International Journal of GEOMATE, Oct., 2016, Vol. 11, Issue 26, pp. 2665-2670
Geotec., Const. Mat. & Env., ISSN: 2186-2982(Print), 2186-2990(Online), Japan

CORAL SAND SOLIDIFICATION TEST THROUGH MICROBIAL CALCIUM CARBONATE PRECIPITATION USING *PARARHODOBACTER* SP.

Md. Nakibul Hasan Khan[1], Suguru Shimazaki[1] and Satoru Kawasaki[2]

[1]Graduate School of Engineering, Hokkaido University, Japan
[2]Faculty of Engineering, Hokkaido University, Japan

ABSTRACT: The coastal erosion has been a problem in associate with manmade construction. The maintenance and management is expensive for repair and rebuild the coast. Compared to the concrete structure coral sand solidification would considered to minimize cost. The present experimental study was conducted to coral sand solidification through microbial carbonate precipitation (MCP) using *Pararhodobacter* sp. Ureolytic bacteria; *Pararhodobacter* sp. was isolated from the beachrock in Nago, Okinawa, Japan. ZoBell2216E as a medium for marine bacteria was used for the culture of the bacteria. Suitability for the use in MCP syringe test, growth properties of the bacteria were observed in various cultural conditions. MCP sand solidification test was carried in 30mL Terumo syringe injected bacterial culture fluid. Strength of the specimen was measured by needle penetration test. The maximum value of the absorbance at bacterial growth curve was constant if the added amount of the medium is 100mL. The correlation coefficient of determination for absorbance measurements and viable cell count measurements was obtained 0.5478. In MCP syringe test, pH of the specimen was decreased and Ca^{2+} concentration was increase with time. The estimated value of unconfined comprehensive strength (UCS) also increased with time. The maximum value of UCS of the specimen was 12MPa. The estimated UCS values of the specimen produced by sand solidification test for 14 days curing time have been achieved.

Keywords: Sand Solidification, Unconfined Compressive Strength, Microbial Carbonate Precipitation, Pararhodobacter sp

1. INTRODUCTION

Erosion of the sandy shore is often used to refer to changes in the coastline due to the collapse of the sediment balance. Coastal erosion has been a significant problem globally due to anthropogenic changes along the coastline. In order to prevent, or at least minimize damage from erosion, a combination of various structures and processes has been traditionally used, including embankments, revetments, jetties, artificial reefs, offshore breakwaters, and sand bypassing [1].

The use of inexpensive alternative materials should be considered, to cope with the increase in costs related to the maintenance and management of concrete structures. In this regards, we focused on alternative materials to replace beachrock to reduce lifecycle costs associated with the currently employed methods. Beachrock forms much more quickly than other sedimentary rocks within the intertidal zone and is composed of coastal sediments that have been cemented together mainly by $CaCO_3$ [2]. By using the processes that solidify beach sand, we hypothesized that could create a highly durable artificial beachrock that would be comparable in efficacy to the existing concrete structures.

For the formation of beachrock various factors have been considered. Among them, Danjo and Kawasaki (2014) [3], focused on the possibility of promoting solidification by microbial processes, specifically urea decomposition by microorganisms. In this study, the microbial induced calcium carbonate precipitation (MICP) method was utilized, which relies on the microbial metabolism of urea that generates carbon dioxide and precipitates $CaCO_3$ [4]. This low environmental impact method was assessed to determine its efficiency as an alternative means to alleviate coastal erosion. In order to create an artificial rock, the present study was conducted with the following two purposes:

(a) To solidify coral sand with an unconfined compressive strength (UCS) of 10 MPa or more, through the MICP method using ureolytic bacteria, *Pararhodobacter* sp..
(b) To examine the growth features of the *Pararhodobacter* sp. which used in the experiment.

2. MATERIALS AND METHODS

2.1 Absorbance Measuring Test

2.1.1 Materials

The test bacterium was *Pararhodobacter* sp., an ureolytic bacterium isolated from the soil near

beachrock in Sumuide, Nago, Okinawa, Japan. This strain was isolated from soil samples using artificial seawater (Akuamarine, Yashima Drug Company, Osaka, Japan) (Table 1). The strain was cultured on ZoBell2216E medium (polypeptone 5.0 g/L, yeast extract 1.0 g/L and $FePO_4$ 0.1 g/L, in artificial seawater, pH 7.6~7.8), which is often used for growth of marine bacteria.

Table 1 Composition of artificial seawater

Reagent	g/20L
$MgCl_2 \cdot 6H_2O$	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

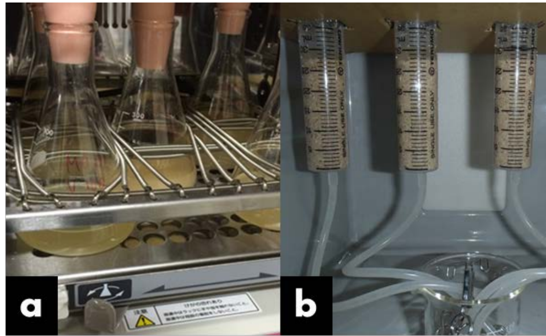

Fig. 1 State of (a) liquid culture and (b) sand solidification tests in the syringe.

2.1.2 Methods

The bacterial strain was cultured in liquid medium under various conditions. The change in cell density over time for each culture condition was quantified by measuring the absorbance (optical density) of the suspension using a spectrophotometer at 600-nm wavelength (OD_{600}) [5], [6]. For each culture condition, the absorbance was read and the viable cell count was determined using the plate dilution method simultaneously. Finally, the growth curve of the strain in each culture condition was obtained to investigate the effects of various conditions on bacterial growth.

2.1.3 Experimental condition

In order to shorten the total time for culturing the bacterial strain used in the experiment, the following three conditions were set up and analyzed, with respect to previous study [1].

(a) The initial mass of the microbe when added to the liquid culture medium was varied: 0.01 g, 0.1 g, or 1.0 g (the previously used mass was 0.1 g).
(b) The volume of liquid culture media was varied: 100 mL, 150 mL, or 200 mL (as opposed to the previously used volume of 100 mL).
(c) The shaking speed at the time of culture was varied: 80 rpm and 160 rpm (the previously used speed was 80 rpm).

2.2 MCP Test in Syringe

2.2.1 Materials

Pararhodobacter sp. and coral sand (the grain size distribution of the coral sand is 0.07 ~ 4.0 mm) from Okinawa, Japan, were used for the syringe solidification test. In addition, artificial seawater (Table 1) was used to recreate similar conditions to those in which the original beachrock was formed.

2.2.2 Methods

First, the bacterium was grown up under various experimental conditions listed above. These cultures, ZoBell2216E culture solution, were shaken for 2 days at 30°C. Then, 40 g of coral sand, dried at 110°C for at least 2 days, was placed in a 35mL syringe (diameter 2.5cm × height 7cm). Subsequently, 16mL of the culture medium (ZoBEll2216E) and 20mL of the solution for consolidation (this was the composition, mainly urea and $CaCl_2$, used for all of the various test conditions) was sequentially injected into the syringe. The solution for consolidation was then injected and drained once a day. The pH values and Ca^{2+} ion concentrations were measured every 3 days. The state of the syringe solidification test is shown in Fig. 1b. After 14 days of curing, the UCS of the specimen was estimated using a needle penetration device (SH-70, Maruto Testing Machine Company, Tokyo, Japan).

2.2.3 Experimental conditions

Considering the effect of conditions on the UCS of specimen, two test conditions were utilized: one with regular and other without regular, the re-injection of the culture solution. For regular test condition, the mass of the bacterial culture used was 0.01 g, 0.1 g, or 1.0 g; the concentrations of urea and $CaCl_2$ (solution for consolidation) used were 0.3 M, or 0.5 M. The standard conditions (0.1 g/0.3 M) were the same as those used by Inagaki *et al.* (2011) [7]. The test period was 14 days. In the experiment where the culture solution was re-injected, the mass of the bacteria (culture solution) was 1.0 g and urea and $CaCl_2$ (solution for consolidation) concentrations were 0.5 M each. The test period was also 14 days, where the culture solution repeatedly injected at 7th day. Both the pH and Ca^{2+} ion concentrations were measured daily.

3. RESULTS AND DISCUSSION

3.1 Absorbance Measuring Test

The absorbance values, which indicate the growth rate of the bacterium, under various culture conditions are described below.

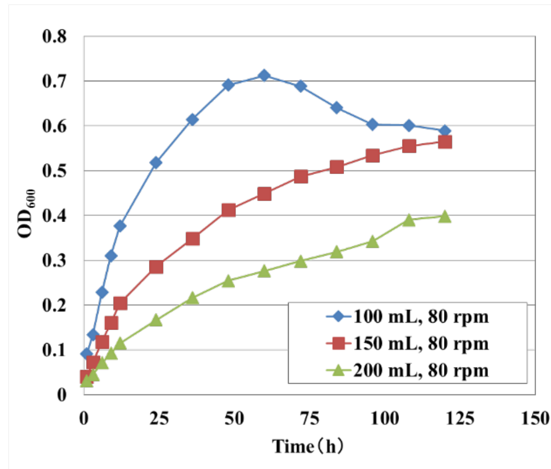

Fig. 2 The absorbance measurement results focused on the amount of liquid medium.

3.1.1 Importance of the volume of the culture solution

The results obtained using various volumes of culture media (100 mL, 150 mL, and 200 mL) and shaking speeds (80 rpm) are shown in Fig. 2 where microbial strain was 0.1g. With regard to shaking at 80 rpm, the absorption values for all three experiments increased with a decrease in the volume of culture medium. However, the absorbance values measured in 150mL and 200mL volumes at 80 rpm experiments were drastically different. In addition, the time required to reach the maximum absorbance was between 48 and 60 h in 100mL. In this case, it appears that the nutrient source (oxygen) was rapidly and homogeneously dispersed in the culture. If homogenization of nutrients and increased supply of oxygen in the culture fluid is assumed important, it is also explains why a decrease in absorbance is observed when the volume of the culture media is increased.

3.1.2 Effects of the amount of strain added and shaking speed

When more bacterial culture was added at the beginning of the experiment, the maximum value of the absorbance was obtained (Fig. 3), where culture solution was used 100mL. Since the absorbance measurement was based on the turbidity of the culture solution, this result is expected. However, as the same concentration of nutrients is available in the media, we predicted that the difference in cell densities would reach a peak regardless of the amount of cells initially added. Unlike the expected test result, regardless of the amount of strain added, the absorbance was nearly constant at approximately 80–100h (Fig. 3). It is difficult to explain the actual cause of the above result; the possible reasons could lie in the nutrients, source of dissolved oxygen and medium, but these factors were not examined. However, when the medium composition was the same, the absorbance read at the various time points were comparable regardless added amount of strain. This result is considered advantageous as it allows for the artificial control of the cell density in the medium used. The absorbance increased with a faster shaking speed, the maximum value of the absorbance was obtained when shaken at 160 rpm in comparison to 80 rpm (Fig. 3). The increase in shaking speed was considered to be effective in shortening overall time needed for the experiment to take place. When shaken at 160 rpm, the absorbance peaked approximately 24h after the start of the culture, indicating that the three-day growth is not necessary in the future.

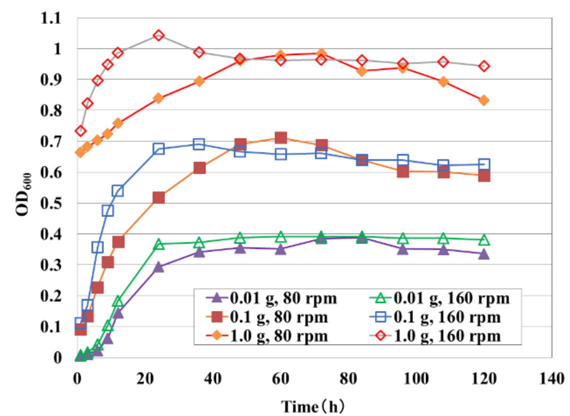

Fig. 3 The absorbance measurement results focused on the amount of strain added and shaking speed.

3.2 MCP Test in Syringe

The liquid culture medium of 100 mL and shake the culture at 160 rpm, which would provide ideal absorbance readings as well as result in optimum bacterial growth was used for syringe test. The pH and temperature for the optimal growth of the bacteria was 7.0–8.5 and 30–40°C, respectively. The above conditions were implemented for all aspects during the solidification test.

3.2.1 Role of pH and Ca^{2+} concentrations

The pH of all specimens during the test period tended to be lower than the pH at the beginning of the experiment (Fig. 4). However, when 1.0 g of the culture was added, regardless of the amount of urea and $CaCl_2$ added, the pH increased from days 3 through 6. The Ca^{2+} concentration was at 2.0 g/L or

less in all cases when measured after 3 days (Fig. 5). After the 6th day, the Ca^{2+} concentration tended to increase markedly when the urea and $CaCl_2$ solution added was at 0.3 M and 0.5 M. The concentration of Ca^{2+}, when the 0.3 M urea and $CaCl_2$ solution were used, did not change over time and continued to be consumed until the Ca^{2+} was present in concentrations above 10.0 g/L. On the other hand, the most dramatic increase in Ca^{2+}concentration when using 0.5 M (0.01 g/0.5 M), during which the consumption of Ca^{2+} at the 12-day time point was reduced to 7.0 g/L. As can be gathered from Fig. 4 and Fig. 5, the Ca^{2+} concentration increases when the pH lowers. The diminution of urea hydrolysis with decreasing cell density is a potential cause of pH reduction. Therefore, in the specimen, if there are high concentrations of urea or $CaCl_2$ at the beginning, the amount of carbon dioxide produced by urea decomposition does not reduce Ca^{2+} consumption, in fact, eventually Ca^{2+} concentration could have increased. Alternatively, the precipitation of $CaCO_3$ for MICP begins at the cell surface of bacteria [8], [9], strains induce precipitation of $CaCO_3$ at a crystal nucleus.

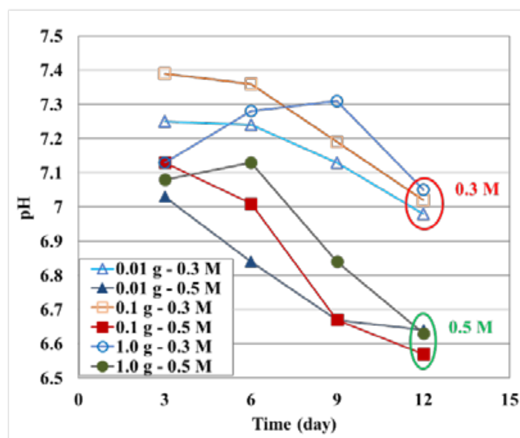

Fig. 4 The pH of the effluent over time course of different test cases in the syringe.

3.2.2 Estimated UCS value

The summarized results of the needle penetration test for each case are shown in Fig. 6. The estimated UCS value of the 0.3 M urea and $CaCl_2$ experiments was approximately 2 MPa, regardless of the added amount of culture added. In the cases of 0.5 M, the estimated UCS value tended to be higher than the case of 0.3 M. When the amount of bacterial strain added was 0.01 g and 0.1 g for 0.5 M urea and $CaCl_2$ solutions, the UCS showed 3~4 MPa. The specimens were significantly solidified in all experiments using 0.5 M, where the estimated UCS at some points exceeded 7 MPa. The estimated UCS tended to decreases from the top to the bottom of the specimen.

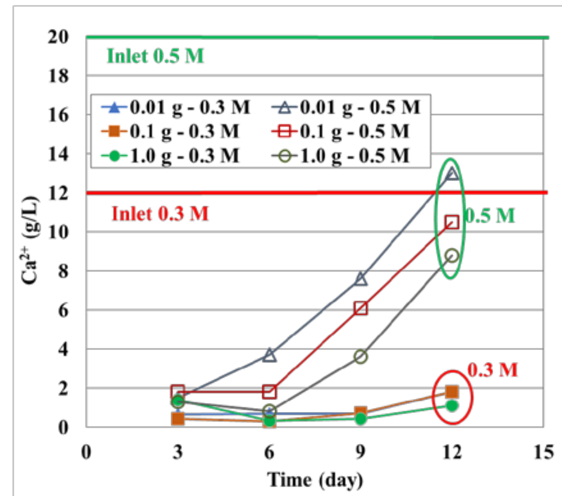

Fig. 5 Ca^{2+} concentration of effluent over time of different test cases in the syringe.

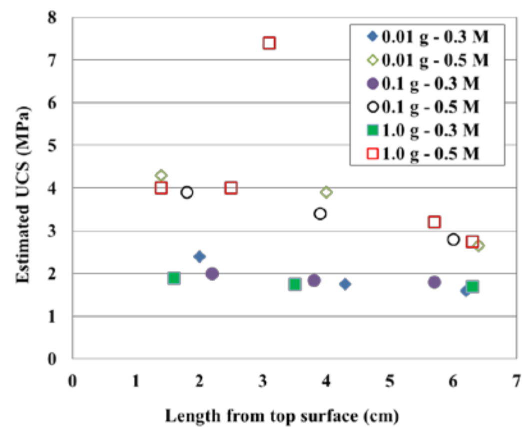

Fig. 6 Needle penetration test results of specimen in syringe in various test conditions.

3.2.3 Relationship between Ca^{2+} concentration and the estimated UCS in the specimen

From Fig. 6, in the case of 0.5 M solutions of urea and $CaCl_2$, the estimated UCS value of the specimen ranged from 3 to 4 MPa; the intensity distribution tended to decrease from the top to the bottom of the specimen. In this case, the Ca^{2+} concentration in the effluent demonstrated a tendency to increase in the latter half of the study period (Fig. 5). The collected effluent was estimated to have a pH and Ca^{2+} concentration of the lower part of the specimen. Furthermore, since this strain was cultured in aerobic conditions, it is possible that oxygen concentration played a role in strain activity. Since it can be considered that the bottom of the specimen approaches anaerobic conditions, the lower portion of the specimen may demonstrate reduced strain activity when compared to the top of the specimen. Therefore, the estimated UCS value of the specimen was expected to decrease from the top to bottom. On the

other hand, from Fig. 6, in the case of 0.3 M urea/$CaCl_2$, the estimated UCS of the specimen were approximately 2 MPa. In addition to the concentration of the urea and $CaCl_2$, the amount of Ca^{2+} in the effluent was generally constant throughout the test period (Fig. 5). The concentration of Ca^{2+} in the original solidification promoting solution was consumed nearly 90% by the precipitate as $CaCO_3$. Therefore, it was expected that the estimated UCS values of the specimen should also remain constant, which is consistent with the results obtained above.

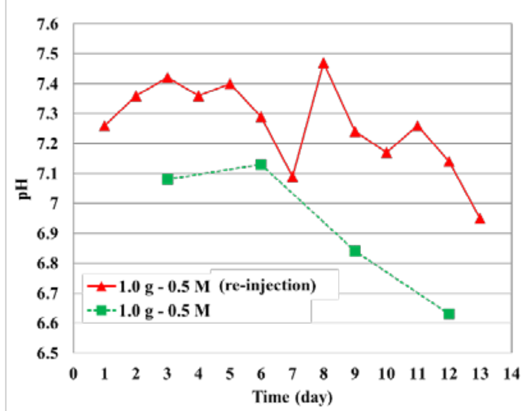

Fig. 7 Changes of pH in the effluent over time in the re-injection test in the syringe.

3.2.4 pH and Ca^{2+} concentration of effluent in the re-injection test

The pH of effluent in the re-injection test (1.0 g of bacterial culture was also re-injected on 7th day) is shown in Fig. 7. In the re-injection test, the pH was maintained higher as compared to the previous test, without re-injection. The pH begins to decrease after 5th day, and at 7th days it was reduced to a value close to the previous test. However, when carrying out the re-injection of the culture on day 7, pH was the maximum value during the study after 8 days thereafter decreased. However, the pH in the re-injected test remained between approximately 6.9~7.5, and did not continue to decrease (Fig. 7). The concentration of the Ca^{2+}on effluent in the re-injection test is shown in Fig. 8. The Ca^{2+} concentration in the re-injection test was approximately constant during the entire test period (Fig. 8). Overall, in the standard tests (without re-injection) the Ca^{2+} concentration increased from the beginning to the end of the experiment, which was not seen when cultures were re-injected.

The results demonstrated in Fig. 7 and Fig. 8, indicate that if the re-injection of the culture was not performed during the study in the syringe solidification test, both the pH and Ca^{2+} concentrations in the effluent decreased and increased, respectively. This trend was observed regardless of the cell density of the liquid culture injected. However, if the re-injection of the culture is completed during the study, the pH was approximately 7.0~7.3, and the Ca^{2+} concentration was constant at approximately 1.0 g/L throughout the course of the experiment (Fig. 7 and Fig. 8). The pH rise of in the effluent due to urease activity of strain increased during re-injection of the culture liquid. At the same time, the Ca^{2+} concentration decreases also to be considered due to the increase in cell concentration. After subsequent re-injection test that had remained high cell concentration in the specimen as compared to the previous test, even longer observed increase and maintenance of pH by urease activity. The precipitation of $CaCO_3$ was increased and persisted, Ca^{2+} concentration remain constant.

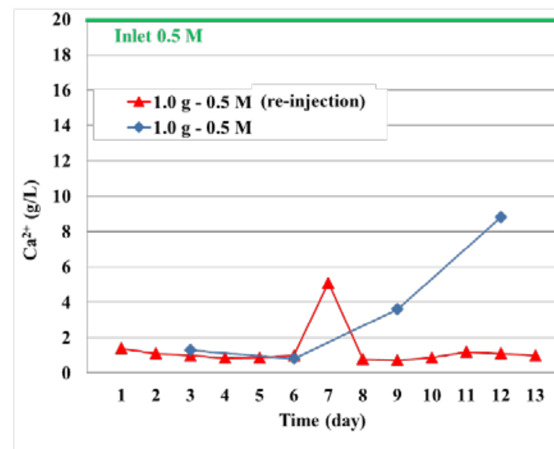

Fig. 8 Ca^{2+} concentration of effluent over time in the re-injection test in syringe.

3.2.5 Estimated UCS value in the re-injection test

In the re-injected 14 days test period, the UCS was approximately 8.0~13.0 MPa (Fig. 9), this value is greater than the maximum value at the time of the previous test. The UCS demonstrated a minor decreasing tendency from the upper half to the lower half of the specimen (Fig. 9). Specimens of immediately after removal of the syringe test and the re-injection syringe test is shown in Fig. 10. In the re-injection test results of the syringe shows the estimated UCS value was double that recorded using the without re-injection test. In addition, we also observed variation in solidification, which had increased the UCS value regardless of the position of the specimen, indicating that the specimen was heterogeneous and lacked problems in solidification. For this reason, there was a possibility that cell density in the specimen was not constant. From Fig. 9, the estimated UCS value in re-injection test was shown by about 2 times the needle penetration test as compared to the without reinjection. By increasing the cell concentration at 7th day, for the amount of precipitation of $CaCO_3$ and trends of Ca^{2+} concentration was kept constant, it is considered that the estimated UCS value increases. Therefore, although the estimated UCS value of the specimen

was expected to be constant from the top to the bottom, variations were observed in the results.

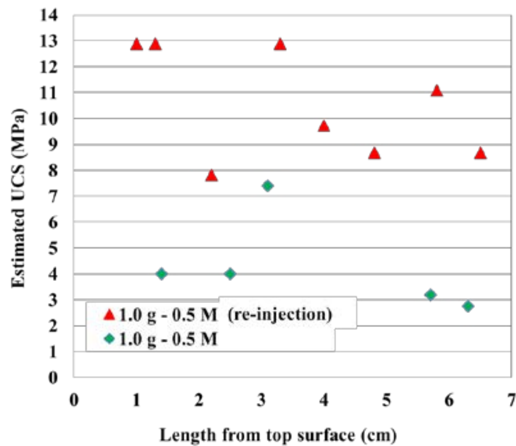

Fig. 9 Needle penetration test results of the re-injection test case in syringe.

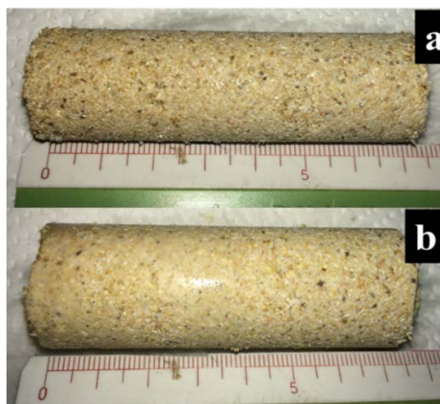

Fig. 10 appearance of specimens immediately after removal of (a) the syringe test and (b) the re-injection syringe test (photo left specimen top).

4. CONCLUSION

In this study, results demonstrated that the specimen solidified up to 13 MPa UCS after 14 days of curing using a microbial strain of *Pararhodobacter* sp.. Based on the findings from this study, for future attempts of the MICP method using this strain to obtain the ideal conditions for solidification in the future, four suggestions are outlined below:

(1) To facilitate solidification in a short period, the urea and $CaCl_2$ concentration should preferably be 0.5 M.

(2) For the well progress of solidification of the specimen, the pH in the specimen should be 7.0 or higher, and the Ca^{2+} concentration should be maintained at1.0 g/L.

(3) When the pH and Ca^{2+} concentrations in the specimen are out of the above range, the process can be improved by injecting more of the culture again.

5. REFERENCES

[1]. T. Danjo, S. Kawasaki, S. Shimazaki and K. Koizuka: Coral sand solidification test using ureolytic bacteria. Geomechanics from Micro to Macro (2013) 1521-1526.

[2]. T. Danjo and S. Kawasaki: A Study of the Formation Mechanism of Beachrock in Okinawa, Japan: Toward Making Artificial Rock. International Journal of Geomate 5(1) (2013) 633–638.

[3]. T. Danjo and S. Kawasaki: Formation Mechanisms of Beachrocks in Okinawa and Ishikawa, Japan, with a Focus on Cements. Materials Transactions 55(3) (2014) 493-500.

[4]. V. S. Whiffin, L. A. Van Paassen and M. P. Harkes: Microbial Carbonate Precipitation as a Soil Improvement Technique. Geomicrobiology Journal 24 (2007) 417-423.

[5]. H. L. Ehrlich: Geomicrobiology. Marcel Dekker, New York (2002).

[6]. J. K. Fredrickson and M. Fletcher: Subsurface microbiology and biogeochemistry. Wiley-Liss, Hoboken, NJ (2001).

[7]. Y. Inagaki, M. Tsukamoto, H. Mori, S. Nakajima, T. Sasaki and S. Kawasaki: A centrifugal model test of microbial carbonate precipitation as liquefaction countermeasure. Japan Geotechnical Journal 6 (2011) 157–167 (in Japanese with English abstract).

[8]. J. T. De Jong, B. M. Mortensen, B. C. Martinez and D. C. Nelson: Bio-mediated soil improvement. Ecological Engineering 36 (2010) 197-210.

[9]. W. De Muynck, N. De Belie and W. Verstraete: Microbial carbonate precipitation in construction materials: A review. Ecological Engineering 36 (2010) 118-136.

International Journal of GEOMATE, Oct., 2016, Vol. 11, Issue 26, pp. 2665-2670.
MS No. 5185 received on June 14, 2015 and reviewed under GEOMATE publication policies.
Copyright © 2016, Int. J. of GEOMATE. All rights reserved, including the making of copies unless permission is obtained from the copyright proprietors.
Pertinent discussion including authors' closure, if any, will be published in Oct. 2017 if the discussion is received by April 2017.
Corresponding Author: Md. Nakibul Hasan Khan