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EXAMINATION OF CALCITE PRECIPITATION USING PLANT-DERIVED UREASE ENZYME FOR SOIL IMPROVEMENT

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ABSTRACT: The enzyme-mediated calcite precipitation (EMCP) method was established as a soil-improvement technique. This study discussed the possibility of using a plant-derived urease enzyme as a bio-catalyst. The urease enzyme was utilized for the hydrolysis of urea, which causes the supply of calcium ions to precipitate calcium carbonate. Several sources that may contain the urease enzyme were examined. Extracts from cabbage and soy pulp, without purification, were utilized as substitutes for commercial urease enzyme products. Initially, the solution was composed of 15 g/L of crude extract and 1 mol/L each of urea and calcium chloride. The precipitation results using commercial products and crude extracts were compared under the same concentration of reagent solution. A mineralogical analysis of the precipitated materials was also conducted. The mineralogy and morphology of the precipitated materials were examined by Fourier-Transformed Infra-Red (FTIR) spectroscopy, X-Ray Diffraction (XRD), and Scanning Electromagnetic (SEM) tests. The precipitation ratios using the crude extracts as replacements for the urease enzyme were found to be more than 60% of the maximum theoretical value. From the mineralogical and morphological analyses, the calcite mineral was confirmed as the precipitated material. The results of this study indicated that crude extracts from cabbage and soy pulp are potential materials for sources of the urease enzyme.

Keywords: Calcite precipitation, Crude extract, Plant-derived urease, Soil improvement

1. INTRODUCTION

Calcite precipitation methods were established for improving the mechanical properties (i.e., shear strength and stiffness) of soil [1]–[8], for reducing the permeability of porous materials [4]–[8], for decreasing the swelling potential of clayey soil, and for mitigating the liquefaction potential [2], [3]. The most popular method among them is the microbially-induced carbonate precipitation (MICP) method. With this method, bacterial cells (e.g., *Sporosarcina pasteurii*) are utilized to hydrolyze urea into ammonium and carbonate ions [7], [9], [10. The produced carbonate ions may be precipitated as calcite crystal in the presence of calcium ions. The calcite crystal can be used to improve the engineering properties of soil, such as shear strength, stiffness, and permeability [4], [10]. However, the evolutions of the mechanical and hydraulic properties of the soil may not be easy to control because it is nearly impossible to constrain the extinction and/or generation of living bacteria in natural environments [8]. Therefore, an alternative to the bacterial method is necessary. Recently, using the urease enzyme itself may be one of the alternatives for producing calcite precipitation. Using the urease enzyme may be more straightforward than using bacteria because the biological treatment does not need to be considered [11].

The urease enzyme has been found in various bacteria, fungi, and higher plants. Several families of common plants, including some varieties of beans, melons, squash, and the pine family, are very rich in urease enzymes [12**]**. This includes jack beans (*Canavalia ensiformis*) and soybean (*Glycine max*) leaves and seeds [13]. The best source of a plant-derived urease enzyme is that obtained from jack beans, which were identified as the first nickel metalloenzyme [13], [14**]**. In 1926, the urease from jack beans was the first enzyme to be crystallized [13**]**. The urease enzyme from jack beans has a maximum catalytic activity in pH 6.0 – 7.0 at a temperature of 65°C and is inactive at temperatures above 70°C [14]–[16]. The urease enzyme is needed to catalyze the reaction of urea hydrolysis to form ammonia (NH_4^+) and carbonate (CO_3^{2-}) ions. The reactions of the calcium carbonate formation are shown in the following equations (1)-(3):

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

$$CaCl_2 \rightarrow CO_2^+ + 2Cl^- \quad (2)$$

$$Ca_2^+ + CO_3^{2-} \rightarrow CaCO_3 \quad (3)$$

The enzyme-mediated calcite precipitation (EMCP) technique has been investigated using commercial products of the urease enzyme from jack beans to enhance the mechanical properties of soil [10], [17]–[19]. This method increases the unconfined compressive strength (UCS) of treated soil ranging from 400 kPa to 1.6 MPa, and reduces the permeability by more than one order of magnitude [19]. Therefore, the utilization of the enzyme itself is very effective for laboratory-scale models [11]. However, because urease purification is expensive, the cost of the urease enzyme may hamper field applications when purchased from commercial suppliers [20].

Several studies have used plant-derived urease enzymes to improve the mechanical properties of soil – an example is the utilization of a crude extract from watermelon seeds [20]–[22]. The reagent solution obtained by mixing urea and the crude extract from watermelon seeds was seen to be effective for inducing the precipitation of a calcium phosphate compound. The maximum UCS obtained through the use of this method is 125.6 kPa after 28 days of curing [21]. Meanwhile, each plant-derived urease enzyme has a limited lifespan - its activity and chemical reaction decrease with time. This limited lifespan is beneficial in soil improvement as the enzyme can degrade naturally and eliminate the long-term environmental effect [23], [24]. Instead of using seeds that have urease activity, many researchers have been investigating new plant species such as leaves. The examination of other sources for urease enzymes is of great importance. The schematic of soil improvement with plant-derived urease is shown in Fig. 1.

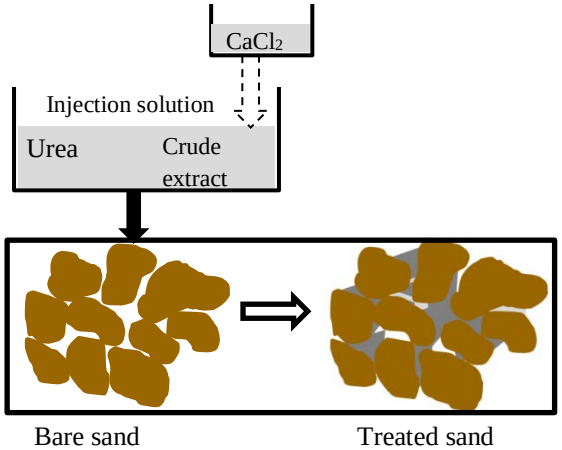


Fig. 1 Schematic of soil improvement using calcite precipitation with plant-derived urease enzyme

The aim of this study is to obtain new sources of urease enzymes as replacements for the purified enzyme. The outer leaves of cabbage, as well as soy pulp, which are both waste materials, were selected in this work and crude extracts of them were examined for the production of calcite precipitation. Test-tube experiments were performed to determine the effect of the crude extracts and to evaluate the material precipitation. Fourier-Transformed Infra-Red (FTIR) spectroscopy, X-Ray Diffraction (XRD), and Scanning Electron Microscope (SEM) tests were conducted to observe the mineralogy and morphology of the precipitated materials.

2. METHODOLOGY

2.1 Extraction of Urease Enzyme

The crude extract from plant-derived urease was obtained by the following procedure. The plant-based raw materials (i.e., outer leaves of cabbage and soy pulp) were finely ground using a mixer, and 1.5 g of the pulp or powder were soaked in 0,1 L of the solution with 20 mM of buffer phosphate and pH 7 as a solvent extraction for 1 hour with occasional agitation. The suspension was then centrifuged, stirred at 40 g for 20 minutes, and a clear or slightly turbid solution without dregs was produced and then filtered using filter paper (pore size 11μm) to remove the undissolved particles of the crude extract. The procedure for the crude extract preparation from the plant-based raw materials is illustrated in Fig. 2.

2.2 Test-Tube Experiments

In these experiments, the precipitation of calcium carbonate was evaluated directly in transparent test tubes. The performed procedure is a modification of the procedures developed by Neupane and Putra [17]-[18]. The urea and $CaCl_2$ were obtained from Kanto Chemical (Tokyo, Japan). The concentration of the reagent solution (Urea-$CaCl_2$) was 1mol/L, which was mixed with 15 g/L of the crude extract. The reagent solution and the crude extract were thoroughly mixed with distilled water with a total solution volume of 30 mL. Curing time was necessary during the process of precipitation. The test tubes were cured at a room temperature of 20°C, while the sample was kept in a box without shaking. The precipitation of $CaCO_3$ was produced over a curing time of 2 weeks, and the solution was filtered with filter paper. The particles deposited on the filter paper and the particles remaining in the tubes were dried at 60°C for 24 hours. The procedure for the test-tube experiments with the crude extracts is

illustrated in Fig. 3.

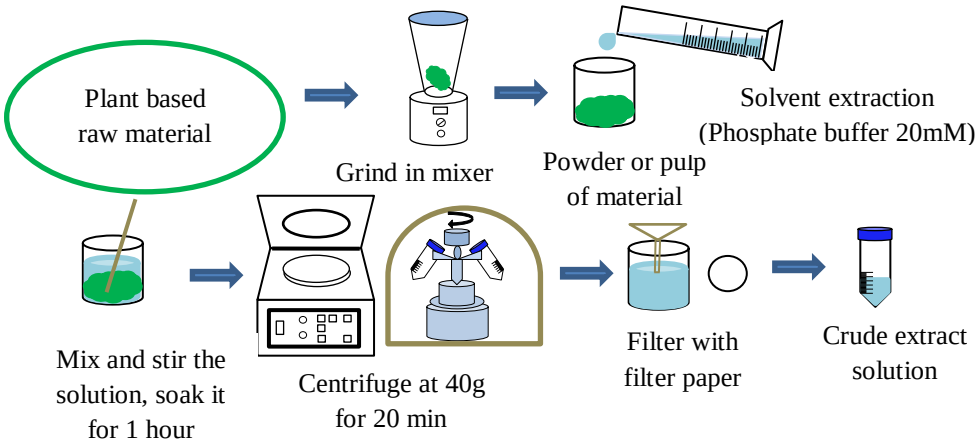


Fig. 2 Procedure for crude extract preparation

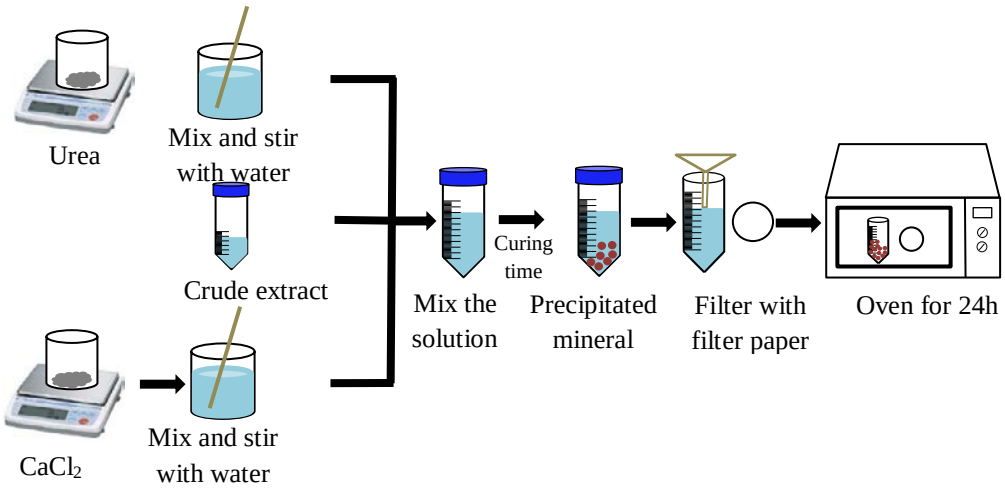


Fig. 3 Procedure for test-tube experiments

The precipitation ratio, which is the ratio of the actual mass of the precipitated materials to the theoretical mass of the maximum precipitation of calcium carbonate, is determined as

$$\textbf{precipitation ratio}\ (\%) = \frac{\textbf{actual mass}\ (a_m)}{\textbf{theoretical mass of}\ CaCO_3(t_m)} \quad (4)$$

$$t_m = C \cdot V \cdot M \quad (5)$$

where a_m is the mass of the precipitated materials obtained from the test (g), t_m is the theoretical mass of $CaCO_3$ (g), C is the concentration of the solution (mol/L), V is the volume of the solution (L), and M is the molar mass of $CaCO_3$ (100.087 g/mol).

The hydrolysis rate was measured immediately after the mixing; a standard curve was provided by determining the conductivity resulting from the complete hydrolysis of several concentrations of urea [26]. The evolution of the resistance with time was measured using an LCR meter KC-555 KDK from Koyo Electronics Industries Co., Ltd, Tokyo, Japan, and the changes in conductivity were determined. The hydrolysis rate was determined by calculating the gradient slope of the conductivity changes versus time, expressed in Equations (6) and (7).

$$Conductance\ (mS) = \frac{1}{R} \quad (6)$$

$$Hydrolysis\ rate\ \left(\frac{u}{g}\right) = \frac{\theta_{ms}}{\theta_{sc}} \cdot v \cdot N \quad (7)$$

where R is the measured resistance (Ω), θ_{ms} is the gradient slope of the measured sample, θ_{sc} is the gradient slope of the standard curve, v is the volume of the sample (L), and N is the final concentration of ammonia (mMol/L).

A comparison of the precipitation ratios between the plant-derived urease enzyme and that of a commercial urease product, purified from jack bean

meal (Kishida Chemical Co., Inc., Japan) with a urease activity of 2.360U/g, was conducted to evaluate the effect of adding the crude extract as a substitute for the urease enzyme. The material precipitation was evaluated in this work according to the different sources of the urease enzyme. The material precipitation was crushed into a fine powder for further characterization. FTIR spectroscopy, XRD, and SEM tests were conducted to analyze and examine the mineralogical substances.

3. RESULTS AND DISCUSSION

The addition of the crude extracts of cabbage and soy pulp as replacements for the urease enzyme for the test-tube experiments may be effective for producing precipitation. The precipitation ratios of various sources of the urease enzyme with a concentration of 1 mol/L of urea-CaCl2 and 15 g/L of urease for several curing times were evaluated. A summary of the precipitation ratios obtained from the experiments is shown in Fig. 4. It should be noted that the reproducibility of the two identical tests has been confirmed for each experimental condition. According to Fig. 4, the average precipitation ratios from the crude extracts of cabbage and soy pulp are more than half of the precipitation ratio of the purified urease (commercial product). The addition of the crude extracts from cabbage and soy pulp should be effective for producing mineral precipitation for soil improvement.

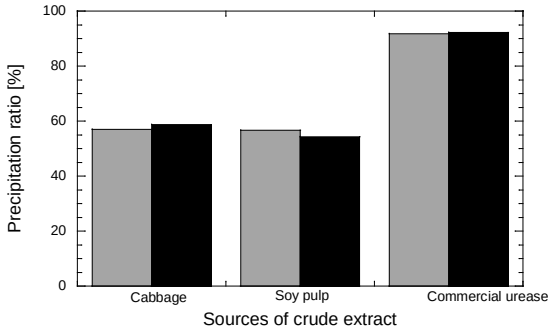


Fig. 4 Results of test-tube experiments for plant-derived urease and commercial urease enzyme

The effect of the crude extracts of cabbage and soy pulp as replacements for the urease enzyme on the hydrolysis rate was also evaluated. The hydrolysis of urea, using a crude extract of cabbage, occurred immediately after the test. However, using a crude extract of soy pulp, the hydrolysis process occurred 30 min after the test. The changes in conductance with time for cabbage and soy pulp are shown in Fig. 5. As is apparent from the figure, the hydrolysis of urea by soy pulp takes longer than that by cabbage.

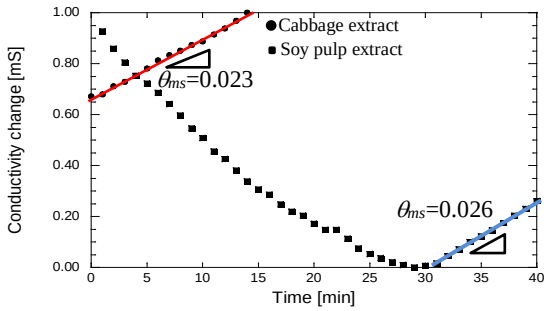


Fig. 5 Evolutions of conductivity with time for crude extracts of cabbage and soy pulp

In order to determine the hydrolysis rate of urea, standard curves were provided by determining the conductivity resulting from the complete hydrolysis of various concentrations of urea. The standard curves are depicted in Fig. 6. The hydrolysis rate of urea was determined using Equation (7). The hydrolysis rates for the crude extracts of cabbage and soy pulp are 93U/g and 104U/g, respectively.

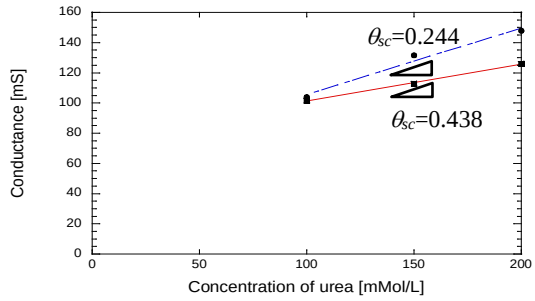


Fig. 6 Standard curves for hydrolysis rate analysis

The precipitated materials were assessed by X-Ray Diffraction (XRD) to confirm more precisely the types of calcium carbonate that had been formed. There are three polymorphs of calcium carbonate: calcite, vaterite, and aragonite. All three polymorphs can occur at the same time in some material precipitation. Calcite is a carbonate mineral and the most stable polymorph of calcium carbonates. Vaterite, like aragonite, is the metastable phase of calcium carbonate. As it is less stable than either calcite or aragonite, vaterite has a higher solubility than either of these phases. It converts to calcite (at low temperatures) or aragonite (at high temperatures: >60°C) [27]. The obtained XRD patterns for the precipitates using cabbage and soy pulp are shown in Fig. 7; the main peak intensities of the precipitated materials are plotted on the curve. The peaks in the XRD test results show that the precipitated materials

are calcite.

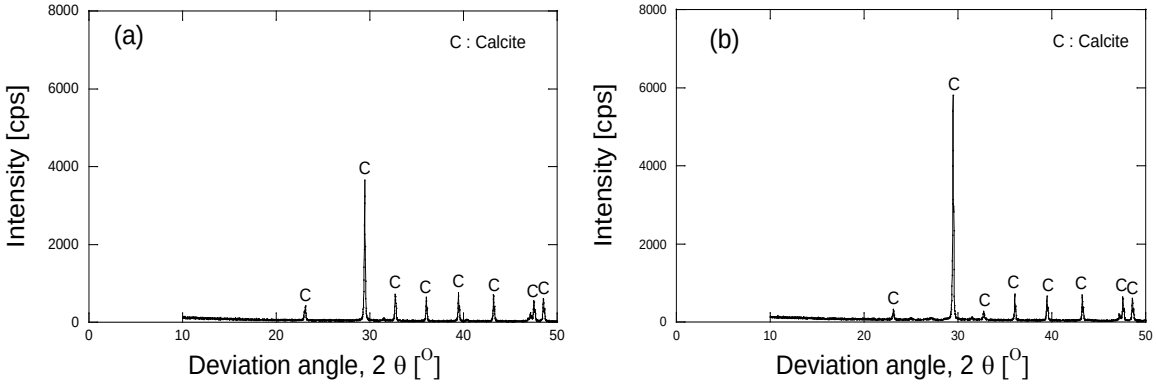


Fig. 7 X-Ray Diffraction (XRD) spectra confirming the polymorph of crystal: (a) cabbage and (b) soy pulp

Fourier-transformed Infra-Red (FTIR) spectroscopy has been performed to detect the characteristic functional groups of the active constituent present in the plant crude extracts. The occurrence of the major absorbance bands specific to calcite was observed using the wave number based on the vibrational frequencies between the bonds of atoms. The FTIR spectra for precipitates, formed by utilizing the crude extracts of cabbage and soy pulp, have been compared with those of the commercial calcite product (Fig. 8). The wavelength (i.e., the wave numbers) used in this study are ultraviolet and visible (UV-Vis), which range from 400 cm^{-1} to 4000 cm^{-1}. The measurement results using the calcite product showed absorption bands in the area of 873 cm^{-1} and 1400 cm^{-1} which resulted from vibrations of C=O [28].

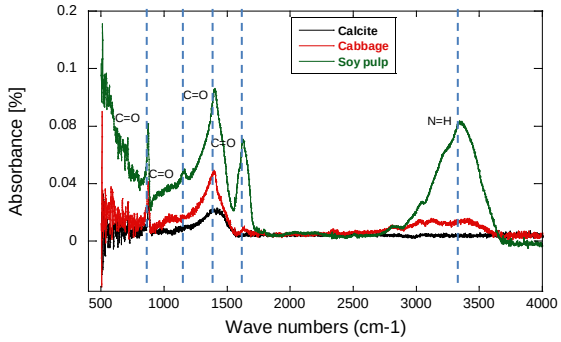


Fig. 8 FTIR spectra for precipitate formed of calcite powder and crude extracts of cabbage and soy pulp

Similar results were also shown for the crude extracts of cabbage and soy pulp. The absorption was seen at wavelengths of 872 cm^{-1} and 1416 cm^{-1} for the crude extract of soy pulp. In addition, there was absorption at a wavelength of 1739 cm^{-1} that showed the C=O bond; it can be derived from organic compounds [29]. The absorption at wavelength 3361 cm^{-1} was the absorption from the NH and OH group; it is due to the crude extract of the natural materials that have been used. Therefore, the NH and OH groups were probably from natural compounds contained in the soybeans as basic material [29].

From the results of the analysis, the crude extract from cabbage showed absorption patterns similar to those of the commercial calcite, as well as those of the crude extract of soy pulp in the lower range of intensity. The difference between the cabbage and the soy pulp was in the wavelength from 3000 cm^{-1} - 3600 cm^{-1}. While the spectra in the crude extract of cabbage at 3600 cm^{-1} may be attributed to the absorption for the OH group, the spectra in the crude extract of soy pulp at the 3000 cm^{-1} - 3300 cm^{-1} indicate the absorption of C-H bonds that may originate from the mixture of alkane, alkene, and aromatic [29].

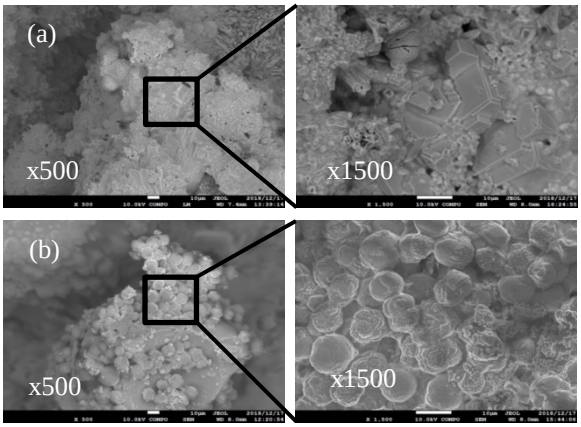


Fig. 9 SEM images of calcium carbonate precipitated: (a) crude extract of cabbage and (b) crude extract of soy pulp

The visualization of the crystal phase can be shown using SEM tests. Fig. 9 presents the SEM images of the precipitated $CaCO_3$ obtained from various sources of the urease enzyme. The results show that the particle size in a crude extract of cabbage is not uniform when compared to that of soy pulp. In Fig. 8(a), the crystal structure image of the precipitated materials shows a rhombohedral shape with different sizes.

On the other hand, Fig. 8(b) shows a spherical shape. Furthermore, crystal agglomeration forms also exist in the precipitated materials when using cabbage and soy pulp. It has been reported in previous research that the delay in the precipitated process may promote the agglomeration forms in the precipitated materials [30], and that the appearance of agglomeration crystals may be inhibited in the crystallization process [31]. The delay in the hydrolysis process when using a crude extract can cause considerable agglomeration. The crystallization process may not be completed even after the curing time of 2 weeks. The above-mentioned results confirmed the usefulness of the urease enzyme that is obtained from certain waste food on the production of $CaCO_3$. However, further experiments will be required to enable the control of the particle size, morphology, and polymorphism of calcite precipitation when using cabbage and soy pulp.

4. CONCLUSION

The possibility of using a plant-derived urease enzyme as a bio-catalyst in the enzyme-mediated calcite precipitation (EMCP) method has been evaluated. The development of alternative sources for the urease enzyme, which are cost-effective and easy to control in the natural environment, is a crucial issue in the sustainability of calcite precipitation techniques. In this study, the applicability of crude extracts of cabbage and soy pulp as the bio-catalysts, instead of the commercial urease enzyme, was examined. The results of the test-tube experiments showed that 40-60% of the precipitated materials can be achieved using cabbage and soy pulp, without purification, as the bio-catalysts. The mineralogical analysis of the precipitated material using FTIR showed that the precipitated material was calcium carbonate; it was identified by several wave numbers. This was due to the effect of the use of the crude extracts. Additionally, XRD confirmed that the polymorph of calcite was promoted. The particle size and SEM analyses showed that the utilization of cabbage and soy pulp results in different sizes and shapes of precipitated materials. The results of this study have indicated that plant-derived urease enzymes, using the crude extracts of cabbage and soy pulp, are potential sources for the urease enzyme in the EMCP method. However, further experiments will be required to examine the mechanical and hydraulic properties of the treated soils.

5. ACKNOWLEDGMENTS

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6. REFERENCES

[1] Akiyama M. and Kawasaki S., Novel Grout Material Comprised of Calcium Phosphate Compounds: In Vitro Evaluation of Crystal Precipitation and Strength Reinforcement. Eng Geol.;125, 2012, pp.119-128.

[2] Ivanov V. and Chu J., Applications of Microorganisms to Geotechnical Engineering for Bioclogging and Biocementation of Soil In Situ. Rev Environ Sci Biotechnol.;7(2), 2008,pp.139-153.

[3] Putra H., Yasuhara H. and Kinoshita N., Optimum Condition for The Application of Enzyme-Mediated Calcite Precipitation Technique As Soil Improvement Method. Int J Adv Sci Eng Inf Technol,7(6), 2017, pp. 2145-2151.

[4] Harkes M.P., Van Paassen L. A., Booster J. L., Whiffin V. S. and Van Loosdrecht M. C. M., Fixation and Distribution of Bacterial Activity In Sand to Induce Carbonate Precipitation for Ground Reinforcement. Ecol Eng.;36(2), 2010, pp.112-117.

[5] Van Paassen L. A., Harkes M. P., Van Zwieten G. A., Van Der Zon W. H., Van Der Star W. R. L., and Van Loosdrecht M. C. M., Scale Up of Biogrout: A Biological Ground Reinforcement Method. Proc 17th Int Conf Soil Mech Geotech Eng Acad Pract Geotech Eng. 2009, pp.3:2328-2333.

[6] Sidik W. S., Canakci H., Kilic I. H. and Celik F., Applicability of Biocementation for Organic Soil and Its Effect on Permeability. Geomech Eng.;7(6), 2014, pp.649-663.

[7] Whiffin V. S, Van Paassen L. A., and Harkes M. P., Microbial Carbonate Precipitation As A Soil Improvement Technique. Geomicrobiol J.;24(5), 2007, pp.417-423.

[8] Yasuhara H., Hayashi K. and Okamura M,. Evolution in Mechanical and Hydraulic Properties of Calcite-Cemented Sand Mediated by Biocatalyst. Geo-Frontiers ,2011.

[9] DeJong J. T., Mortensen B. M., Martinez B. C., and Nelson D. C., Bio-Mediated Soil Improvement. Ecol Eng.;36, 2010, pp.197–210.
[10] Martinez B. C., DeJong J. T., Ginn T. R., Montoya B. M., Barkouki T. H., Hunt, C., Tanyu B. and Mayor. D., Experimental Optimization of Microbial-Induced Carbonate Precipitation For Soil Improvement. J Geotech Geoenviron Eng.;139, 2013, pp.587–598.
[11] Knorr B., Enzyme-Induced Carbonate Precipitation for The Mitigation of Fugitive Dust. A thesis degree Master Sci Arizona State Univ. 2014.
[12] Sirko A. and Brodzik R., Plant Ureases: Roles and Regulation. Acta Biochim Vol. 47, 2000, Pp. 1189-1195.
[13] Sumner J. B., The Isolation And Crystallization Of The Enzyme Urease. J Biol Chem Vol. 69. 1926, Pp. 435-441.
[14] Sumner J. B., Urease. In: Sumner J.B., The Enzyme, Academic Press New York. 1951, Vol. 1, Pp. 873-892.
[15] Frankenberger W. T. Jr., and Tabatabai M. A., Amidase and Urease Activity In Plants. Plant Soil. Vol. 64. Issue 2, 1982, pp. 153-166.
[16] Lai C. M. and Tabatabai M. A., Kinetic Parameters of Immobilized Urease. Soil Biol Biochem. Vol. 24, 1992, Pp. 225-228.
[17] Putra H., Yasuhara H. and Kinoshita N., Applicability of Natural Zeolite for NH-Forms Removal in Enzyme-Mediated Calcite Precipitation Technique. Geosci. Vol. 7, Issue 3, 2017.
[18] Neupane D., Yasuhara H., Kinoshita N. and Unno T., Applicability of Enzymatic Calcium Carbonate Precipitation as a Soil-Strengthening Technique. Geotech Geoenviron Eng ASCE. Vol. 139, 2013, Pp2201–2211.
[19] Yasuhara H., Neupane D., Hayashi K. and Okamura M., Experiments and Predictions of Physical Properties of Sand Cemented by Enzymatically-Induced Carbonate Precipitation. Soil Found. Vol. 52 (3), 2012, Pp. 539-549.
[20] Dilrukshi R. A. N., Nakashima K., and Kawasaki S., Soil Improvement using Plant-Derived Urease-Induced Calcium Carbonate Precipitation. Soils Found. Vol. 58, Issue 4, 2018, Pp. 894-910.
[21] Dilrukshi R. A. N., Watanabe J. and Kawasaki S., Sand Cementation Test using Plant-Derived Urease and Calcium Phosphate Compound. Mater Trans. Vol. 56, Issue 9, 2015, Pp. 1565-1572.
[22] Dilrukshi R. A. N., Watanabe J. and Kawasaki S. Strengthening of Sand Cemented with Calcium Phosphate Compounds using Plant-Derived Urease. Int J GEOMATE. Vol. 11, Issue 3, 2016, Pp. 2461-2467.
[23] Marzadori C., Miletti S., Gessa C. and Ciurli S., Immobilization of Jack Bean Urease on Hydroxyapatite: Urease Immobilization in Alkaline Soils. Soil Biol Biochem. Vol. 30, 1998,Pp. 1485–1490.
[24] Pettit N. M., Smith A. R. J., Freedman R. B. and Burns R., Soil Urease: Activity, Stability, and Kinetic Properties. Soil Biol Biochem. Vol. 8, 1976, Pp. 479–484.
[25] Putra H., Yasuhara H., Kinoshita N. and Hirata A., Application of Magnesium to Improve Uniform Distribution of Precipitated Minerals in 1-M Column Specimens. Geomech Eng. 2017. Vol. 12, Issue 5, Pp. 803-813.
[26] Whiffin V. S., Microbial $CaCO_3$ Precipitation for The Production of Biocement. Murdoch Univ. 2004.
[27] Ming N. and Ratner B.D., Differentiating Calcium Carbonate Polymorphs by Surface Analysis Techniques–an XPS and TOF-SIMS Study. Surf Interface Anal. Vol. 40, Issue 10, 2008, Pp. 1356–1361.
[28] Dixon J., Minerals in Soil Environments. Second Edition. Madison, Wisconsin, USA: Soil Science Society of America. 1989.
[29] Uddin J., Macro to Nano Spectroscopy. InTech; http://intechopen.com/books/macro-to-nano-spectroscopy/organic-compounds-ft-ir-spectroscopy, 2012.
[30] Putra H., Yasuhara H., Kinoshita N., Neupane D., Lu C. W., Effect of Magnesium as Substitute Material in Enzyme-Mediated Calcite Precipitation for Soil-Improvement Technique. Front Bioeng Biotechnol. Vol.4:37, 2016.
[31] Rasyidi F.A., Tumanggor J., Yuni N. P., The Effect of Crystallization Time with Cooling Prices on Ammonium Sulphate Crystal Growth from The Solution. J Tek Kim. Vol. 15, 2008, Pp. 9-16.

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