Euro-Mediterr J Environ Integr (2016) 1:2
DOI 10.1007/s41207-016-0003-8

ORIGINAL PAPER

# Evaluation of sediment solidification ability using in situ microbial functions in Ichkeul Lake, Tunisia

**Toshiro Hata[1] · Mitsuteru Irie[2] · Atsushi Kawachi[3] · Taichi Tebakari[1]**

Received: 17 February 2016 / Accepted: 18 August 2016 / Published online: 8 September 2016


**Abstract** The merit of exploiting ecosystems is debated globally. There have been harmful effects due to water resource management of the world natural heritage site at Lake Ichkeul, Tunisia. These concerns have increased the interest in the production of natural minerals. Natural minerals, such as calcite, are produced from in situ microorganisms and are categorized as construction materials. They are considered to be a viable alternative new resource. This study focused on the physical and biological properties of sediments from Ichkeul Lake and the potential of calcite precipitation from in situ microorganisms in these sediments. The results of field investigations focused on high-salinity aquatic plants (*Ruppia cirrhosa*) that were distributed in the eastern portion of the lake. These results show that the area around the Tinja channel had increased salinity in the dry season. The element contents were measured with portable X-ray fluorescence (XRF). Ichkeul Lake sediments had higher calcium content than standard Japanese samples. This suggests that microbial carbonate precipitation is applicable to Ichkeul Lake. Calcite precipitation techniques, such as acceleration of urease-producing bacteria, were studied. Pre-cultivation tests focused on increasing urease activity rates from in situ bacteria. Solidification of sediment resulting from enhanced calcite precipitation rates through use of urease-producing bacteria was examined. All of the sediment samples from Ichkeul Lake were able to precipitate calcite. The results of the field investigation and laboratory tests indicated that the area around the mouth of the Joumine River showed an enhanced calcite precipitation rate after a cultivation period of 1 month under high-salinity conditions.

**Keywords** Microbial carbonate precipitation (MCP) · In situ microorganisms · Sediment · Ichkeul Lake

✉ Toshiro Hata
t-hata@pu-toyama.ac.jp

Mitsuteru Irie
irie.mitsuteru.fu@u.tsukuba.ac.jp

Atsushi Kawachi
kawachi.atsushi.fp@u.tsukuba.ac.jp

Taichi Tebakari
tebakari@pu-toyama.ac.jp

[1] Department of Environmental Engineering, Toyama Prefectural University, 5180, Kurokawa, Imizu, Toyama 939-0398, Japan

[2] Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, Japan

[3] Faculty of Life and Environmental Sciences, ARENA (Alliance for Research on North Africa), University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, Japan

## Introduction

Ichkeul Lake is located in northern Tunisia (Fig. 1). The Ichkeul National Park (lake and wetland) has been listed as a World Heritage Site since 1980 and is an important stop for migratory birds. However, the park was listed on the World Heritage Sites in the Danger list from 1996 to 2006 because the construction of several large dams changed the ecological balance of the lake and wetlands [5, 8].

Microbial carbonate precipitation (MCP) is a bacteria-based bio-mineralization process that has been investigated extensively in sand, clay, and sediment solidification [4, 11, 13, 14]. In this study, we developed new sediment solidification techniques to control sediment surface strength and reduce the re-suspension of turbidities using

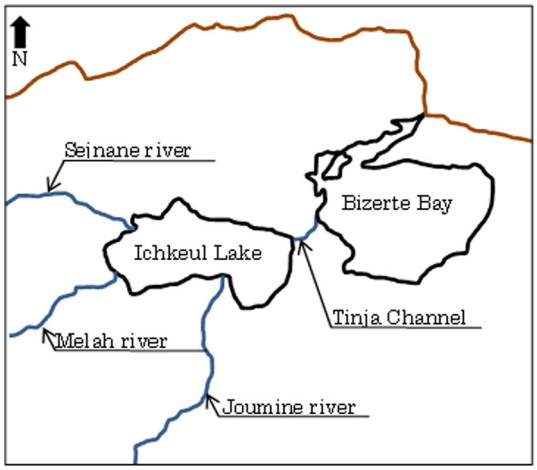


**Fig. 1** Location *map* of Lake Ichkeul showing its tributaries and the Tinja channel

microbial carbonate precipitation. The authors propose restoration of the lake and wetland bottom conditions, based on microbial functions that can control the microorganisms, benthos, and aquatic plants specifically for the benefit of migratory birds (i.e., restoring the pre-1980s natural environment of the lake). These techniques include enhancement of the natural calcium carbonate (calcite) crystallization process [7, 10].

The reactions that produce $CaCO_3$ from Ca, $CO_2$, and $H_2O$ are given by Eqs. 1, 2, and 3.

Improvement of soil strength:

Hydrolysis of urea:

$$CO(NH_2)_2 + 2H_2O \longrightarrow 2NH_4^+ + CO_3^{2-} pH(\uparrow). \quad (1)$$

Precipitation of calcium carbonate (calcite):

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

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

These reactions require urease-producing bacteria as well as sources of urea and calcium.

The microbial carbonate precipitation (MCP) technique involves hydrolysis of urea by urease-producing bacteria. When this process occurs in the pores of unconsolidated sand, a sandstone-like natural material called beach rock is created.

High-urease *Bacillus pasteurii*, which can enhance the calcite precipitation rate under alkali conditions and increase soil strength, has been isolated from land areas [1]. Another type of urease-producing bacteria, *Pararhodobacter* sp. Strain SO1, has been isolated from Japanese sediment on Okinawa Island, Japan. These bacteria can increase sand strength and form beach rock [12].

Many studies have investigated bio-augmentation and bio-stimulation for sand applications (e.g., [6]. This is primarily because not all areas contain bacteria with good potential for soil cementation and because of the uncertainty of applying this technique in clay and silt (e.g., sediment).

In this study, we conducted sediment cementation tests using local urease-producing microbes shown to enhance sediment strength.

The key benefits of the field investigation and laboratory tests are: (1) characterization of aquatic plant distribution, physical, chemical, and biological properties of sediments in Lake Ichkeul, and evaluation of the MCP process; (2) identification of bacteria useful for sediment solidification in areas near the lake floor in Lake Ichkeul in Tunisia; and (3) discovering that bacteria originating from the sediment having the potential to promote cementation and to coat the seabed surface.

The objectives of this study were to enhance sediment strength in Ichkeul Lake and to assess the influence of some test conditions related to the natural ecosystem, like germination of low-salinity aquatic plants that can help lake recovery with regard to providing an appropriate habitat for a migratory bird stopover point.

## Materials and methods

### Sediment sampling

Six sediment samples were collected (No. 1 to No. 6) in March 2015 (wet season) and again in September 2015 (dry season). These samples were analyzed with a cultivation test to evaluate the activity of urease-producing bacteria and for physical properties, such as vane shear strength, measured with column tests. Sediment samples were collected with a core sampler (diameter 5 cm, height 20 cm). Approximately, 500 g of sediment was collected from the sampler, using an acetone-rinsed Ekman–Birge grab. Sediments were stored at room temperature in an airtight container until use. The sediment and aquatic plant sampling points are shown in Fig. 2.

### Aquatic plant sampling

Distribution maps of aquatic plants in Ichkeul Lake have been compiled in prior research [2, 3]. Plant samples were taken from shoots of two species around the lake. Aquatic plant samples were collected by boat from eight locations at the water surface in September 2015 (dry season). The aquatic plant sampling points (Points A to H) are shown in Fig. 2. The plants were dried quickly on a plant drying mat and then classified; all samples were identified as belonging to either *Potamogeton* or *Ruppia* genera.

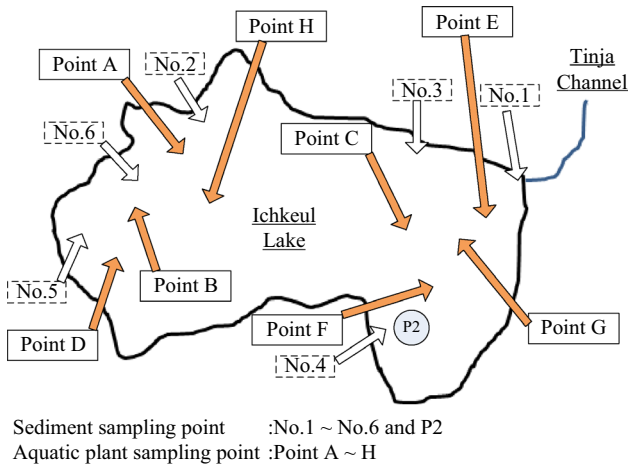


**Fig. 2** Locations of sediment sampling

## Laboratory tests outline

A flow diagram for the three types of laboratory tests performed on the sediments is shown in Fig. 3. The pre-cultivation test evaluated urease activity with various combinations of yeast extract and urea (nitrogen source). Urease activity was measured in all pre-cultivation sediment samples. A comparison between initial urease activity and the value after cultivation determined the in situ sediment solidification potential. Samples with positive results underwent a calcite precipitation test to evaluate the calcite precipitation rate under various calcium chloride and urea concentrations. A combination of nutrients and a calcium source was selected according to the calcite precipitation tests, and this combination was used with the sediment samples to accelerate the enzyme activities of microorganisms and enhance the strength of the sediment surface.

Next, vane shear strength was measured to evaluate sediment solidification effects under microbial carbonate precipitation. In the actual restoration work on Ichkeul Lake, microbial monitoring was also carried out to ensure that conditions were optimal for the entire microbial community in general and for the specific microorganisms in particular.

*Pre-cultivation (enhancement of urease-producing bacteria population) test*

A cultivation test was carried out on each sample for 1 month. A sediment sample (0.3 g) was added to a bottle containing 30 mL of culture medium that contained urea (0.15 mol/L) and yeast extract (0.05 g/L). A room temperature of 20 °C was used as the static culture condition. After 7, 14, and 28 days, water samples were extracted, and the ammonium ion concentration was measured. Ammonium and calcium ion concentrations were analyzed with an ion chromatograph (PIA-100, Shimadzu Corp.) with a Shim-Pack IC-C3(S) ion exchange column. Urease activity was measured using Whiffin's method [15].

*Calcite precipitation tests*

Calcite precipitation samples were prepared as follows: (1) the solidification medium (Table 1) was added to 500 mL distilled water; (2) 30 mL of the liquid solidification medium and 0.3 g of the sediment sample were added to 50 mL vials; (3) the headspace in the vials was purged with pure air; and (4) the vials were rapidly sealed with Viton rubber caps and incubated for 28 days at 20 °C. Samples were extracted at 7-day intervals. Duplicate vials were prepared for each sampling time, so a duplicate sample could be used for analysis. The calcium ion concentration was analyzed using an atomic absorption photometer and the theoretical value was calculated from calcium consumption rates of bacteria with no added sediment. Microbial DNA was extracted from the 0.3 g sediment samples using a Power Soil DNA Isolation kit (MO BIO Laboratories). Urease encoding genes (ureC) were detected using the real-time polymerase chain reaction (PCR) method. The DNA fragment samples (including 16S rDNA) were amplified by the universal PCR primer set (GC-341F and 534R), which focused on the 16S rDNA V3 region for denaturing gradient gel electrophoresis (DGGE) analysis [9]. PCR conditions were as follows: 95 °C for 10 min, 94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min (35 cycles). The final temperature was then kept at

**Fig. 3** Laboratory test stages

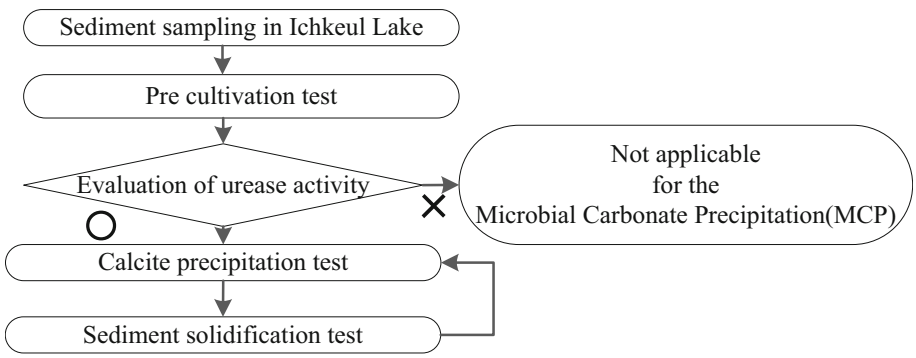

**Table 1** Composition of the solidification medium (1 L)

| Items | Value (g) |
|---|---|
| Nutrient broth | 3.00 |
| $NH_4Cl$ | 10.00 |
| $CO(NH_2)_2$ | 9.00 |
| $CaCl_2$ | 16.64 |

4 °C to the end of the PCR machine run time. DGGE gel was prepared to obtain an 8 % acrylamide gel with a denaturant gradient of 30–60 %. The DGGE electrophoresis conditions were 130 V for 5 h using the D-Code system (Bio-Rad Laboratories).

*Sediment solidification tests*

Samples for the sediment solidification test at sampling point P2 were prepared as follows: (1) sediment moisture content was adjusted to within twice the liquid limit value for the homogenized sediments; (2) 300 g of sediment was added to the column equipment and left to stand for 3 days to consolidate the sediments; (3) the column was connected to an overflow tube; (4) the pre-cultivation medium that enhanced the urease-producing bacteria of the in situ sediments was injected (0.3 mL/min) in the sample; and (5) the solidification medium was injected at three times the pore volume into the test specimen. The columns were incubated for 21 days at constant temperature and moisture (25 °C, 50 % moisture). Water analysis samples were collected after 0, 7, 14, and 21 days at the outlet of the column. Duplicate water samples were prepared for each sampling time so that the control sample could be used for analysis, to calculate the consumption rate from microbial functions. After three injection cycles, vane shear strength was measured at the surface, middle, and bottom of the column using the Japan Geotechnical Society standard method (JGS1411). A schematic of the column test is shown in Fig. 4.

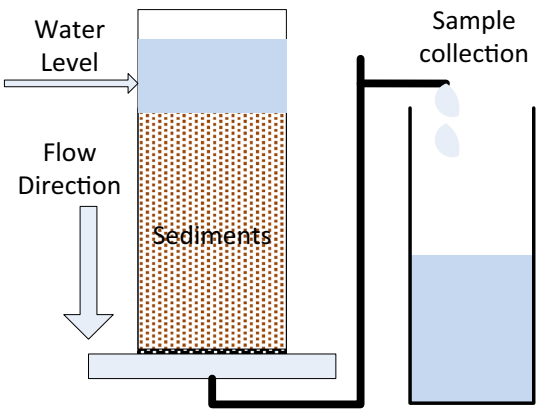


**Fig. 4** Overview of the sediment solidification tests

After the vane shear strength test, we added 300 mL deionized water to the column to wash the sediment pores. Measurement of $CaCO_3$ mass was performed as follows: (1) oven drying (110 °C, 24 h) to stop microbial metabolism; (2) extraction of sediment samples and measurement of weight; (3) sediment sample washing with 1 N HCl to dissolve precipitated carbonates ($CaCO_3$); and (4) measurement of calcium ion concentration in the rinsed samples to calculate the calcite precipitation rate. Duplicate samples from each position (surface, middle, and bottom) were prepared so that the whole sample could be used.

## Results and discussion

### Chemical and physical properties of Lake Ichkeul sediments

Sediment physical properties and element ratios obtained using X-ray fluorescence (XRF) are shown in Table 2. Soil density and loss on ignition are slightly higher than those of standard Japanese lake sediments from the center of the main island, with similar concentration and grain size distributions. The calcium content ratio is four times higher than in typical Japanese lake sediments. This indicates that the volume of the solidification medium that acts as a calcium source can be reduced when applying these methods for Lake Ichkeul calcite precipitation. The grain size distribution at point P2 (Fig. 2) as measured by the standard method of the Japanese Geotechnical Society (JGS 0131) is shown in Fig. 5. The D50 value (the mean grain size for a particular type of soil) is 0.00103 mm. Results indicate that the P2 sediment is classified as clay (according to the American Association of State Highway and Transportation Officials) and fine (according to the US Geological Survey and ASTM International). The sediment from Ichkeul Lake is classified as having low permeability ($10^{-7}$ to $10^{-9}$ cm/s). The slightly higher value for loss on ignition than standard Japanese sediments indicates high amounts of organic compounds are contributed by the tributary. These results indicate that many types of microorganisms (aerobic and anaerobic) can survive in the lake sediments. The diversity of microorganisms in Lake Ichkeul sediments will be evaluated by DNA analysis (PCR-DGGE or MiSeq amplicon) in future work.

### Distribution of aquatic plants

The results of aquatic plant identification are shown in Table 3, indicating species distributions. A map of the distribution of *Potamogeton* and *Ruppia* genera is shown in Fig. 6. The dominant species in the high-salinity area is *Ruppia cirrhosa*. The *Ruppia* distribution area is

**Table 2** Physical properties and element ratios of Ichkeul Lake and Japanese lake sediments

| Physical properties | Ichkeul Lake | Japanese lake | Element ratio (%) | Ichkeul Lake | Japanese lake |
|---|---|---|---|---|---|
| Dry density $\rho_d$ (g/cm$^3$) | 2.743 | 2.672 | Si | 43.40 | 58.20 |
| Liquid limit $W_l$ (%) | 103.6 | 118.2 | Al | 20.43 | 16.33 |
| Plastic limit $W_p$ (%) | 28.0 | 42.1 | Ca | 11.78 | 2.54 |
| Plastic index IP | 75.6 | 76.1 | Mg | 5.77 | 3.95 |
| Ignition loss (%) | 17.7 | 10.10 | Fe | 3.28 | 5.58 |

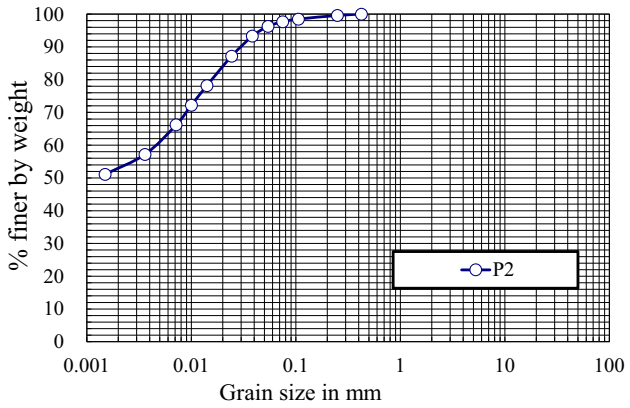


**Fig. 5** Grain size distribution curve

**Table 3** Distribution of aquatic plant species

| Point | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| *Potamogeton genus* | 3 | 3 | 2 | 3 | 0 | 1 | 0 | 3 |
| *Ruppia genus* | 0 | 0 | 0 | 0 | 3 | 1 | 3 | 0 |
| Unclassified | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| Total | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

concentrated to the south of the Tinja channel (near the Joumine water inlet area). This area consists of mixed freshwater and seawater. The distribution of *Ruppia* is in high-salinity conditions on the bottom and in surface water [such as 9–11 practical salinity unit (PSU)]. The distribution of *Potamogeton* is in lower salinity conditions (almost 7–9 PSU). The *Ruppia* distribution area is spreading in a more southerly direction than that found in previous work.

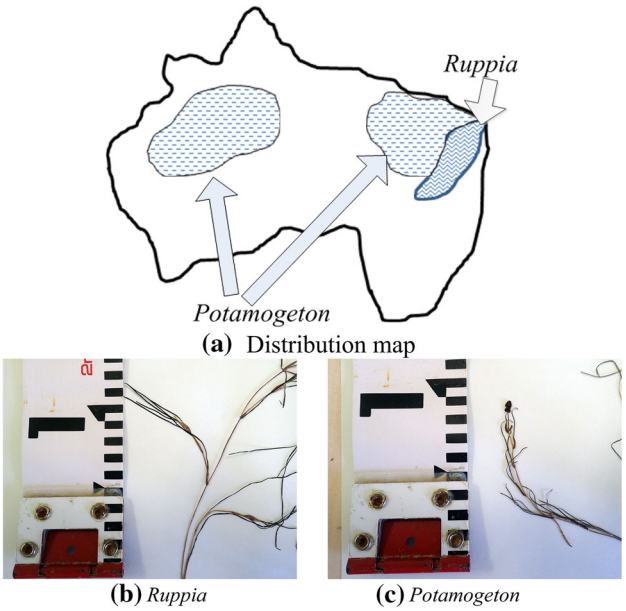


**Fig. 6** **a** Distribution map, **b** *Ruppia* and **c** *Potamogeton* samples

## Results of laboratory tests

### *Results of pre-cultivation tests*

The trends for ammonium ion concentration and pH during the test period are shown in Figs. 7 and 8, and urease activity results are shown in Fig. 9. Ammonium ion concentrations increased over time, except in sample No. 3. These results indicate that all of the sediment samples contained urease-producing bacteria. The ammonium ion concentration in sample No. 3 decreased between the 14th and 28th days of cultivation. The urease activity results exhibited similar trends. These results indicate that nitrification occurred during the test period. In these laboratory

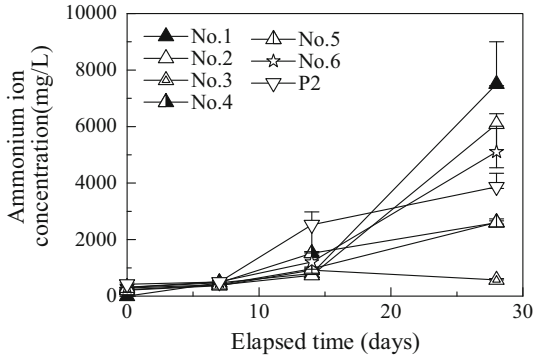


**Fig. 7** Ammonium ion concentration trends for sediment samples

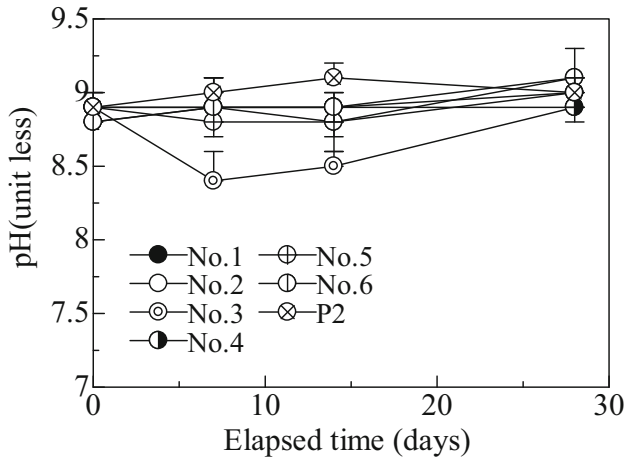


**Fig. 8** The pH value trends for sediment samples

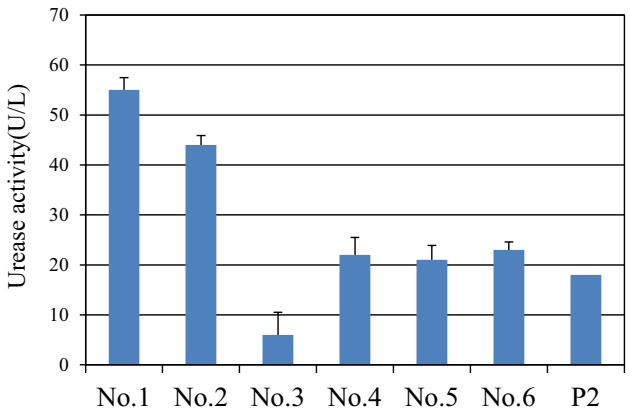


**Fig. 9** The urease activity values for sediment samples

tests, the initial urea concentration was 0.15 mol/L. This condition was applicable in both the low- and high-salinity sediments for enhancement of urease-producing bacteria activity.

There is a clear relationship between ammonium ion concentration and urease activity (Fig. 10). The Lake Ichkeul sediments showed a trend of increased urease activity

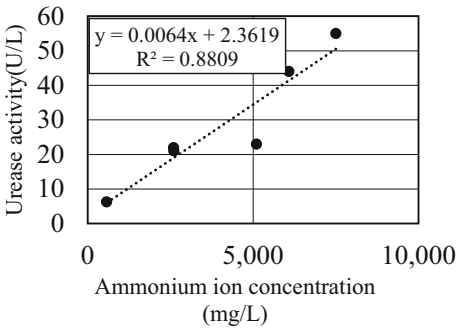


**Fig. 10** Relationship between urease activity and ammonium ion concentration

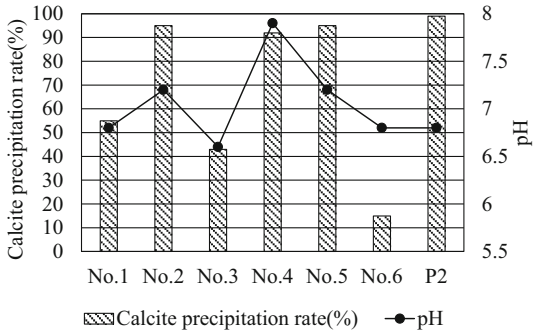


**Fig. 11** Relationship between the calcite precipitation rate and pH

as ammonium ion concentration increased. The trend was notable at the start of culture and after the pre-cultivation test. After 1 month of culture, urease activity increased and the ammonium ion concentration rose, owing to hydrolysis of urea.

*Results of the calcite precipitation tests*

The calcite precipitation rates (mass of calcite/dry mass sediments) and measurements of pH after 7 days are shown in Fig. 11. The pH was neutral, except in sample No. 4, which was taken from the Joumine River estuary. Previous work showed that the Joumine River sediment can be strengthened using the calcite precipitation method for about 1 month [13]. These data indicate that the Joumine River can supply urease-producing bacteria from the upstream area (e.g., the Joumine water reservoir). The calcite precipitation rates and numbers of ureC gene copies for each sample site are shown in Fig. 12. Sample No. 6 shows the lowest number of ureC gene copies.

The PCR-DGGE analysis results are shown in Fig. 13. Each unique band suggested the existence of a different species of microorganism. A comparison of the bands for samples No. 1 (high-salinity area), No. 4, P2, and No. 6 showed different bacterial communities. The No. 4 and P2 samples were taken in different seasons (dry and wet) at the

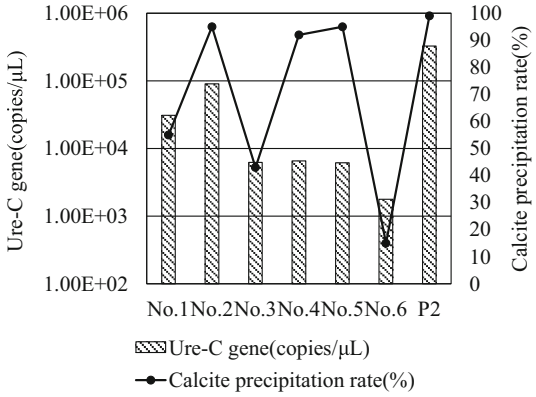


**Fig. 12** Relationship between the ureC gene and calcite precipitation rates

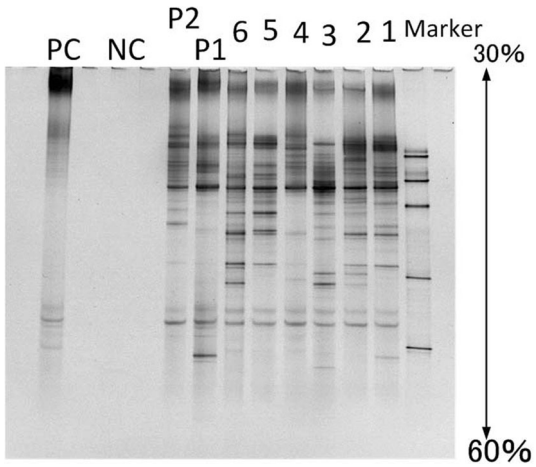


**Fig. 13** Gel image of polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis of the microbial community at each point

same location. These bands documented changes in the microbial community from the dry to the wet season. These results suggest that the calcite precipitation effect changes seasonally. Samples No. 1 and No. 6 showed different band patterns, indicating that salinity influenced the sediment microbial community during calcite precipitation. The microorganism community structure will be analyzed through base sequence identification for each band in a future work.

*Results of the sediment solidification tests*

Ammonium ion ($NH_4^+$) concentration and pH changes during the sediment solidification test period are presented in Fig. 14. The relatively stable pH values suggest that weak alkali pH conditions were maintained during the test period. The ammonium ion concentration increased after 7 days. This shows that urea hydrolysis was undertaken by urease-producing bacteria. Additionally, the increase in

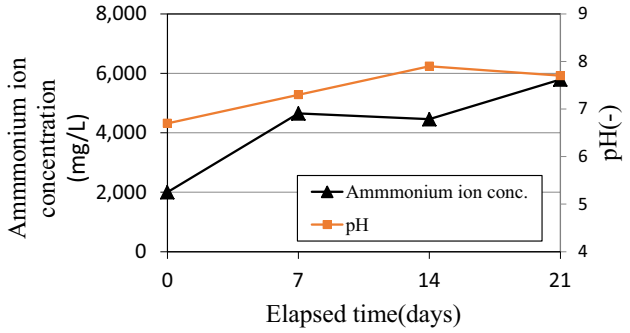


**Fig. 14** Ammonium ion concentration and pH trends

ammonium ion concentration after 7 days indicates that the chemical reactions of urea hydrolysis and calcite precipitation started under weak alkali pH (around pH = 8.0) conditions.

The values for vane shear strength, obtained using the Japan Geotechnical Society standard method (JGS1411), are presented in Fig. 15. These tests compared initial conditions and sediment solidification with microbial function conditions. Urea hydrolysis activity was enhanced by growth of urease-producing bacteria, and vane shear strength increased from the top to the bottom of the columns. These results show that microbial enhancement of sediment strength depends on the position of the sediment in the column.

The mass of $CaCO_3$ from microbial production after the sediment solidification test is shown in Table 4. The $CaCO_3$ content increased to 1.78 % of sediment dry weight at the top of the column. The $CaCO_3$ contents by sediment dry weight are 0.96 and 0.83 % at the middle and bottom of the column, respectively. The calcite precipitation rate

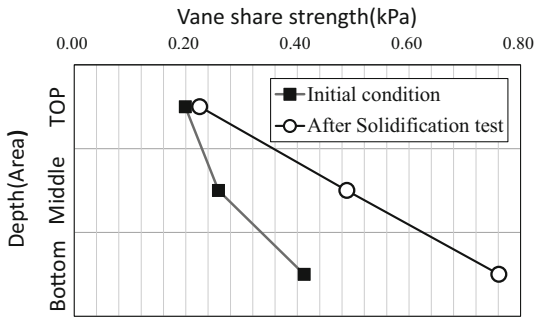


**Fig. 15** Results of vane shear strength test

**Table 4** Results of calcite precipitation (sediment solidification test)

| Area | Calcite precipitation (%) |
|---|---|
| Top | 1.78 |
| Middle | 0.96 |
| Bottom | 0.83 |

decreases from the top to the bottom of the column. This result suggests that the flow direction of the solidification medium is an important factor for carbonate precipitation based on urea hydrolysis.

## Conclusions

In this study, field sampling (of sediments and aquatic plants) and laboratory tests were conducted to verify the effects of MCP in the Ichkeul National Park World Heritage Site, Tunisia. The main conclusions of this study are as follows:

(1) *Ruppia cirrhosa* distribution is concentrated south of the Tinja channel in the dry season.
(2) All sediment samples demonstrated enhanced MCP potential following approximately 1 month of cultivation.
(3) The addition of urea and yeast extract increases urease production.
(4) After a pre-cultivation period, all culture media containing Lake Ichkeul sediments could precipitate 0.15 mol of calcite in about 1 month.
(5) PCR-DGGE results suggest that specific microbes produce calcium carbonate.
(6) MCP can enhance sediment strength after about 1 month of cultivation.

Future research should verify classification of all sediment microbes based on gene analysis and isolation of the specific microorganisms in Ichkeul Lake.

**Acknowledgments** This research was partially supported by a Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research (A) 15H02634.

**Compliance with ethical standards**

**Funding** This study was funded by JSPS KAKENHI Grant Number 15H02634.

**Conflict of interest** The authors declare that they have no conflicts of interest.

## References

1. Qabany Al, Soga K (2013) Effect of chemical treatment used in MICP on engineering properties of cemented soils. Geotechnoew 63(4):331–339
2. Alain T, Charles B (1994) Aquatic bird populations as possible indicators of seasonal nutrient flow at Ichkeul Lake, Tunisia. Hydrobiologia 279(280):149–156
3. Casagranda C, Charles B, Francour P (2005) Trophic flows in the macroinvertebrate community of a Mediterranean brackish lagoon, Lake Ichkeul(Tunisia), using a functional model, International Workshop on "The Protection of Coastal and Environment" Proceedings, 153–1625
4. Fujita Y, Taylor JL, Gresham TL, Delwiche ME, Colwell FS, Mcling TK, Petzke LM, Smith RW (2008) Stimulation of microbial urea hydrolysis in groundwater to enhance calcite precipitation. Environ Sci Technol J 42(8):3025–3032
5. Irie M, Kawachi A, Tarhouni J, Ghrabi A (2011) Development of sedimentation and characteristics of sediment on the reservoir in Tunisia. J Jpn Soc Civ Eng Ser B1 (Hydraul Eng) 67(4):163–168
6. Burbank M, Weaver T, Green T, Barbara W, Ronald C (2011) Precipitation of calcite by indigenous microorganisms to strengthen liquefiable soils. Geomicrobiol J 28(4):301–312
7. Harkes MP, van Paassen LA, Booster JL, Whiffin VS, van Loosdrecht MC (2010) Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement. Ecol Eng 36:112–117
8. Irie M, Kashiwagi K, Ujiie K, Nisiri I, Bouguerra S, Tarhouni J (2012) Feasibility of exploitation of the sediment in the reservoirs for the sustainability of surface water resource in Tunisia. J Jpn Soc Civ Eng Ser G (Environ Res) 68(6):41–46
9. Muyzer G, Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16SrRNA. Appl Environ Microbiol 56(3):695–700
10. Jiang NJ, Yoshioka H, Yamamoto K, Soga K (2016) Ureolytic activities of a urease-producing bacterium and purified urease enzyme in the anoxic condition: Implication for subseafloor sand production control by microbially induced carbonate precipitation (MICP). Ecol Eng 90:96–104
11. Al-Thawadi SM (2011) Ureolytic bacteria and calcium carbonate formation as a mechanism of strength enhancement of sand. J Adv Sci Eng Res 1(1):98–114
12. Danjo T, Kawasaki S (2016) Microbially induced sand cementation method using *Pararhodobacter* sp. strain SO1, inspired by beachrock formation mechanism. Mater Trans 57(3):428–437
13. Toshiro H, Mitsuteru I (2015) Feasibility study of the Ichkeul lake environmental remediation technology by using the Joumine water reservoir sediments. J Jpn Soc Civ Eng Ser G (Environ Res 71(4):125–133
14. Ivanov V, Chu J, Stabnikov V, Li B (2015) Strengthening of soft marine clay using bioencapsulation. Mar Georesour Geotechnol 33(4):320–324
15. Whiffin VS (2004) Microbial CaCO3 precipitation for the production of biocement, Sc.D. Thesis, School of Biological Sciences and Biotechnology, Murdoch Univ