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A preliminary study on the enrichment of indigenous ureolytic and nitrifying bacteria in beach sand: implication for coastal erosion control

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ABSTRACT

Ureolytic induced calcite precipitation (UICP) as an emerging method to mitigate coastal erosion has been studied recently. While exogenous ureolytic bacteria have been used widely for UICP, they may not be compatible with local environment and ecosystem. In this study, indigenous bio-stimulation was used to enrich native ureolytic bacteria from beach sand to achieve UICP. YE (yeast extract) and YEU medium (yeast extract with urea) were used as enriching media in this study. The solution enrichment tests were conducted up to 72 hours. The pH value, ammonium concentration, ureolytic activity, and viable bacterial colony number were measured to determine the enrichment effectiveness. This study also performed a preliminary investigation of ammonium treatment, a by-product of ureolytic UICP, by native nitrifying bacteria in beach sand. The findings suggested that both indigenous ureolytic and nitrifying bacteria were stimulated with appropriate enrichment media.

Keywords: UICP, bio-stimulation, enrichment, indigenous bacteria, nitrifying bacteria

1 INTRODUCTION

In many coastal regions, the mechanical properties of sandy soil are unstable and can be easily subjected to wave or tide induced erosion. Coastal erosion is a common engineering problem along the shorelines worldwide. Over 70% of the Earth's sandy beach environment can be impacted by coastal erosion (Bird, 1985). Wave induced coastal erosion, whether in foredunes or cliffs, mainly depends on the sea level rise. Although runup elevations of wave and the morphology of the fronting beach have important impacts on the coastal erosion (Ruggiero et al., 2001), other ecological factors such as human activities also largely intensify the erosion nowadays (Adger et al., 2005). Permanent loss of beach sand can bring devastating damage to the coastal communities and infrastructures. Therefore, it is needed to find an effective and environmental-friendly way to mitigate the coastal erosion.

Various methods have been developed in practice to prevent coastal erosion. Artificial beach nourishment has been used for decades and have positive feedbacks on coastal development (Armstrong et al. 2016), though it is also a costly method (Van Rijn, 2011). Other types of hard engineering structures include seawalls (Mimura & Nunn, 1998), breakwater (Browder et al., 2000), coastal cells (Hansom et al., 2004) and massive sea block system. However, environmental and ecological parameters are usually not considered in these hard structures.

In recent decades, ureolytic induced calcite precipitation (UICP) has been explored for applications in civil and environmental engineering. When providing necessary nutrients, ureolytic bacteria convert urea into carbonate and ammonium. With sufficient calcium ions, calcite precipitation is generated, which can serve as cementation between loose granular soil particles. Using exogenous urease-produce bacteria like *sporosarcina pasteurii* to achieve bio-cementation has been studied by some researchers (Wang et al., 2011, Jiang et al., 2022, Keykha et al., 2018). However, the ureolytic bacteria is ubiquitous in soil. Therefore, the indigenous bio-stimulation method to obtain ureolytic bacteria can be feasible for in the coastal environment (Wang et al., 2022). Ammonium is a by-product of the ureolysis process, which

greatly limits the use of UICP in sensitive environment. Nitrifying bacteria can convert ammonium into nitrate and thus may serve as an effective way to eliminate ammonium.

In this study, the indigenous stimulation of ureolytic and nitrifying bacteria was conducted. Both generic (YE) and selective (YEU) media were prepared to stimulate ureolytic bacteria from Kailua beach sand. In addition, ammonium chloride enrichment medium was used to stimulate indigenous nitrifying bacteria. A series of parameters (pH, concentration of ammonium and nitrate ions, ureolytic activity and viable bacterial colony number) were measured to evaluate the enrichment effectiveness.

2 MATERIAL AND METHODS

2.1 Soil and enrichment media

In this study, the calcareous beach sand from intertidal zone of Kailua Beach on the east of O'ahu Island, Hawaii was used. Twigs, shells, roots and large rocks were first cleared out of the collected sand samples. The samples were then stored at 4℃ to keep it fresh. Figure1 shows the particle size distribution of the Kailua calcareous beach sand.

Three different media were used in this study. A generic medium YE (yeast extract), where 20g yeast extract was dissolved in 1L distilled water, and a selective YEU (yeast extract with urea) medium, where 20g yeast extract and 170mM urea were dissolved in 1L distilled water, were used as enriching solution for indigenous ureolytic bacteria stimulation. Ammonium chloride (NH_4Cl) enriching media (0.0714mol/L, concentration of NH_4^+-N: 1000mg/L, 5mL trace elements) was prepared to stimulate indigenous nitrifying bacteria and to test ammonium reduction effectiveness. All chemicals were autoclaved at 121℃, 17-20 psi for 35-40 minutes separately and then mixed with each other when cooled down to the room temperature.

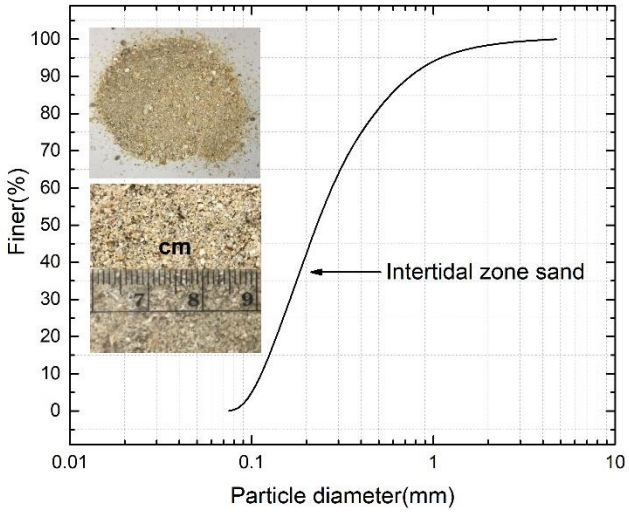

Figure 1. *Particle size distribution curve of the calcareous Kailua Beach sand*

2.2 Solution enrichment test for ureolytic bacteria

The solution enrichment test was conducted in 50mL conical-bottom centrifuge tubes under aerobic condition. Fresh Kailua Beach sand (1g) was mixed with YE and YEU media (50mL). The soil-solution mixture was shaking-incubated at 200rpm and 30℃ ambient temperature for 72 hours (Wang et al., 2020). Triplicate tests were conducted to ensure result reliability.

2.3 Solution enrichment test for nitrifying bacteria

Fresh Kailua Beach sand (3g) was mixed with 150mL NH_4Cl enriching media in autoclaved serum bottles, which were then incubated at 30°C and 200rpm in a shaking incubator for 30 days under aerobic condition.

2.4 Testing measurements

During the solution enrichment test, ammonium concentration, pH, viable bacterial colony number, ureolytic activity, and ammonium elimination were measured at 6h, 12h, 24h, 48h, and 72h respectively. More specifically, the modified Nessler's Method was applied to determine ammonium concentration using spectrophotometer (supplied by VWR®) at 425nm wavelength. The ureolytic activity was obtained from the difference of EC (electrical conductivity) at the 1st and 9th minute after the mix of 1mL enriched bacterial solution and 9mL urea solution (1.5M). The nitrate concentration was measured based on the ultraviolet spectrophotometric method at 220nm wavelength. Plate counting method was used to quantify the viable bacterial colony number by spreading 100μL bacterial solution on the solid YE agar media (20g yeast extract, 15g agar in 1L distilled water).

3 RESULTS AND DISCUSSION

3.1 Ammonium concentration

The amount of ammonium ions can directly reflect the rate of ureolysis in the enriching solution. The variations of ammonium concentration in YE and YEU conditions are shown in Figure 2.

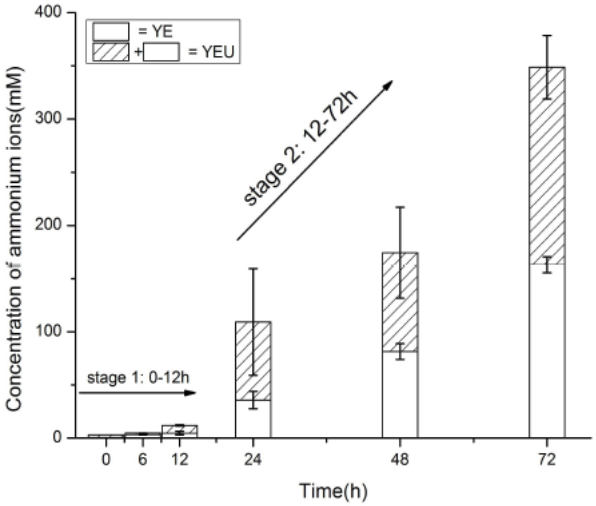

Figure 2. *Evolution of concentration of ammonium ions with time.*

In general, more ammonium ions were generated in YEU bacterial solution than YE. Three stages can be defined based on the variations of ammonium concentration in 72 hours. From 0 to 12h (defined as stage 1), the concentration of ammonium increased slightly from 0.5mM to 3.4mM in YE solution and from 2.9mM to 22mM in YEU solution. From 12h to 72h (defined as stage 2), the concentration of ammonium increased dramatically to 168mM and 349mM respectively, which were 58 and 16 times more than the increasing counterpart at the end of stage 1.

The rapid accumulation of ammonium ions in stage 2 in the YEU solution was accounted for the hydrolysis of urea. Although no urea used as a stimulus in the YE solution, the concentration of ammonium still increased marginally in stage 2, which was attributed to the bacteria induced degradation of yeast extract. Ammonium ions were generated as a waste product of bacterial metabolism.

3.2 The pH variation

The time dependent variation of pH is shown in Figure 3. In general, the pH experienced three phrases. In the first 12 hours, solution pH dropped 0.5 and 0.4 for YE and YEU respectively, which was possibly caused by the generation of CO_2 in the respiration process of heavily bacterial reproduction. During this period, the increase of ammonium ions was limited (Figure 2), which indicated that only small amount of urea was hydrolyzed. Therefore, the respiration process dominated the pH change in the first 12 hours.

For the YE solution, pH increased from 6.4 to 8.0 in the following 60 hours, caused by the decomposition of organic nitrogenous compounds (come from yeast extract) by bacteria. For the YEU solution, the pH increased to 9.0, which was mainly attributed to the generation of ammonium caused by the hydrolysis of urea. This can be also confirmed by the intense increasing of ammonium ions after 12 hours.

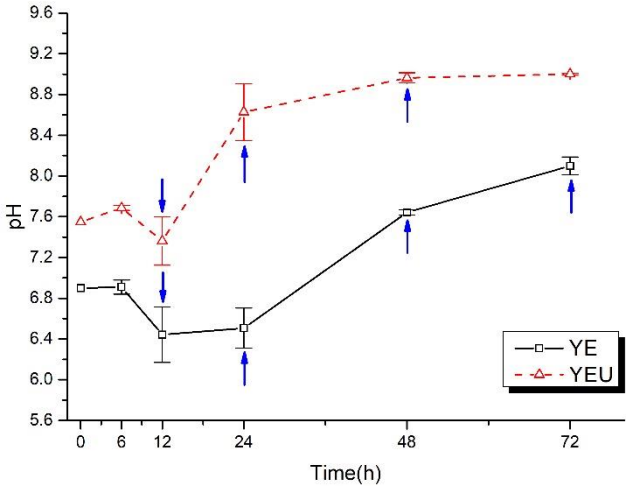

Figure 3. *Variation of pH with time*

3.3 Viable bacterial colony number

Plate counting method can estimate live bacterial number in the enriching solution. The variation of viable bacterial colony number with time is shown in Figure 4.

In both YE and YEU solution, the number of colonies increased significantly (from 3×10^6 to 6×10^9 CFU per gram soil) within 24 hours. It was apparent that bacteria reproduced heavily in the first 24 hours. The decrease of pH observed at 12h (Figure 3) could also be explained by the massive bacterial reproduction during this time.

From 24h to 72h, a noticeable decrease of bacterial colony number was observed in YEU solution. This was possibly due to the alkaline environment caused by the hydrolysis of urea. It could inhibit the growth of non-ureolytic bacteria, however, the number of bacterial colonies still increased after 24h in a relatively low rate.

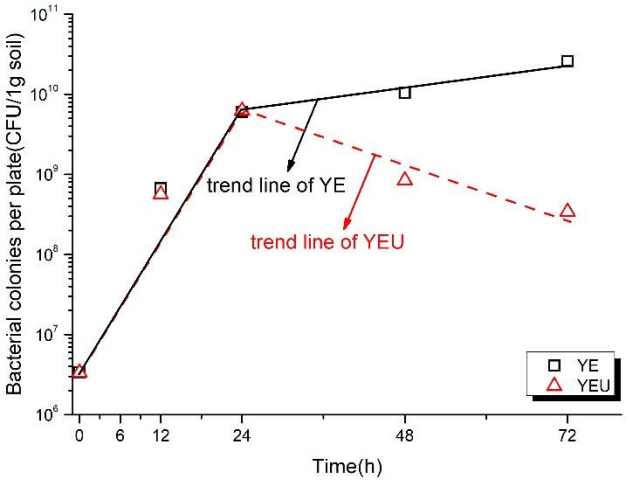

Figure 4. *Variation of viable bacterial colony number with time*

3.4 Ureolytic activity

The ureolytic activity indicates the rate of hydrolysis of urea in the solution. The results are shown in Figure 5. It is found that there was no obvious increase of ureolytic activity within 72 hours in YE solution, indicating that few ureolytic bacteria were stimulated. Conversely, the trend of ureolytic activity in YEU

solution was consistent with the concentration of ammonium (Figure 2). In the first 24 hours, the ureolytic activity increased very slowly, from 0 to 0.01 mM completely hydrolyzed urea/min. However, it was followed by a significant increase from 24h to 72h, indicating that massive ureolytic bacteria could be stimulated. In addition, the difference of concentration of ammonium ions between YEU and YE at 48h was 93mM, which was not far away from 100mM. Therefore, the depletion of urea might be the major factor for the low growth of ureolytic activity between 48 and 72h.

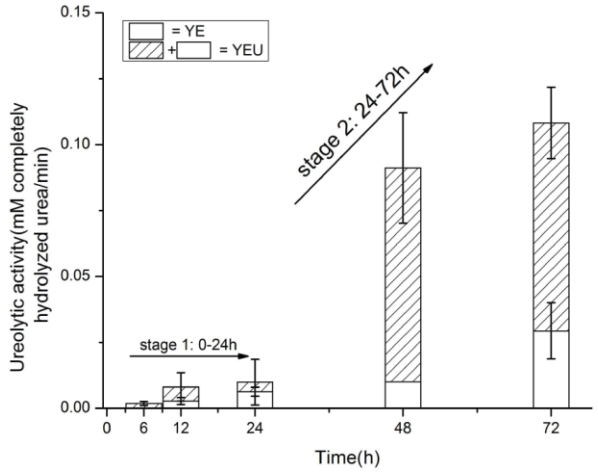

Figure 5. *Variation of viable bacterial colony number with time*

3.5 Ammonium elimination

Nitrification is a process of nitrogen compounds oxidation, during which ammonium is converted into nitrite and nitrate by a series of bacterial catalysis process, written as Equation (1) and (2).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ \quad (1)$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad (2)$$

Figure 6 shows the variation of the total NH_4^+-N concentration with time in the nitrification test. In the first 15 days, the rate of ammonium reduction in the enrichment solution was relatively low, indicating that nitrifying bacteria were not significantly stimulated. From 15 to 30 days, however, the concentration of NH_4^+-N dropped rapidly from 350 to 100 mg/L/per gram of sand. It means that over 70% ammonium ions were converted to either NO_2^- or NO_3^-.

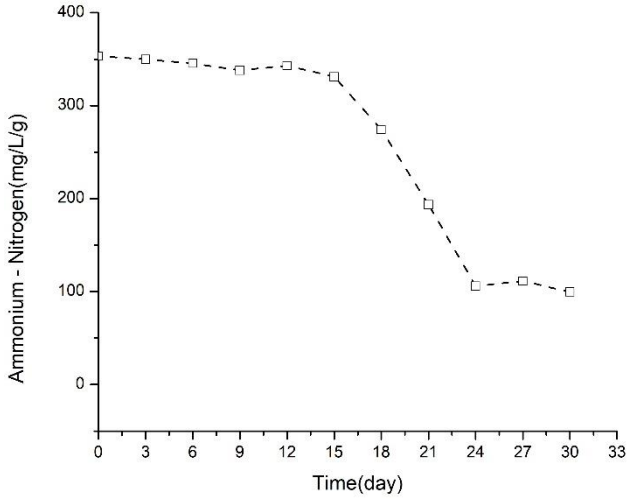

Figure 6. *Variation of NH_4^+-N concentration in ammonium ions with time*

Figure 7 shows the variation of NO_3^--N concentration converted from ammonium ions (Figure 6). In the first 15d, very few nitrate ions were generated. However, the concentration of nitrate ions increased

significantly from 15 to 30 days. The changing trend of Figure 6 and Figure 7 illustrated that the indigenous nitrifying bacteria in Kailua Beach sand could be stimulated effectively using the NH_4Cl enriching medium after 15 days of enrichment.

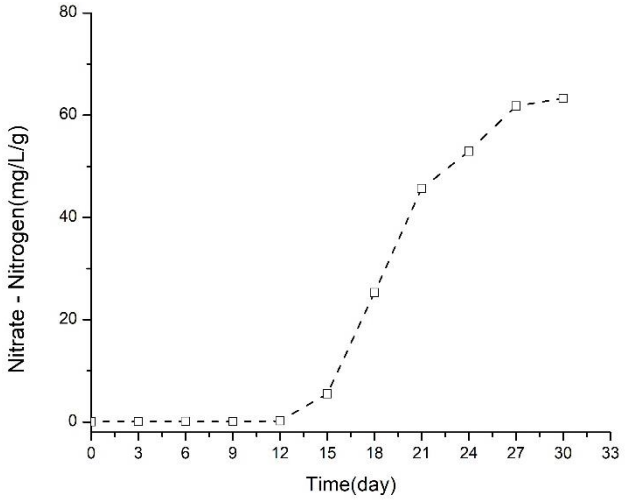

Figure 7. *Variation of NO_3^--N concentration in nitrite and nitrate ions with time*

4 CONCLUSIONS

The following conclusions are drawn from this study:

(1) The indigenous ureolytic bacteria could be significantly stimulated in the presence of YEU medium; YE medium was not effective at stimulating ureolytic bacteria.
(2) The ureolytic activity increased significantly from 24h to 48h, indicating that ureolytic bacteria reproduced rapidly during this time. Moreover, the significant increase of ureolytic activity occurred when a specific viable bacterial colony number could be reached.
(3) The indigenous nitrifying bacteria could be stimulated effectively in the NH_4Cl enriching medium. Ammonium ions were converted into nitrate ions in the process of nitrification.

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