzymatic induced carbonate precipitation using yellow soybeans for soil stabilization. *KSCE J. Civ. Eng.* 24 [7], 2026-2037. https://doi.org/10.1007/s12205-020-1659-9.

12. Dilrukshim, R.A.N.; Nakashima, K.; Kawasaki, S. (2018) Soil improvement using plant-derived urease-induced calcium carbonate precipitation. *Soils. Found.* 58 [4], 894-910. https://doi.org/10.1016/j.sandf.2018.04.003.

13. Gao, Y.; He, J.; Tang, X.; Chu, J. (2019) Calcium carbonate precipitation catalyzed by soybean urease as an improvement method for fine-grained soil. *Soils. Found.* 59 [5], 1631-1637. https://doi.org/10.1016/j.sandf.2019.03.014.

14. Imran, M.A.; Nakashma, K.; Kawasaki, S. (2021) Bio-mediated soil improvement using plant derived enzyme in addition to magnesium ion. *Crystals.* 11 [5], 516. https://doi.org/10.3390/cryst11050516.

15. Putra, H.; Simatupangm, M.; Yanto, D.H.Y. (2021) Improvement of organic soil shear strength through calcite precipitation method using soybeans as bio-catalyst. *Crystals.* 11 [9], 1044. https://doi.org/10.3390/cryst11091044.

16. Khodadadi, H.T.; Kavazanjian, E.; van Paassen, L.; Dejong, J. (2017) Bio-grout materials: a review. Grouting 2017, July, Honolulu, HI, USA. 9-12. https://doi.org/10.1061/9780784480793.001.

17. Putra, H.; Yasuhara, H.; Kinoshita, N. (2017) Applicability of natural zeolite for NH-forms removal in enzyme-mediated calcite precipitation technique. *Geosciences.* 7 [3], 61. https://doi.org/10.3390/geosciences7030061.

18. Keykha, H.A.; Mohamadzadeh, H.; Asadi, A.; Kawasaki, S. (2018) Ammonium-free carbonate-producing bacteria as an ecofriendly soil biostabilizer. *Geotech. Test. J.* 42 [1], 19-29. https://doi.org/10.1520/GTJ20170353.

19. Zhao, Y.P.; Gao, T.Y.; Jiang, S.Y.; Cao, D.W. (2004) Ammonium removal by modified zeolite from municipal wastewater. *J. Environ. Sci.* 16 [6], 1001-1004.

20. Ji, Z.Y.; Yuan, J.S.; Li, X.G. (2007) Removal of ammonium from wastewater using calcium form clinoptilolite. *J. Hazard. Mater.* 141 [3], 483-488. https://doi.org/10.1016/j.jhazmat.2006.07.010.

21. Almajed, A.; Tirkolaei, H.K.; Kavazanjian, E.; Hamdan, N. (2019) Enzyme induced biocementated sand with high strength at low carbonate content. *Sci. Rep.* 9 [1], 1-7. https://doi.org/10.1038/s41598-018-38361-1.

22. Mohsenzadeh, A.; Aflaki, E.; Gowthaman, S.; Nakashma, K.; Kawasaki, S.; Ebadi, T. (2022) A two-stage treatment process for the management of produced ammonium by-products in ureolytic bio-cementation process. *Int. J. Environ. Sci. Technol.* 19 [1], 449-462. https://doi.org/10.1007/s13762-021-03138-z.

23. Querol, X.; Moreno, N.; Umaña, J.T.; Alastuey, A.; Hernández, E.; Lopez-Soler, A.; Plana, F. (2002) Synthesis of zeolites from coal fly ash: an overview. *Int. J. Coal. Geol.* 50 [1-4], 413-423. https://doi.org/10.1016/S0166-5162(02)00124-6.

24. Ryu, T.G.; Ryu, J.C.; Choi, C.H.; Kim, C.G.; Yoo, S.J.; Yang, H.S.; Kim, Y.H. (2006) Preparation of Na-P1 zeolite with high cation exchange capacity from coal fly ash. *J. Ind. Eng. Chem.* 12 [3], 401-407. Retrieved from https://www.cheric.org/PDF/JIEC/IE12/IE12-3-0401.pdf.

25. ASTM D2487 (2000) Standard practice for classification of soils for engineering purposes (unified soil classification system). ASTM D2487, ASTM International, West Conshohocken, PA, USA.

26. ASTM D2166 (2005) Standard test method for unconfined compressive strength of cohesive soils. ASTM D2166, ASTM International, West Conshohocken, PA, USA.