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*The paper was published in the proceedings of the 3<sup>rd</sup> International Symposium on Coupled Phenomena in Environmental Geotechnics and was edited by Takeshi Katsumi, Giancarlo Flores and Atsushi Takai. The conference was originally scheduled to be held in Kyoto University in October 2020, but due to the COVID-19 pandemic, it was held online from October 20<sup>th</sup> to October 21<sup>st</sup> 2021.*

## A preliminary study of carbonate sand stabilization by bio-stimulation based MICP method

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### ABSTRACT

Microbiologically Induced Calcite Precipitation (MICP) has been proposed to improve the weak sandy soil ground via bio-augmentation or bio-stimulation. Compared with bio-augmentation, bio-stimulation approach has more advantages since indigenous microbes are more likely to survive and effectively generate MICP. This paper reports a preliminary experimental study of investigating the mechanism of bio-stimulation and shear strength behavior of bio-cemented carbonate sand via a series of column enrichment and soil direct shear tests. The carbonate sand was firstly treated by enrichment media containing nutrient solution and urea, and then treated by cementation solution containing urea and calcium chloride. After treatment, direct shear tests were conducted under four normal stress (25 kPa, 50 kPa, 100 kPa and 200 kPa). The results indicate that indigenous ureolytic bacteria can be enriched in soil effectively, and the shear strength can be improved significantly.

**Keywords:** biostimulation, indigenous bacteria, ureolysis, direct shear

## 1 INTRODUCTION

The carbonate sands are often considered as weak and unstable materials. They normally have low strength, poor water retention and are sensitive to compaction (Bruand et al., 2005). These adverse engineering properties make them difficult to manage in the construction field.

Microbiologically induced calcite precipitation (MICP) is a new ground improvement method that can be potentially applied for carbonate sand stabilization. The most popular MICP mechanism is through ureolysis (DeJong et al., 2010; Dhami et al. 2017). Compared with other conventional chemical sand stabilizers such as cement, and lime (Bahmani et al., 2014; Di Sante et al., 2015), MICP is a less destructive, energy-saving and more sustainable method.

In most previous studies, well-known existing ureolytic bacterial strains such as *Sporosarcina pasteurii*, *Bacillus megaterium* and *Bacillus sphaericus* (Jiang et al., 2014, 2017; Jiang and Soga, 2017; Lian et al., 2006) are used as sources for ureolysis reaction. Meanwhile, the ureolytic activity has been found widely distributed in natural soils, (Mobley and Hausinger, 1989), which makes it possible to enrich indigenous ureolytic bacteria for MICP. Bio-stimulation approach to generate MICP shows a superior advantage over the bio-augmentation approach using exogenous bacteria due to the better compatibility with natural environment and potential

less risk to local ecosystem (Burbank et al., 2011; Gomez et al., 2018). By percolation method, Cheng and Cord-Ruwisch (2012) achieved the treatment down to 1m depth with satisfied homogeneity. For the treatment in further deeper area, the pressurized injection can be applied to overcome the immobilization of enrichment media due to the great gravitational stress from above.

Previous studies show that the MICP through bio-augmentation is effective in improving shear strength and liquefaction resistance of sandy soils (DeJong et al., 2006; Mortensen et al., 2011; Jiang et al., 2017). However, the research on the microbiological and geotechnical properties of bio-cemented carbonate sand subjected to bio-stimulation treatment is limited. In this study, a local carbonate sand from a Hawaii coastal site was subjected to bio-stimulation treatment using a yeast extract (YE) based enrichment solution. The chemical and biological parameters including the concentration of ammonium and urea, and ureolytic activity were measured or calculated during bio-stimulation. Additionally, bio-cemented carbonate sand specimens were prepared using bio-stimulation approach and subjected to direct shear test to investigate their shear responses.

## 2 MATERIALS AND METHODS

### 2.1 Soil and enrichment solution

The carbonate sand was sampled from Waikiki beach, Oahu, Honolulu, Hawaii. The particle size distribution

curve is shown in Fig. 1. The sand was collected from the shallow depth (0 to 5 cm), which was in the oxic zone, instead of collecting from the deeper zone, in which the anaerobic condition may reduce the amount of native ureolytic bacteria. The small twigs, leaves or roots were removed from the samples. The sands were then sieved passing the No.10 sieve (opening size 2 mm) to remove large granular particles.

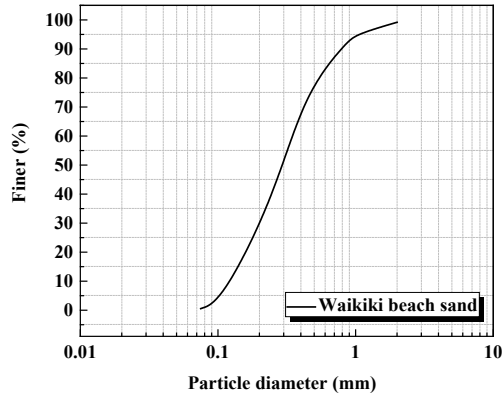


Fig. 1. Particle size distribution curve of carbonate beach sand.

To simplify the enrichment medium, only 20 g/L yeast extract was used as nutrient source for bio-stimulation. The enrichment solution was amended with urea so that it was more selective for ureolytic bacteria. A total of four urea concentrations were adopted (i.e., 50, 100, 170 and 300 mM). 0.5 M urea and calcium chloride with 0.2 g/L YE were used as the cementation solution to produce bio-cementation in the carbonate sand.

## 2.2 Sample preparation and treatment procedures

The samples to examine bio-stimulation efficiency were prepared in PVC tubes with a height of 10.4 cm and inner diameter of 5.2 cm. A layer of polyvinyl film was wrapped at the bottom of the PVC pipe before soil filling and was fixed by a rubber band. 300 g sand was firstly mixed thoroughly with 45 mL enriching solution in a stainless-steel basin, then the mixture was filled into the tubes in three layers. Duplicate sand columns were made to ensure the results reliable. The set-up of sand column system is presented in Fig. 2(a). A control group was also made, in which the carbonate sand was mixed with 45 mL distill water.

The bio-cemented carbonate sand specimens were prepared in brass rings with a height of 2.54 cm and inner diameter of 6 cm, as shown in Fig. 2(b). The relative density of the carbonate sand was 11%. 100 g sand was mixed with 14 mL enrichment solution, and then compacted into the brass rings in three layers. The samples were then firstly subjected to 2-day bio-stimulation in which only enrichment solution was used. Then, 40 mL ( $\approx 1.5$  pore volume) cementation solution was flushed through the sand specimens via gravity for 5 consecutive days. One flushing was conducted each day. After treatment, the samples were oven-dried at 110 °C overnight prior to the direct shear test. The horizontal shear rate of direct shear test was set to

1mm/min. The detailed geotechnical characteristics of the soil specimens are shown in Table 1.

## 2.3 Measurements

All samples were placed at room temperature ( $21 \pm 1^\circ\text{C}$ ). For the bio-stimulation samples in PVC tubes, sand was poured out of the PVC tubes for the biological and chemical measurements at the end of each curing age (i.e., 0, 6, 12, 18, 24, 48, 72 hours), including ammonium/urea concentrations and ureolytic activity. For the bio-cemented sand samples in brass rings, after the completion of flushing treatment, the shear strength response (shear stress, volumetric strain) was investigated through the direct shear test.

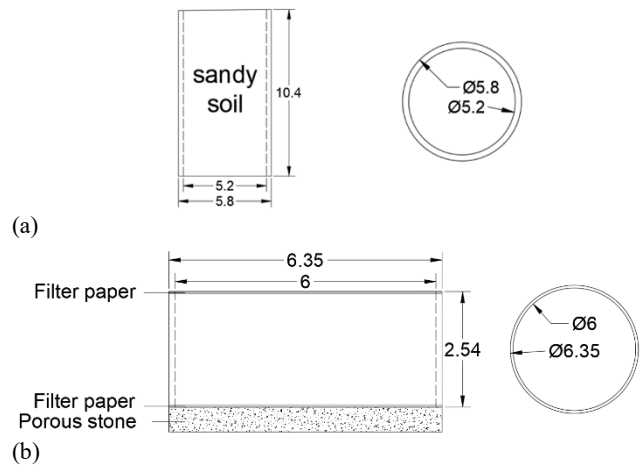


Fig. 2. The set-up of the system (cm).

Table 1. The geotechnical characteristics of samples.

Characteristic	Value
Soil classification (ASTM D2487)	SP
Coefficient of curvature, $C_c$	0.816
Coefficient of uniformity, $C_u$	2.5
Specific gravity, $G_s$	2.7 g/cm <sup>3</sup>
Maximum void ratio, $e_{max}$	0.736
Minimum void ratio, $e_{min}$	0.271
Relative density, $D_r$	11%

Handheld pH meter was used for the pH value monitoring. The ureolytic activity was calculated from the increase of EC measured at the 1st and 9th minute after the soil containing 1 mL pore fluid was mixed with 7 mL urea solution (1.5 M) (Whiffin, 2004). The concentrations of urea and ammonium within soil were determined spectrophotometrically via measuring the absorbance at 425 and 422 nm wavelength, respectively (Greenburg et al. 1992; Knorst et al. 1997). The viable cell number was recorded by the plate counting method. The pore fluid was extracted using 99 mL sterilized water from 1 g soil shaking for 15 min. After appropriate dilution, the 100  $\mu\text{L}$  suspended solution was spread on the solid YE agar medium (20 g/L YE, 15 g/L agar) and incubated overnight for counting.

### 3 RESULTS AND DISCUSSION

#### 3.1 Urea and ammonium concentrations during bio-stimulation

The concentrations of urea and ammonium are direct indicators of the degree of ureolysis based on the Equation (1).



The concentrations of urea and ammonium during the bio-stimulation test are shown in Fig.3. In general, most urea could be hydrolyzed after 48 hours in all cases. It should be noted that the rate of ureolysis was different. The highest rate appeared between 24 h and 48 h. Moreover, a faster rate of ureolysis could be seen in the higher initial urea concentration case. Correspondingly, the ammonium concentration increased with time and reached the peak between 24 h and 48 h. After that, a decline trend could be observed, which might be related with the liberation of ammonia gas ( $\text{NH}_3$ ) converted from ammonium ions ( $\text{NH}_4^+$ ) dissolved in the pore fluid due to the more alkaline condition. Similarly, higher initial urea concentration produced more ammonium.

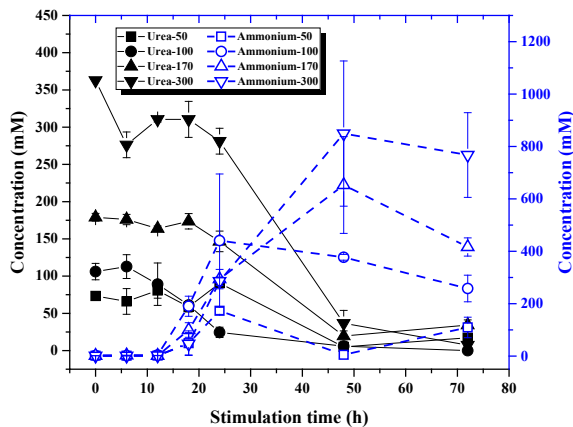


Fig. 3. The variation of urea and ammonium concentration with time.

It should be mentioned that the ratio of urea consumption and ammonium generation did not exactly follow the stoichiometric relationship, which is 1:2 according to the Equation 1. This was like due to the oxidative deamination process in the soil. YE as nutrient provider contained large amount of glutamic acids, which could facilitate the oxidative deamination reaction. The amine group was removed from the amino acid and converted to ammonia (Stewart et al. 1995). Nevertheless, when urea started to be hydrolyzed substantially, the ammonium concentration increased significantly accordingly. It was also interesting to see that the ammonium concentration declined when ammonium reached the peak. This was possible attributed to the nitrifying bacteria that were enriched at the end of the test due to the presence of the ammonium ions. The ammonia was harmful to the ecosystem. Enriching nitrifying bacteria to mitigate the produce of ammonia could be a strategy and

needs further investigation.

#### 3.2 Ureolytic activity during bio-stimulation

The ureolytic activity directly reflects the rate of ureolysis in the soil column. It is also an important indicator of the amount of microbial urease presenting in the soil. In general, higher ureolytic activity corresponds to more activated microbial urease. Figure 4 shows the evolution of ureolytic activity with time for all cases.

It was obvious that there was rather limited ureolytic activity in the control case. Few ureolytic bacteria could be enriched in the soil without proper enrichment solution. For the cases with urea, higher urea concentration could induce higher ureolytic activity. In the 100, 170 and 300 mM cases, the development of ureolytic activity could be divided into two stages. At the first stage, the ureolytic activity increased significantly. After that, it slowed down and kept relatively stable. However, it should be noted that although the peak ureolytic activity could reached around 12 mM/min/mL in the 300 mM case, the value dropped to 3.75 mM/min/mL at 72 hours, which was close to the value in the 170 mM case. The variation of ureolytic activity was in general consistent with that of urea and ammonium concentrations (Fig.3). Once the urea started to be consumed and then be hydrolyzed completely, the increase of ureolytic activity slowed down and kept relatively stable between 24 h and 48 h.

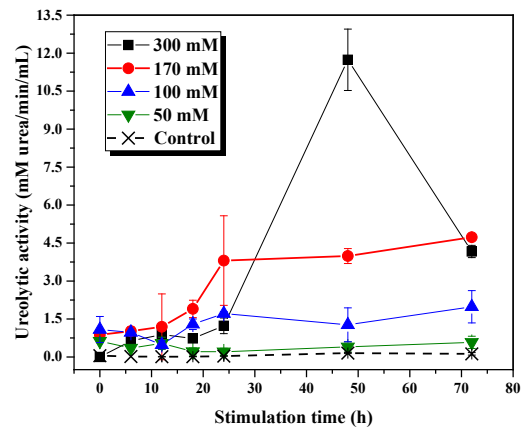


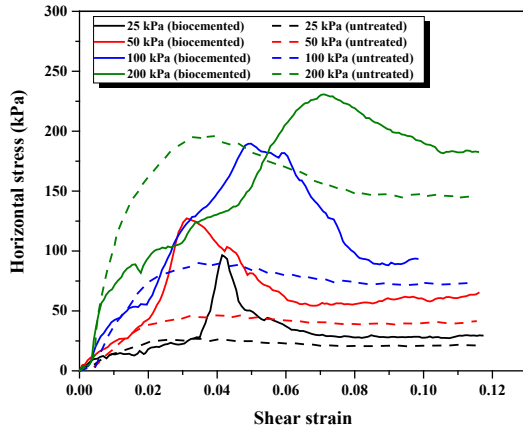
Fig. 4. The variation of ureolytic activity with time.

#### 3.3 Direct shear test for bio-cemented carbonate sand

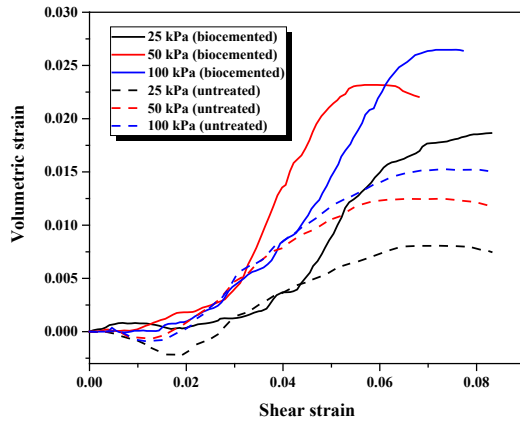
According to the bio-stimulation test results, 170 mM urea was finally used to prepare the direct shear test specimens, as its capability to generate ureolytic activity was similar to 300 mM urea. Figure 5 shows the stress-strain relationship and Mohr-Coulomb failure envelop of the bio-cemented carbonate sand under four different normal stresses.

From Fig.5(a), it could be seen that, compared with untreated carbonate sand, the shear strength of bio-cemented carbonate sand saw a dramatic increase. More specifically, the peak and residual strength of bio-cemented sand was increased by 17% – 252% and 18.9% – 34% respectively under the normal stress range implemented in this study, indicating that substantial cementation was

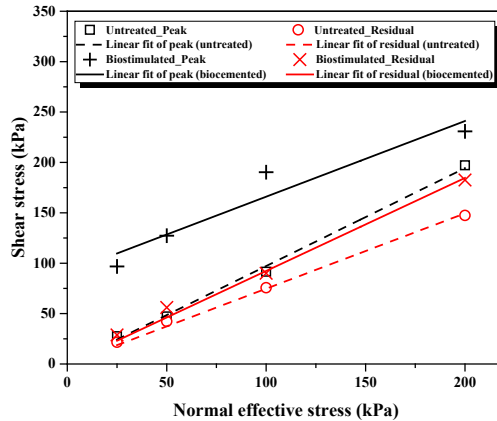
generated in the sand after bio-stimulation of indigenous ureolytic bacteria. Moreover, the softening behavior of the specimens became less pronounced under higher normal stress (200 kPa). Beyond the peak point, the shear stress decreased gradually to the residual state, which was resulted from the breakage of bonding of cementation (Dejong, 2007). The higher residual shear strength in bio-cemented cases was possibly due to (1) a much denser state resulted from the formation of calcite filling in voids (Dejong, 2007); and (2) the increase of particle angularity (Dejong et al., 2010).



(a)



(b)



(c)

Fig. 5. Results of direct shear test. (a) shear stress-normal stress; (b) dilation; (c) failure envelope.

Figure 5(b) shows the volumetric strain versus shear

strain. Under 25, 50 and 100 kPa normal stress, the sample did not show much obvious initial contractive behavior compared with the untreated samples, which was due to the bonding of cementation inside between sand grains. The increase of interlocking mass coming from calcite precipitation promoted a more dilative behavior for the bio-cemented samples than untreated cases (Saxena et al., 1988).

The failure envelope based on the Mohr-Coulomb theory is shown in Fig.5(c). The linear peak failure envelop of bio-cemented sand was well above that of untreated sand. But the two envelopes tended to converge under higher normal stress. In other words, the effect of bio-cementation on increasing peak shear strength is diminished under higher normal stress, which is consistent with those reported in previous studies (Nafisi et al., 2020). Mechanistically, the frictional interaction and dilatancy depends on normal stress, while the contribution from cementation is independent from normal stress (Nafisi et al., 2020). Under lower normal stress, the contribution of cementation is more dominant than that of frictional interaction and dilatancy. Thus, the peak strength of bio-cemented sand is significantly higher. However, under higher normal stress, the effect of frictional interaction and dilatancy become more dominant while the influence of cementation is diminishing. Therefore, the peak strength of cemented and uncemented sand becomes closer. In a strict sense, it can be seen from Fig.5(c) the failure envelope of bio-cemented sand is actually not a straight line as uncemented sand, which is also pointed out by Nafisi et al. (2020). The effect of cementation is variable with the increasing normal stress level. Consequently, the analysis of cohesion and frictional angle based on Mohr-Coulomb model is not applicable here in this paper, and it is necessary to develop a more accurate non-linear model.

## 4 CONCLUSIONS

In this study, MICP was achieved through bio-stimulation on Hawaii carbonate sand. A series of bio-stimulation enrichment test and direct shear test were performed to illustrate the feasibility of bio-stimulation induced MICP for sand stabilization. Main conclusions are shown as follows:

1. During the bio-stimulation process, 170 mM urea concentration was sufficient to enrich the indigenous ureolytic bacteria. After 24 to 48 hours, the ureolytic activity could reach the peak and then became stable.
2. After bio-cementation treatment, both peak and residual shear strength were improved in the bio-cemented carbonate sand.
3. The shear stress of both peak and residual state increased more significantly under lower normal stress than higher one.

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