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Enzyme-Induced Struvite Precipitation in Soils: Crystal Characterization and Soil Improvement

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ABSTRACT: The ammonium ions generated from the bio-cementation method, such as microbial-induced carbonate precipitation (MICP) and enzyme-induced carbonate precipitation (EICP) pose environmental risks including eutrophication and ammonia gas emissions. Recently, some researchers have studied the conversion of the ammonium ions in the urea hydrolysis into struvite crystals ($MgNH_4PO_4 \cdot 6H_2O$) and proved the permeability reduction at the struvite-treated soil specimen, bonding slightly the particles. The objective of this study is to propose a novel enzyme-based soil improvement approach that immobilizes ammonium ions by precipitating them as struvite crystals, decreasing the requirement of significant effort and resources for the cultivation and maintenance of microorganisms. Under slightly acidic initial pH, a series of solution tests were conducted at constant levels of magnesium and phosphate ions, varying urea and urease concentrations. The higher urea concentration reached pH conditions suitable for struvite formation within 4 days at all urease levels. In contrast, the lower urea concentration did not achieve the necessary pH during the same timeframe at all urease levels. At the higher urea concentration, greater urease levels produced a markedly faster increase in solution pH, which may reduce particle size. XRD and SEM analyses confirmed struvite formation under the higher-urea condition at all urease levels, whereas the lower-urea condition yielded a mixture of struvite and newberyite. Applying the optimal recipe to sand columns yielded an approximately two-fold increase in shear stiffness and a reduction in hydraulic conductivity. These findings highlight the potential of this approach as an environmentally sustainable alternative to traditional bio-cementation methods.

KEYWORDS: Urea hydrolysis, Ammonium ion, Struvite crystallization, Supersaturation, Shear stiffness.

1 INTRODUCTION

Bio-cementation is an effective ground improvement method for enhancing strength and stiffness (DeJong et al., 2010; Kim et al., 2023) and reducing permeability (Al Qabany & Soga, 2013; Whiffin et al., 2007). In the most studied variants of bio-cementation, microbial-induced carbonate precipitation (MICP) and enzyme-induced carbonate precipitation (EICP), calcium carbonate ($CaCO_3$) forms via urea hydrolysis. The hydrolysis of urea favors $CaCO_3$ precipitation via two concurrent effects: (i) an increase in total carbonate content through the production of dissolved inorganic carbon and (ii) a rise in pH resulting from ammonia (NH_3) generation (Equation (1)).

$$\begin{aligned}
&CO(NH_2)_2 + 2H_2O \xrightarrow{urease} 2NH_3 + CO_2 \\
&CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \\
&2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^- \qquad (1) \\
&HCO_3^- + OH^- \leftrightarrow CO_3^{2-} + H_2O \\
&Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3\ (S)
\end{aligned}$$

Despite the advantages of MICP/EICP in soil improvement, the NH_4^+ ions as by-products can cause environmental issues, including eutrophication in ecosystems and potential NH_3 gas emissions (Behera & Sharma, 2010). Because greater $CaCO_3$ precipitation is typically associated with increased mechanical strength and lower hydraulic conductivity, substantial NH_4^+ by-products are likely to be potentially released during or after ground improvement treatment.

To mitigate the concentration of NH_4^+ produced during or after the reaction, several control strategies have been investigated in recent years. Crane et al. (2022) utilized the calcium-rich zeolites as an NH_4^+ adsorbent and Ca^{2+} source for EICP process. Lee et al. (2019) applied the rinsing method by pouring cation-enriched solutions into the soil column. Gowthaman et al. (2023) proposed enzyme-induced calcium phosphate precipitation (EIPP), suggesting the significant reduction of NH_4^+ by adjusting the ratio of urea and Ca^{2+}. Besides, Gowthaman et al. (2022) introduced the magnesium (Mg^{2+}), and phosphate (PO_4^{3-}), ions in the effluent of bio-cementation to immobilize the NH_4^+ as struvite crystals. Furthermore, some researchers formed struvite crystals during urea hydrolysis as a pre-treatment strategy and demonstrated the permeability reduction with slightly the particle bonding in sand column (Yu et al., 2022; Yu & Yang, 2023).

Struvite (magnesium ammonium phosphate hexahydrate; $MgNH_4PO_4 \cdot 6H_2O$) is a white crystalline substance, formed as Mg^{2+}, PO_4^{3-}, and NH_4^+ react in 1:1:1 molar ratio, as described by Equation (2) (for n = 0, 1, 2, where n depends on the solution pH).

$$Mg^{2+} + NH_4^+ + H_nPO_4^{3-n} + 6H_2O \leftrightarrow MgNH_4PO_4 \cdot 6H_2O\ (s) + nH^+ \qquad (2)$$

Bacterially promoted struvite formation occurs primarily via the microbial decomposition of nitrogenous organic matter, which raises pH and NH_4^+ concentration and establishes conditions conducive to struvite precipitation. Although this technique has demonstrated effectiveness in soil improvement, the requirement for substantial effort and resources to cultivate microorganisms constitutes a major limitation.

This study proposes a novel soil-improvement technique that formed struvite via enzyme-based urea hydrolysis. Batch solution experiments were conducted to investigate struvite precipitation under varying urea and urease concentrations. The precipitates were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), and nitrogen quantification by elemental analysis. The effect of struvite formation induced by free urease on soil behavior was evaluated through measurements of shear-wave velocity and hydraulic conductivity both during and after treatment. Overall, the findings highlight the potential of bio-mediated struvite precipitation as ground-improvement method with practical engineering relevance.

2 MATERIAL AND EXPERIMENTAL STUDY

2.1 *Chemical materials*

Unless otherwise indicated, all chemicals were purchased from Sigma-Aldrich. Reagents included magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$; M0250), potassium dihydrogen phosphate (KH_2PO_4; P8709), urea (U5378), and urease (U1500). The initial pH (pH_0) of the treatment solution was adjusted by using sodium hydroxide (NaOH; DuckSan).

2.2 *Solution tests*

In order to investigate the struvite precipitation under varying urea and urease concentrations, solution tests were conducted. Two solutions were prepared. Solution A contained the phosphate source (KH_2PO_4), urease, and NaOH for pH_0 adjustment, while Solution B contained Mg^{2+} (as $MgCl_2{\cdot}6H_2O$) and urea. The two solutions were mixed at a 1:1 volume ratio, after which pH was recorded at regular intervals for more than three days. At the end of the reaction period, 1 mL of supernatant was withdrawn to determine the NH_4^+ concentration using an ammonium photometer.

The precipitates were oven-dried at 40 °C for 48 h and characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM). The quantity of struvite was additionally estimated indirectly from the nitrogen content of the dried solids by elemental analysis. A schematic illustration of the test procedure is provided in Figure 1.

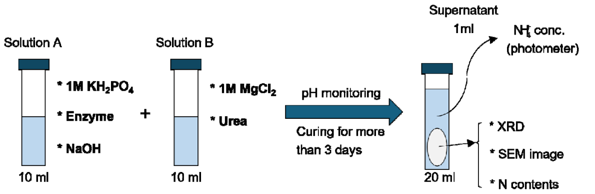

Figure 1. Schematic illustration for solution test procedures.

2.3 *Sand material*

Jumunjin sand, collected from the coastal area of Jumunjin, Republic of Korea, was employed to evaluate the effectiveness of struvite precipitation for modifying soil properties. According to the Unified Soil Classification System (USCS), the material is a poorly graded sand (SP) with a specific gravity of 2.65. The mean particle size (D_{50}) is 0.542 mm, the uniformity coefficient, Cu = 1.94, and the coefficient of gradation, Cc = 1.09. The maximum and minimum void ratios are $e_{max} = 0.897$ and $e_{min} = 0.600$, respectively.

2.4 *Shear-wave measurements in sand columns*

To quantify changes in shear stiffness induced by struvite formation, shear (S-) wave velocity was measured using bender elements installed in a custom polycarbonate oedometer cell. A pair of bender elements was mounted in the top and bottom caps; a 50 Hz square-wave excitation was applied at the top element, and the received signal at the bottom element was filtered through a filter amplifier (bandwidth 100 Hz – 1 MHz). Specimens were prepared to a relative density of 70%, and the injected volume of the optimized treatment solution was set to one pore volume corresponding to this density. Struvite precipitation was allowed to proceed for 5 days under an axial confining stress of approximately 25 kPa to ensure intimate contact between sand grains and the embedded elements. S-wave velocity (V_S) was computed as the tip-to-tip distance divided by the first-arrival time (taken at the initial deflection of the received waveform). Measurements were recorded continuously over the 5-day treatment period.

After measuring the V_S, a constant head hydraulic conductivity test was conducted as step 2. Distilled water was passed through the treated specimen under constant water pressure. The amount of water discharge was recorded by an electronic balance at 5-second intervals. The measurement was stopped once the difference between two consecutive weight readings stabilized. The stabilized difference between two consecutive weight readings was converted to volume using the density of water. Experimental setup for measuring V_S and hydraulic conductivity is shown in Figure 2.

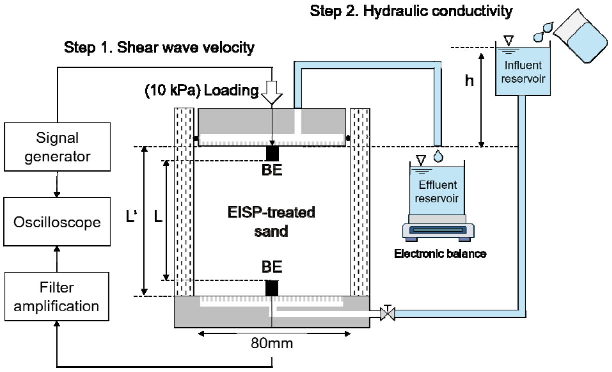

Figure 2. Experimental setup to measure V_S (Step 1) and hydraulic conductivity (Step 2) of struvite-treated specimen.

3 RESULTS

3.1 *pH evolution and precipitation behavior*

pH evolution with reaction time under the various urea and urease combinations are presented in Figure 3. In the absence of urease, the pH decreased irrespective of urea concentration, a trend attributable to newberyite crystallization in which Mg^{2+} reacts with PO_4^{3-} and releases H^+ (Equation (3)).

$$Mg^{2+} + H_2PO_4^- + 3H_2O \rightarrow MgHPO_4 \cdot 3H_2O\,(s) + H^+ \qquad (3)$$

With urease present at the lower urea level, pH exhibited a slight initial drop followed by a rise that stabilized near 6 (Figure 3a). By contrast, at the higher urea level the pH increased continuously to nearly 8 for all urease concentrations (Figure. 3b). Numerous studies indicate that struvite forms most readily at pH ≈ 7.5−8.0 (Kim et al., 2017; Wang et al., 2005). Consistent with Equation (1), urea hydrolysis elevates both solution pH and NH_4^+ concentration, thereby increasing supersaturation with respect to struvite. Once the pH approaches 6, co-precipitation of struvite and/or newberyite can occur. These precipitation reactions release H^+ (Equations (2) – (3)), which suppresses pH increase. Consequently, the terminal pH represents a dynamic balance between pH elevation driven by urea hydrolysis and pH depression associated with crystal formation. At the higher urea concentration, the hydrolysis-induced alkalinization outweighs the acidifying effect of precipitation, producing a continued rise in pH.

In all cases, increasing urease concentration accelerated the pH rise. This response reflects a higher urea hydrolysis rate at greater urease levels, which speeds the production of NH_4^+ and increases OH-.

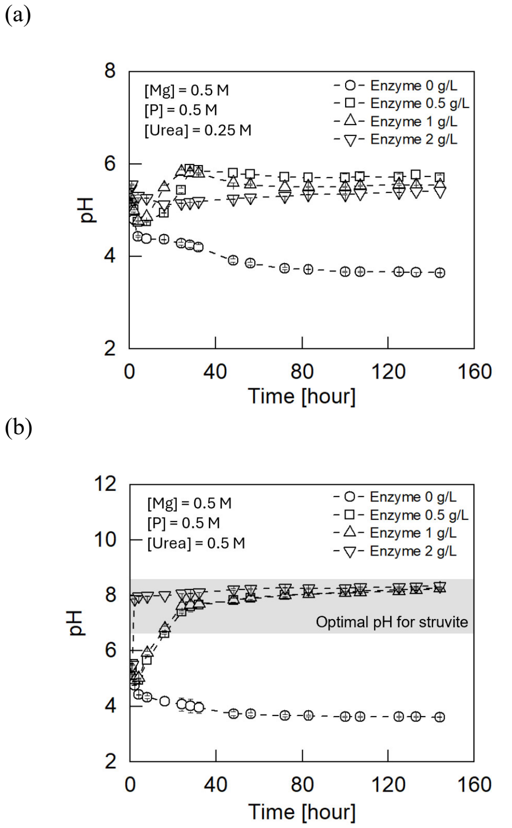

Figure 3. Solution pH evolution over time for (a) low urea concentration (0.25 M) and (b) high urea concentration (0.5 M).

3.2 *Precipitate characterization*

Representative SEM images are shown in Figure 4. In the low urea condition, the solids exhibited a combination of pseudo-octahedral crystals, consistent with morphologies reported for newberyite, and coffin-like prismatic crystals characteristic of struvite (Figure 4a). In contrast, only struvite-type morphologies were observed under the high urea condition (Figure 4b).

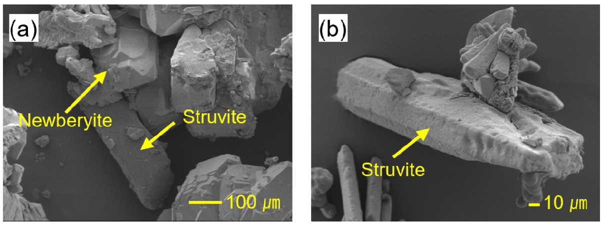

Figure 4. SEM images of crystals formed under each case: (a) low urea concentration (0.25 M) and (b) high urea concentration (0.5 M).

Figure 5 presents the diffraction patterns of the precipitates for all test conditions. In the absence of urease, newberyite was identified under both urea concentration, consistent with the observed pH decrease. At the low urea level, mixed phases of newberyite and struvite were detected for most urease dosages, with the exception of the 0.5 g/L urease case, which yielded struvite alone (Figure. 5a). Under the high urea case, struvite was confirmed across all urease concentrations (Figure 5b).

Elemental analysis of the precipitates indicated that, at low urea concentration, all urease dosages produced an ammonium-to-solid conversion (precipitation efficiency) of approximately 30 %. Under the high-urea condition, the efficiency increased to about 70 % for every urease level tested. Based on these outcomes, the high-urea solution with 0.5 g/L urease was selected as the optimal treatment recipe.

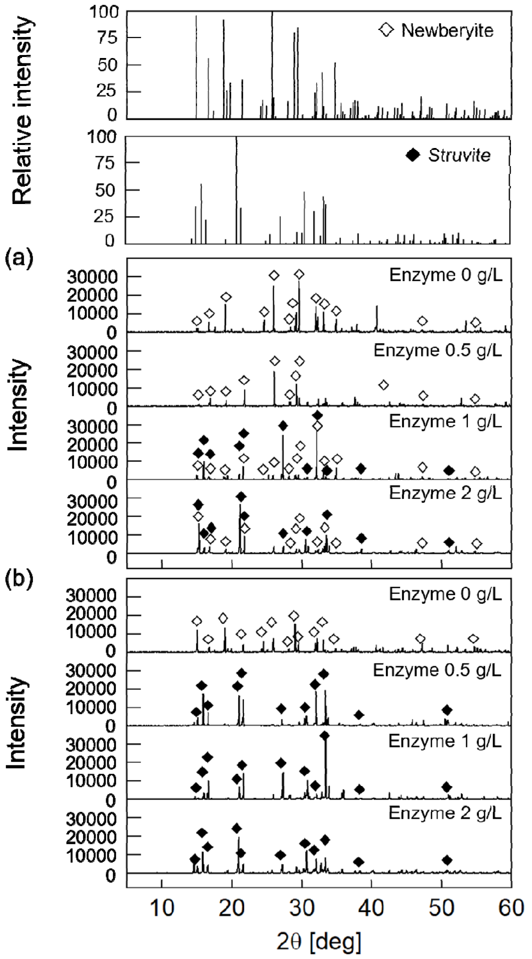

Figure 5. Stacked XRD patterns of crystals formed under (a) low urea concentration (0.25 M) and (b) high urea concentration (0.5 M).

3.3 *Improvement of Shear Stiffness in Soil Column*

Figure 6 illustrates the temporal change in shear waveform and velocity within the struvite-treated sand column. A progressive decrease in first-arrival time was observed, indicating a continual increase in V_S over the treatment period (Figure 6a). As struvite precipitation advanced, V_S rose from 398 to approximately 545 m/s and then reached a plateau near 545 m/s. This result indicates the struvite crystal increases contact area between soil particles. This increase corresponds to an almost two-fold enhancement in the shear stiffness of the specimen.

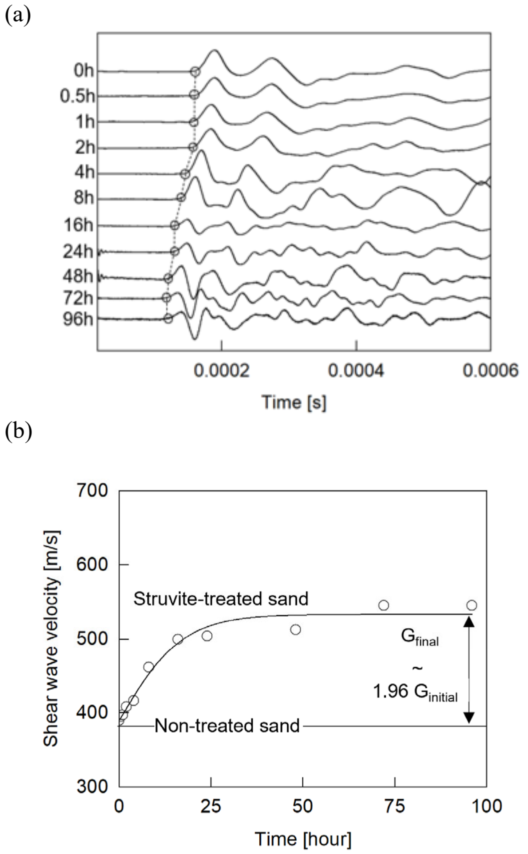

Figure 6. Evolution of (a) shear waveform and (b) shear wave velocity during the struvite precipitation in the sand specimen.

Moreover, subsequent to the S-wave tests, the hydraulic conductivity of the struvite-treated sample decreased markedly by approximately two orders of magnitude, dropping from 6 X10^{-2} cm/s to 6 X10^{-4} cm/s.

4 CONCLUSIONS

Ammonium by-products generated from urea hydrolysis can drive eutrophication and, through pH-dependent speciation, convert to un-ionized ammonia (NH_3) gas that poses human-health risks. Enzyme-induced struvite precipitation was investigated by varying urea and urease concentrations at slightly acidic initial pH. Higher urea levels enabled attainment of the pH window favorable for struvite, and the precipitates were confirmed as struvite by XRD and SEM. In sand treated via struvite formation, shear stiffness increased by approximately two-fold, coupled with a two-orders of magnitude reduction in hydraulic conductivity relative to the untreated specimen.

5 ACKNOWLEDGEMENTS

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