Effect of temperature on precipitation of calcium carbonate for biogrout

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1. INTRODUCTION

Natural soil contains approximately 10^7 to 10^9 microorganisms per gram. Considering this large number of soil microorganisms, it is expected that soil and rock engineering may be able to develop a novel eco-friendly technology to improve the strength or sealability of soil and rock. The authors have been performing fundamental tests in order to develop a novel conceptual grout, hereafter denoted as biogrout. Biogrout which utilizes calcium carbonate ($CaCO_3$) or silica (SiO_2) is the material to plug the voids of soil or rock by metabolism of microorganisms, and this paper focuses on the biogrout using $CaCO_3$. The precipitation of $CaCO_3$ is significantly influenced by metabolism of microorganisms living in soil or rock and other factors; organic matters, nitrogen sources, inorganic salt, water, temperature, pH, pressures, oxygen (O_2) and carbon dioxide (CO_2). In our previous studies, permeability and strength tests have been performed under intermediate temperature (25℃).

Considering injection of biogrout to voids of soil or rock in places with a cold climate like Hokkaido in Japan, it is important to know the influence of temperature for biogrout. Therefore, we investigated $CaCO_3$ precipitation under wide-ranging temperatures (5~35℃) using the soils which were sampled from various sites in Japan. Also, the number of microorganisms was measured before and after the precipitation tests to investigate what kind of microorganisms have an effect on $CaCO_3$ precipitation. Although a number of problems will need to be addressed, these test results indicate that temperature affects the metabolism of microorganisms and it is possible to use biogrout in areas with a cold climate.

2. MECHANISMS OF BIOGROUT

The biogrout, which utilizes $CaCO_3$ precipitation, is a solution including Ca^{2+} and organic nutrition and the like to plug voids of soil or rock. Namely, biogrout is injected in their voids, and then $CaCO_3$ is precipitated by the combination of Ca^{2+} and CO_2 made by metabolism of microorganisms. Consequently,

$CaCO_3$ plugs voids of soil or rock, and their solidification or sealability is enhanced. For instance, reaction formulas for microbial metabolism of glucose ($C_6H_{12}O_6$) and precipitation of $CaCO_3$ are shown in Eq. (1) to (3).

$C_6H_{12}O_6+6O_2 \rightarrow 6CO_2+6H_2O$ (Aerobic) Eq. (1).

$C_6H_{12}O_6 \rightarrow 2CO_2+2C_2H_5OH$ (Anaerobic) Eq. (2).

$Ca^{2+}+CO_2+H_2O \rightarrow CaCO_3+2H^+$ Eq. (3).

3. MATERIALS AND METHODS

3.1 Precipitation Tests on $CaCO_3$

Soil samples obtained from Horonobe, Sapporo, Obihiro, Okayama and Okinawa in Japan were used throughout the study. These soil samples were extracted in the range from 20 to 30 cm in depth from July to September with a scoop, which was sterilized by ethanol, and then put in containers sterilized. After soil samples were put in containers, these samples were kept at a constant temperature of 4℃ within incubators until the precipitation tests started.

The tests have been conducted to investigate the influence of temperature on $CaCO_3$ precipitation in the laboratory. The ratio of each ingredient which constitutes biogrout is the same as the previous experiments [1].We prepared 100 ml Tris-HCl solution, which contained the following ingredients; calcium nitrate ($CaNO_3 \cdot 4H_2O$), 2.36g; $C_6H_{12}O_6$, 3g, to make Ca^{2+} concentration of the solution 0.1mol/l. 8ml of the solution was put into test tubes, and left to rest in incubators which were set to 5, 15, 25, 35℃. After 24 hours, the test tubes were taken out from the incubators, and 3g of the soil sample was put in each test tube, and then the tubes were put into the incubators again. One, two, four and eight weeks later, the test tubes were taken out from the incubators and filtered by the membrane-filter method whose bore diameter is 0.2μm. Finally we measured Ca^{2+} concentration and pH of the filtrate.

3.2 Measurement of the number of microorganisms

To investigate what kind of microorganisms have an effect on $CaCO_3$ precipitation, the number of soil microorganisms in test tubes of Sapporo and Okinawa samples were measured and categorized into three groups; bacteria; actinomycetes; molds and yeasts by the dilution plate methods before the precipitation tests on $CaCO_3$ and 8 weeks after the tests started. The test tubes of these soil samples were put in a incubator which was set to 25℃.

4. RESULTS AND DISCUSSION

4.1 Precipitation Tests on $CaCO_3$

Figs. 1 to 5 show the relationships between Ca^{2+} concentration and elapsed time. Here, we should note that the reduction of Ca^{2+} concentration means precipitation of $CaCO_3$ from the biogrout solution. The followings 1 to 4 are descriptions founded from the test results.

1. Ca^{2+} concentration of the filtrate of all samples tend to decrease with the days, namely, it is thinkable that $CaCO_3$ precipitation of all samples increases with the days at 5, 15, 25 and 35℃.
2. Samples of Horonobe, Okayama and Sapporo which were maintained at intermediate temperatures (25 and 35℃) had a larger Ca^{2+} concentration than equivalent samples maintained at low temperatures (5 and 15℃) after 8 weeks. These results suggest that temperature highly affects the metabolism of microorganisms.
3. Ca^{2+} concentration of the filtrate of Obihiro samples is not affected by temperature, while the temperature affects on Ca^{2+} concentration of samples of other sites (i.e. Horonobe, Sapporo, Okayama and Okinawa).
4. Ca^{2+} concentration of the filtrate of Okinawa samples increased over a period of a few weeks because of Ca^{2+} elution from soil.

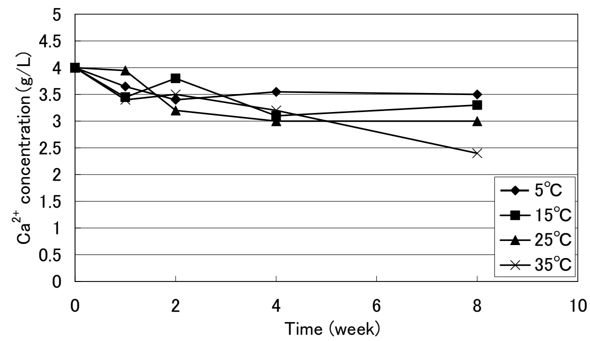


Fig. 1 Relationship between Ca^{2+} concentration and elapsed time (Horonobe).

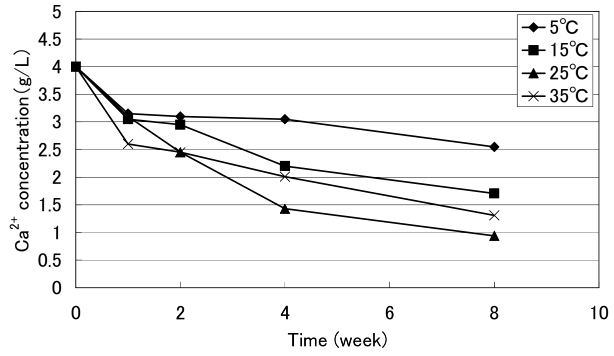


Fig. 2 Relationship between Ca^{2+} concentration and elapsed time (Sapporo).

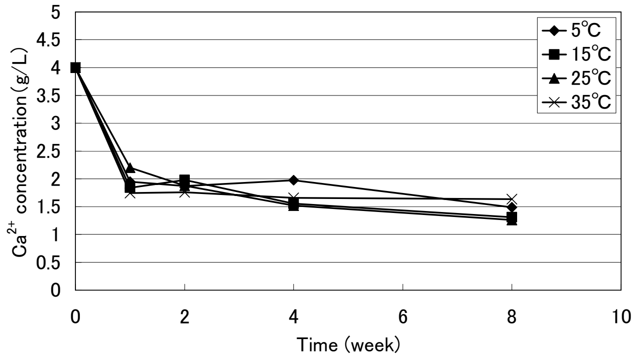


Fig. 3 Relationship between Ca^{2+} concentration and elapsed time (Obihiro).

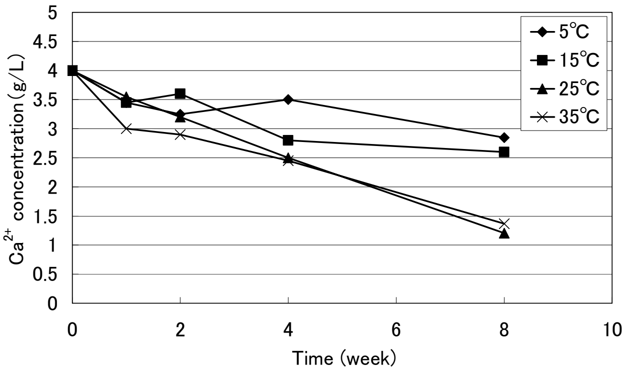


Fig. 4 Relationship between Ca^{2+} concentration and elapsed time (Okayama).

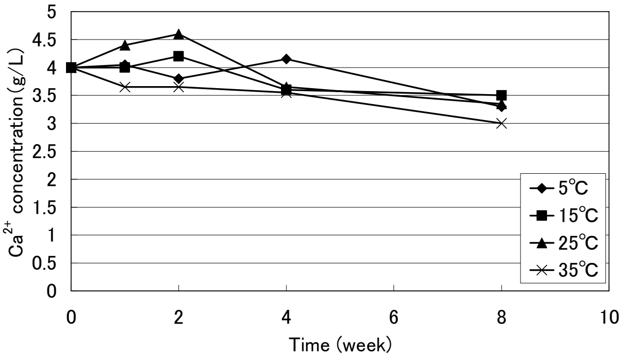


Fig. 5 Relationship between Ca^{2+} concentration and elapsed time (Okinawa).

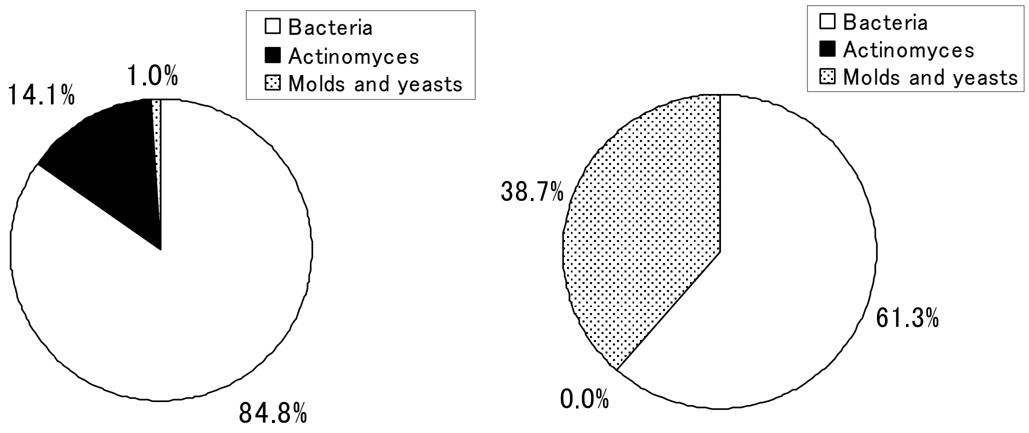
4.2 Measurement of the number of microorganisms

Table 1 shows the number of soil microorganisms, and Figs. 6 and 7 illustrate the ratio between bacteria, actinomycetes, and molds and yeasts in test tubes of Sapporo and Okinawa samples. As shown in Table 1, the number of molds and yeasts increases from 0.81×10^5 cfu/g to 2.9×10^5 cfu/g in a test tube of Sapporo sample, and increases from 0.28×10^5 cfu/g to 1.2×10^5 cfu/g in a test tube of Okinawa sample over a period of 8 weeks. Therefore, it is possible that molds and yeasts have an affect on the reduction of Ca^{2+} concentration.

Table 1. The number of soil microorganisms (Sapporo and Okinawa).

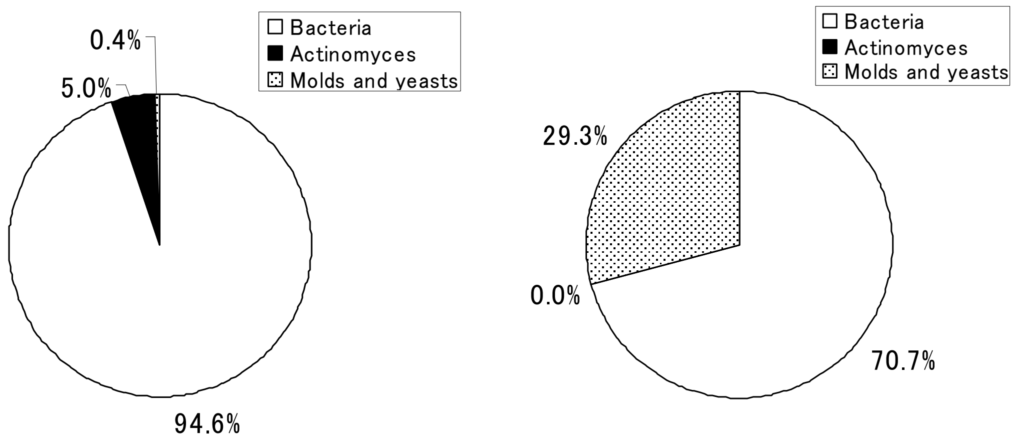
	Sapporo		Okinawa	
	Initial state	8 weeks later	Initial state	8 weeks later
Bacteria	66	4.6	61	2.9
Actinomycetes	11	0	3.2	0
Molds and yeasts	0.81	2.9	0.28	1.2

(unit: $\times10^5$ cfu/g)



(a) Initial state (b) 8 weeks later

Fig. 6 The ratio between bacteria, actinomycetes, and molds and yeasts (Sapporo).



(a) Initial state (b) 8 weeks later

Fig. 7 The ratio between bacteria, actinomycetes, and molds and yeasts (Okinawa).

5. CONCLUDING REMARKS

In this paper, a series of $CaCO_3$ precipitation tests was performed, and the number of soil microorganisms was measured using soil microorganisms from various sites in Japan. As a result, it was confirmed that Ca^{2+} concentration in the test tubes of all samples decreased at 5, 15, 25 and 35℃. Furthermore, it is conceivable that molds and yeasts have an influence on the reduction of Ca^{2+} concentration in the test tubes. Though more experiments need to be performed, injecting biogrout into voids of soil or rock in places with a cold climate is expected to enhance their solidification or sealability, regardless of location.

ACKNOWLEDGEMENTS

The authors are grateful to the Ministry of Education, Culture, Sports, Science and Technology (MEXT) for the financial support of Grant-in-Aid for Scientific Research (Grant No. 18300304, Satoru KAWASAKI).

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