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Feasibility Study of Native Ureolytic Bacteria for Biocementation Towards Coastal Erosion Protection by MICP Method

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Abstract: In recent years, traditional material for coastal erosion protection has become very expensive and not sustainable and eco-friendly for the long term. As an alternative countermeasure, this study focused on a sustainable biological ground improvement technique that can be utilized as an option for improving the mechanical and geotechnical engineering properties of soil by the microbially induced carbonate precipitation (MICP) technique considering native ureolytic bacteria. To protect coastal erosion, an innovative and sustainable strategy was proposed in this study by means of combing geotube and the MICP method. For a successful sand solidification, the urease activity, environmental factors, urease distribution, and calcite precipitation trend, among others, have been investigated using the isolated native strains. Our results revealed that urease activity of the identified strains denoted as G1 (*Micrococcus* sp.), G2 (*Pseudoalteromonas* sp.), and G3 (*Virgibacillus* sp.) relied on environment-specific parameters and, additionally, urease was not discharged in the culture solution but would discharge in and/or on the bacterial cell, and the fluid of the cells showed urease activity. Moreover, we successfully obtained solidified sand bearing UCS (Unconfined Compressive Strength) up to 1.8 MPa. We also proposed a novel sustainable approach for field implementation in a combination of geotube and MICP for coastal erosion protection that is cheaper, energy-saving, eco-friendly, and sustainable for Mediterranean countries, as well as for bio-mediated soil improvement.

Keywords: microbially induced carbonate precipitation; ureolytic bacteria; urease activity; biomineralization; coastal erosion protection; artificial beachrock

1. Introduction

At present, coastal erosion is a major problem all over the world. Traditional measures consisting of hard and soft defense protection systems are adopted to protect from this disintegration. All of these coastal design and structures have been identified as being expensive to build and sustain because of the scarcity of materials, energy, time, cost, and environmental concern. Therefore, sustainable and eco-friendly materials and systems are in demand for the protection against coastal erosion. Countermeasures against coastal erosion protection have been studied by many researchers [1], including hard and soft structures focusing on urea degradation bacteria. However, all these methods have an adverse effect on the surrounding landscape, environment, and ecosystem [2]; moreover, eco-sustainability is another major concern.

As an alternative method, earlier studies used the microbially induced carbonate precipitation (MICP) technique focusing on ureolytic bacteria within the field of geotechnology and civil engineering [3]. Among them, T. Danjo and S. Kawasaki conducted several studies in Ishikawa and Okinawa, Japan focusing on beachrock formation mechanism [4,5]. They found significant information to protect against coastal erosion using the MICP method, which they called "artificial beachrock". Moreover, for coastal erosion protection, the attention of using hard and soft structures, such as geo-tube, beach drainage system, vegetation, sand fencing, artificial reefs, and green belts, are dominating with progressive knowledge [6]. Recently, geo-tube technology has converted from being a substitute construction technique to a key solution of choice in a more advanced way for preventing water logging conditions, as well as coastal erosion protection [7]. The traditional geotextile tube technology for coastal erosion protection is not sustainable because of the penetration of UV (Ultraviolet) rays that could accelerate to damage the geosynthetic product very easily; moreover, it is rather difficult to recover or repair, which would be considered as costly [8]. Therefore, this technology needs to be improved and, hence, we proposed a novel approach to protect coastal erosion through a combination of MICP and geotube, which is more eco-friendly, cost-effective, and more sustainable.

There are various metabolic pathways leading to MICP, and urea hydrolysis is the most prominent for the formation of beachrocks [8,9]. The reactions occurring in this mechanism are represented by the following equations (Equations (1)–(3)), wherein urea hydrolysis occurs by urease enzyme and produces ammonium ion and carbamate, mentioned in Equation (1), and subsequently, the carbamate ion is hydrolyzed to form an additional ammonia ion and bi-carbonate, shown in Equation (2) [10,11].

$$CO(NH_2)_2 + H_2O \overset{Urease}{\rightarrow} H_2NCOO^- + NH_4^+, \quad (1)$$

$$H_2NCOO^- + H_2O \rightarrow HCO_3^- + NH_3. \quad (2)$$

Then, calcium carbonate is formed and finally precipitated, which plays a major role for binding the sand particles. In the presence of Ca^{2+} ions, calcium carbonate is then formed and precipitated, which is effective for the binding of sand particles and for the filling of micro-space:

$$Ca^{2+} + HCO_3^- + NH_3 \rightarrow CaCO_3 \downarrow + NH_4^+. \quad (3)$$

In the MICP method, precipitation of carbonate minerals is an important factor that occurs through extracellular or intracellular pathways by a sequence of chemical reactions and physiological pathways, which are a key factor for the making of solidified sand that is close to natural beachrock, and could be effective for various bioengineering applications [12]. Therefore, for successful implementation of the MICP method in the bio-geoengineering field, environmental factors such as temperature, pH, duration of bacterial cultivation, and crystal precipitation tendency need to be assessed.

In the MICP method, most of the earlier studies addressed ureolytic bacteria as a specific foreign microorganism, and there was a major concern about the adverse effects (for example, competition for nutrients with other species, survival capacity in the new environment, dormancy or sudden shock) on the surrounding environment due to the presence of a large number of foreign species [11–14]. Therefore, to overcome this adverse effect, this study aimed to reduce the impact on ecosystems by isolating urea degradation bacteria from local marine sand (Greece), targeting for coastal erosion protection in that specific area (Greece), and later on we will focus on other areas where coastal erosion is a major problem. Isolated native strains were evaluated for urease activity as whole-cell, supernatant and cell pellets and subsequently different environmental parameters were investigated. On the basis of the results, the sand solidification test (syringe) was also performed, and the degree of solidification was quantitatively evaluated by the needle penetration test. Furthermore, for the practical use of this technology, we proposed a novel approach that is sustainable and eco-friendly, one we aim to apply in future for coastal erosion protection in the targeted area.

Moreover, most of the research on MICP has been limited to land ureolytic bacteria [15–18], focusing on the urea hydrolysis mechanism and on the efficacy of calcite production, and little consideration has been drawn for marine ureolytic bacteria and their subsequent application [5,18–20]. However, some of the previous studies and scope of these studies are mentioned in Table 1. Nonetheless, in the present study, we selected marine ureolytic bacteria isolated from a coastal region in Greece, which has the most comprehensive coastlines among the Mediterranean countries and coastal erosion in this region is severe. In order to protect this long coastal shoreline from erosion, the application of the proposed MICP method was suggested towards the creation of artificial beachrock using native ureolytic bacteria because of its long-term sustainability, considering the local coastal climatic environment. Previously, it was revealed that the formation mechanism of beachrocks is greatly influenced by marine ureolytic bacteria, which show a great affinity for $CaCO_3$ precipitation and can be sustained for a long time, which is a key factor for the MICP method. Hence, we isolated three ureolytic bacterial species (16s rDNA analysis) from the Greek coastal area: *Micrococcus* sp., denoted as G1; *Pseudoalteromonas* sp., denoted as G2; and *Virgibacillus* sp., denoted as G3. We selected native ureolytic bacteria (from Greece) due to their sustainability (considering the temperature, pH, etc.) and high urease activity, which is very important for the making of artificial beachrock by the MICP method.

Table 1. Microorganism studied for microbially induced carbonate precipitation (MICP).

Ureolytic Bacteria	Type	References
Sporosarcina pasteurii	Land	[1,10,18]
Bacillus cohnii	Land	[14]
Bacillus subtilis	Land	[12]
Micrococcus sp.	Marine	This study
Pseudoalteromonas sp.	Marine	This study
Virgibacillus sp.	Marine	This study

The main objectives of this study were to find out an appropriate or feasible condition and method that would be very useful in making artificial beachrocks for coastal erosion protection in Greece and in Mediterranean countries, along with using geotextile tube technology by the MICP method in an inexpensive, eco-friendly, and sustainable way. In future, we aim to apply our findings using our proposed method in the field scale through considering durability study, rainfall effects, low-cost reagent (preferable waste material), and so on. Moreover, the information of this study could play an important source of information for commercial applications in protecting against coastal erosion and for other bio-engineering applications.

2. Methodology

2.1. Isolation and Bacteria Cell Culture

The ureolytic bacterial (G1, G2, and G3) species were isolated from the coastal area of Porto Rafti and Loutraki in Greece (Figure 1). The soil was sampled from each peripheral beachrock in Greece. After screening, the bacterial species were identified by 16s rDNA gene analysis. The genome extraction was done using 100 µL of Mighty Prep reagent taken into a 1.5 mL microtube. From the colonies on the plate, the cells were removed with a sterilized platinum loop, suspended in the microtube, heated at 95 °C for 10 minutes, and then centrifuged at 12000 rpm for 2 minutes. The supernatant of the suspension centrifuged was transferred to another tube and used as a template for PCR (polymerase chain reaction).

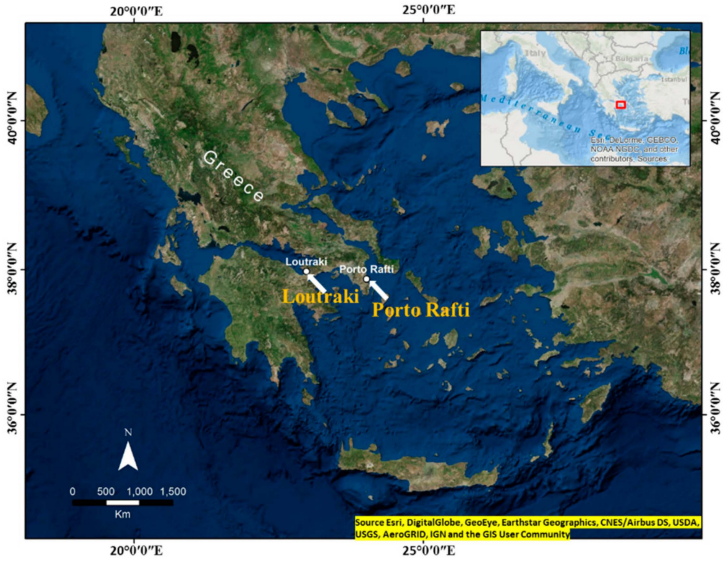


Figure 1. Map of Greece showing the sample collection site (Porto Rafti: N 37° 52′ 57.1′′, E 24° 00′ 51.1′′ and Loutraki: N 37° 57′ 02.3′′, E 22° 57′ 37.7′′).

After successful PCR amplification, DNA extraction was carried out by an extraction kit (Nippon Genetics Co. Ltd.). The six types of primers used in this experiment were 9F, 515F, 1099F, 1541R, 1115R, and 536R. Finally, for the identification of the isolated bacterial species 16s rDNA, base sequence analysis (about 1500 bp) was performed. Finally, BLAST (Basic Local Alignment Search Tool) analysis was performed by the database DB-BA12.0 (Techno Suruga Laboratory, Japan). ZoBell2216 culture solution (hi-polypeptone 5.0 g/L, yeast extract 1.0 g/L, and $FePO_4$ 0.1 g/L mixed with seawater (artificial), maintained at pH 7.6–7.8) was used as the main culture medium for the growth of the species. The bacterial cells were pre-cultured (5 mL) in the ZoBell2216 solution at 30°C for 24 h by shaking at 160 rpm. The preculture (1 mL) was inoculated into 100 mL of fresh ZoBell2216 medium and incubated at 30°C with shaking at 160 rpm. Subsequently, it was continuously cultivated for 10 days. During the cultivation, the bacterial cell growth was determined (OD_{600}) by a UV-VIS spectrophotometer (V-730, JASCO Corporation, Tokyo, Japan).

2.2. Assessment of Urease Activity

Urease activity of the cultured bacterial cells was assessed at different pH and temperature levels following the indophenol method [21,22] where ureolysis occurred directly by the bacterial cell in a solution. The collected sample solution (1 mL) from the cell culture was added to 100 mL of the stratum solution containing 0.3 M urea and 0.1 M EDTA (Basic Local Alignment Search Tool) buffer with phenol-nitroprusside. The reaction composition was stirred using a water bath and kept at a steady temperature and pH. At the sampling point, 10 mL solution was sampled and 0.1 mL of 10 M NaOCl was added to the solution to prevent quick enzymatic hydrolysis. The composed solution was incubated subsequently for 10 min at 50–60 °C. The urease activity test was carried out to evaluate the effect of the reaction solution conditions, by 48 h cultured cells at distinctive temperatures ranging from 10 to 60 °C, and the pH was 6, 7, and 8 (adding EDTA buffer solutions at 30 °C). The urease activity of the discharged urease from the culture supernatants was also investigated. Cultured bacteria cell culture (1.2 mL) was centrifuged by 10,000 rpm for 5 min at 10 °C, and then the supernatant (1 mL) was used to examine urease activity as per the following method. Urease activity of the sampled supernatant would correspond to the discharge of urease. The urase activity of bacterial cell pellets and their relationship to bacterial cell growth was also evaluated.

To remove the supernatant, a 40 mL sample was collected from the bacterial cell culture tube and centrifuged by 10,000 rpm, for 5 min at 10 °C. To get a solution from the resuspended bacterial cell, the cell pellet was washed by 0.1 M Tris and HCl buffer for repeated times. The urease activity assessment

was conducted considering different OD_{600} values. The urease activity of the cell lysate, soluble and insoluble portion of the cell lysate, and finally the whole bacterial cell was also investigated.

After 48 h culture of the cell, 10 mL sample solution was collected and centrifuged by following the same procedure as mentioned earlier. The sample solution was resuspended to remove the culture supernatant with 500 mL sonication buffer (0.1 M NaCl, 20 mM Tris/HCl, and 1 mM EDTA) and was named as the whole-cell. The soluble cell lysate was prepared using the disrupted bacterial cell using ultrasonication for 5 min with 130 W, and time interval was 1 min. Remaining bacterial cell-debris was also collected by centrifugation for 20 min at 4 °C with 8000 rpm, which was insoluble to the solution. The urease activity assessment was also conducted for both soluble and insoluble cases, followed by the indophenol method.

2.3. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis

SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) was conducted using 10 mL bacterial cell culture solution. Using the ultrasonication method (5 min and 130 W) the cultured bacterial cell was broken and the collected cell was resuspended with 500 mL of buffer solution (sonication). The collected solution (soluble) was moved to a tube for SDS-PAGE analysis after centrifugation for 20 min at 4 °C with 8000 rpm. In the meantime, the precipitate segment of cell lysate was dissolved with denaturation buffer (6 M urea with 50 mM Tris/HCl). The collected insoluble solution was also assessed by SDS-PAGE method. Finally, after electrophoresis, the formed gel was washed with CBB solution (Coomassie Brilliant Blue) by Nacalai Tesque, Inc., Kyoto, Japan.

2.4. Microbial $CaCO_3$ Precipitation Test

The selected strains (G1, G2, and G3) were cultured with a ZoBell2216 solution (1.0 g/L of yeast extract, 5.0 g/L of hi-polypeptone, and 0.1 g/L of $FePO_4$) mixed with artificial seawater (Table 2) with a controlled pH of 7.6–7.8, until the maximum cell growth was observed. The cell growth (OD_{600}) was measured by a UV-VIS spectrophotometer (V-730, JASCO Corporation, Tokyo, Japan). In the meantime, 0.5 M $CaCl_2$ and 0.5 M urea were put in a test tube up to 10 mL. The bacterial culture solution was centrifuged (12,000 rpm) for 10 min. The supernatant and cell pellets were divided and the bacterial cell concentration (OD_{600}) was adjusted using distilled water. The adjusted bacterial cell concentration was then added to the test tube and put in a shaker at 30 °C for 48 h. Finally, the precipitation (white crystals) was observed and the precipitated crystals (centrifuged at 12,000 rpm for 10 min) was separated from the culture solution by a filter paper. Precipitated crystal tubes were kept in an oven drier for 24 h at 100 °C and then the final weight was taken to calculate the total crystal precipitation amount.

Table 2. Composition of artificial seawater.

Reagent	Concentration (g/L)
$MgCl_2 \cdot 6H_2O$	222.23
$CaCl_2 \cdot 2H_2O$	30.7
$SrCl_2 \cdot 6H_2O$	0.85
KCl	13.90
KBr	2.00
H_3BO_3	0.54
NaCl	490.68
Na_2SO_4	81.88

2.5. Sand Solidification Test

In order to check the ability of sand solidification of the isolated strains, a small-scale sand syringe solidification test was conducted. First, the isolated strains were grown in ZoBell2216E culture medium and shaken continuously at 30 °C for 2 days.

Then, 40 g of dried (110 °C for 2 days) Mikawa sand was loaded in a 35 mL syringe (height: 7 cm and diameter: 2.5 cm). Subsequently, 16 mL of the bacterial culture solution (ZoBEll2216E) and 20 mL of the consolidation solution (Table 3) was serially injected into the syringe showing in Figure 2a. The syringe was flushed after 2 h, leaving approximately 2 mL of the solution close to the surface of the sand. The consolidation solution was injected and drained every day for 21 days. The testing conditions are presented in Table 4. The pH values and Ca^{2+} concentrations from the outlet were measured afterwards. The condition of the syringe solidification test and grain size distribution of Mikawa sand is presented in Figure 2b. After 21 days, the UCS of the sample was measured by a needle penetration device (SH-70, Maruto Testing Machine Company, Tokyo, Japan).

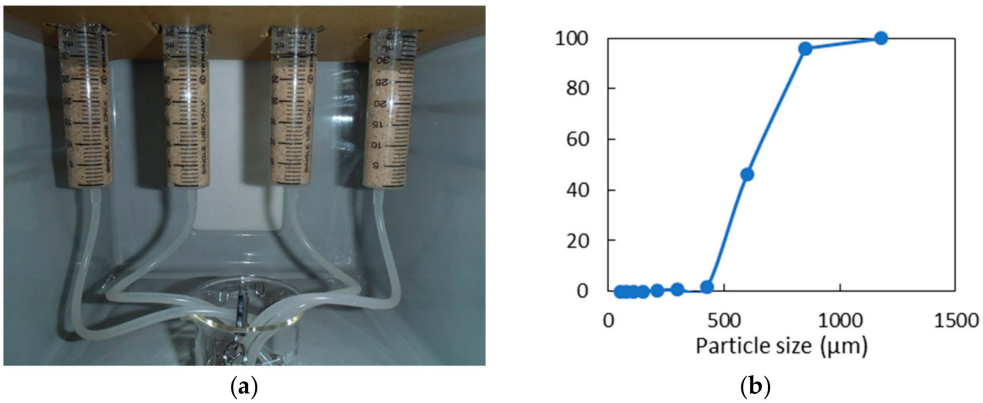


Figure 2. The state of (**a**) sand solidification test (syringe) and (**b**) particle size distribution of Mikawa sand.

Table 3. Composition of sand solidification solution.

Composition	Concentration (g/L)
$CO(NH_2)_2$	30.0
$CaCl_2$	55.5
Nutrient broth	3.0
$NaHCO_3$	2.12
NH_4Cl	10.0

Table 4. Testing conditions for sand solidification test (syringe).

Test Period	Incubation Time	Temperature	Solidification Solution Concentration	Nutrient Injection Interval	Solidification Solution Injection Interval
21 days	24 h 48 h 72 h	30 °C	0.5 M	- 7 days -	- 1 day -

3. Results and Discussion

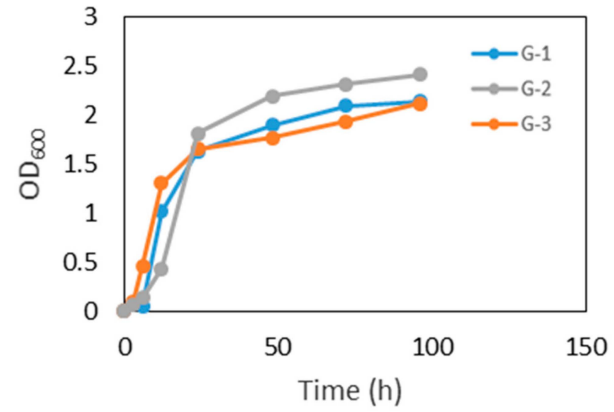
3.1. Cell Concentration (Isolated Species) and Urease Activity

According to the BLAST analysis result, the identified species are shown in Table 5. Urease activity is closely related to the bacterial cell growth and it was revealed that a high bacterial cell concentration enhanced the amount of calcite precipitated by the MICP method. Moreover, it was concluded that the urea hydrolysis rate was directly proportionate to the concentration of bacteria, which is an important factor for the success of the MICP application [23,24]. On the basis of these findings, the generation of urease from the isolated species could be a growth correlated framework that favors for sand solidification. Figure 3a represents the cell growth, whereas Figure 3b,c illustrate the urease activity of the G1, G2, and G3 species as whole-cell, cell pellet, and supernatant, respectively. From Figure 3a, it can be observed that initially the bacterial cell growth increased with time and then

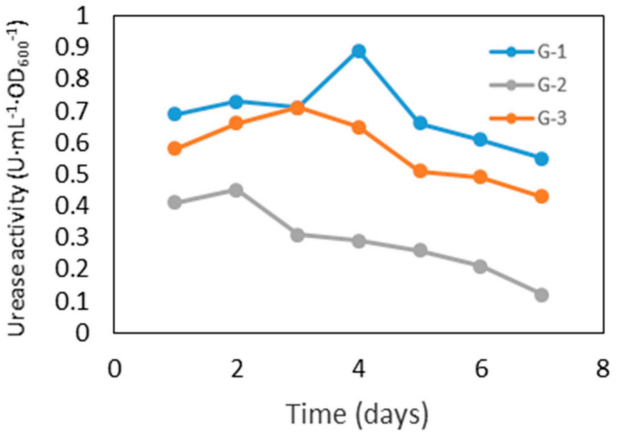
became stable and, conversely, the urease activity increased initially and then decreased, making a bell-shaped profile in between 3–6 days for the G1, G2, and G3 species.

Table 5. Identified strains from the collected soil sample.

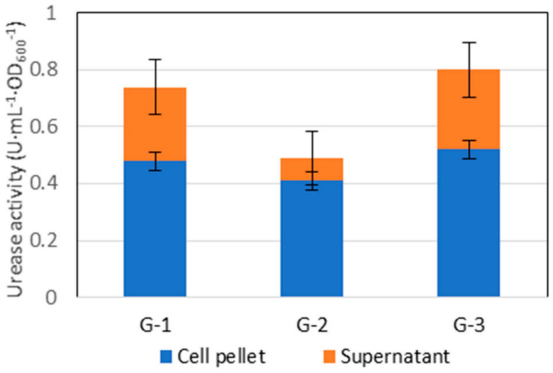
Code Name	Species
G1	*Micrococcus* sp.
G2	*Pseudoalteromonas* sp.
G3	*Virgibacillus* sp.



(**a**)



(**b**)



(**c**)

Figure 3. The results showing the (**a**) cell growth and (**b**) urease activity of the isolated strains including the (**c**) cell pellets and supernatant.

It was shown that the urease activity of the bacterial whole cell, cell pellet, and supernatant was closely related to the presence of protease in the cell [25]. However, it is well established that urease activity of different bacteria species depends on each species, as well as some environmental factors, which is similar to this study. Earlier studies have also reported that the urease from a few species such as *Pararhodobacter* sp. Appeared to have very high stability with extensive cultivation [26]. However, our results revealed that the G2 species of bacteria showed a dissimilar tendency for urea hydrolysis even under extended cultivation compared to the G1 and G3 species. The possible reasons for varying the urease activity might be osmotic pressure and resuspension of the cell, which might release enzyme into the solution from cells. However, further study needs to investigate the actual reasons.

The findings of this study are consistent with earlier findings and could contribute as useful information for the sand solidification process by MICP, considering the bacteria population increase via centrifuge and/or applying the re-injecting method of bacteria before urease activity becomes null.

3.2. Impact of pH to Urease Activity

Figure 4 represents urease activity with different pH conditions at a certain temperature (30 °C) for G1, G2, and G3 species. The results exposed a nearly bell-shaped outline showcasing the maximum activity around pH 7 for all of the three species.

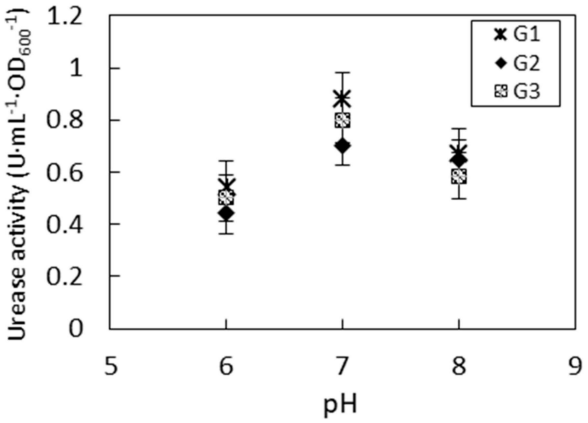


Figure 4. Impact of pH for the urease activity of the reaction solution at 30 °C.

Following the same conditions, the G1 species showed the highest urease activity compared to the G2 and G3 species. Previous research showed that the MICP process favored an initial pH environment of 6.5–9.3 (neutral to basic condition). Additionally, it was revealed that, in the case of some species such as *Pararhodobacter* sp., considerable loss of urease activity was observed with acidic (lower than pH 6) and alkaline (higher than pH 9) conditions attributed to denaturation of protein [24]. It was also reported that the urease activity of some ureolytic bacterial species favored lightly alkaline conditions [25–27]. Thus the pH value played an important role for the bacteria transportation and adhesion to obtain homogeneously improved strength of treated soils. Therefore, the results of this study could also be useful information for obtaining a successful sand solidification process for identifying the optimum pH condition for native species.

3.3. Impact of Temperature to Urease Activity

Urease activity with various temperature conditions is shown in Figure 5 for the G1, G2, and G3 species. High urease activity was observed at a temperature above 30 °C (G1 species) and then gradually decreased and showed an almost bell-shaped profile because of cell denaturation at a higher temperature, except in the case of G2 and G3 species. The divergent tendency of urease activity (nearly 40–60 °C) was also observed by Fujita et al. [26]. However, there are few data about the urease

activity of marine ureolytic bacteria in Greece. Even though the urease activity of G1, G2, and G3 species are lower than that of *Pararhodobacter* sp., the results obtained in this study would be innovative and adventitious information for the sand solidification method through microbial carbonate precipitation because the use of native ureolytic species (isolated from marine area). In future, it may be possible to use G1, G2, and G3 species for sand solidification, which could help to manufacture artificial beachrock following the MICP technique. This result could also be adventitious in Greece for coastal erosion control purposes by increasing bacterial population either by centrifugation and/or urease enzyme extraction by ultrasonication and/or adding bacterial populations several times during the sand solidification process before urease activity completely declined. Similar results were also found for the strains such as *Deleya venusta* and *Pararhodobacter* sp. [26–28]. Previously, it was also found that urease activity influenced to $CaCO_3$ precipitation at temperatures from 10 to 60 °C, which is similar to the findings of this study. However, the effectiveness of environmental factors on urease activity of the negative species with variation temperatures was not examined. Therefore, on the basis of these experimental findings, the G1, G2, and G3 species showed high urease activity in the different range of temperatures that could reflect the importance of isolating the ureolytic bacterial species from the local environment and their subsequent application for sand solidification and soil improvement.

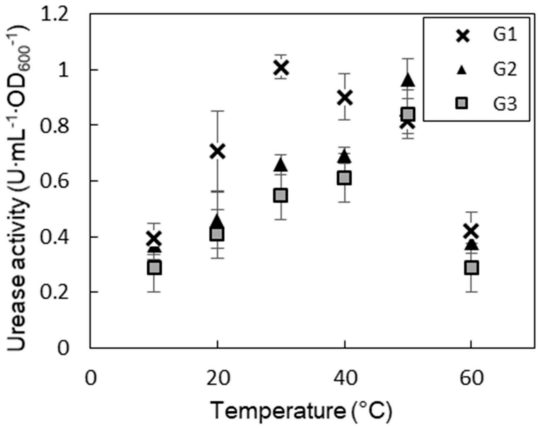


Figure 5. Results showing the urease activity of the isolated species at different temperatures.

These results could be very useful for the sand solidification by the MICP method in Mediterranean coastal regions considering the local environmental conditions because the isolated ureolytic bacterial species are well adapted to the local environmental conditions. These results could also be very advantageous if we want to focus on soil or ground improvement techniques by bio-stimulation or bio-augmentation method [29], and we must consider the native ureolytic bacterial species which are well adapted with the local environment and could ensure long term sustainability.

3.4. SDS-PAGE Analysis

The SDS-PAGE analysis was performed to confirm the expression proteins for both soluble and insoluble fractions of the strains cell lysate from the isolated native G1, G2, and G3 species, which is vital in detecting the location of the reaction in a mixture of sand/soil particles. Figure 6 represents the SDS-PAGE results for the soluble and insoluble portion of the bacterial cell suspensions.

It has been studied that ureases from bacterial cell are composed of two small subunits (8–12 kDa) with one subunit being larger [30], and the results of SDS-PAGE analysis exhibited several bands of nearly 15 kDa and 60 kDa [26,29]. These results indicated that the bacterial urease could be expressed as either insoluble protein or as a soluble membrane protein, which also corresponds to the results [26,31].

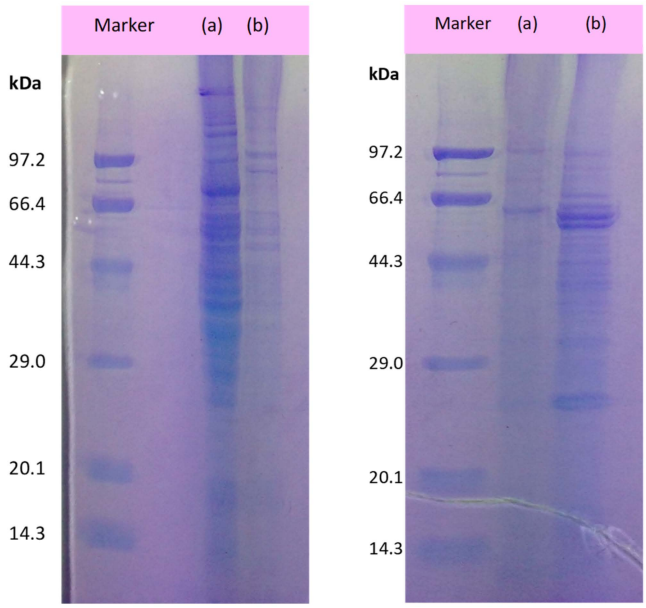


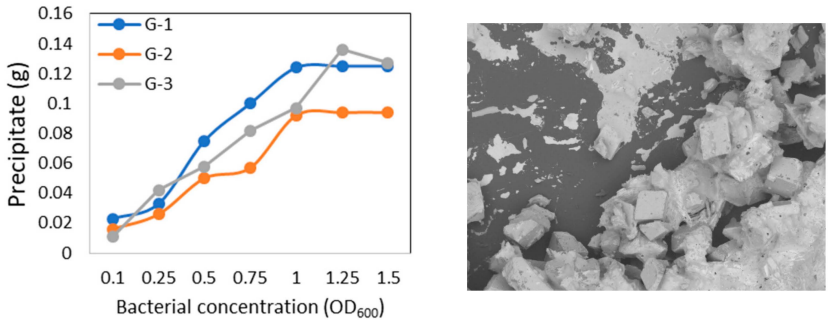
Figure 6. Confirmation of protein (enzyme) expression of the (**a**) soluble and (**b**) insoluble portion of bacterial cell lysate of the isolated species.

These results showed that the variation of urease activity depended on cell lysate, cell membrane, and whole-cell by focusing on the urea hydrolysis by native bacterial strain.

Thus, on the basis of these results, urease could exist as a soluble protein or likely as a soluble membrane protein. We found that the protein was not released into the culture solution but was separated from the bacterial cell membrane into the solution during ultrasonication because the crystal precipitation was greatly influenced by the bacterial cells. Therefore, the findings in this study support in figuring out the urease accumulation characteristic and extraction of the enzyme by sonication, which could be effective for the efficacy of the soil treatment/ground improvement by MICP and/or enzymatic process in practical field application for coastal erosion protection, especially in the coastal area of Greece.

3.5. Microbial $CaCO_3$ Precipitation Test

Earlier studies showed that it is possible to control the strength of the treated sand by regulating the amount and/or volume of precipitated minerals. It has also been reported that various environmental factors, including temperature, pH, bacterial size, and cell concentration, control the amount of calcite precipitation during the MICP process [31–33]. Therefore, it is very important to investigate the trend in $CaCO_3$ precipitation for individual bacterial species because the $CaCO_3$ acts as the main binder material between the substrate particles for soil improvement during the MICP process. The $CaCO_3$ precipitation trend for the G1, G2, and G3 species is shown in Figure 7a. From the figure, it is clear that $CaCO_3$ precipitation is closely related to the OD_{600} values, which supports previous studies [34]. From our studies, was also observed that higher OD_{600} values influenced the $CaCO_3$ precipitation (Figure 7b) amount. Under the same conditions, the G1 and G3 species had the advanced ability of precipitating a higher amount of $CaCO_3$ compared to the G2, leading to a higher possibility of sand solidification. At the same time, the rate of the precipitated $CaCO_3$ decreased and tended to be constant with a further increase of the bacterial cell concentration. However, this study suggests that the variation in the $CaCO_3$ precipitation trend was related to the bacterial population of individual species, which supports earlier studies.

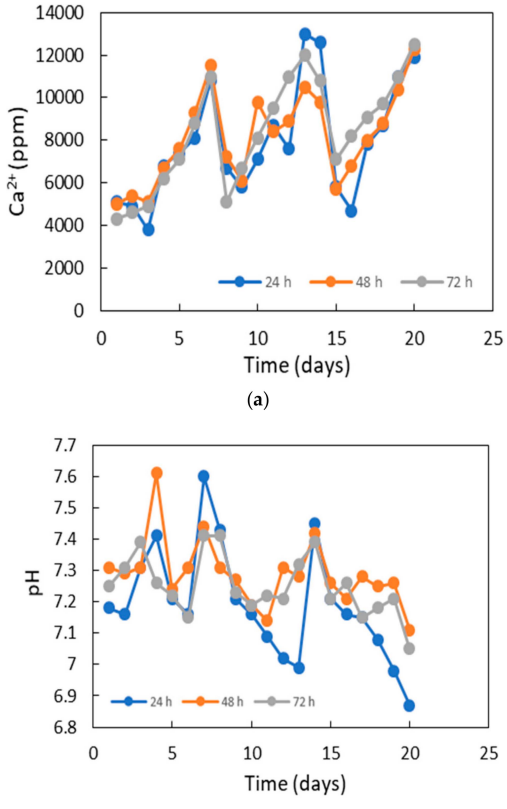


(**a**) (**b**)

Figure 7. (**a**) $CaCO_3$ precipitation trend of the isolated strains and (**b**) SEM images of the precipitated $CaCO_3$ of G1 species.

3.6. Potentiality for Sand Solidification and Design for the Application

The results of the temporal variation of Ca^{2+} and the pH effluent is presented in Figure 8a,b, respectively. From the figure, it is shown that the Ca^{2+} discharge liquid was seen to continue to rise from around 4 days from bacterial implantation and then decreased with time (Figure 8a). The Ca^{2+} concentration increased, indicating that the hydrolysis effect of the urea-degrading bacteria in the syringe no longer worked, and it was presumed that the injection of regular cells was required. The pH tended to increase and then decrease with time (Figure 8b) which indicated the variation of urea hydrolysis inside the syringe.



(**a**)

(**b**)

Figure 8. (**a**) Temporal variation of Ca^{2+} and (**b**) pH in the effluent from solidification solution in days.

The outcomes of the needle penetration test are summarized individually and shown in Figure 9. From the figure, it was observed that maximum USC around 1.8 MPa was obtained at the middle portion for the G3, G1, and G2 species. The top portion was moderately solidified (UCS around

1.3 MPa) and the bottom portion was solidified bearing UCS around 1.4 MPa (for G3). This was because maximum urea hydrolysis possibly occurred at the middle portion and precipitated crystal might have had more contact point to bind sand particles at this stage [35]. Another reason could be the variation of aerobic and anaerobic conditions in between the bottom portion and beneath the portion of the sample that might be responsible in decreasing the urease activity and/or crystal precipitation. Therefore, further investigation is required to improve the solidification condition.

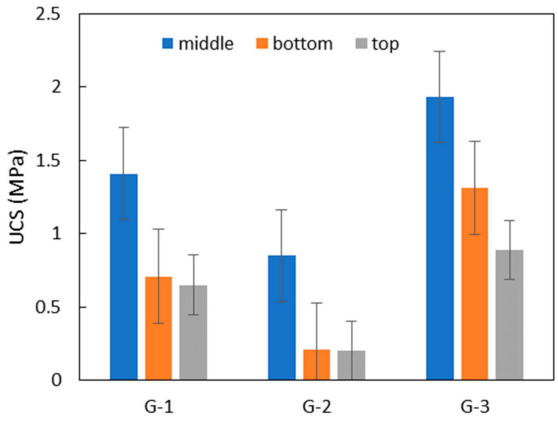


Figure 9. Comparison of estimated unconfined compressive strength (UCS) in each strain.

It is well known that the traditional geotubes are widely utilized for flood control, to prevent waterlogging conditions, and for coastal erosion protection. However, all of these conventional methods are not sustainable because of the various of UV rays that come from sunlight damage the geotube after a certain period of time, and protection of soil might be hampered and thus, consequently, the re-installing cost for geotube will be increased [36]. Nonetheless, the re-installing cost could be minimized by using the MICP method and the geotube following our proposed novel approach (Figure 10). By implementing this novel approach, it would be possible to strengthen the coastal structure because the geotube and solidified sand MICP will protect against the UV rays, helping it to remain for a longer period and being more sustainable than the conventional method. In our proposed implementation method, the total cost will be much lower compared to traditional methods, and local ureolytic bacteria will be used, which is more eco-friendly and will ensure long term sustainability of the coastal structures. This method can also be useful in a wider range of civil engineering implementations including paving, construction, and drainage.

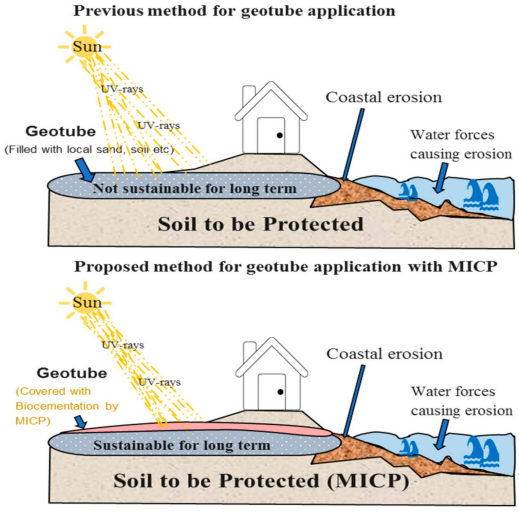


Figure 10. Proposed coastal erosion protection method.

4. Conclusions

From our experimental results, we demonstrated that the G3 species showed a potentially advanced growth rate and urease activity when compared to the G1 and G2 species. Nonetheless, the environmental parameters also had a remarkable consequence on the bacterial urease activity. Moreover, from the SDS-PAGE analysis, it was revealed that urease could be released as a soluble protein or conceivably as a membrane protein (soluble) in/on the bacterial cell, but the exact location of urease activity could not be detected. We also concluded that urease activity for G1, G2, and G3 species relied on the environmental parameters. Additionally, urease was not secreted into the culture solution but accumulated in and/or on the cell, and the urease activity was also observed in resuspended cells. Indeed, the unconfined compressive strength (UCS) of our solidified sand was lower than when compared to conventional structure. Moreover, its capability could play an important role as a new eco-friendly design for coastal erosion protection in combination with geotube in a more sustainable way. This research showed a positive result leading to the enhanced possibility of application in coastal erosion protection by assimilation of knowledge from microbial mechanism and design applications considering the engineering viewpoint. Future investigations will be focused on studying strength improvements, enhanced durability, and feasibility at a small or medium scale, which are essential for implementation as an ideal substantial design. Moreover, these results could be applied towards the creation of artificial beachrocks by the MICP method for coastal protection in Mediterranean countries, as well as for bio-mediated soil improvement.

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