（第3号様式）(Form No. 3)

学 位 論 文 要 旨
Dissertation Summary

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論　文　名: **Optimization of Enzyme-Mediated Calcite Precipitation for Soil**
(Dissertation Title) **Improvement Technique**

Enzyme-mediated calcite precipitation (EMCP) as a soil-improvement technique has been studied as an alternative method for improving the engineering properties of soil. The increase in unconfined compressive strength ranging from 400 kPa to 1.6 MPa depending upon the amount precipitated calcite are achieved and the permeability of the improved samples is reduced by more than one order of magnitude (Neupane et al., 2015, 2013, Yasuhara et al., 2012, 2011). This technique employs enzyme of urease to dissociate urea into ammonium and carbonate ions. The produced carbonate ions are precipitated as calcite crystals in the presence of calcium ions. The reaction of the urea hydrolysis and the calcite formation are shown in **Equations (1)-(3).**

$$CO(NH_2)_2+2H_2O \xrightarrow{\text{urease}} 2NH_4^{+}+CO_3^{2-} \qquad \textbf{(1)}$$

$$CaCl_2 \rightarrow Ca^{2+}+2Cl^{-} \qquad \textbf{(2)}$$

$$Ca^{2+}+CO_3^{2-} \rightarrow CaCO_3\downarrow\text{(precipitated)} \qquad \textbf{(3)}$$

An enzyme-reagent mixed solution (i.e., purified urease and $CaCl_2$-urea), which produces the precipitated calcite after the chemical reaction, is injected into the soil. The precipitated calcite may provide bridges between the grains of sand, restricting their movement, and hence, improving the stiffness and the strength of the soil (Yasuhara et al., 2011). A schematic of the whole process listed above and grouting mechanism expected are illustrated in **Figure 1.**

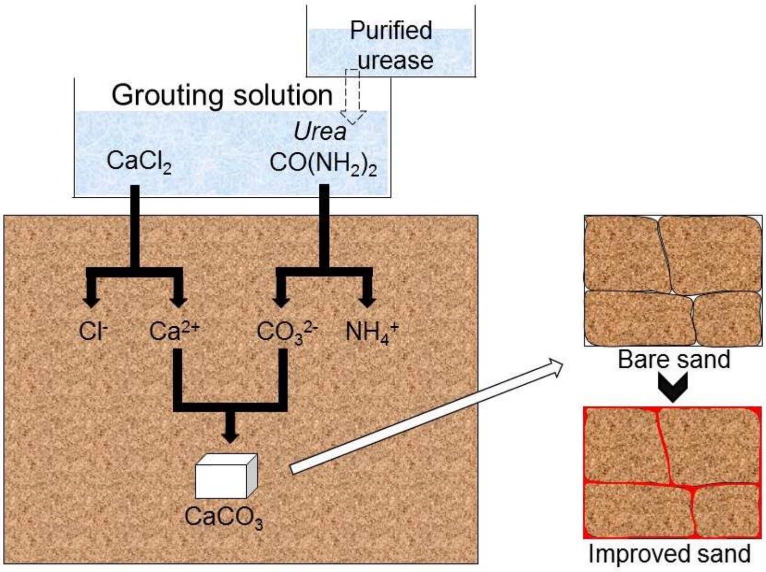

Figure 1: Schematic of calcite precipitation process on EMCP technique

In this study, the precipitation of calcite is evaluated directly in the transparent polypropylene (PP) tubes. Various combinations of reagent and enzyme are mixed thoroughly and allowed to react. As seen in the schematic in **Figure 2**, reagent and urease are prepared separately. Various combinations of urease are thoroughly mixed in distilled water for 2 mins and filtered using a filter paper (pore size of 11 μm) to remove the undissolved particle of urease. The purified urease and the reagent solution (i.e., urea and $CaCl_2$) are mixed thoroughly in the PP tubes, in a total solution volume of 30mL.

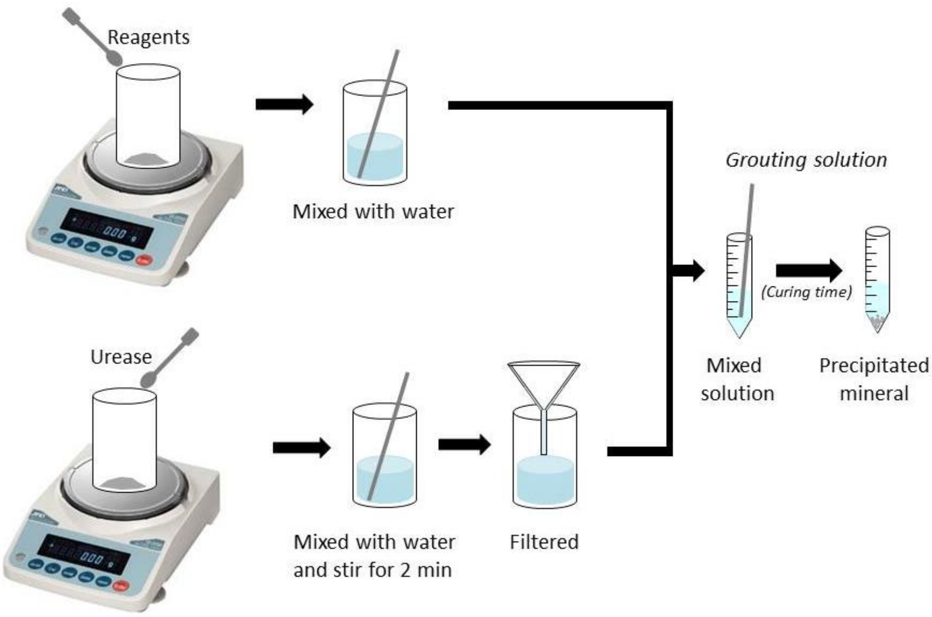

Figure 2: Schematic of precipitation test

Test tubes are kept in a box without shaking at a room temperature of 20°C. After the curing time, the grout solution is filtered through filter paper (pore size of 11 μm). The particles deposited 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 minerals is evaluated by combining the precipitated minerals deposited in the test tubes with the materials remaining on the filter paper. Two identical tests are performed for each condition to check the reproducibility. The mass of the precipitated minerals is shown as the precipitation ratio, namely, the ratio of the actual mass of the precipitated minerals to the theoretical mass of the maximum precipitation of $CaCO_3$. The theoretical mass of $CaCO_3$ (g) is evaluated as $C·V·M$, where C and V represent the concentration of the solution in moles per liter and the volume of the solution in liters, respectively, and M is the molar mass of $CaCO_3$ of 100.087 g/mol. The actual mass is the mass of the precipitated materials in grams evaluated from the tests.

In order to evaluate applicability of EMCP as a soil-improvement technique, the evolutions in the strength and permeability of the treated sample are examined through unconfined compressive strength (UCS) tests and permeability test, respectively. Soil specimens are prepared in PVC cylinders and treated with concentration-controlled solutions composed of reagent-enzyme. Polyvinyl chloride (PVC) cylinders (5 cm in diameter and 10 cm in height) are used to prepare the sand samples. The fixed volume of the solution is injected into the prepared sand specimens. The injected volume is controlled by the number of pore volumes (PV), one PV being ~75 mL. Firstly, dry silica sand is poured into the PVC cylinders to obtain a relative density of 50%. Secondly, one PV of the grout solution is poured into the PVC cylinders from the top. After the curing time, the treated specimens are removed from the PVC cylinders. The surface of the treated samples is flattened before the UCS tests are conducted. The schematic of UCS test is illustrated in **Figure 3.**

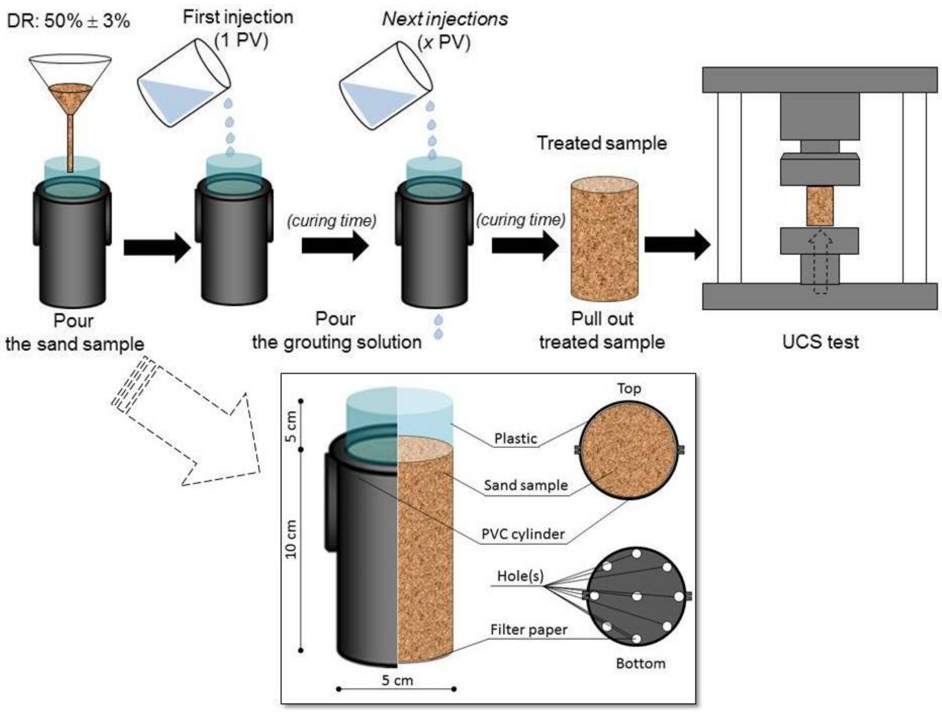

Figure 3: Schematic of UCS tests

The permeability tests are conducted to examine the effect of the precipitated minerals on the hydraulic conductivity of the treated sand. An acrylic cylinder (5 cm in diameter and 10 cm in height) is used to conduct the tests. The procedure of the sample preparation for the permeability tests is equivalent to that for the UCS test. It is the same for the sample preparation, density of sand, the grouting solution and number of pore volumes (PV). The schematic for the permeability test is showed in **Figure 4**.

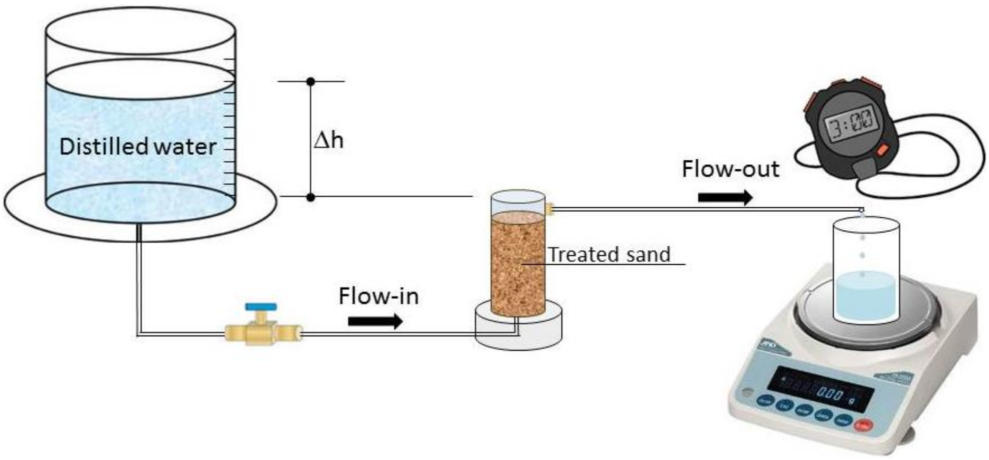

Figure 4: Schematic of permeability tests

The acid leaching method is used to evaluate the amount of precipitated calcite between the sand samples (Neupane et al., 2013; Yasuhara et al., 2012, 2011). In this process, the treated sand is washed with distilled water to dissolve the salt materials and then dried in an oven at a temperature of 100^{o}C for 24 hrs. The dried sand is weighed and washed with 0.1 mol/L of HCl several times until air bubbles no longer appeared. Filter paper (pore size of 11 μm) is used to minimize the lost mass of sand during the ishing process. The sand is dried again, and the final weight is taken. The dry weight lost during the acid leaching is evaluated and assumed to be the weight of the precipitated materials. The reactions taking place are expressed by **Equation (4).**

$$CaCO_3 + 2HCl \rightarrow CaCl_2 + CO_2 + H_2O \qquad \textbf{(4)}$$

Finally, by comparing the relation between the numbers of injections, the mineral mass, and the UCS within the treated specimens obtained in this study, the effects on the mechanical properties are explicitly investigated.

A series of experiments is conducted in this study. First series of experiment is conducted to evaluate the applicability of magnesium chloride as the substituted material in EMCP technique. Magnesium chloride was newly added to the grouting solutions composed urea, urease and $CaCl_2$ to control the reaction rate and to enhance precipitation amount. The second series of experiments is conducted to examine the applicability of magnesium chloride as delaying agent to control the precipitation process and improve the homogenous distribution of the precipitated minerals within soil. Sand specimens are prepared in 1-m PVC cylinders and are treated with the obtained grouting materials. Thus, the distribution of the precipitated minerals within the sand samples is evaluated. The third series of experiments is conducted to evaluate the effectivity of magnesium sulfate as a substitute material in EMCP technique. Magnesium sulfate is added to the grouting solutions composed urea, urease and $CaCl_2$ to increase the precipitated amount and promote the formation of aragonite and gypsum. The fourth series of experiments is performed to evaluate the applicability an ammonium removal method of ion exchange using natural zeolite to reduce the concentration of ammonium ion in EMCP technique. The natural zeolite of *mordenite* is added to the prepared grouting solutions, and the evolution of concentration of ammonium ion is evaluated. The effects of the utilization of natural zeolite during the grouting preparation on the EMCP parameters such as amount, pH, mineralogical substance of the precipitated minerals, and the improvement in the strength of treated soil are also evaluated.

Whole series of the experiment has the specific and valuable finding in order to optimize the application EMCP as a soil improvement technique. The findings in this study are listed below.

1. Application of magnesium chloride as the substituted material in the EMCP technique is indeed found to increase the precipitated amount and delay the reaction rate of the precipitation process.
2. The presence of magnesium chloride in the grouting material is found to be able to delay the reaction rate and hence, improves the uniformity distribution of the precipitated materials within 1-m column sample.
3. Utilization of magnesium sulfate in the grouting materials is found to significantly increase the efficiency of precipitated ratio in a high concentration of reagent (1.0 mol/L) and promotes the formation of aragonite and gypsum in addition to calcite. The production of the high amount of precipitated materials causes a sufficient strength, which can be obtained by a limited of number of injections.
4. Application of ammonia removal method of ion exchange using zeolite in EMCP technique is found as a potential method to be a solution for the environmental issue in the application of EMCP as a soil improvement technique. A significant reducing in the ammonium ion is obtained without compromising in the efficacy of EMCP as a soil improvement technique.