Materials Transactions

Microbially Induced Sand Cementation Method Using *Pararhodobacter* sp. Strain SO1, Inspired by Beachrock Formation Mechanism

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To develop an alternative ground improvement technique in coastal areas based on bio-stimulation, we investigated sand cementation using bacteria that have been shown to enhance beachrock formation. We conducted cementation tests using *Pararhodobacter* sp. strain SO1, a local ureolytic bacteria originating from the sand near beachrock in Okinawa, Japan. Specifically, we attempted to cement sand specimens to unconfined compressive strength (UCS) of several MPa and establish the influence of several test conditions (curing temperature, injection interval of cementation solution, Ca^{2+} concentration and sodium malate concentration in the cementation solution, and test period) on the UCS. Column specimens were cemented up to UCS of 10 MPa after 28 days under the conditions (curing temperature; 30°C, injection interval; 1 day, Ca^{2+} concentrations in cementation solution; 0.3 M). Multiple regression analysis showed that the relevant conditions for UCS were test period, D (days), and Ca^{2+} concentration of the cementation solution, C_{ca} (M). The prediction formula for UCS, q_{ud} (MPa), was experimentally determined to be $q_{ud} = 48.3C_{ca} + 0.456D - 19.51$. Overall, the results of this study will contribute to the application of a new technique for coastal sand improvement and bio-stimulation. [doi:10.2320/matertrans.M-M2015842]

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1. Introduction

With growing concerns regarding the global environment, environmentally friendly methods for ground improvement are needed. Accordingly, microbially induced sand cementation methods have been investigated for their potential application in environmentally friendly soil improvement techniques.

The biomediated soil improvement technique can be divided into two primary strategies; namely, bio-augmentation, in which the required microbes are injected into the soil, and bio-stimulation, which involves stimulation of microbes already present in the soil.[1)] Bio-augmentation enables control of microbial concentrations and the velocity of cementation; however, it poses a risk to the ecosystem because of the addition of non-native microbes and is expensive. Bio-stimulation is generally preferred over bio-augmentation because native bacteria are used; however, it is difficult to control the growth rate and induce uniform soil cementation using this technique.

Sporosarcina pasteurii, which produces highly active urease, is commonly used for soil improvement by bacteria.[2–6)] This ureolytic bacterium stimulates urea hydrolysis, which leads to production of $CaCO_3$ between sand particles, resulting in sand cementation (eq. (1)–(3)).

$$CO(NH_2)_2 + 3H_2O \rightarrow 2NH_4^+ + 2OH^- + CO_2 \quad (1)$$

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

$$Ca^{2+} + HCO_3^- + OH^- \rightarrow CaCO_3 + H_2O \quad (3)$$

Aqua marina,[7)] NO-A10 strain isolated from the soil in Niwase, Okayama,[8)] *Bacillus sphaericus*[9)] and *Bacillus* sp. strain VS1[10)] have been shown to induce sand cementation using the same mechanism. Yeast metabolic activity has also been reported to be useful for induction of sand cementation.[11)]

Although many studies have investigated bio-augmentation, few have investigated bio-stimulation.[12,13)] This is primarily because not all areas contain bacteria with good potential for cementation of soil, and no universal method that can be applied in a wide variety of locations has been developed. However, bio-stimulation is generally more environmentally friendly than bio-augmentation; accordingly, additional studies of this technique are warranted.

Moreover, investigations of microbially induced soil improvement have mainly focused on land areas, while few have investigated coastal areas,[7,14)] even though many coasts worldwide suffer from erosion. Concrete barriers such as breakwaters and processes such as sand bypassing have been traditionally used to prevent erosion. However, the production of cement for making concrete releases large amounts of CO_2, while a large amount of materials is required for sand bypassing.

As an alternative countermeasure against coastal erosion, we considered the use of artificial rocks produced using beachrock formation mechanisms. Beachrock is a coastal sedimentary rock formed during a shorter period (several decades to several thousand years) than other sedimentary rocks. Some beachrocks have been cemented to unconfined compressive strength (UCS) of a few dozen MPa.[15,16)] Beachrocks are formed by several mechanisms, including bacterial action. Therefore, application of such formation mechanisms could be used to cement coastal sediments at the site of erosion to artificial rock using local materials (e.g., bacteria, seawater). In addition, the cracks of existing concrete constructs could be repaired by precipitation of cement contained in the artificial rock. Hence, artificial rock could be an economical and environmentally friendly countermeasure against coastal erosion.

In this study, we conducted sand cementation tests using local bacteria shown to enhance beachrock formation. The strong points of this study are as follows: (1) Identification of the bacteria useful for soil improvement in areas near

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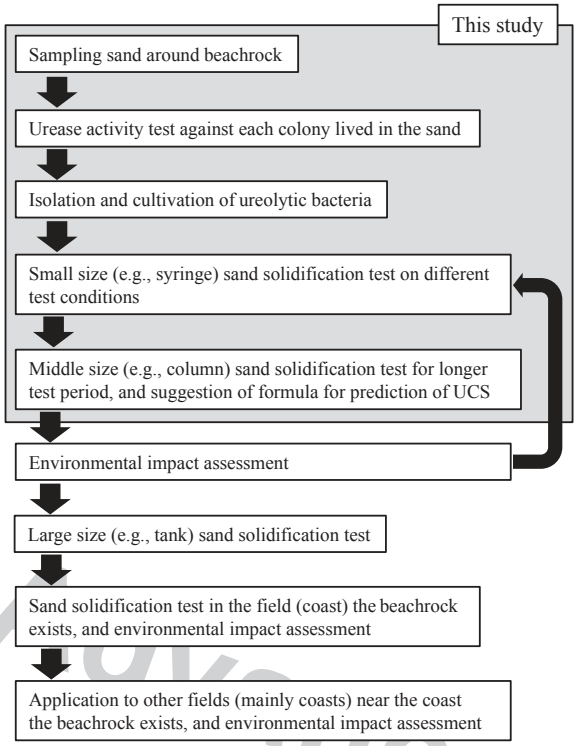

Fig. 1 Flowchart of the study design. The steps carried out in this study are highlighted in gray.

Fig. 2 Beachrock in Sumuide, Okinawa, Japan.

Table 1 Composition of artificial seawater.

Reagent	(g/20 L)
$MgCl_2 \cdot 6H_2O$	222.23
$CaCl_2 \cdot 2H_2O$	30.7
$SrCl_2 \cdot 6H_2O$	0.85
KCl	13.89
$NaHCO_3$	4.02
KBr	2.01
H_3BO_3	0.54
NaF	0.06
NaCl	490.68
Na_2SO_4	81.88

beachrock would be easier than random search from any soil. (2) The bacteria originating from the sand near beachrock have the potential to promote cementation of sand to the UCS of beachrock. (3) The bacteria have the potential for application to not only the coast where the bacteria were collected but also nearby areas connected through seawater; thus, reducing the risk of microbial pollution. To the best of our knowledge, this is the first paper to describe coastal ground improvement based on bio-stimulation.

A flowchart of the study design is shown in Fig. 1. This study comprises the first half of the steps shown; from isolating ureolytic bacteria to cementation of middle size (e.g., column) sand specimens. Ureolytic bacteria,[16] cyanobacteria[17] and heterotrophic bacteria in Heron Island[18] have been reported to enhance = beachrock formation. Because sand cementation using ureolytic bacteria has been well established, we initially focused on ureolytic bacteria to advance a field application technique as early as possible. Urea is supplied from biodegradation of dead fish, as well as urine from mammal, amphibian, and fish.[19] Accordingly, urea is found in many coastal areas, including those in which beachrocks have formed and those containing artificial rocks.

The objectives of this study were to cement sand specimens to UCS of several MPa using local ureolytic bacteria collected from sand near the beachrock and to assess the influence of some test conditions referred to the environment around beachrocks on the UCS. In addition, the UCS mentioned above was set as an initial target value to develop artificial rock to prevent coastal erosion. This UCS is approximately one fifth that of concrete constructs and beachrocks. The results presented herein provide fundamental and important knowledge that will enable development of new methods of bio-stimulation and coastal soil improvement.

2. Materials and Methods

2.1 Isolation of ureolytic bacterium

Sand near the beachrock in Sumuide, Nago, Okinawa, Japan (Fig. 2) was collected into sterile test tubes, transported to the laboratory and refrigerated at 4°C. This beachrock was previously reported to have potentially been formed by ureolytic bacteria.[16] Subsequently, 1.0 g of the sand was mixed with 10 mL of autoclaved artificial seawater (Aquamarine, Yashima Drug Company, Osaka, Japan; Table 1), then diluted 10^1–10^4 times with the artificial seawater. Next, 10 µL of each dilution was added to ZoBell 2216E agar medium (for marine bacteria). After incubation at 30°C for 3 days, about 30 colonies were isolated from one of the plates.

A urease activity test was then conducted against the colonies to identify ureolytic bacteria. The each colony (diameter: 2–3 mm) was mixed with 20 mL of solution (20 mL/L cresol red solution, 0.4 g/L cresol red with distilled water and 25 g/L $CO(NH_2)_2$ with distilled water) in a 20 mL bottle. The samples were then sealed, mixed by shaking and incubated at 45°C for 2 hours. To determine whether or not the colonies have urease activity, we observed the color of the solution after 2 hours. Cresol red changes from yellow to purple when the pH changes from 7.2 to 8.8; therefore, we

measured the pH of samples that had turned purple at the end of the incubation period. Finally, the bacterium that induced the greatest increase in pH of the solution was identified by sequencing their 16S rDNA and comparing the results to sequences available in the Apollon DB-BA 9.0 database, GenBank, DDBJ (DNA DataBank of Japan) and EMBL (European Molecular Biology Laboratory). The ureolytic bacterium was used for all subsequent cementation tests.

2.2 Syringe cementation test

To determine the influence of test conditions referring to the environment around the beachrock, which the aforementioned bacteria were collected from the sand near, on the UCS of sand samples, we conducted 11 small size cementation tests over a short period as described below.

First, 100 mL ZoBell 2216E medium solution (polypeptone 5.0 g/L, yeast extract 1.0 g/L, and $FePO_4$ 0.1 g/L with artificial seawater) was inoculated with 0.1 g of the ureolytic bacterium isolated by the above test, then incubated at 30°C with gentle shaking at 80 rpm for 3 days. Next, 40 g of coral sand collected from Namihira, Okinawa, Japan (Fig. 3) was dried at 110°C for 2 days and then placed in a 35 mL syringe (diameter, $\varphi = 2.5$ cm). Subsequently, 16 mL (more than the estimated 14 mL initial pore volume in the sample) of the bacterial culture, and 20 mL of the cementation solution (Table 2) were sequentially added to the syringe and drained, leaving about 2 mL of solution above the surface of the sand = to maintain wet conditions. After curing, the cementation solution was added and drained at fixed intervals. The Ca^{2+} concentration and pH of the drainage were also measured to determine temporal variations of these parameters in the samples. The setting of the syringe cementation test is shown in Fig. 4. Each test condition is described in Section 2.4.

After 14 days of curing, the needle penetration inclination (N_P) of each syringe sample ($\varphi = 2.5$ cm, height, $h = 7$ cm) was measured using a needle penetration device (SH-70, Maruto Testing Machine Company, Tokyo, Japan), and the UCS was estimated from the N_P based on the following regression equation (eq. (4)) (correlation coefficient: 0.941, x: N_P (N/mm), y: UCS (MPa)) determined from 114 natural rock samples and 50 improved soils with cement.

$$\log(y) = 0.978 \log(x) + 2.621 \quad (4)$$

In addition, samples were observed microscopically and elemental measurements were made by scanning electron microscopy (SEM) (SuperScan SS-550, Shimadzu Corporation, Kyoto, Japan) and energy dispersive X-ray spectroscopy (EDX) (SEDX-500, Shimadzu Corporation).

2.3 Column cementation test

To cement the sand specimen to UCS of several MPa, we attempted larger size cementation tests under six test conditions with longer test periods than the syringe tests. The method used for the column tests was similar that used for the syringe test; however, different volumes of specimen and solution were used, test conditions were different, and the test was conducted over a longer time.

First, 100 mL ZoBell 2216E medium solution was inoculated with 0.1 g of the ureolytic bacterium isolated by the above test and then shaken at 80 rpm for 3 days at 30°C. Next, 300 g of dried coral sand was added to a column ($\varphi = 5$ cm), after which 120 mL (more than the estimated 110 ml of the initial pore volume in the sample) of the bacterial culture and 150 mL of the cementation solution (Table 2) were sequentially added to the column and drained, leaving approximately 1 cm of solution above the surface of the sand ($h = 11.5$ cm) to maintain wet conditions. The cementation solution was then added and drained at fixed intervals using the same method that was used for the syringe test, after which the Ca^{2+} concentration and pH of the drainage were measured. The column test is shown in Fig. 5, and each test condition is described in Section 2.4.

After each test period, the UCS of the specimens ($\varphi = 5$ cm, $h = 10$ cm) was measured using an Instron universal testing machine 5586 (Instron Japan Co., Ltd., Japan) and a needle penetration device. In addition, the microbial

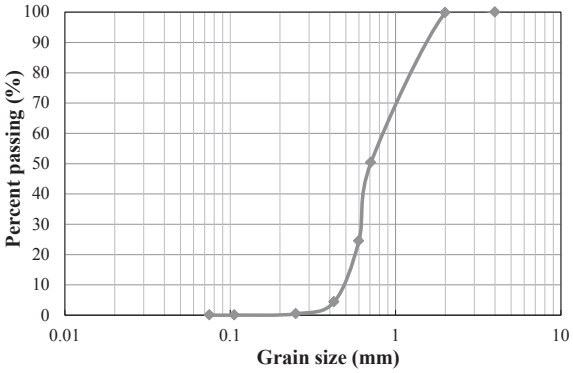

Fig. 3 Grain size distribution of coral sand.

Table 2 Standard composition of cementation solution (solvent: artificial seawater).

Reagent	Content (g/L)
Nutrient broth	3.00
NH_4Cl	10.00
$NaHCO_3$	2.12
Urea, $CO(NH_2)_2$	18.02
$CaCl_2$	33.3

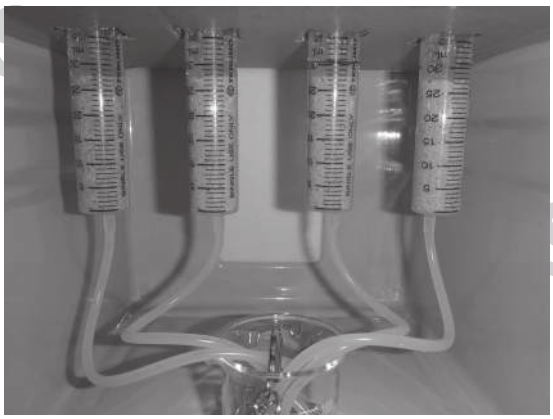

Fig. 4 Syringe cementation test.

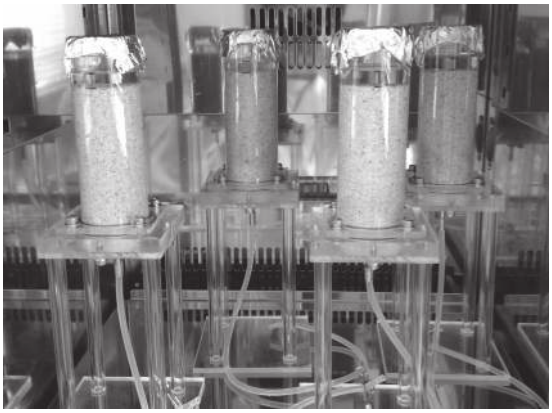

Fig. 5 Column cementation test.

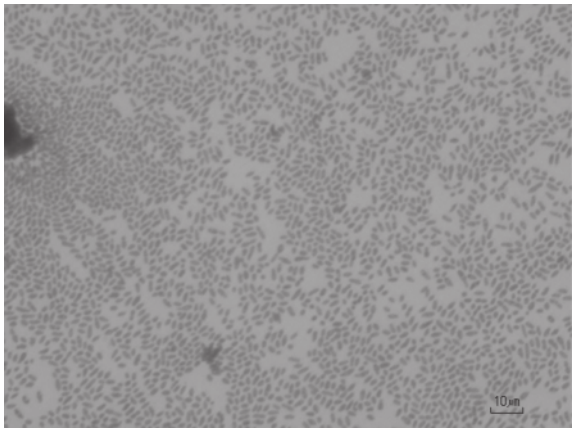

Fig. 6 Gram stain of *Pararhodobacter* sp. strain SO1.

Table 3 Test conditions for syringe and column cementation tests.

Case No.	Temperature (°C)				Injection interval (days)			Cementation solution		Test period (days)	
	20	25	30	35	1	0.5	2	Urea and $CaCl_2$ (M)	Sodium malate (g/L)	Syringe	Column
1			×		×			0.3	0	14	10, 14, 21, 26, 28
2	×				×			0.3	0	14	—
3		×			×			0.3	0	14	14, 28
4				×	×			0.3	0	14	—
5			×			×		0.3	0	14	14, 28
6			×				×	0.3	0	14	28
7			×		×			0.1	0	14	—
8			×		×			0.15	0	—	28
9			×		×			0.2	0	14	—
10			×		×			0.3	0.1	14	—
11			×		×			0.3	1	14	—
12			×		×			0.3	5	14	14, 28

population in the specimens was enumerated using ZoBell 2216E agar medium after 7 days of curing at 30°C.

2.4 Test conditions

To determine the influence of test conditions on the UCS of sand samples, we subjected the samples to the conditions shown in Table 3. Case 1 in Table 3 was chosen as the standard condition based on a study by Inagaki *et al.*[20] and the local condition around the beachrock which bacteria were collected from the sand near.

Curing temperatures, injection intervals, Ca^{2+} concentrations (urea and $CaCl_2$ concentrations) and sodium malate concentrations in the cementation solution, and test periods were varied with different test conditions. These conditions were selected based on simplification and efficiency of the sand cementation technique, as well as the environment around the beachrock. It was expected that temperature would affect bacterial growth and metabolism, injection interval, Ca^{2+} concentration, and test period would be correlated with the amount of $CaCO_3$ precipitation, and sodium malate would stimulate the precipitation of high-Mg calcite (HMC), which is the cement of beachrock in the area from which the bacteria were isolated.[16]

3. Results

3.1 Isolation of ureolytic bacterium

The results of the urease activity test revealed that *Pararhodobacter* sp. strain SO1 ("SO" from Sumuide, Okinawa) increased the pH of the solution to the highest value (9.1). *Pararhodobacter* sp. are Gram-negative, rod-shaped, aerobic, chemoorganotrophic bacteria, that are moderately halophilic.[21] As shown in Fig. 6, the bacterium was approximately 1 μm in diameter and 3 μm in length.

3.2 Syringe cementation test

The UCS estimated by the needle penetration test of samples (Cases 1–4) that were cured at different temperatures (20°C–35°C) increased by approximately 0.5 MPa with each 5°C increase (Fig. 7). Moreover, the sample cured at 20°C was cemented only at the surface (0.5 cm thick).

The estimated UCS of samples (Cases 1, 5, and 6) that were injected with the cementation solution at different intervals (0.5, 1, and 2 days) decreased with increasing injection intervals (Fig. 8). Moreover, the Ca^{2+} concentration of the injectate (cementation solution) was about 12,000 ppm, while the concentration of the drainage of Case 1 (1 day

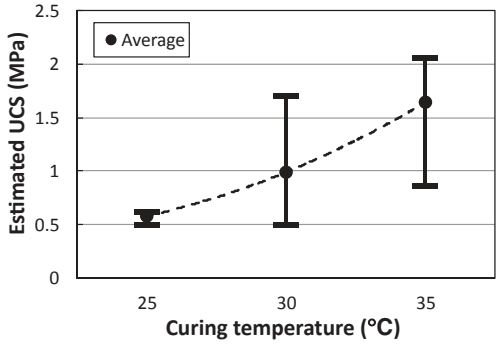

Fig. 7 Relationship between curing temperature and unconfined compressive strength, UCS estimated by the needle penetration test (Cases 1–4).

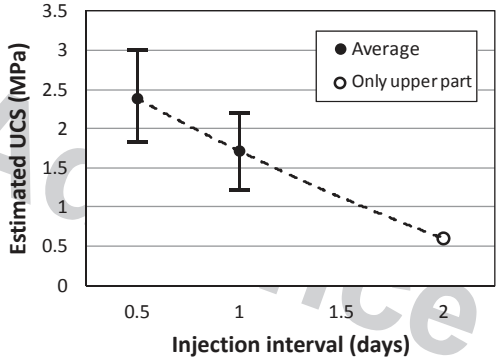

Fig. 8 Relationship between injection interval and estimated UCS (Cases 1, 5, and 6).

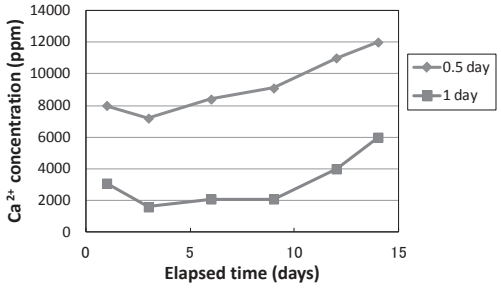

Fig. 9 Temporal variation in Ca^{2+} concentration of each drainage (Cases 1 (1 day interval) and 5 (0.5 day interval)).

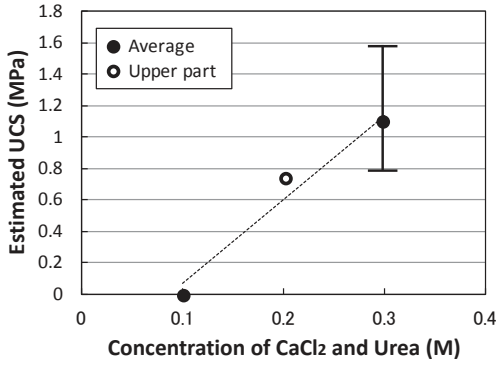

Fig. 10 Relationship between concentrations of $CaCl_2$ and urea and estimated UCS (Cases 1, 7, and 9).

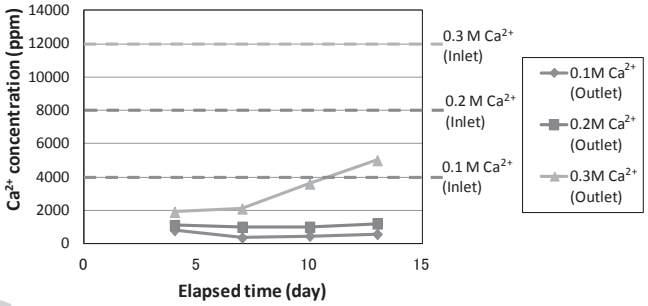

Fig. 11 Temporal variation in Ca^{2+} concentration of each drainage (Cases 1, 7, and 9).

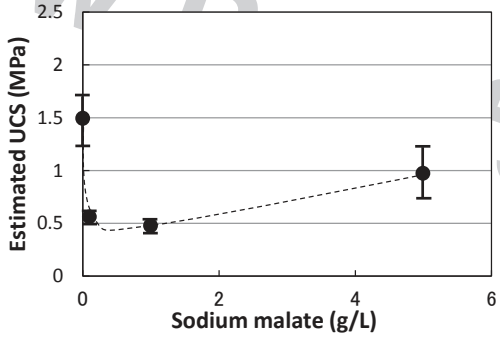

Fig. 12 Relationship between sodium malate concentration and the estimated UCS (Cases 1, and 10–12).

interval) was lower than that of Case 5 (0.5 day interval) (Fig. 9).

As shown in Fig. 10, the estimated UCS of the samples (Cases 1, 7, and 9) increased in response to injection of cementation solutions containing increasing concentrations of $CaCl_2$ and urea (0.1, 0.2, and 0.3 M). The sample for Case 7 was not cemented, while only the upper part of that of Case 9 was cemented. Moreover, the Ca^{2+} concentrations of the drainages of the samples tended to be higher when higher concentrations were present in the injection solutions (Fig. 11).

As shown in Fig. 12, the estimated UCS decreased greatly when 0.1 g/L sodium malate was added, but decreased to a lesser extent when higher sodium malate concentrations were used. As shown in Fig. 13, the surface of the Case 1 sample (no addition of sodium malate) was covered with needle-like precipitates, whereas the surface of the Case 12 sample was covered with micritic precipitates. Moreover, Cases 1 and 12 were composed of different elements (Fig. 14). The shape and elemental components of the precipitate of the Case 1 sample suggested that it was aragonite, which was confirmed by X-ray diffraction (XRD). The shapes of Cases 10 and 11 differed from those of Case 1, while their elemental compositions were similar to that of Case 12.[22)]

3.3 Column cementation test

The UCS of the specimens cured under standard condition (Case 1) showed an almost exponential increase with time (Fig. 15). The specimens were cemented up to 10 MPa UCS after 28 days. This is the first successful report of cementing sand up to 10 MPa using a marine bacteria and the second report of cementing up to this value using bacteria in general, after the results obtained using *Sporosarcina pasteurii* (UCS of 12.4 MPa[4)]). Cases 5 and 12 showed the second highest

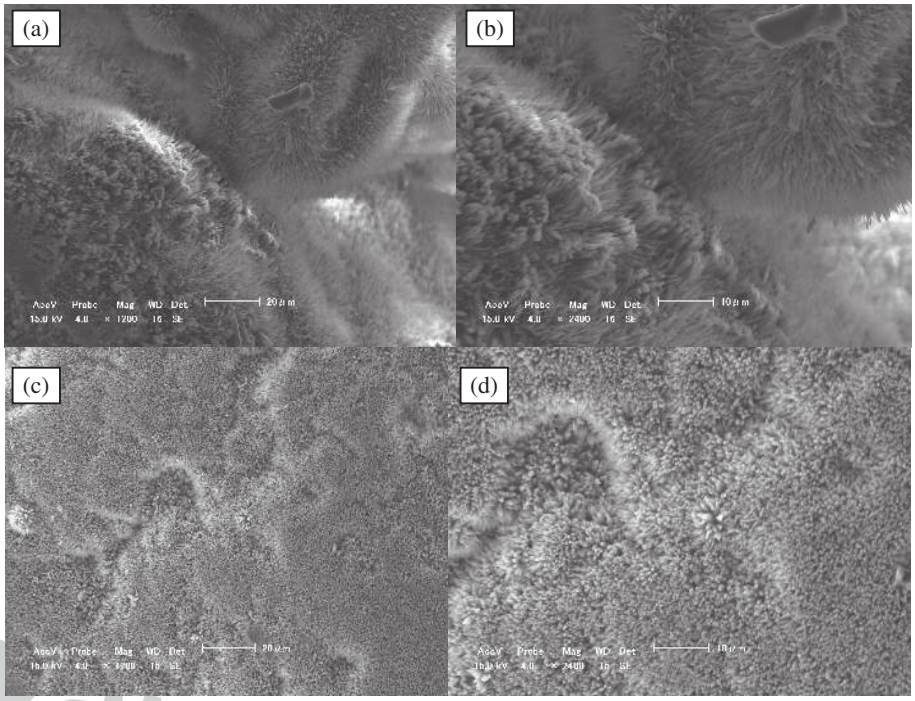

Fig. 13 SEM images of (a), (b): Case 1 (×1200, ×2400), (c), (d): Case 12 (×1200, ×2400).

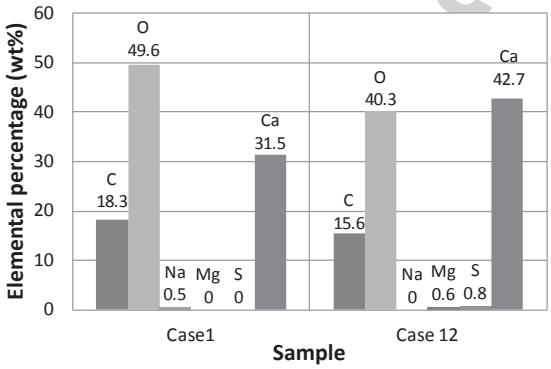

Fig. 14 Elemental percentages of Fig. 13(a) and (c) determined using EDX.

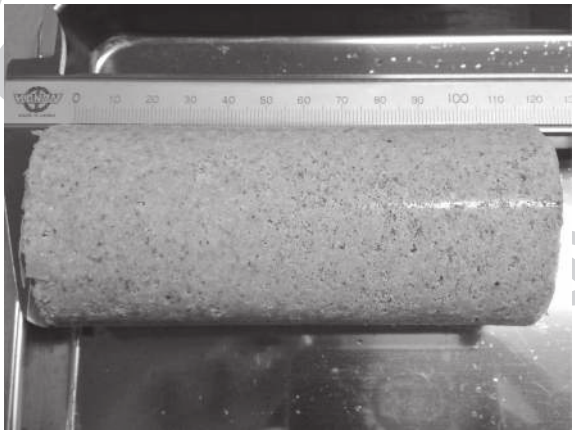

Fig. 16 Column specimen of Case 1 after 28 days of curing (the top of the specimen is positioned on the left and the bottom is positioned on the right).

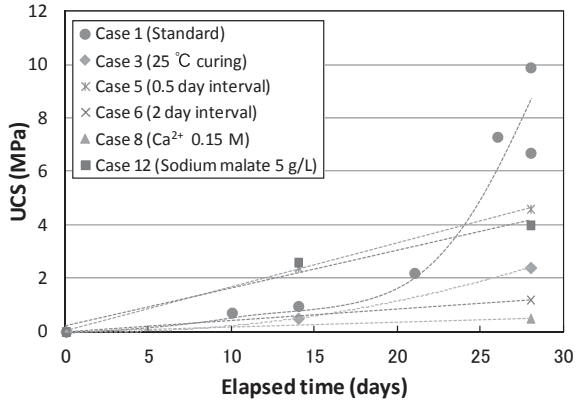

Fig. 15 Temporal variation in the UCS of each column specimen.

increases in USC, as well as a linear increase over time to about 4 MPa after 28 days. Cases 3, 6, and 8 showed lower USC after 28 days in descending order.

After 14 days of curing, the UCS of Cases 1, 3, and 5 in the column test were as large as those in the corresponding syringe test. However, the UCS of Case 12 in the column test was twice as large as that in the syringe test.

After 14 days, the upper parts of each specimen were more cemented than the lower parts; however, the cementation was generally homogeneous after 28 days. The specimen of Case 1 after 28 curing days (UCS: about 10 MPa) is shown in Fig. 16.

The microbial populations ranged from 10^6 to 10^9 CFU/mL before and after curing (Fig. 17). In detail, when the initial microbial populations were 10^8–10^9 CFU/mL, the final microbial population decreased to 1/3–1/40 times that of the initial concentration, while an initial concentration of 10^6–10^7 CFU/mL led to a 3–9 fold increase in the final bacterial concentration.

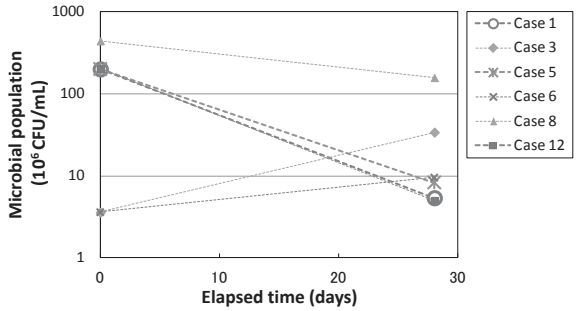

Fig. 17 Temporal variation in microbial populations of each column specimen.

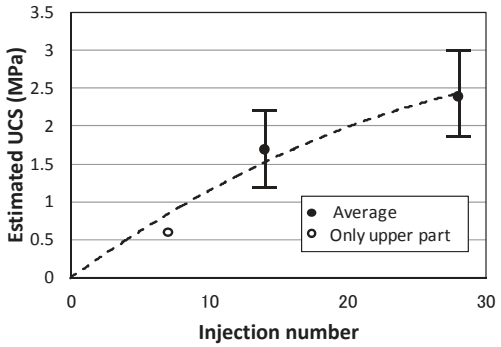

Fig. 18 Relationship between injection number and estimated UCS (Cases 1, 5, and 6).

4. Discussion

4.1 Influence of test conditions on the UCS of sand samples

(1) Curing temperature

The UCS likely increased with increasing curing temperature (Fig. 7) because of its effects on bacterial growth and $CaCO_3$ solubility. Foesel *et al.*[19] reported that the optimal growth temperature of *Pararhodobacter aggregans* was 30°C–40°C. Therefore, the optimal growth temperature of *Pararhodobacter* sp. strain SO1 is likely to be the same, because a genus is a taxonomic group that contains one or more species and species of same genus share one or more capital common properties.[23] As a result, ureolysis would increase with increased temperature in response to the increased bacterial growth. Moreover, the solubility of $CaCO_3$ is lower at higher temperature, which could lead to an increase in the precipitation of $CaCO_3$ produced by ureolysis at higher temperatures.

(2) Injection interval

The estimated UCS was positively correlated with the injection number (Fig. 18). While the injection number was proportional to the amount of Ca^{2+} added, the optimum injection interval for increasing the UCS from an economic standpoint was not clear. Therefore, the total $CaCO_3$ precipitation content was estimated from the measured Ca^{2+} concentration of each drainage (Cases 1 and 5) as shown in Fig. 9.

Because we measured the Ca^{2+} concentration of the drainage only at 1, 3, 6, 9, 12, and 14 days, the concentrations at other days were assumed to be the same as for the previous time point. The daily precipitated Ca^{2+} concentration (C_{pn} (ppm)) was considered to be the difference between the Ca^{2+} concentration of the injectate (C_i: 12000 ppm) and that of the drainage (C_{on} (ppm)) divided by the injection interval (x (days)) (eq. (5)).

$$C_{pn} = (C_i - C_{on})/x \tag{5}$$

The initial void volume (V_{v0} (cm^3)) was 13.9 cm^3, as calculated from the specimen volume (V_t: 28.5 cm^3), the dry mass of sand in the specimen (m_{sd}: 40.0 g), and the sand particle density (ρ_s: 2.74 g/cm^3) (eq. (6)).

$$V_{v0} = V_t - m_s/\rho_s = 28.5 - 40.0/2.74 = 13.9 \tag{6}$$

The daily amount of $CaCO_3$ precipitation (m_{pn} (g)) between $n-1$ and n elapsed days was calculated by eq. (7). The molecular masses of $CaCO_3$ and Ca^{2+} were 100.1 g and 40.1 g, respectively.

$$m_{pn} = (C_{pn}/1000 \times V_{vn-1}/1000) \times 100.1/40.1 \tag{7}$$

The void volume at n elapsed days was determined by eq. (8). For this equation, the density of $CaCO_3$ (ρ_{CaCO_3} (g/cm^3)) was considered to be equivalent to that of aragonite (2.93 g/cm^3) because the precipitate was aragonite. Moreover, the $CaCO_3$ precipitation from $n-1$ to n days (X_{pn} (g/g sand)) was calculated using eq. (9).

$$V_{vn} = V_{v0} - \sum_{i=0}^{n}(m_{pi}/\rho_{CaCO_3}) \tag{8}$$

$$X_{pn} = m_{pn}/m_{sd} \tag{9}$$

The temporal variation in total $CaCO_3$ precipitation was calculated from X_{pn}, and is shown in Fig. 19. The total $CaCO_3$ precipitation of the sample injected at 1 day intervals was higher than that of the sample injected at 0.5 day intervals.

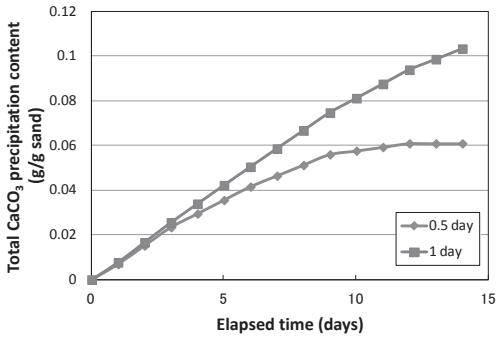

Fig. 19 Temporal variation in total $CaCO_3$ precipitation content (Cases 1 (1-day interval) and 5 (0.5-day interval)).

We next investigated why the estimated UCS was higher with shorter injection intervals, while the total precipitation was lower at shorter injection intervals. Cheng *et al.*[9] reported that the precipitation in a sample treated at 20% saturation was lower than that in a sample treated at 100% saturation, but that the former had a higher UCS because the cement precipitated preferentially around the contact points of sand particles under a lower degree of saturation. In our experiments, the injection number at 0.5 day intervals was two times greater than that at 1 day intervals. Therefore, at 0.5 day intervals, the chance for bacteria that live in the pore

solution to be washed out and remain on the sand surfaces was two times higher than that at 1 day intervals. When bacteria are on the sand surface, ureolysis and $CaCO_3$ precipitation is stimulated at the surface, including around the contact points between sand particles. Although this suggests that the $CaCO_3$ precipitation content of the sample injected at 0.5 day intervals would be lower than that of the sample injected at a 1 day interval, the UCS was higher for samples injected with the 0.5 day interval because the $CaCO_3$ precipitated preferentially around the sand contact points.

In contrast, the column tests revealed that after 28 curing days (Fig. 15) the UCS of specimens injected at 1 day intervals was higher than that of specimens injected at 0.5 day intervals. This might have occurred because cemented points between sand particles and/or $CaCO_3$ precipitate increased at 1 day intervals relative to 0.5 day intervals and the void volume became lower at 1 day intervals because the precipitation content was consistently higher. Future studies should be conducted to analyze the precipitation sites by thin section observation.

(3) Ca^{2+} concentration

The UCS were higher when the cementation solution contained a higher concentration of urea and $CaCl_2$ (Fig. 10). To determine the reason for this, the $CaCO_3$ precipitation of each sample was calculated as described in Section 4.1 (2) (Fig. 11). In addition, the initial Ca^{2+} concentration (C_i (ppm)) of Cases 1, 7, and 9 (0.3, 0.1, and 0.2 M Ca^{2+}) were 12000, 4400, and 8400 ppm, respectively.

As shown in Figs. 20 and 21, the average estimated UCS increased from 0.2 MPa to 1.2 MPa, while the total $CaCO_3$ precipitation increased from 8.5% to 10%. Cheng *et al.*[9] reported a similar trend for the relationship between total $CaCO_3$ precipitation and UCS. One of the reasons for the large increase in UCS with small increases in total precipitation content may be that the small difference in daily precipitation content led to a large difference in the UCS. Indeed, to generate a cement with a UCS of more than 1 MPa after 14 days of curing, 0.8–0.9% (0.008–0.009 g/g sand) of daily precipitation content would be necessary during the first week.

(4) Sodium malate concentration

To investigate the influence of sodium malate on the cement and the UCS, the composition of each cement was identified. The cement consisted of aragonite when no sodium malate was added. In contrast, the morphology of the cement changed as sodium malate was added, and micritic precipitates covered the sand particles (Fig. 13(d)). This morphology was similar to that of high Mg calcite (HMC).[24] HMC is a polymorph of $CaCO_3$ that contains more than 1.2 mass% $MgCO_3$.[25] Moreover, the $MgCO_3$ contained in the cement was calculated to be 2.1 mass% (>1.2 mass%) based on the Mg percentage in Fig. 14 (Case 12). However, the true amount was more than 2.1 mass% because this SEM-EDX experiment was conducted after carbon evaporation, so the true carbon percentage was lower and the other elemental percentages were higher than the values shown in Fig. 14. Based on the morphology and elemental percentages of the cement in Case 12, this cement could be HMC.

Kitano[26] reported that $CaCO_3$ in the form of calcite is very easy to precipitate from a solution, while organic matter like

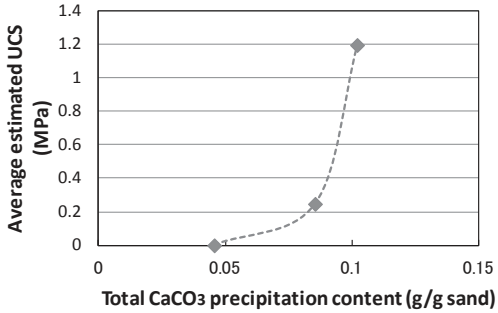

Fig. 20 Relationship between the total $CaCO_3$ precipitation content and average estimated UCS (Cases 1, 7, and 9).

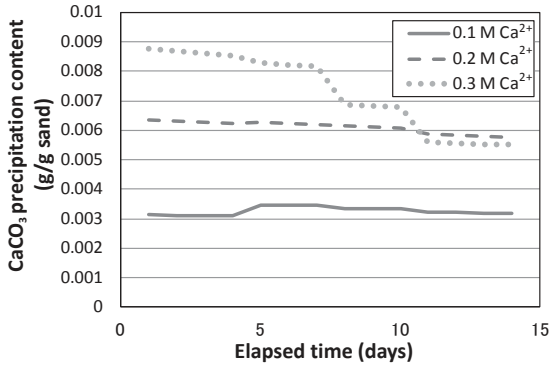

Fig. 21 Temporal variation in daily $CaCO_3$ precipitation content (Cases 1, 7, and 9).

citrate or malate forms complexes with Ca^{2+} and reduces the rate of carbonate precipitation. Moreover, $CaCO_3$ in the form of aragonite tends to be formed in solutions containing Mg^{2+}. However, in a solution with higher concentrations of above organic matter than Mg^{2+}, only $CaCO_3$ in the form of calcite precipitates. Furthermore, HMC precipitates from solutions containing organic matter such sodium malate. The HMC also contains a higher amount of $MgCO_3$ when a higher concentration of the above organic matter is present.

Therefore, the addition of sodium malate likely led to the precipitation of different cements and varying UCS for the following reasons. When no sodium malate was added, aragonite precipitated because the cementation solution contained Mg^{2+}. However, when 5.0 g/L sodium malate was added into the cementation solution, HMC, which has the form of calcite, precipitated because the concentration of sodium malate was higher than the Mg^{2+} concentration (1.33 g/L). Additionally, the addition of sodium malate decreased the precipitation rate because of complexation. As a result, the UCS became smaller when sodium malate was added.

4.2 Comparison of bacterial sand cementation techniques of this study and previous studies

To compare bacterial sand cementation technique of this study with those of previous studies, we compared the relationship between the total $CaCO_3$ precipitation and the UCS of sand specimens cemented by each bacterium.

First, the total $CaCO_3$ precipitation content of each of our column specimens was estimated from the measured Ca^{2+}

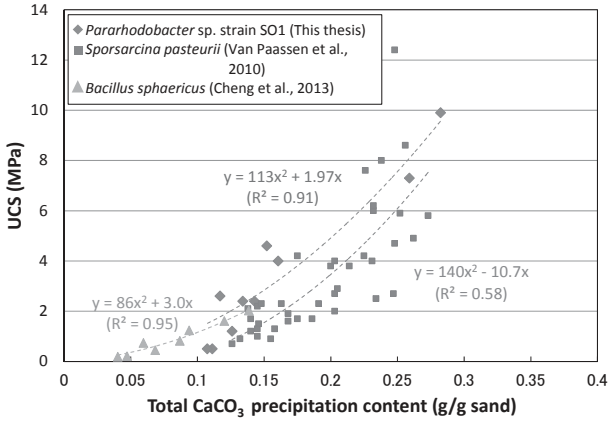

Fig. 22 Relationship between total $CaCO_3$ precipitation content and UCS of sand specimen cemented by effect of each bacterium (van Paassen *et al.*, 2010; Cheng *et al.*, 2013).

concentration of each drainage based on the calculation method described in Section 4.1 (2). The Ca^{2+} concentrations (C_i (ppm)) of all cases were 12000 ppm, except for Case 8 (6400 ppm). The density of $CaCO_3$ (ρ_{CaCO_3}) for all cases was 2.93 g/cm^3, except for that of Case 12, which was 2.71 g/cm^3. This is because the cements formed in all cases were aragonite, except for that of Case 12, which was HMC. The relationship between the total $CaCO_3$ precipitation content and the UCS of sand specimens cemented by *Pararhodobacter* sp. strain SO1 is shown in Fig. 22. Equation (10) describes the relationship between the total $CaCO_3$ precipitation content (x) and the UCS (y).

$$y = 113x^2 + 1.97x \quad \text{(determination coefficient: 0.91)} \quad (10)$$

The results for other bacteria are shown in Fig. 22.[4,9] *Sporosarcina pasteurii* is the bacterium that has been most widely applied for investigation of sand improvement using bacteria. *Bacillus sphaericus* was isolated by Al-Thawadi and Cord-Ruwisch.[27]

The UCS of the specimens prepared using *Pararhodobacter* sp. strain SO1 was higher than that of specimens generated using *Sporosarcina pasteurii*, even though these specimens contained the same amount of total $CaCO_3$ precipitate (Fig. 22). However, because these specimens contained different kinds of sand and cement, and were cured under different conditions, it is unclear which bacteria is better for sand cementation. Conversely, the different amounts of total precipitation could explain the different UCS of the specimens produced using *Bacillus sphaericus* or the other two bacteria. In addition, these findings are similar to those for sand cemented by enzymatically induced carbonate precipitation.[28]

Therefore, our results indicate that the *Pararhodobacter* sp. strain SO1, which originates from the sand near the beachrock, has the potential for soil improvement. The potential is comparable to that of bacteria used in previous studies, such as *Sporosarcina pasteurii*.[3,4]

4.3 Suggested formula for prediction of UCS

Multiple regression analysis was conducted in this study to analyze the relative importance of each test condition to the UCS, and to determine experimentally a formula that can predict UCS as a useful reference for future cementation tests and field tests.

Table 4 Results of the multiple regression analysis of data from the column cementation test.

	Partial regression coefficient	Standard error	t	P-value
Intercept	−23.4	12.25	−1.908	0.0928
Injection interval (days)	−2.82	1.905	−1.478	0.1776
Temperature (°C)	0.461	0.355	1.296	0.231
Ca^{2+} concentration (M)	34.5	16.40	2.10	0.0685
Sodium malate concentration (g/L)	−0.0907	0.355	−0.255	0.805
Test period (days)	0.275	0.0930	2.95	0.01831

In this study, multiple regression analysis was conducted using the results of the column cementation test because it produces conditions closer to those seen in the field than the syringe test with respect to specimen size and UCS. In this analysis, the column test conditions were set as explanatory variables and the measured UCS was the objective variable. The following relational expression (eq. (11)) and Table 4 were generated by this analysis.

$$q_{ud} = -2.82I_i + 0.461T + 34.5C_{ca} - 0.0907C_{so} + 0.275D - 23.4 \quad \text{(determination coefficient: 0.62)} \quad (11)$$

q_{ud}: Predicted UCS (MPa)
I_i: Injection interval (days)
T: Curing temperature (°C)
C_{ca}: Ca^{2+} concentration in cementation solution (M)
C_{so}: Sodium malate concentration in cementation solution (g/L)
D: Test period (days)

In Table 4, the partial regression coefficient indicated the coefficient of each multiple regression equation, which is set so that the theoretical value is close to the measured value. Additionally, the standard error was determined as follows: only one set of all intended experiments was conducted, but it was assumed that several sets were conducted. The frequency distributions of the partial regression coefficients and the constant term were then obtained by multiple regression analyses against each set of all intended experiments. The standard deviation of the normally distributed histogram obtained by calculating the frequency distributions is the standard error. The t value was obtained by dividing the partial regression coefficient by the standard error. From the t values, the degree of importance of each explanatory variable to the objective variable can be judged. The P-value was twice as much as the upper probability of the t value on the t distribution. In this study, a $P \leqq 0.01$ and $0.01 < P \leqq 0.05$ was considered to indicate that both explanatory variables are important, because the significance levels were 1% and 5%, respectively, while a $P > 0.05$ indicated that the explanatory variable was not important.

As shown in Table 4, only the test period was significant. Based on each t value, the test period had the highest degree of importance followed by Ca^{2+} concentration, injection interval, curing temperature, and concentration of sodium

Table 5 Results of the multiple regression re-analysis of data from the column cementation test.

	Partial regression coefficient	Standard error	t	P-value
Intercept	−19.51	5.19	−3.758	0.01981
Ca^{2+} concentration (M)	48.3	13.83	3.49	0.0251
Test period (days)	0.456	0.1053	4.33	0.01233

malate, in descending order. In addition, the determination coefficient of eq. (11) was not high.

To suggest a more reliable formula for prediction of the UCS than eq. (11), the more important explanatory variables against the objective variable were selected. Because the P-values of the test period and the Ca^{2+} concentration were less than 0.05 and comparatively closer to 0.05, respectively, these conditions were selected as explanatory variables, and the UCS of specimens generated at a curing temperature of 30°C, injection interval of 1 day, and a sodium malate concentration of 0 g/L were used as objective variables. The results of this multiple regression re-analysis are shown in eq. (12) and Table 5.

$$q_{ud} = 48.3C_{ca} + 0.456D - 19.51 \quad \text{(determination coefficient: 0.85)} \qquad (12)$$

The determination coefficient of eq. (12) was 0.85, and the P-values of both explanatory variables (Table 5) were lower than 0.05. Therefore, eq. (12) can be considered a reliable formula for prediction of UCS. However, it should be noted that this formula is only reliable for samples generated using the curing temperature, injection interval, and concentration of sodium malate, described above. In addition, the conditions of the explanatory variables can change within the range of our column tests. Hence, further cementation tests need to be performed to develop a reliable formula for prediction of UCS under more varied conditions. Nevertheless, eq. (12) will be useful for further cementation tests and field tests.

5. Conclusions

To cement sand specimens to UCS of several MPa using local ureolytic bacteria from sand around the beachrock, and to consider the influences of various test conditions on the UCS, we conducted coral sand cementation tests. The main findings of our study are as follows:

(1) The column specimens (5 cm diameter × 10 cm height) were cemented up to 10 MPa UCS after 28 days under a curing temperature of 30°C, injection interval of cementation solution of 1 day, and 0.3 M Ca^{2+} and 0 M sodium malate in cementation solution.

(2) Multiple regression analysis revealed that the relevant test conditions influencing the UCS of the specimen were test period (D (days)) and Ca^{2+} concentration of the cementation solution (C_{ca} (M)). The formula for prediction of the UCS (q_{ud} (MPa)) was $q_{ud} = 48.3C_{ca} + 0.456D - 19.51$.

The results of this study will contribute to the application of new techniques for bio-stimulation and coastal sand improvement. In the future, environmental impact assessments, tank cementation tests and field tests are needed to enable widespread application of this soil improvement technique using bacteria that have enhanced beachrock formation.

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