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Bio-cementation by using microbially induced carbonate precipitation technique by using sporosarcina pasteurii with oxidizing agent supplements

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KEYWORDS

Bio-cementation
Bacteria
Biomineralization

ABSTRACT

In recent years, the natural biomineralization of bacterial metabolism to form calcium carbonate has drawn researchers and engineers' attention to exploring various applications for industrial purposes. Generally, microbially induced carbonate precipitation (MICP) is a biogeochemical process that can be applied to strengthen materials, including soil and sand structure, to overcome the chemical soil stabilization technique's limitations. In this study, Sporosarcina pasteurii was used to form $CaCO_3$ by ureolytic MICP for soil-sand reinforcement in LAB-scale. As preliminary results, the increasing of $CaCO_3$ crystals over curing time was an essential parameter for creating strong bonding between the soil-sand particles. Besides, the positive results in the water permeability test with the materials analysis as SEM, XRD demonstrated bacterial bio-cementation of sand specimens. Also, experimental results about oxidizing agent supplements show a positive effect on improving MICP capacity. Despite many challenges, bio-mineralization or bio-cementation is a promising technique for soil-sand stabilization in geotechnical engineering and building material applications, leading to sustainable development.

1. Introduction

Microbially induced carbonate precipitation (MICP) is a biogeochemical process that can be applied to strengthen materials by densifying the structure. From the beginning to recent years, MICP has been studying by many researchers, scientists, and engineers all over the world for a wide range of applications not only in construction materials but also in agriculture, soil, and geomaterials [1–4]. As an approach for sustainable development, soil stabilization and strength improvement using bio-geotechnical (across between biological technique and geological technique) method are focused through many projects and research. Since the previous results in significant improvement in soil's physical and geotechnical properties, MICP using bacteria shows promising ability for large-scale applications. By the forming of $CaCO_3$ to bind the separated components together (bio-cementation) in soil structure (Figure 1), the mechanical resistance can be increased with less detrimental effect for the environment than chemical treatment method. In fact, bio-cementation can occur naturally since time immemorial through bio-deposition of sand over a long period of time. Also, the structure can become more stable and compact for many years without any significant human-maintenance. Among various mechanism of MICP, the hydrolysis of urea by microbial catalysis, especially bacteria to form carbonate is usually applied for cementation and strength improvement. In addition, the biological mechanisms to $CaCO_3$ can be associated not only with the strength improving of soil, but also with the reduction of atmosphere carbon dioxide. At the beginning stage, the urease degradation enzyme initiates the process to breakdown urea and raise local pH. $CaCO_3$ crystals are then precipitated through the absorption of Ca^{2+} into the bacterial cell wall. Depend on the nutrient source and curing conditions, the reaction times of MICP effect can range from hours to a few days [4]. Some types of bacteria can be used, such as *Bacillus cohnii* [5], *Bacillus pasteurii* [6], *Bacillus pseudofirmus* [7], *Bacillus subtilis* [8–10]. As a preliminary study, *Bacillus subtilis* [9, 11] was used as a treatment technique for sand and soil-sand materials in LAB-scale. Materials properties as density, flexural strength, mineral composition, and microstructure observation were tested and confirm the effect of applying MICP on bio-cementation separate sand particles. In addition, soil isolated *Bacillus* species were tested sand stiffening ability using syringe set-up with daily nutrient addition [11]. This treatment method for soil and sandy-soil show benefits with simple technique and preparation. However, there still be a lack of knowledge on bio-mineralization under different environments. On the other hand, increasing the capacity of calcite precipitation was focused. Therefore, *Sporosarcina pasteurii* was found and studied as high-effect MICP microoganism. Experimental results confirmed the high ability of *Sporosarcina pasteurii* for $CaCO_3$ precipitation by its high urease enzyme enzymatic activity. Besides, an important factor was found that can improve bio-cementation through increase urea hydrolysis by

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oxygen supplement. Based on that, this study will investigate how the oxidizing agents and curing time affect the growth and precipitation of bacteria *Sporosarcina pasteurii* and the properties of sandy soil specimens.

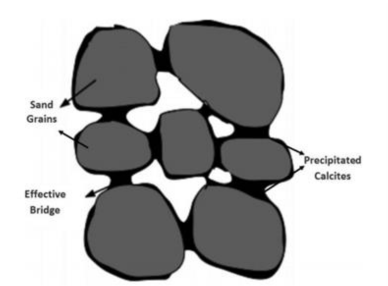


Figure 1. Illustration for bio-cementation for soil/sand by MICP [12].

2. Materials and Experiments

River sand was used to investigate the bio-cementation of bacteria in binding separated particles. 0.3 mm sand was chosen with the similar to the actual soil sample in the Mekong Delta. Mix 50 g sand with 12 mL of calicum carbonate precipitation (CCP) medium, 0.6 g of *Sporosarcina pasteurii* biomass, and $Na_2CO_3.H_2O_2$ with the concentration ranging from 0 to 150 mg/L before filling the syringe carefully (volume = 60 mL) without compression. At each syringe (V = 60 ml, d_{outlet} = 4.5 mm), 50 g of sand is inserted. Water (20 ml) is applied to the top of the syringe and flows through the sand volume by gravity and capillary. The time of this process is recorded. The precipitation of $CaCO_3$ were detected by using X-ray diffraction (XRD), optical microscope, and scanning electron microscopy (SEM).

After 7 and 14 days of curing, water permeability was evaluated by measuring the height-change of the sand column. Intense pressure using 25 mL water (in syringe) injection in 10 seconds was applied to break the structure into a sand column to estimate the bio-cementation level. The parameters include the volume of water leakage, the weight of losing sand, and the height of the sand column after the test were measured and calculated.

Results and discussions

2.1. Bio-cementation effect through water flow test

Figure 2 shows a significant reduction in both water leakage and the washed-away sand for specimens with oxidizing agent supplement (group B50, B100) compared to reference specimen (group R), except group B150. The improvement of MICP could cause this result by increasing the bacterial activity with oxygen. However, the positive effect stopped with group B150, suggesting the limit proportion for oxidizing agent supplements.

As shown in Figure 3a, with water pressure on the top of the syringe, the sand column changed its height from the initial value during the water flow test. In both curing time cases, reference specimen without bacteria showed the highest value compared to the bacterial MICP treated specimens. Even slightly higher when the oxidizing agent up to 150 mL (group B150), sand column remained its height higher than the untreated one. The bacterial bio-mineralization caused a positive effect on the remaining shape ability (Figure 3b). White material that appeared on the surface of sand specimens binding the sand particles could also cause the high density of sand column. In the MICP treated specimen, the sand particle contacts were cemented by precipitated $CaCO_3$, which leads to the aggregation of sand particles, resulting the increase in mean size of substrate.

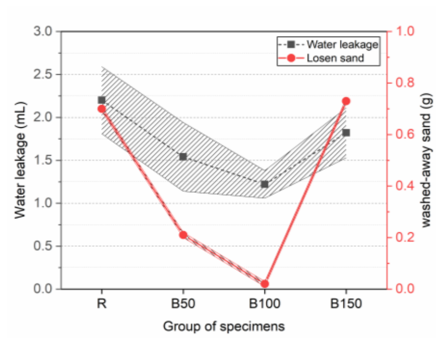
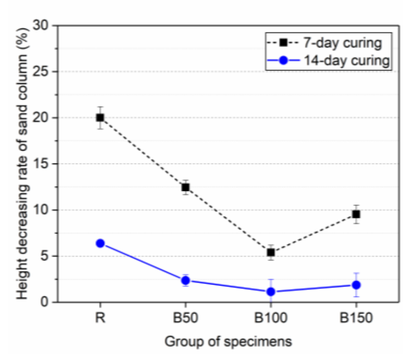
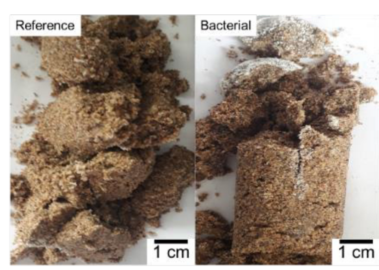


Figure 2. Water leakage through the sand column and the weight of sand washed away from the syringe. R: reference specimen; B50, B100, B150: specimens with supplied oxidizing agents 50, 100, and 150 mg/L corresponding.



(a)

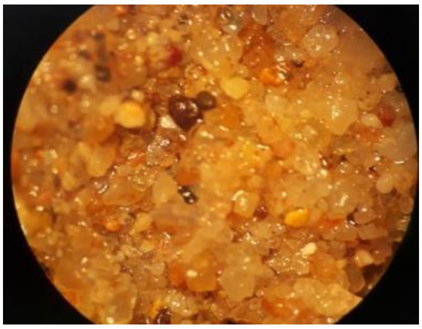


(b)

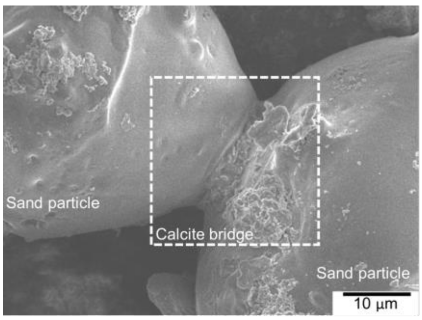
Figure 3. (a) Height decreasing rate of sand column specimens (a). R: reference specimen; B50, B100, B150: specimens with supplied oxidizing agents 50, 100, and 150 mg/L corresponding. (b) Reference sand specimens without bacteria (left) and bacterial treated specimens after mold removing (right).

2.2. Microstructure analysis

After MICP treatment, the bio-cementation effect caused the large-size sand grain from initially separated particles, which can clearly be observed (Figure 4a). Also, SEM image shows a thin layer of precipitated material covered the sand grains and created bridges among them. The crystal morphology observed in the SEM (Figure 4) and the EDS pattern confirmed the presence of calcite. Relatively smaller-size precipitated crystals compared to that had been observed in the study using *Bacillus subtilis*. Note that dissolved organic matter, by its negative charge, might interact with Ca^{2+} and inhibit the growth of $CaCO_3$ crystals.



(a)



(b)

Figure 4. Visual observing image of sand specimens after bio-cementation (a). SEM micrographs of sand particles after bio-cementation using supplied oxygen with *Sporosarcina pasteurii* (b).

3. Conclusion

It can be seen that treatment using *Sporosarcina pasteurii* with oxidizing agent bio-cementation showed a better response than the previous technique. The addition of oxidizing agents provided a better condition that can maintain the high-capacity of MICP by *Sporosarcina pasteurii*. Through the experimental results with sand columns, strong intergranular bridging could be considered the major contributor to erosion resistance. Separated and unstable particles in the soil-sand system can be expected to be cemented and stabilized significantly. The presented results in this study indicate the promising potential of the MICP technique for bio-cementation and to stabilize and improve surface soil structure.

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