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## **Microbially mediated sand solidification using calcium phosphate compounds**

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## **Abstract**

To evaluate the potential utility of a new biogROUT based on calcium phosphate compounds (CPC) (CPC biogROUT), we conducted unconfined compressive strength (UCS) tests and scanning electron microscope (SEM) observations of sand test pieces made from CPC biogROUT. To produce CPC biogROUT, we used soil extracts including microorganisms derived from two soils differing in pH, three amino acids, and urea as a pH increasing reactant. Temporal increase in pH was observed in slightly acidic soil by the addition of ammonia sources. On the other hand, there was no significant increase in pH in slightly alkaline soil except for urea. In most cases, the UCS of the test pieces made from CPC biogROUT with soil extracts from acidic soil was higher than that without the addition of ammonia sources. Whisker-like crystals of CPC were identified by SEM observation of test pieces with UCS of over 50 kPa. These results suggest that CPC biogROUT has sufficient strength as a countermeasure for liquefaction and that amino acids can be made available as new pH-increasing reactants for CPC biogROUT. In addition, they suggest that either CPC biogROUT or CPC chemical grout alone, or a combination of the two grouts, can be used depending on the various properties of grounds and soils.

**Key words:** grouting material, biogrout, calcium phosphate compound, unconfined compression strength, ammonia, amino acid

## **1. Introduction**

Major cities in Japan are located on an alluvial plain and a number of settlements in these cities are vulnerable to disasters such as earthquakes. Ever since liquefaction was observed in the Niigata earthquake of 1964 (Ohsaki, 1966), the damages caused by liquefaction have been confirmed in many earthquakes such as the great East Japan earthquake of 2011. There is an urgent need for seismic reinforcement, including countermeasures for liquefaction (The Japanese Geotechnical Society, 2011).

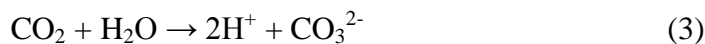
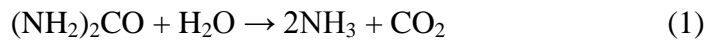
In recent years, grout materials have been developed to control ground permeability and to reinforce the ground with bacterially produced cement material (DeJong et al., 2006; Whiffin et al., 2007; Ivanov and Chu, 2008; Hata et al., 2009; Harkes et al., 2010; Kawasaki et al., 2010; Mukunoki et al., 2010; Van Paassen et al., 2010; Inagaki et al., 2011). Biogrout refers to a new ground improvement technology based on biological activity (Van Paassen et al., 2009). In biogrouting, a moderate microbial reaction is expected to reduce the solidification speed as compared to a chemical reaction that causes rapid solidification. A number of mineral formation mechanisms have primarily

been considered for biogrout:  $\text{CaCO}_3$  precipitation using urea and ureolytic bacteria (Harkes et al., 2010);  $\text{CaCO}_3$  precipitation using glucose and yeast (Kawasaki et al., 2006); siloxane bond formation using glucose and yeast (Terajima et al., 2009); and iron- or manganese-compound precipitation using iron-oxidizing bacteria (Weaver et al., 2011). Soil and rock vary infinitely in their physical, chemical, and biological properties. To apply biogrout to various soils and rocks, it is important to develop new mechanisms of cement material precipitation. Toward this end, we are carrying out fundamental studies by focusing on calcium phosphate compounds (CPC) as novel grout materials (Akiyama and Kawasaki, submitted for publication; Fig. 1).

Research on CPC precipitation and solidification is proceeding in the field of medical and dental science. Fernández et al. (1998) reported that the unconfined compressive strength (UCS) of CPC reaches over 10 MPa under normal temperature and pressure conditions. CPC has two unique characteristics: pH dependency of the solubility and a self-setting mechanism (Tung, 1998). We discovered that grout with only CPC (“CPC chemical grout”) increased the UCS of sand test pieces with time and that the volume of precipitated CPC crystal increased with increasing pH (Akiyama and Kawasaki, submitted for publication). The results demonstrated the possibility of technically developing “a CPC biogrout” in which an increase in biological pH brings about crystal

precipitation of CPC from a low-pH injection solution. With these properties, CPC biogROUT offers the advantage of controlling the solidification time; furthermore, the strength of the ground can be increased by using a combination of CPC chemical grout for rapid solidification and CPC biogROUT for long-term solidification.

CaCO<sub>3</sub> precipitation using urea and ureolytic bacteria—the most common mechanism of biogROUT involving the use of pH-increasing reactions—occurs as follows: hydrolysis of urea (Eq. (1)), pH elevation by NH<sub>3</sub> production (Eq. (2)), dissolution of CO<sub>2</sub> (Eq. (3)), and CaCO<sub>3</sub> precipitation (Eq. (3)) (Whiffin et al., 2007; De Muynck et al., 2010; DeJong et al., 2010; Harkes et al., 2010).



However, injection of foreign ureolytic bacteria (*Sporosarcina pasteurii*) involves many problems such as the obtainment of approvals and licenses, public acceptance, and the necessity to monitor microbial ecology for safety (METI Web site, 2005). Despite the improving efficiency, the industrial production of urea requires an ammonia production process that involves fossil fuel consumption and CO<sub>2</sub> emission (Rafiqul et

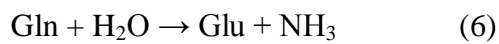
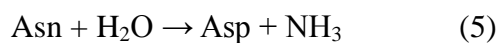
al., 2005). An increase in the demand for fertilizers may cause the consumption of urea to increase worldwide. In this study, a fundamental concept is established for soil improvement: the activation of indigenous bacteria without the injection of foreign microbes. Furthermore, we focus not only on urea but also on novel ammonia sources (amino acids) as pH-increasing reactants to develop adaptable grout materials for a variety of soils and rocks.

In this study, we carried out a fundamental laboratory test of a novel grout called CPC biogROUT. A schematic of the study design is shown in Fig. 2. First, we estimated the decomposing potential of amino acids as candidates for novel ammonia sources by conducting a pH-increasing test. Second, on the basis of the results, we estimated the strength by conducting UCS tests and scanning electron microscope (SEM) observations of test pieces cemented by CPC chemical grout and CPC biogROUT. It is said that liquefaction does not occur if the USC of soil and ground is between approximately 50 and 100 kPa during an earthquake (Port and Harbour Institute, 1997; Yamazaki et al., 1998; Matsuda et al., 2008). We set 50 to 100 kPa as the target UCS range for the CPC chemical grout and CPC biogROUT.

## **2. Selection of ammonia sources**

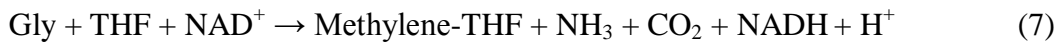
In this study, we focused on ammonification—the process of amino acid deamination after hydrolysis of protein by heterotrophic bacteria—as the pH-increasing reaction (Galloway, 2005). Amino acids can also be made from high-protein organic waste at low cost using bacteria. Therefore, we chose amino acids and urea as ammonia sources. In other words, we adopted the pH-increasing mechanism of microbial ammonia production in soil. To our best knowledge, there are no instances in which indigenous microorganisms and ammonia sources other than urea have been used in actual biogrowth. The chemicals and enzymes involved in the ammonification and degradation of amino acids are shown in Tables 1 and 2.

We identified three amino acids as new ammonia sources from among the 20 that mainly constitute a protein (Alberts et al., 2009). First, asparagine (Asn) and glutamine (Gln), each with two amidogens and an initially acid pH, were chosen for their high efficiency of ammonia production. These amino acids release ammonia according to Eqs. (5) and (6) to produce aspartate (Asp) and glutamate (Glu), respectively, as by-products. These by-products are then taken up by soil microorganisms and the citric acid cycle after conversion to oxaloacetic acid and oxoglutaric acid (Magasanik, 1982).





Second, we also selected the simplest amino acid glycine (Gly), which has an initially acid pH and high solubility in water. After it is taken up by the cell, ammonia is released via the glycine cleavage reaction (Kikuchi and Hiraga, 1982) shown in Eq. (7).



These three amino acids are easy to obtain, safe to handle, and widely used as food additives in Japan. Furthermore, they can be decomposed by soil bacteria (Wheeler and Yemm, 1958; Sato, 1983; Frankenberger Jr. and Tabatabai, 1991a, 1991b). Accordingly, here we used asparagine, glutamine, and glycine as sources of ammonia, in addition to urea, which has been used in many foregoing studies.

### **3. Chemical and biological properties of soil used in this study**

To utilize the indigenous bacteria in soil for the pH-increasing and UCS tests, two soils differing in pH were sampled from each of two agricultural farms (Soil-H and Soil-R). The chemical property (chemical composition of soils and ion concentration of water extracted solution from soils) and biological property (microbial populations of the soils) are shown in Table 3. Sampling from agricultural farms is economical; in addition, the obtained samples contain a large biomass with many kinds of bacteria (Brady, 1990). Soil-H, which was sampled from experimental farms in Hokkaido (Hokkaido

University), is an acidic soil similar to many other agricultural acid soils in Japan (Shindo, 1997). The alkaline soil, originated from unconsolidated lime-rich rock (Jayasinghe et al., 2010), was sampled from an experimental farm on the main island of Okinawa (University of the Ryukyus) as a control. The two soils show the following basic properties.

- 1) pH: Soil-H is acidic whereas Soil-R is alkaline.
- 2) Chemical composition of soils: Though there is approximately similar composition each other, the percentage of CaO in Soil-R is a little larger than that in Soil-H.
- 3) Ion concentration of soil extracts: Higher levels of nitrate and sulfate ions are detected in Soil-H than in Soil-R. Soil-R is rich in Ca and bicarbonate.
- 4) Microbial population: In both soils, approximately  $10^6$  bacteria were detected by YPD (including yeast extract, peptone, and dextrose) media. In addition, decomposers of the four kinds of ammonia source ranged between  $10^4$  and  $10^5$ . There were no significant differences between the soils. Based on these properties, we used the two soils in the pH-increasing test in Sections 4 and 5.

#### **4. Experiment to increase pH by ammonia production**

#### **4.1. Materials and methods**

Four ammonia sources and two soils were used to evaluate the ammonia production potential, pH-increasing reaction, and ammonia concentration (Table 4).

The ammonia sources were prepared at the concentrations of 0.01 mol/L (hereafter M), 0.1 M, and 1.0 M. Asparagine and glutamine could not be used to prepare 1.0 M solutions owing to their low solubility in water; therefore, nearly saturated solutions were prepared at concentrations of 0.18 and 0.23 M, respectively (Chemical book Web site, 2011, Fürst et al., 1997). Each solution (32.5 mL) was carefully poured into 25 g of each soil in a plastic centrifuge tube (Fig. 3). To avoid the effect of suspending of sand particles on microbial population and pH, two-layer design comprised of ammonia source solution in upper layer and lower the solution-saturated soil in lower layer were maintained during incubation term. The samples were left to stand at 20 °C. The pH was measured by inserting electrode of pHSpeare (Eutech Instruments Pte., Ltd., Singapore) into the upper layer of ammonia source solution 0, 1, 2, 7, 14, 21, 28, 35, or 42 days later (day 0 was when the reaction started) without disturbing the soil and solution. This pH device is designed for measuring the pH of solids and semisolids including gels (e.g., Wilson et al., 2010). The ammonium concentration in the supernatant was determined using PACKTEST WAK-NH<sub>4</sub> (Kyoritsu Chemical-Check Laboratory, Corp., Tokyo,

Japan). Solution concentrations beyond the measurement range of PACKTEST were diluted and determined as needed. The values measured by PACKTEST were converted from ppm to M. The detection limit was 0.2 ppm.

In addition, to grasp the increasing range of pH by production of ammonia and carbon dioxide from ammonia sources and to evaluate the pH-increasing potential of their decomposers, the final pH of the supernatant was analyzed by using the typical geochemical code PHREEQC (Parkhurst, 1995) with phreeqc.dat of thermodynamic database. Factors affecting the pH-increasing reaction in this study were discussed by comparing actual measurements and theoretical calculations.

## **4.2. Results**

The following four trends were identified on the basis of the differences in the type and concentration of the ammonia source solution:

1) Ammonia sources over 0.1 M (Fig. 4-B, C, E, and F): For all sources, a temporally increasing trend in the pH was measured in Soil-H, whereas the pH remained constant for deionized water. On the other hand, the pH of the Soil-R supernatant was lower than that of deionized water for all ammonia sources except urea. For 0.1 M of an ammonia source in Soil-H, the pH was higher in the three amino acids than in urea two days after

initiating the reaction.

2) 1.0 M of an ammonia source (Fig. 4-C and F): The pH of the supernatant increased up to 9.5 by the addition of urea in both soils.

3) 0.01 M of an ammonia source (Fig. 4-A and D): Two days after reaction initiation in Soil-H, the pH of the supernatant with an amino acid exceeded that with urea. Although the pH in urea attained the pH level in amino acids between 14 and 21 days later, it decreased thereafter. The final pH 42 days after reaction initiation was about 7 in amino acids and 5 in urea. There was no significant trend in the pH of the supernatant of Soil-R, except when urea was added. As in Soil-H, the final pH of urea-added supernatants in Soil-R was less than that of supernatants with an amino acid.

4) The ammonia concentration 42 days after reaction initiation (Table 5): The concentrations of ammonium were below 2.8 mM (50 ppm) for ammonia sources of 0.01 M. The addition of an ammonia source of 0.1 or 1.0 M increased the ammonium concentration to over 0.1 M (2000 ppm). In particular, the concentration reached ~0.4 M (7500 ppm) with the addition of urea.

### **4.3. Discussion**

The pH of the supernatant increased with ammonia production only in acidic soils

(Soil-H). On the other hand, there were some supernatants in which the pH decreased in comparison with deionized water in alkaline soil (Soil-R), despite the detection of similar concentrations of ammonium. This latter result suggests that the addition of ammonia sources to alkaline soils such as Soil-R suppresses the pH-increasing reaction. Furthermore, these results show that a reaction rate regarding ammonia production depends on the applied soils and suggests that closer attention should be paid to the chemical properties of the soil during the actual construction of biogrout.

The addition of over 0.1 M of urea increased the pH more efficiently than that of other ammonia sources. One reason is that the hydrolysis of 1 mole of urea releases 2 moles of ammonia (Eq. (1)). For 1.0 M of urea (Fig. 4-C and F), the pH in both soils ultimately achieved a steady state at over 9.0. Given that the entire hydrolysis of 1 mole of urea releases 2 moles of ammonia and 1 mole of  $\text{CO}_2$ , the theoretical final pH by PHREEQC was 9.5 in the supernatant of both soils (Table 6). This result suggests that urea was hydrolyzed to the theoretically attainable maximum pH in 14 days and that urea is a candidate reactant for strongly increasing the pH.

At 0.1 M, however, the pH of Soil-H two days after reaction initiation was higher in amino acids than in urea, and a similar trend was observed at a concentration of 0.01 M. These results seemed to be caused by the difference of ammonia production rate among

four decomposers and indicate that amino acids exhibit a stronger pH-increasing effect than urea by controlling the reaction time and concentration of the solution.

The actual pH was lower than the theoretical result in every case except 1.0 M of urea (Table 6). There are a number of possible reasons for this result. First, by-products (aspartate and glutamate) may show acidity without being consumed by bacteria. Second, the pH may decrease owing to the dissolution of CO<sub>2</sub> derived from bacteria. Third, the buffering capacity due to the humic materials and clay mineral of soil (Brady, 1990) may suppress the increase in pH. In addition, the increase in pH was reversed after 28 days for a 0.01 M ammonia source, even with urea in Soil-R. The ammonia concentration in this case significantly decreased relative to that observed with the addition of 0.1 M 42 days later. These results suggest that nitrate production by ammonia oxidizers in soil (Galloway, 2005) exceeds ammonia production. Consequently, the pH reduction is accelerated for 0.01 M ammonia sources.

In conclusion, bacteria that decompose amino acids and urea were found to be activated in both soils, and pH elevation could be induced in acidic soils by adding ammonium sources and in alkaline soil by using urea. Under the acidic soil condition (Soil-H) with 0.01M of ammonia source and with 0.1M of that limited within two weeks in this study, amino acids were found to increase the pH more effectively than

urea. These results suggest that amino acids can be used as new sources of ammonia and, thus, as pH-increasing reactants in CPC biogROUT and other types of biogROUT involving the use of  $\text{CaCO}_3$ .

From the above results, we chose a concentration of 0.1 M for an ammonia source, as discussed in the next section, because the final pH was not significantly different from that with 1.0 M of an ammonia source (except urea) and the low amount of total reagent would have less environmental impact.

## **5. UCS test and SEM observation of test pieces cemented using CPC chemical grout and CPC biogROUT**

### **5.1. Materials and Methods**

A soil extract was used as a source of microorganisms (Fig. 5). The soil extract was made by shaking a mixture of 50 g of soil and 450 mL of sterilized deionized water at 200 rpm manually and vertically. The soil extract was left to stand for 10 min to allow large and high-density soil particles to sink naturally, and then 400 mL of the supernatant was sampled. The soil extract included extremely small soil particles (11.5 g dry weight/L), humic substances, and bacteria that did not sink for 10 min.



The CPC solution for the grout was made by dissolving reagent powders of a phosphate source (diammonium phosphate, DAP) and calcium sources (calcium nitrate, CN; calcium acetate, CA), which were weighed in advance, to the soil extract. Based on a previous study, the compositions of DAP:CN = 1.0M:0.5M (Cases 2-1 and 2-4) and DAP:CA = 1.5M:0.75M (Cases 2-3 and 2-6), which yielded the largest UCS in each calcium source, were adopted as the CPC solutions. In addition, the composition of DAP:CA = 1.0M:0.5M (Cases 2-2 and 2-5) was used as the reference composition. Ammonia sources were added to the CPC solution at a concentration of 0.1 M to achieve the maximum effect at low concentration (see Section 4). According to a previous study (Akiyama and Kawasaki, submitted for publication), cylindrical test pieces ( $\phi = 5$  cm,  $h = 10$  cm) were made using 320.09 g of Toyoura sand and 73.3 mL of CPC solution (Table 7). The test pieces were cured in an airtight container with high humidity for 28 days at 20 °C. Subsequently, the UCS of test pieces was measured at an axial strain rate of 1 mm/min by the UCS apparatus T266-31100 (Seikensha Co., Ltd, Japan). Two test pieces for each cure time were used in the UCS test. The pH of test pieces was calculated as an average of three measurements (top, bottom, and middle of test piece) using pHSppear (Eutech Instruments Pte., Ltd., Singapore). Test pieces made from the CPC solution alone, the CPC solution made by dissolving the reagent powders

into soil extract, and the CPC solution made by dissolving the reagent powders and each of ammonia sources into the soil extract were designated as “Chem,” “Cont,” and the name of each ammonia source (Asn, Glu, Gly, and Urea), respectively (Table 8). A comparison of Chem and Cont was required to evaluate the effect of the soil extract on the UCS brought about by the CPC chemical grout. This study was mainly focused on the effect of addition of ammonia source. Fragments of the UCS test pieces were observed by an SEM (SuperScan SS-550, Shimadzu Corporation, Kyoto). A fragment was naturally dried at 20 °C for a few days and carbon-coated by a carbon coater (Quick Carbon Coater SC-701C, Sanyu Electron Co., Ltd., Tokyo). SEM observations were carried out at an accelerating voltage 15 kV.

## **5.2. Results**

The results of UCS test, stress ( $\sigma$ )-strain ( $\epsilon$ ) curves, and SEM images of test pieces are shown in Figs. 6, 7, and 8–10, respectively. The following five trends were recognized based on differences in the kind and concentration of the ammonia source solution:

- 1) For CA, there was no significant difference between the UCS values of Chem and Cont. For CN, Cont showed a lower UCS than Chem.

2) Comparing Cont and ammonia sources in Soil-H, UCS values increased by adding an ammonia source. Asn in Case 2-2 and Gly in Case 2-3 showed larger UCS values than Chem or Cont.

3) In Soil-R, Gln and Urea of Case 2-5 showed higher UCS values than Chem or Cont. On the other hand, in Case 2-6, the UCS of Gly was lower than with other ammonia sources.

4) Increases in the UCS with increasing pH were recognized in the comparisons between Cont and the ammonia sources of Cases 2-1 and 2-4, in which CN was used as a calcium source.

5) Whisker-like crystals were observed in the fragments of test pieces for Cases 2-3 and 2-6, which mostly showed larger UCS values than Cases 2-1, 2-2, 2-4, and 2-5. The test pieces for Cases 2-3 and 2-6 had a stress ( $\sigma$ )-strain ( $\epsilon$ ) curve with clear a peak around 1%.

### **5.3. Discussion**

#### **5.3.1 UCS properties of sand test pieces due to CPC chemical grout and CPC biogrout**

Viewed from the CPC composition, the UCS values of Cases 2-3 and 2-6 (DAP:CA = 1.5M:0.75M) were larger than those of Cases 2-1 and 2-4 (DAP:CN = 1.0M:0.5M) or 2-2 and 2-5 (DAP:CA = 1.0M:0.5M) (Fig. 6). In addition, the strain at the peak of the curve in Cases 2-3 and 2-6 (Fig. 7C) shifted leftward ( $\epsilon = 1\%$ ) as the UCS increased, and exhibited clearer peaks than Cases 2-1 and 2-4 (Fig. 7A) or 2-2 and 2-5 (Fig. 7B), for which  $\epsilon$  was around 2%. This observation indicates that CPC chemical grout and CPC biogROUT can achieve the UCS values (50–100 kPa) needed to avoid liquefaction by an appropriate choice of CPC composition. In particular, in Case 2-3 of Soil-H, the addition of glycine increased the UCS of Chem and Cont (Fig. 7C), while maintaining the abovementioned strength. On the contrary, Gly in Case 2-6 of Soil-R showed the lowest value among the test pieces fabricated by the combination of DAP:CA = 1.5M:0.75M. The results indicate the necessity of considering the appropriate combination of CPC solution and ammonia source adaptable for the difference of soil. For example, the effective application of ground improvement for Soil-H is the CPC biogROUT that consists of CPC (DAP:CA = 1.5M:0.75M) and Gly, whereas that for Soil-R is the CPC chemical grout with the same CPC solution.

Improvements of the UCS by CPC biogROUT beyond that by CPC chemical grout were shown only at 28 days of cure time. Considering the more moderate nature of the

microbial reaction (compared to that of the chemical reaction) and the temporally increasing trend of UCS in CPC, data beyond 28 days is needed for test pieces made by CPC biogROUT. Based on such data, the solidification speed and final UCS suitable for the ground improvement site can be controlled.

### **5.3.2 Increasing pH using ammonia sources**

In this study, it was initially assumed that increasing the pH by microbial ammonia production from ammonia sources would result in an increase of CPC precipitation and UCS. With increasing pH, for Case 2-1 of Soil-H and Case 2-4 of Soil-R, the UCS in Urea was larger than that in Cont and other CPC solutions with an ammonia source. In Section 4, the extent of pH increase in Urea was also larger than that in other ammonia sources. These results indicate that urea is a strong candidate for an ammonia source in the utilization of CPC biogROUT. In Soil-H, the UCS of Asn in Case 2-2 and Gly in Case 2-3 exceeded the UCS values of both Urea and Cont. This observation indicates that there are more effective cases with ammonia sources other than urea, and that potential sources such as other amino acids, polypepton, etc., can provide further options for pH-increasing reactants in CPC biogROUT.

Except for the addition of urea, no significant pH increase was recognized with the

addition of other ammonia sources in Section 5. Even in Urea, test pieces for Case 2-1 (Fig. 6A) showed lower rate of pH increase than those for Case 1-2 (Fig. 4B) (Section 4). The conditions under which a significant pH increase was not observed can be enumerated as follows:

1) After CPC precipitation, the rate of ammonia oxidation consuming ammonia and releasing nitrate exceeded that of ammonia production, and suppressed the pH increase (Eq. (8)).



2) Given that the silanol group on the subsurface of sand shows slight acidity due to dissociation of the proton (Eq. (9)), the equilibrium reaction of sand particles suppressed the pH increase.



3) The CO<sub>2</sub> released by microbial respiration with ammonia production completely suppressed the pH increase (Eq. (10)).



4) The microbial population and activity in the soil extract from soil were insufficient for inducing a measurable increase in pH in test pieces.

Verification of the above conditions should be carried out in further studies; at the same time, this situation shows that there is room for improvement in how to induce pH increase and the possibility of greater UCS improvement than in this study. In addition, the utilization of not only ammonia sources but also glucose should be investigated to incubate indigenous microorganisms.

### **5.3.3 SEM observation of CPC crystal**

In test pieces of Soil-H, plate-like crystals were recognized for DAP:CN = 1.0M:0.5M (Fig. 9A) and DAP:CA = 1.0M:0.5M (Fig. 9B) by SEM observation. On the other hand, whisker-like crystals were observed for DAP:CA = 1.5M:0.75M (Fig. 9C). Test pieces of Soil-R also exhibited mostly similar crystals corresponding to the same composition of CPC (Fig. 10A, B, and C). These results were consistent and harmonious with SEM

observations in the previous study (Fig. 8A, B, and C, Akiyama and Kawasaki, submitted for publication). Gly in Case 2-6, however, displayed mainly plate-like crystals (Fig. 10C, Gly). Considering that Gly in Case 2-6 had a lower UCS than other test pieces, the induction of whisker-like CPC crystals is apparently one of the necessary conditions for adequate UCS.

#### **5.3.4 Technical and practical outlook of CPC chemical grout and CPC biogROUT as grouting materials**

This study, together with a previous study, indicates that a new grouting material based on CPC has superior functionalities in terms of not only attaining the target UCS but also handling in actual applications as follows:

1) An advantage of CPC chemical grout and CPC biogROUT is that they are recyclable as fertilizer and grout material (Akiyama and Kawasaki, submitted for publication). In contrast, the UCS achieved by CPC is currently less than that of concrete (over 10 MPa). However, if re-reclamation and re-excavation of the ground is expected, it is desirable to reduce the strength of ground as much as possible, considering the drilling required to reuse the CPC. In other words, given that both CPC chemical grout and CPC biogROUT



showed UCS values of 50–100 kPa, CPC potentially satisfies the following two conditions: it can function as a countermeasure of liquefaction and exhibit a high work efficiency of recycling.

2) The chemical properties of soils (in particular the pH) depend on the chemical properties of the mother rock and the amount of rainwater (Brady, 1990). The results of this study, in which two kinds of soil (slightly acidic and alkaline soils) were used, are expected to provide new recipes for ground improvement worldwide, e.g., CPC biogROUT for acidic soils (high rainfall area, peatland, etc.) and CPC chemical grout for alkaline soils (arid area, limestone area, etc.). In addition, multiple combinations of ammonia sources and the combination of both CPC grouts can be used flexibly for a variety of situations.

3) Test pieces that showed UCS values of over 50 kPa had whisker-like crystals (Figs. 8, 9, and 10). This indicates that the effective choice of grout material in accord with the soil and ground properties can be determined by evaluating whisker-like crystal production in advance using a small amount of soil and CPC solution.

The CPC biogROUT in this study, which utilizes indigenous soil microorganisms and amino acids as an ammonia source instead of urea, is a quite new technological

development that cross-sectionally unites the knowledge domains of medicine, dentistry, science, agriculture, and engineering. In addition, the pH-increasing reaction based on amino acids can be applied to existing biogrout that precipitates  $\text{CaCO}_3$  by utilizing urea and urealytic bacteria. These characteristics (including the functionalities stated above) indicate that this technological development has the scalability and versatility to be adapted to various grounds and soils.

## **6. Conclusions**

In this study, we conducted a fundamental laboratory experiment on CPC biogrout by using soil extract that include microorganisms derived from two soils differing in pH and amino acids as new ammonia sources. Especially in the case of use of soil extract from acidic soil (Soil-H), the UCS of test pieces fabricated by CPC biogrout was larger than that of test pieces fabricated by CPC chemical grout without an ammonia source; in addition, the maximum UCS was observed in the test piece with calcium acetate as a calcium source and glycine as an ammonia source. The main achievement of this technological development of a new grouting material, based on the results of this and a previous study, is to have obtained the knowledge and outlook in regards to the following five points.

- 1) Both CPC biogROUT and CPC chemical grout display sufficient strength (UCS of 50–100 kPa) as a grouting material to counteract liquefaction.
- 2) Indigenous microorganisms for the construction site can be made available for CPC biogROUT without the injection of foreign microorganisms.
- 3) Amino acids instead of urea can be made available for CPC biogROUT.
- 4) The effect of adding ammonia sources appears to be higher in acidic soil than in alkaline soil.
- 5) CPC biogROUT and CPC chemical grout can be used separately or in combination depending on the various properties of grounds and soils.

We intend to increase the size of the test sample for strength while considering the compatibility between the CPC compositions, additional ammonia sources, and properties of grounds and soils. Furthermore, we will examine the temporal variation in the microbial population, community structure, and species involved in the degradation of ammonia sources. In addition, we will analyze the precipitation process of whisker-like crystals and its underlying mechanisms.

## **Acknowledgements**

We are grateful to Shimeno Aoi and Mona Ariyama of Hokkaido University for the SEM images of CPC and the microbial population measurements in water-extracted solution from soil, respectively.

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Table 1. Chemical formulas and codes of materials used in this study.

Materials	Chemical formula	Code
Urea	$(\text{NH}_2)_2\text{CO}$	—
Asparagine	$\text{NH}_2\text{COCH}_2\text{CH}(\text{COOH})\text{NH}_2$	Asn
Aspartic acid	$\text{HOOCCH}_2\text{CH}(\text{COOH})\text{NH}_2$	Asp
Glutamine	$\text{NH}_2\text{CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NH}_2$	Gln
Glutamic acid	$\text{HOOC}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NH}_2$	Glu
Glycine	$\text{NH}_2\text{CH}_2\text{COOH}$	Gly
Tetrahydrofolic acid	$\text{C}_{19}\text{H}_{23}\text{N}_7\text{O}_6$	THF
Methylene-tetrahydrofolic acid	$\text{C}_{20}\text{H}_{23}\text{N}_7\text{O}_6$	Methylene-THF
Oxidized nicotinamide adenine dinucleotide	$\text{C}_{21}\text{H}_{27}\text{N}_7\text{O}_{14}\text{P}_2$	$\text{NAD}^+$
Reduced nicotinamide adenine dinucleotide	$\text{C}_{21}\text{H}_{29}\text{N}_7\text{O}_{14}\text{P}_2$	NADH

Table 2. Enzymes related to ammonia release in this study.

Equation No.	Reaction	Enzyme	Enzyme commission No.
(1)	Urea hydrolysis	Urease	3.5.1.5
(5)	Asparagine hydrolysis	Asparaginase	3.5.1.1
(6)	Glutamine hydrolysis	Glutaminase	3.5.1.2
(7)	Glycine cleavage system	Glycine dehydrogenase	1.4.4.2
		Aminomethyltranspherase	2.1.2.10
		Dihydrolipoyl dehydrogenase	1.8.1.4

Table 3. Chemical/biological properties of experimental soils in this study.

Chemical/biological property	Soil-H (Hokkaido Univ. in Sapporo, Hokkaido)	Soil-R (Univ. of Ryukyu in Nishihara, Okinawa)
pH	5.8	7.6
Chemical composition (%)		
Fe <sub>2</sub> O <sub>3</sub>	13.5	13.2
MnO	0.4	0.2
TiO <sub>2</sub>	0.6	0.8
CaO	2.6	5.7
K <sub>2</sub> O	1.9	3.1
Al <sub>2</sub> O <sub>3</sub>	11.5	10.9
SiO <sub>2</sub>	69.5	66.1
Ion concentration in water extracted solution from soil (ppm, (mM))		
Na <sup>+</sup>	3.6 (0.2)	1.1 (0.0)
NH <sub>4</sub> <sup>+</sup>	0.3 (0.0)	0.1 (0.0)
K <sup>+</sup>	8.6 (0.2)	2.8 (0.1)
Mg <sup>2+</sup>	2.2 (0.1)	0.7 (0.0)
Ca <sup>2+</sup>	9.3 (0.2)	16.5 (0.4)
Cl <sup>-</sup>	2.7 (0.1)	0.9 (0.0)
NO <sub>3</sub> <sup>-</sup>	37.6 (0.6)	11.7 (0.2)
PO <sub>4</sub> <sup>3-</sup>	0.0 (0.0)	0.0 (0.0)
SO <sub>4</sub> <sup>2-</sup>	13.8 (0.1)	2.5 (0.0)
HCO <sub>3</sub> <sup>-</sup>	4.3 (0.1)	39.5 (0.6)
Microbial population (cfu/g soil)		
YPD plate	1.5×10 <sup>6</sup>	2.5×10 <sup>6</sup>
0.1 M Asn	8.5×10 <sup>4</sup>	4.5×10 <sup>4</sup>
0.1 M Gln	3.5×10 <sup>5</sup>	4.5×10 <sup>5</sup>
0.1 M Gly	1.0×10 <sup>5</sup>	6.5×10 <sup>4</sup>
0.1 M Urea	1.2×10 <sup>5</sup>	2.0×10 <sup>4</sup>

Table 4. Case number in experiment to increase pH by ammonia production.

Case No.	Soil	Concentration of ammonia source
Case 1-1	Soil-H	0.01 M
Case 1-2	Soil-H	0.1 M
Case 1-3	Soil-H	1.0 M (0.18 M Asn, 0.23 M Gln)
Case 1-4	Soil-R	0.01 M
Case 1-5	Soil-R	0.1 M
Case 1-6	Soil-R	1.0 M (0.18 M Asn, 0.23 M Gln)

Table 5. Ammonia release from ammonia source by soil microorganisms.

Reaction solution	NH <sub>3</sub> concentration			
	Soil-H		Soil-R	
	ppm	<i>m mol/L</i>	ppm	<i>m mol/L</i>
Deionized water	<0.2	<0.01	<0.2	<0.01
0.01 M Asn	50	2.8	35	1.9
0.01 M Gln	50	2.8	20	1.1
0.01 M Gly	1	0.1	15	0.8
0.01 M Urea	15	0.8	10	0.6
0.1 M Asn	2000	111.1	2000	111.1
0.1 M Gln	2000	111.1	2000	111.1
0.1 M Gly	150	8.3	500	27.8
0.1 M Urea	2000	111.1	1500	83.3
0.18 M Asn	2000	111.1	2000	111.1
0.23 M Gln	2000	111.1	2000	111.1
1.0 M Gly	100	5.6	500	27.8
1.0 M Urea	7500	416.7	7500	416.7



Table 6. Theoretical final pH in reaction solution by PHREEQC and actual value by measurement.

Ammonia source	Mole concentration released from 1 mole of ammonia source		Theoretical value of pH in reaction solution to initial concentration of ammonia source				
	CO <sub>2</sub>	NH <sub>3</sub>	0.01	0.1 M	0.18	0.23	1.0 M
			M		M	M	
Deionized water							
Asparagine	0	1	10.8	11.3	11.4	—	—
Glutamine	0	1	10.8	11.3	—	11.5	—
Glycine	1	1	7.9	7.8	—	—	7.8
Urea	1	2	9.4	9.4	—	—	9.6
Soil-H							
Asparagine	0	1	10.6	11.1	11.4	—	—
Glutamine	0	1	10.6	11.1	—	11.5	—
Glycine	1	1	7.8	7.8	—	—	7.8
Urea	1	2	9.3	9.4	—	—	9.5
Soil-R							
Asparagine	0	1	10.5	11.1	11.6	—	—
Glutamine	0	1	10.5	11.1	—	11.3	—
Glycine	1	1	7.8	7.8	—	—	7.8
Urea	1	2	9.3	9.4	—	—	9.5

Table 7. Wet density of test pieces.

Case No.	Combination of P and Ca solution for CPC	Soil	Wet density $\rho_t$ (g/cm <sup>3</sup> )
Case 2-1	DAP:CN = 1.0M:0.5M	Soil-H	$1.851 \pm 0.025$
Case 2-2	DAP:CA = 1.0M:0.5M	Soil-H	$1.799 \pm 0.023$
Case 2-3	DAP:CA = 1.5M:0.75M	Soil-H	$1.780 \pm 0.021$
Case 2-4	DAP:CN = 1.0M:0.5M	Soil-R	$1.868 \pm 0.013$
Case 2-5	DAP:CA = 1.0M:0.5M	Soil-R	$1.838 \pm 0.025$
Case 2-6	DAP:CA = 1.5M:0.75M	Soil-R	$1.777 \pm 0.020$

Table 8. Composition of experimental solution for fabricating test pieces.

Name of solutions	Composition of experimental solution
Chem	CPC
Control (Cont)	CPC + Soil extract
Asn	CPC + Soil extract + 0.1 M Asparagine
Gln	CPC + Soil extract + 0.1 M Glutamine
Gly	CPC + Soil extract + 0.1 M Glycine
Urea	CPC + Soil extract + 0.1 M Urea

## Figure captions

Fig. 1. Flowchart of entire study. The steps carried out are highlighted in gray.

Fig. 2. Design and flow of present study.

Fig. 3. Two-layer design of pH-increasing test.

Fig. 4. Temporal variation in pH in soil solution with ammonia source. For asparagine and glutamine, nearly saturated solutions (C and F) were prepared at concentrations of 0.18 and 0.23 M, respectively, because their low solubility in water prevented the preparation of 1.0 M solutions.

Fig. 5. Procedure of making soil extract in this study.

Fig. 6. Effect of adding ammonia sources on unconfined compression strength (UCS) of test pieces cemented with CPC.

Fig. 7. Stress ( $\sigma$ )-strain ( $\epsilon$ ) curves of test pieces cemented with CPC.

Fig. 8. SEM images ( $\times 2000$ ) of test pieces cemented with CPC chemical grout (Chem).

Fig. 9. SEM images ( $\times 2000$ ) of test pieces cemented with CPC biogROUT using water-extracted solution from acidic soil (Soil-H).

Fig. 10. SEM images ( $\times 2000$ ) of test pieces cemented with CPC biogROUT using water-extracted solution from alkaline soil (Soil-R).

Figure 1

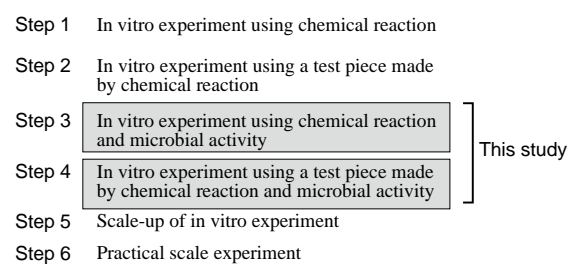


Figure 2

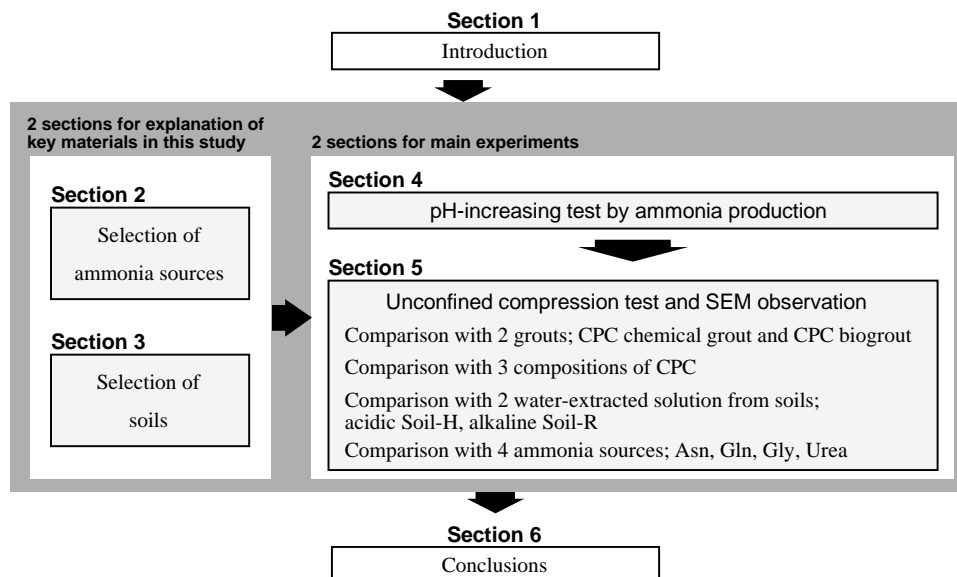


Figure 3

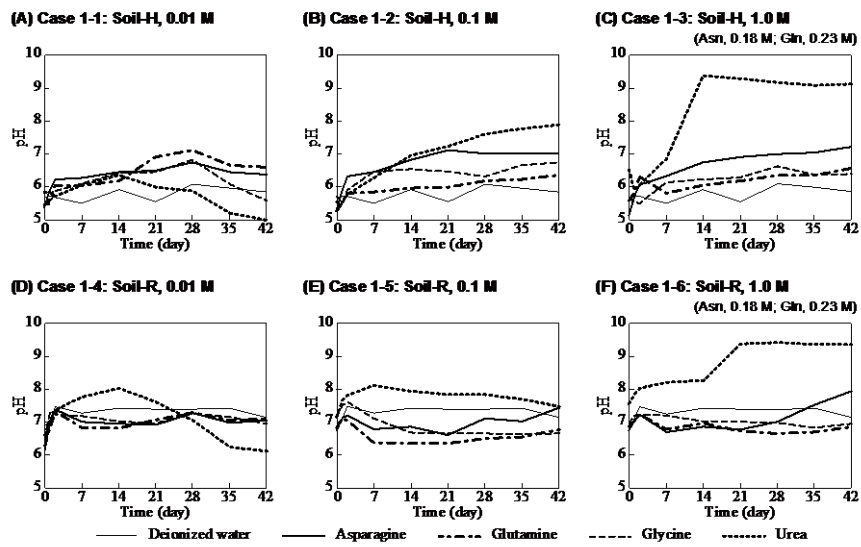




Figure 4

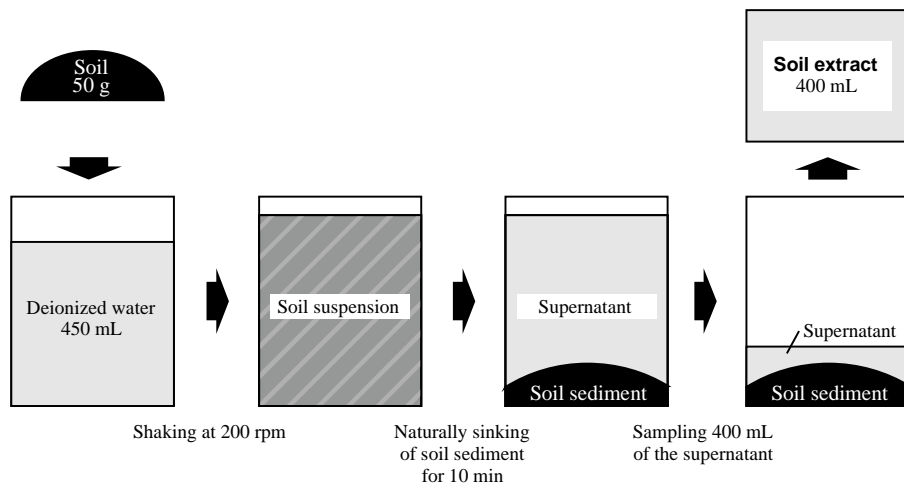


Figure 5

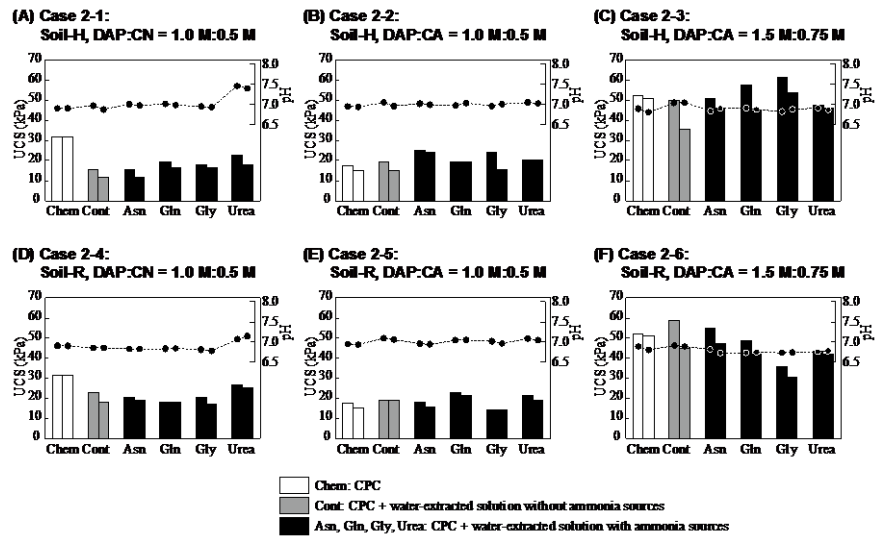


Figure 6

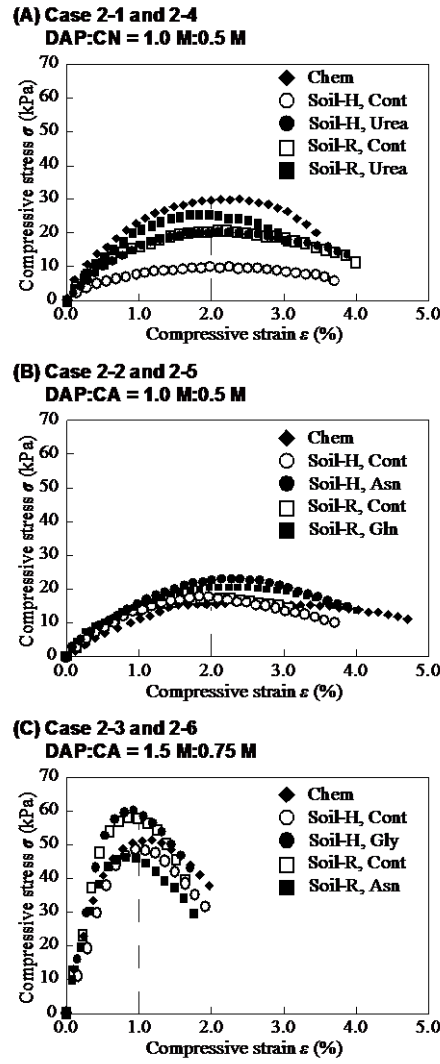
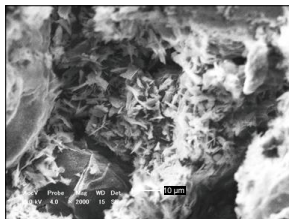
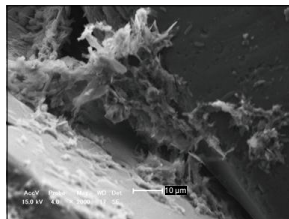


Figure 7

(A) DAP:CN = 1.0 M:0.5 M



(B) DAP:CA = 1.0 M:0.5 M



(C) DAP:CA = 1.5 M:0.75 M

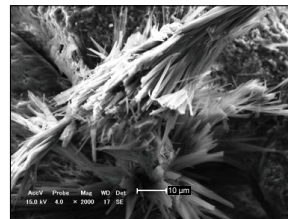
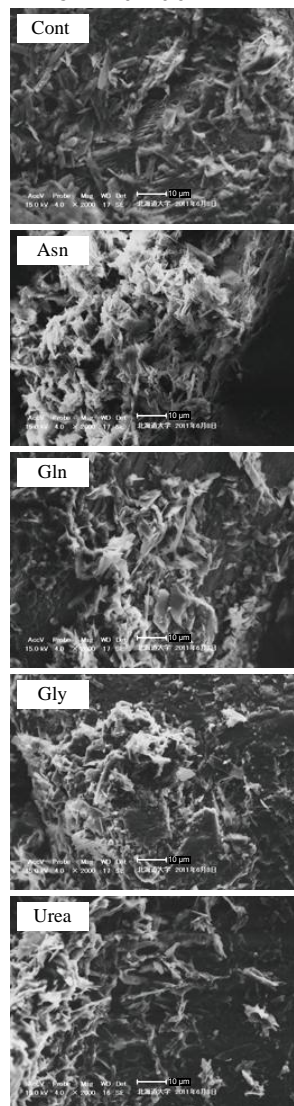
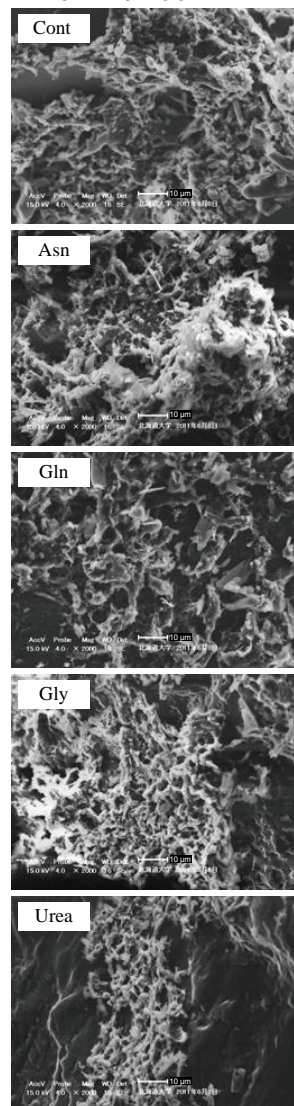


Figure 8

(A) Case 2-1  
Soil-H  
DAP:CN = 1.0 M:0.5 M



(B) Case 2-2  
Soil-H  
DAP:CA = 1.0 M:0.5 M



(C) Case 2-3  
Soil-H  
DAP:CA = 1.5 M:0.75 M

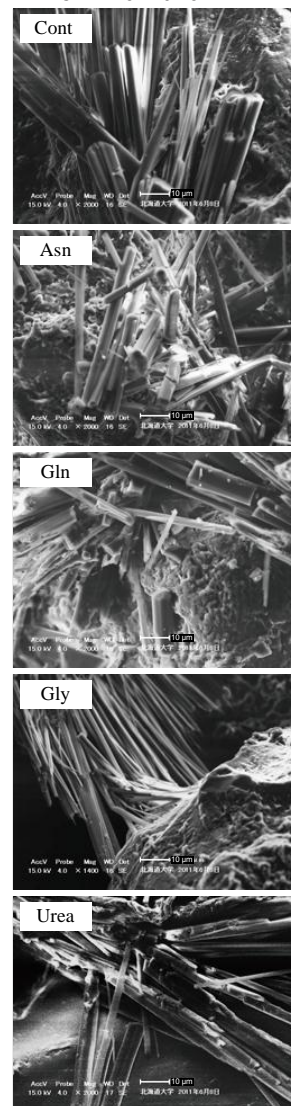


Figure 9

