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Effect of soil surface characteristics on denitrification activity and induced calcium carbonate precipitation on the surface

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ABSTRACT

Soil microorganisms in nature are known to be adhering to any surfaces and forming aggregates of the cells on the surface. It is said that almost 80–90% of the microorganisms in soil and water are attached to solid surfaces, rather than being free-living cells in aqueous phase. This study evaluated soil surface characteristics contributing to microbial induced calcium carbonate precipitation known as MICP by denitrification. Compared to artificial silica sand, microbial denitrification activity tended to be accelerated in the presence of natural sand, suggesting that the presence of impurities other than quartz contributed to microbial activity. Microbial metabolic reactions that accelerate crystal nucleation play an important role in calcium carbonate crystal formation. And, the microbial cells activate their metabolism by settling on the particle surface, which is the foundation for nucleation. The fixation of the microbial cells to the soil particles is selective, and the molecular structure and surface charge properties of the particle are expected to contribute to the selective fixation. Soil particle coating with ferrihydrite, an amorphous iron hydroxide is expected to promote attachment of microorganisms to the particle surface and calcium carbonate crystal formation on the surface.

Keywords: microbial induced carbonate precipitation (MICP), denitrification, calcium carbonate, sand, microbial induced desaturation and precipitation (MIDP), soil surface characteristics

1 INTRODUCTION

Many studies have been reported on ground improvement techniques utilizing the metabolic functions of the microorganisms as bio-geo-environmental engineering research in the last decades. In addition to sand cementation using urease-positive bacteria frequently called microbial induced carbonate precipitation (MICP), the related studies and techniques have developed over the past few decades, including soil remediation using different type of bacteria like sulfate-reducing bacteria and denitrifying bacteria, and concrete self-healing technology using the catalytic functions of microorganisms (DeJong et al., 2016; Zhu and Dittrich, 2016; Heveran et al., 2020). This is due to the recent requirement of the sustainable development in materials and techniques for civil engineering.

This study has been working on the research for soil improvement technique using nitrate-reducing bacteria extracted from natural soils, the so-called microbial induced desaturation and precipitation (MIDP)（Nakano, 2018; Pham et al., 2016）. There are a number of bacteria capable of reducing nitrate, denitrifiers, such as *Alcaligenes, Bacillus, Denitro bacillus, Thiobacillus, Pseudomonas, Spirillum, Micrococcus, and Achromobacter* (Karatas, 2008; Zhu and Dittrich, 2016). Denitrifiers are known as heterotrophic bacteria, and the most of them are facultative anaerobic commonly found in various natural environments such as soil, marine and freshwater sediment, as well as in wastewater treatment systems. Denitrifying bacteria are facultative anaerobes and they can respire using oxygen under aerobic conditions. In other words, denitrifying bacteria do not necessarily require denitrification for survival. And, the presence of denitrifying bacteria does not imply the presence of denitrification.

Trough several experiments in this study, it is revealed that the denitrifying activity could be controlled by the surface condition of the soil. Soil microorganisms in nature are known to be adhering to any surfaces and forming aggregates of the cells on the surface. It is said that almost 80–90% of the microorganisms in soil and water are attached to solid surfaces, rather than being free-living cells in aqueous phase (Nannipieri et al., 2003; Hong et al., 2018). Mills (2003) differentiates between attached and free-living microbes, and states that attached microbes are the most abundant microorganisms in

soil environment. Microorganisms are able to acquire nutrient sources efficiently by adhering on the optimal surface environment like solid particles and plant bodies (Chenu & Sotzky, 2002). Based on this fact, the surface conditions of soil particles could influence microbial activity when substances for cementation such as calcium carbonate are produced in the soil through microbial reactions, which ultimately determines the degree of the soil strength improvement. This paper shows how the surface characteristics would affect microbial induced calcium carbonate precipitation by denitrification, so called MICP and MIDP. That would be one finding that can enhance the efficiency of the ground improvement, and at least we all should know that to explore this research topic.

2 MATERIALS AND METHODS

2.1 Soil samples

Following soil samples were used in this study for the comparison in denitrification and the associated calcium carbonate precipitation; (1) Toyoura sand, (2) beach sand, (3) silica sand and (4) natural zeolite. Toyoura sand is natural sand from Yamaguchi prefecture, Japan, that is produced only by washing, drying, and sieving without artificial crushing by Toyoura Keiseki Kogyo K.K., Japan. It has a naturally occurring shape and the grains are rounded. Beach sand was collected from Wakimisaki Beach, Nagasaki prefecture, Japan. Wakimisaki beach is known for its beach rock deposits as a natural monument of the prefecture. Silica sand (unknown collection locality) and natural zeolite (Shimane, Japan) were purchased from a general market store, and the grain size for both was adjusted by grinding and sieving. Figure 1 shows the grain size distribution curves of the samples. The average grain size was 0.20, 0.65, 0.10 and 0.18 mm for Toyoura sand, beach sand, silica sand and zeolite respectively. Natural sands such as Toyoura sand and beach sand are expected to contain feldspar, carbonate, sulfate, iron oxide, organic matter, and other trace impurities in addition to quartz. Some trace impurities are possibly sedimented on the surface of the bigger particles and then they could not be valued in the grain size distribution analysis. Based on the chemical compositions by XRF and ignition loss under 800°C for the samples (Table 1), it is assumed that the impurities content is particularly high in beach sand while silica sand is mostly composed of quartz and contains few impurities. In the XRF analysis, the FP method was applied for quantifying elements, divalent iron was converted to trivalent iron, total Fe was expressed as Fe_2O_3, the target elements were described as oxides, and the total amount was normalized to 100%, which is the standard description of soil chemical compositions in soil science.

Table 1. *Chemical compositions by XRF and ignition loss of soil samples.*

%	Toyoura	Beach	Silica	Zeolite
SiO_2	84.36	66.27	87.11	73.99
Al_2O_3	6.85	13.18	8.34	14.97
K_2O	2.68	1.19	3.42	1.97
TiO_2	2.67	0.94	0.49	0.35
Fe_2O_3	2.43	8.39	0.43	2.52
CaO	0.54	3.95	-	2.23
MgO	0.24	3.10	-	0.74
Trace element	0.23	2.98	0.21	3.23
Ignition loss (800°C, %)	0.34	2.10	0.62	6.81

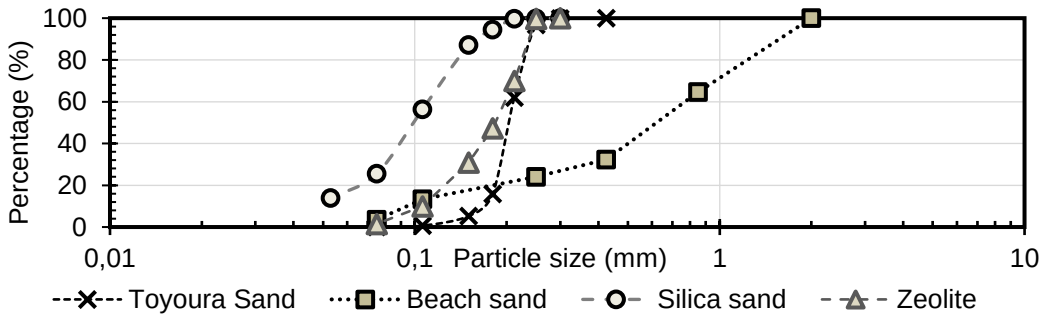


Figure 1. *Grain size distribution curves of soil samples.*

2.2 Isolation of nitrate reducing bacterial from sludge

Nitrate-reducing bacteria were extracted from natural sediments collected in Japan. Approximately 30 g of the wet sediment and 100 ml of liquid medium were mixed in a sterile plastic bag, sealed, and placed in an incubator at 37°C for 3 days. The suspension was centrifuged (3500 rpm, 5 min), and 50 ml of the supernatant was added to a new plastic bag containing 100 ml of sterile liquid medium and incubated at 37°C for 5-7 days. This procedure was repeated again, and the resulting medium was coated on SCD agar medium (Nissui Pharmaceutical, Japan) and incubated at 37°C for 2 days. One colony appeared on the agar plate was inoculated in 25 ml of sterile liquid medium and incubated at 37°C for 5-6 days. The bacterial medium obtained in this way was diluted with sterile liquid medium to adjust the number of microbial cells and substrate concentration, and then used in the following culture experiments. The liquid medium used in this study was a mixture of calcium nitrate, calcium acetate, and sodium acetate (pH = 7.0-7.2).

The 16S rDNA sequence analysis for an isolated single colony on the agar plate revealed *Mesobacillus thioparans* (BMP-1) to be the most probable species of the bacteria with the highest identity = 99.8%. Members of *Mesobacillus* was transferred from the genus *Bacillus* (Patel & Gupta, 2020). The identified species are non-pathogenic bacteria at Biosafety Level 1 in the Biosafety Guidelines of Japanese Society for Bacteriology. For the identification, full-length 16S rRNA gene sequence was compared with all other sequences available in DDBJ/ENA/GenBank (The International Nucleotide Sequence Databases) and DB-BA17.0 (The Sequence Database by Techno Suruga Laboratory, Shizuoka Japan).

2.3 Evaluation of denitrification activity and induced calcium carbonate precipitation by nitrate reducing bacteria in the presence of soil samples

2.3.1 Bacterial culture experiments

Five grams of the weighed soil sample (dry soil mass) and 30 ml of bacterial liquid medium were placed in a 30 ml centrifuge plastic tube and incubated statically in an incubator at 37°C. The number of microbial cells in the culture medium was adjusted to 2×10^7– 4×10^7 cells/ml. At regular intervals, solute concentrations (NO_3-, Ca^{2+}, pH) in the liquid medium were measured using ion sensors for NO_3- and Ca^{2+} (HORIBA) and a pH electrode (TOA DKK).

2.3.2 Bacterial cell counts

Bacterial cell counts were conducted by distinguishing between bacteria independently existing in the liquid culture medium (here defined as free-living bacteria) and bacteria adhering to the soil sample (defined as attached bacteria) in the soil suspensions after the culture experiments. After the incubation period, the culture medium and soil samples were separated by centrifugation at 3500rpm for 5 mins, and the number of bacterial cells in the liquid medium was measured as free-living bacteria. The soil sample was washed three times with ultrapure water, transferred to a 100 ml triangular flask, and sonicated by BRANSONIC® -CPX3800H (40 kHz, 45mins) with 30 ml of the diluted liquid medium, and the supernatant was transferred to a 100 ml volumetric flask. New liquid medium was added to the remaining soil and the ultrasonication was repeated twice. The total volume of supernatant collected in the flask was adjusted to 100 ml, and the number of microbial cells in the supernatant was measured as attached bacteria. A bacteria counter (A161, SLGC) was used to measure the number of microorganisms, and each test solution was diluted accordingly to determine the number of cells. Since most microbial cells in the liquid medium were observed to be active, the cells were counted without distinguishing between live and dead cells.

2.3.3 Soil surface observation

The surface of the soil samples after the culture experiments was observed using a 3D digital microscope (RH-2000, MXB2500REZ, HIROX) and Scanning electron microscope SEM-EDX (SU3500, Hitachi High-Technologies) in low-vacuum mode.

2.4 The effect of soil surface treatment to microbial induced carbonate precipitation

Toyoura sand, a natural sand, is expected to contain trace amounts of secondary minerals. One of the objectives of this study is to evaluate the effects of these impurities on microbial reactions, but it is very

difficult to qualify and quantify them. Therefore, this study attempted several surface treatments by acid washing or coating sand surface to control the surface condition. Surface treatment methods are described below. *Acid treatment*: Toyoura sand was stirred with 1 mol/L nitric acid solution for 24 hours, then washed repeatedly with distilled water until the pH of the suspension reached neutral (pH~6.8), and then air-dried at room temperature 25°C. Hereinafter referred to as ***Toyo-Acid***. *Ferrihydrite-coating*: Surface coating of Toyoura sand with iron hydroxide mineral, ferrihydrite was performed following the method of Stahl & James (1991). The resulting sample was designated as a ferrihydrite-coated sample (hereinafter referred to as ***Toyo-Ferri***). *Gelatin mixing*: A very thin gelatin solution (approximately 0.1%) was mixed with liquid medium for the incubation experiment below. Hereinafter referred to as ***Toyo-Gel***.

Microbial culture experiments were conducted on the surface-treated Toyoura sands above using the same method as described in 2.3.1. Also, observation of the soil surface after incubation was conducted in the same manner as in 2.3.3.

3 RESULTS AND DISCUSSIONS

3.1 Soil-induced differences in denitrification activity and calcium carbonate precipitation

Figure 2 shows the results of a centrifuge tube culture experiment conducted to examine differences in denitrification activity depending on the soil samples. The denitrification rate was faster for beach sand, zeolite, Toyoura sand, and silica sand, in that order. Beach sand and zeolite, in particular, showed a rapid denitrification immediately after the addition of microorganisms, and the added nitrate of 40 mmol/L was reduced to below the detection limit in 5 days. The same microorganisms were added to each sample, but the blank sample, to which no soil was added, showed little change in nitrate concentration and no denitrification progressed during the incubation period of up to 22 days. This indicates that the microorganisms promote denitrification due to the presence of soil particles. In the coexistence of any of the samples, calcium ions also decreased with the progress of denitrification, suggesting that the reaction in the equations below ((1)-(3)) is progressing. In the equation (1), the coefficient for each compound **n** is indicated as Y_n since it varies with the reaction rate. And Biomass is simply expressed as $CH_{1.8}O_{0.5}N_{0.2}$ following Heijnen & Kleerebezem (1999).

$$Y_{Ace}\ C_2H_3O_2^- + Y_{NO_3}\ NO_3^- + Y_H\ H^+ \rightarrow 1.0\ CH_{1.8}O_{0.5}N_{0.2} + Y_{CO_2}\ CO_2 + Y_{H_2O}\ H_2O + Y_{N_2}\ N_2 \quad (1)$$

$$CO_2 + H_2O \rightarrow HCO_3^- + H^+ \quad (2)$$

$$HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + H^+ \quad (3)$$

The pH of the liquid medium during the incubation period increased from an initial pH = 7.0-7.2 to pH = 7.8, 8.5, 8.4, 7.8, and 7.7 for zeolite, beach sand, Toyoura sand, silica sand, and blank, respectively, at the end of incubation. As shown in equation (1), the pH increases as the denitrification reaction proceeds, and the pH again shows a decreasing trend with the precipitation formation of calcium carbonate in equations (2) and (3), and both reactions eventually reach equilibrium according to the

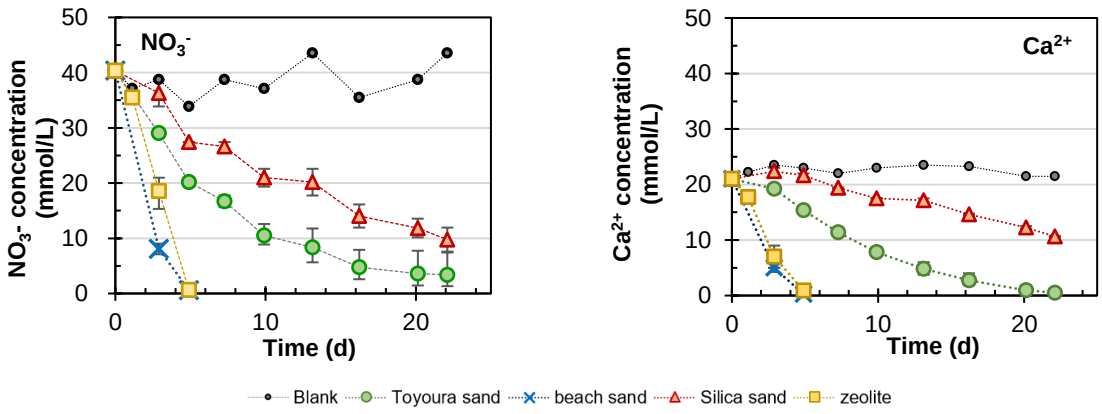


Figure 2. *Nitrate concentration as an indicator of denitrification activity with each sample over time (left). Calcium concentration over time associated with denitrification (right).*

concentration balance between nitrate and calcium ions (and also carbon sources). The reason why the pH increase

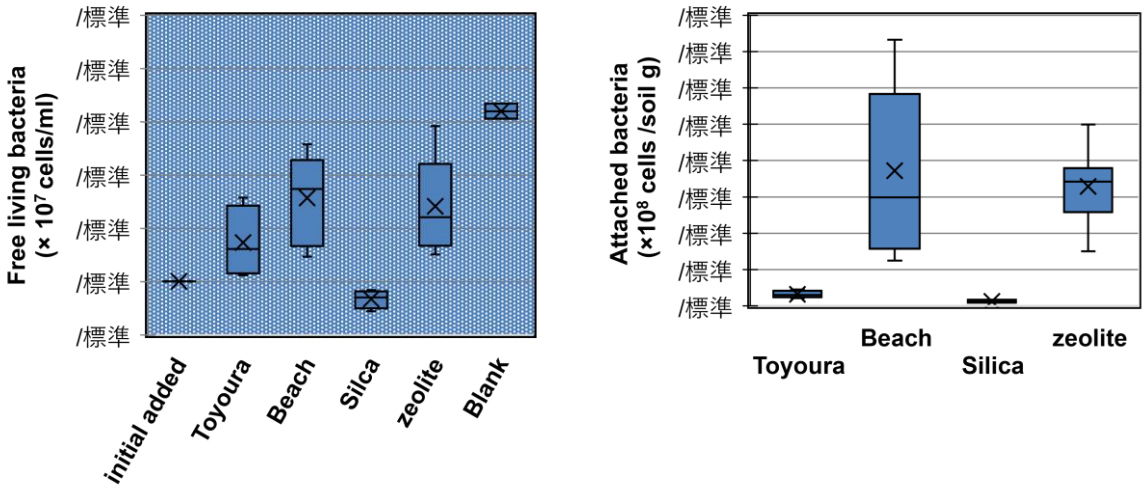


Figure 3. *Free-living bacteria in liquid medium before and after incubation (left). Attached bacteria in soil after incubation (right)*

accompanying the progress of denitrification in zeolite is lower than in other soil samples may be due to the pH buffering ability of zeolite. And, the pH increase in blank in which no denitrification reaction is observed can be attributed to respiration and metabolism necessary to maintain their life of the microorganisms.

Microbial growth was observed in all samples in the culture medium after the incubation experiments (Figure 3). While the concentration of microorganisms added at the start of incubation was 5×10^7 cells/ml, the average concentration of free-living bacteria after the end of incubation was 12.9, 12.1, 8.7and 3.4×10^7 cells/ml, in the order of beach sand, zeolite, Toyoura sand and silica sand, respectively. The number of microorganisms suspended in the solution tended to increase in the samples with faster denitrification rates. However, microbial growth was observed even in blank samples where denitrification did not proceed, indicating that the target microorganisms do not necessarily require denitrification for survival. On the other hand, the number of bacterial cells attached to the soil was overwhelmingly large for beach sand and zeolite, with 37.2 and 32.9×10^8 cells/g soil, respectively, while it was 3.2 and 1.3×10^8 cells/g soil for Toyoura sand and silica sand, respectively. These results indicate that the bacteria grow by attaching to the surface of soil particles, thereby increasing their denitrification activity. Also, the number of cells attached to different types of soil varied, suggesting that the microorganisms may selectively attach to specific particle surfaces or that the particle surfaces on which the microorganisms can settle are limited.

Electron microscopic observations of each sample after the culture experiment showed that the precipitation of small calcium carbonate crystals in various places on the surface of Toyoura sand (Figure 4). As Figure 4 (left) shows, all of these crystals precipitated in slight contact with the bigger particles. In beach sand, on the other hand, calcium carbonate precipitated as if it was stuck to the surface of the bigger particles (Figure5). In this case, the crystals were rounded in shape, unlike those in the Toyoura sand. As shown in the EDX results on Figure 6 (left), low concentrations of calcium were widely detected on the particle surface of the zeolite, and in some areas, high concentrations of calcium were detected. As Figure 6 (right) shows, in the areas where high concentrations of calcium were detected, small crystal was observed in the form of attached crystals on the surface of the zeolite. Unlike beach sand, the shape of those crystals was angular. No clear calcium carbonate crystal was observed in silica sand, although all calcium added was consumed after incubation.

Calcium carbonate CaCO3 is known as the polymorphism. CaCO3 has three anhydrous crystalline polymorphs: hexagonal vaterite, orthorhombic aragonite, and rhombohedral calcite. In the pure system, vaterite usually forms polycrystalline spheres, aragonite forms needles, and calcite forms equant rhombs (Sand et al, 2012). Given this fact, aragonite was deposited in Toyoura sand and vaterite in the beach sand. And these differences in crystal structure can be attributed to differences in the microbial activity rate and the surface condition of the soil particles. XRD is an accurate and simple technique of

analyzing the crystal structure of calcium carbonate. However, the culture experiments in this study used low calcium concentrations (initial Ca^{2+} = 0.022-0.037 mol/L) rather than the high calcium concentrations

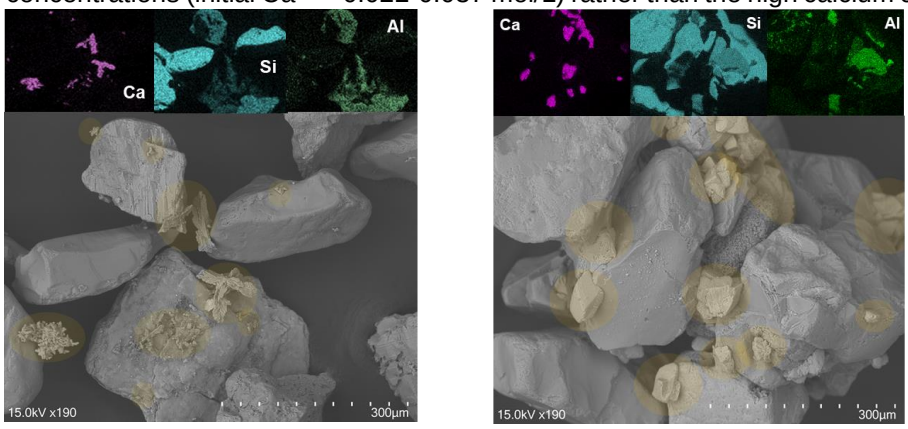


Figure 4. *Soil surface of sand samples after microbial reaction and distribution of calcium, silicon and alminium* on the surface *detected by SEM-EDX: (left) original Toyoura sand, (right) Toyoura sand with acid treatment (**Toyo-Acid**).*

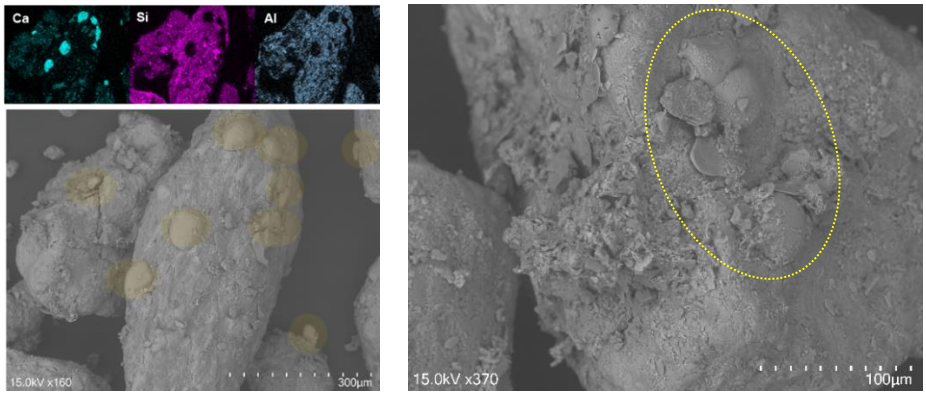


Figure 5. *Soil surface of beach sand after microbial reaction and distribution of calcium, silicon and alminium on the surface detected by SEM-EDX (left), Shape of calcium carbonate crystals commonly found in beach sand after the reaction (right).*

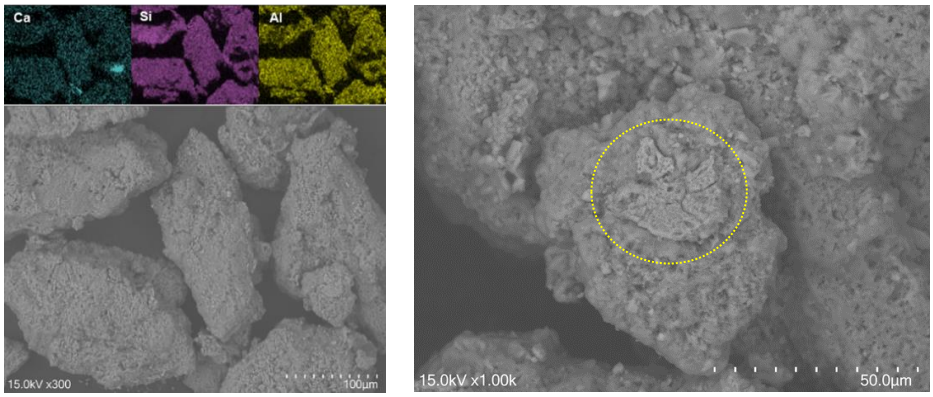


Figure 6. *Soil surface of zeolite after microbial reaction and distribution of calcium, silicon and alminium on the surface detected by SEM-EDX (left), Shape of crystals with high Ca concentration found in the zeolite surface after the reaction (right).*

often employed in MICP reactions using urease positive bacteria (typically 0.5-1.0 mol/L), so the maximum amount of calcium carbonate will be around 0.5-0.9% by mass. In addition, the distribution of induced calcium carbonate crystals is heterogeneous and some of them are placed on the bigger soil particles, as can be seen from the SEM images. Therefore, standard XRD could not identify the clear peaks indicating calcium carbonate minerals because the peaks of other primary minerals (especially quartz and feldspars) appeared very strongly, while the peaks of calcium carbonate were ambiguous.

The large specific surface area and surface charge of zeolite due to its unique crystalline structure may be the reason for the activated denitrification in zeolite. The number of attached microbes was much larger in the zeolite sample than in Toyoura sand and silica sand, which can be attributed to the larger specific surface area and the higher cation exchange capacity (CEC). The CEC of the zeolite used in this study is 1944 $mmol_c\ kg^{-1}$, while that of Toyoura sand is only 7 $mmol_c\ kg^{-1}$. It is already known in the field of water treatment engineering that the addition of zeolites accelerates denitrification. Fernández et al. (2008) showed that the biofilm formation of bacteria living in the sludge contributes to the purification efficiency of activated sludge used in water treatment. Biofilms developed in sludge play a role in fixing microorganisms in water, and microorganisms are activated under stable conditions. The starting point of the biofilm formation is the fixation of microbial cells on solid surfaces. The zeolite used in this study is mainly composed of mordenite, which has a complex pore structure with large and small pores of less than 1 nm (Kubota and Tatsumi, 2006). The microbes with 1-5 μm of their average size cannot physically penetrate the small pores of the zeolite. However, the edges of the crystals, which also have a complex structure and charge characteristics, can be surfaces for the microbes to attach. Under the conditions of this experiment, ion-exchange of divalent calcium ions on the zeolite may have served as a bridge to promote surface attachment of negatively charged microbial cells. Chenu and Stotzky (2002) explained the importance of soil characteristics as the controlling factors for the activity of microorganisms in soil. They also described the colloidal fraction with large surface area and permanent and/or variable charges like most clay minerals, oxyhydroxides and organic matters as surface-active particles. In the case of beach sand, it is highly likely that impurities such as iron oxide hydroxides, carbonates, sulfates, and organic matter were mixed or adhered to the sand surface in addition to the main component of quartz sand, and that these secondary components promoted microbial activity. The silica sand, which consisted mostly of quartz, had lower microbial activity than the other samples, and there was almost no calcium carbonate crystal precipitation on the surface of the soil particles, suggesting that the availability of a surface environment for microbial attachment affected microbial activity and crystal precipitation.

3.2 The effect of soil surface treatment to microbial induced carbonate precipitation

Figure 7 shows the incubation results to see the effect of the treatments for Toyoura sand on denitrification; acid treatment, ferrihydrite coating, and gelatin mixing. All of the pre-treatments of the sand surface showed accelerated denitrification. The pH of the liquid medium during the incubation period increased from an initial pH = 7.0-7.1 to pH = 9.7, 9.7, 9.8, 8.2, 7.5 and 7.3 for Toyoura sand, Toyo-Acid, Toyo-Ferri, Toyo-Gel, Gel-0.1% (gelatin only without sand), and blank, respectively, at the end of incubation.

By the acid treatment, soluble solid components such as carbonate could have been removed from the sample while quartz (SiO_2) was acid-stable. Feldspar (WZ_4O_8 (W=Na,K,Ca,Ba; Z=Si,Al)) is also known to be sensitive to chemical weathering, such as acid hydrolysis, and weathered plagioclase and potassium feldspar are known to be left as clay minerals (typically kaolinite). In addition, when trace clay minerals and metal oxides and hydroxides with variable charge were included as the impurities, the amount of their positive surface charge increases on the acidic side. As already mentioned, most microbial colloids are negatively charged. If this acid treatment of the soil particles changed the charge characteristics of the particle surface and increased the positive charge, it is possible that the inter-particle distance between the microbial cells and the soil particle surface was reduced, thereby promoting surface settlement of the microbes. The crystal precipitation of calcium carbonate in *Toyo-Acid* after denitrification was also significantly different compared to the untreated Toyoura sand (Figure 4). The crystals were larger than those in the untreated sand, and showed a rhombus or square shape. There were many crystals precipitated throughout the sample, and these crystals precipitated between large soil particles, binding them together. This difference in crystal formation with and without acid treatment is thought to be due to the fixation of microorganisms on the surface of the particles, which is the foundation for crystal nucleation. I believe that more microbes accumulated on the surface of the particles and became growth-active, thereby concentrating carbonate ions and promoting nucleation of calcium carbonate, resulting in the formation of larger crystals. Alteration of surface charge of the soil particles by the surface treatments is a matter for future study.

The ferrihydrite coating also showed an activity-promoting effect similar to that of the acid treatment. Ferrihydrite, amorphous iron hydroxides have a large specific surface area due to their amorphous micro-nanostructure. In general, the larger the specific surface area, the greater the surface charge and the more electronically complex the surface environment. In particular, iron oxide hydroxides adsorb not

only cations but also anions and organic matter on their surfaces due to their unique charge properties (Fink et al., 2016). In addition, ferrihydrite is easily bioavailable and used as an electron acceptor substrate for cultivating iron-reducing microorganisms, while the microbial reduction of crystalline iron

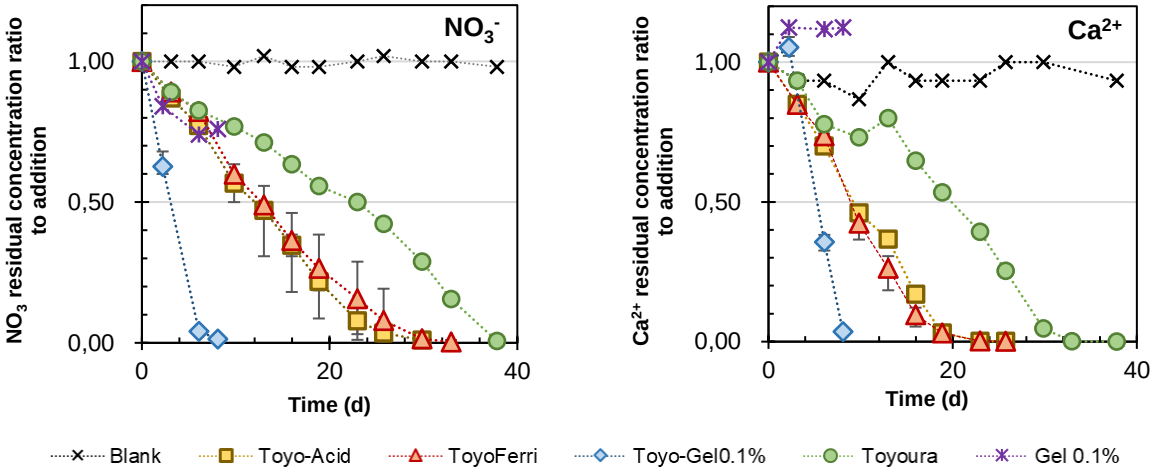


Figure 7. *Nitrate reduction rate as an indicator of denitrification activity with each sample over time (left). Calcium consumption rate over time associated with denitrification (right).*

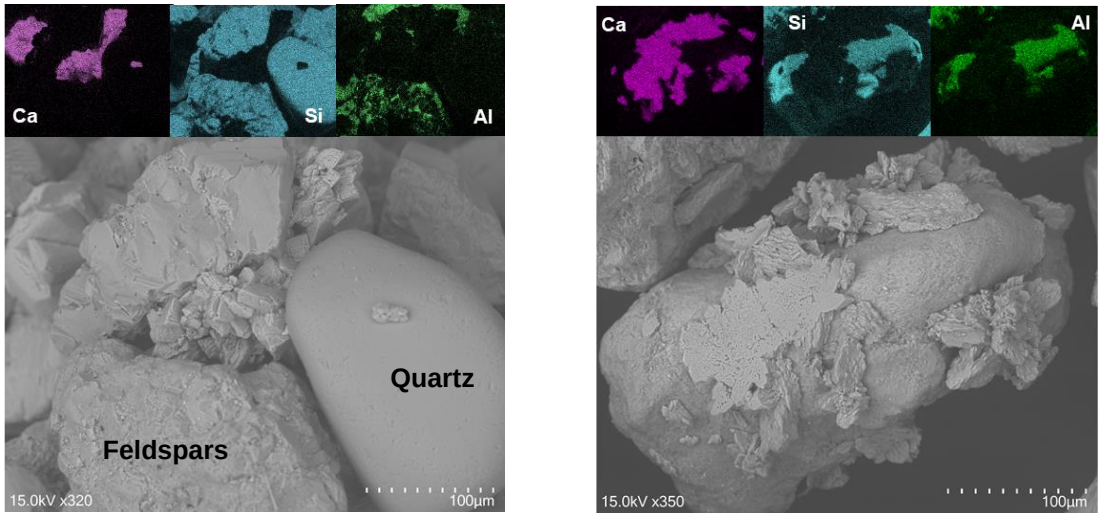


Figure 8. *Soil surface of Toyoura sand after microbial reaction and distribution of calcium, silicon and alminium on the surface detected by SEM-EDX: (left) Toyoura sand with acid treatment (**Toyo-Acid**), (right) Toyoura sand with 0.1% gelatin (**Toyo-Gel**).*

oxides such as goethite and hematite is known to be very weak (Hori, 2019). The presence of iron hydroxide on the particles may have a function of energy transfer as an electron acceptor between microorganisms and hydroxides resulting in promotion of the denitrification.

As shown in Figure 7, denitrification was not promoted by Gel-0.1%, in which gelatin was only present in the culture medium, but was promoted by Toyo-Gel, in which Toyoura sand and gelatin coexisted. The effect of gelatin is only apparent when there are soil particles as a foundation for microorganisms to bond to. Gelatin is derived from the hydrolysis of collagen. It is biodegradable, biocompatible, cheap, and has good hydrophilicity, making it the polymeric component for the construction of the biological scaffold due to the lower molecular weight compared to collagen, and simpler space conformation which allows the cell binding sequences for cell ingrowth on the material. Therefore, it retains many functional groups which are recognised by microbial cells (Mo et al. 2000, Yang et al. 2016, Chan & Lim, 2019). After the incubation, many calcium carbonate crystals were observed widely on a single particle surface in *Toyo-Gel* (Figure 8 (right)). This was more pronounced than in other samples, suggesting that the adhesive effect of gelatin was effective in precipitating calcium carbonate crystals on the soil surface. At 0.1% Gel (no sand added), denitrification proceeds slowly, but no calcium consumption is observed

(Figure 7). Therefore, it can be said that calcium carbonate crystal formation requires a foundation for nucleation, and a surface environment in which microorganisms can settle on that foundation will contribute to promote the nucleation of calcium carbonate precipitation.

The interface between the soil surface and the microbial cell may exhibit electrostatic interactions depending on their surface charge characteristics, and/or it may be influenced by the physical shape and hydrophilicity or hydrophobicity of the particle surface. Most environmental microorganisms are rarely found floating alone in water. Rather, it is known that many microorganisms accumulate and settle at particle interfaces that are favorable for their survival and form biofilms, thereby efficiently acquiring energy sources while growing and activating themselves. Many environmental microorganisms are considered unculturable by conventional microbiological cultivation methods, but may become culturable if they are provided with any favorable surface environment that is effective for their growth.

4 CONCLUSIONS

Soil surface characteristics contributing to microbial induced calcium carbonate precipitation by denitrification were studied. Compared to artificial silica sand, microbial denitrification activity tended to be accelerated in the presence of natural sand, suggesting that the presence of impurities other than quartz contributed to microbial activity. Microbial metabolic reactions that accelerate crystal nucleation play an important role in calcium carbonate crystal formation. In addition, microbial cells activate their metabolism by settling on the particle surface, which is the foundation for nucleation. The fixation of the microbial cells to the soil particles is selective, and the molecular structure and surface charge properties of the particle are thought to contribute to the selective fixation. Soil particle coating with ferrihydrite, an amorphous iron hydroxide, or polymetric gelatin is expected to promote attachment of microorganisms to the soil surface and calcium carbonate crystal formation. Establishing more effective surface conditions for the target microorganisms by learning their interaction with soil particles may enable to propose more efficient ground improvement processes using microbial functions.

5 ACKNOWLEDGEMENTS

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