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Video Article

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Abstract

The goal of this article is to develop an immersion method to improve the microbially induced calcite precipitation (MICP) treated samples. A batch reactor was assembled to immerse soil samples into cementation media. The cementation media can freely diffuse into the soil samples in the batch reactor instead of cementation media being injected. A full contact flexible mold, a rigid full contact mold, and a cored brick mold were used to prepare different soil sample holders. Synthetic fibers and natural fibers were selected to reinforce the MICP-treated soil samples. The precipitated $CaCO_3$ in different areas of the MICP-treated samples was measured. The $CaCO_3$ distribution results demonstrated that the precipitated $CaCO_3$ was distributed uniformly in the soil sample by the immersion method.

Video Link

The video component of this article can be found at https://www.jove.com/video/60059/

Introduction

As a biological ground improvement technology, microbially induced calcite precipitation (MICP) is capable of improving engineering properties of soil. It has been used to enhance the strength, stiffness, and permeability of soil. The MICP technique has gained much attention for soil improvement worldwide[1,2,3,4]. Carbonate precipitation naturally happens and can be induced by nonpathogenic organisms that are native to the soil environment[5]. The MICP biogeochemical reaction is driven by the existence of ureolytic bacteria, urea and a calcium-rich solution[5,6]. *Sporosarcina pasteurii* is a highly active urease enzyme that catalyzes the reaction network towards precipitation of calcite[7,8]. The urea hydrolysis process produces dissolved ammonium (NH^{4+}) and inorganic carbonate (CO_3^{2-}). The carbonate ions react with calcium ions to precipitate as calcium carbonate crystals. The urea hydrolysis reactions are shown here:

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad (1)$$

$$C_a^{2+} + CO_3^{2-} \rightarrow C_aCO_3\,(s)\downarrow \quad (2)$$

The precipitated $CaCO_3$ can bond the sand particles together to improve the engineering properties of MICP-treated soil. The MICP technique has been applied in various applications, such as improvement of strength and stiffness of soil, repair of concrete, and environmental remediation[9,10,11,12,13,14,15].

Zhao et al.[16] developed an immersion method to prepare MICP-treated samples. A full contact flexible mold made of geotextile was used in this method. The precipitated $CaCO_3$ distributed uniformly throughout their MICP-treated samples. Bu et al.[17] developed a rigid full contact mold to prepare MICP-treated beam samples by an immersion method. The MICP-treated sample prepared by this method using a rigid full contact mold can form the suitable beam shape. The MICP-treated sample was divided into four and the $CaCO_3$ contents were measured. The $CaCO_3$ content ranged from 8.4 ± 1.5% to 9.4 ± 1.2% by weight, which indicated that the $CaCO_3$ distributed uniformly in the MICP-treated samples by the immersion method. These MICP-treated samples also achieved better mechanical properties. These MICP-treated bio-specimens reached a 950 kPa flexure strength, which was similar to that of 20- 25% cement-treated samples (600- 1300 kPa). Li et al.[10] added randomly distributed discrete fiber into the sandy soil and treated the soil by the MICP immersion method. They found that the shear strength, ductility, and failure strain of MICP-treated soil were enhanced obviously by adding appropriate fiber.

The immersion method for MICP has been continually improved[10,16,17]. This method can be used to prepare MICP-treated soil samples and MICP-treated prefabricated building materials, such as bricks and beams. Different geometry dimensions of sample preparation mold were developed. Fibers were added in the MICP-treated samples to enhance their properties. This detailed protocol was intended to document the immersion methods for MICP treatment.

Protocol

NOTE: All relevant material used in the following procedures are non-hazardous. Personal protective equipment (safety glasses, gloves, lab coat, full length pants, closed-toe shoes) are still needed.

1. Preparation of bacteria solution

1. Preparation of growth medium (NH_4-YE medium)
   NOTE: The components of growth media per liter of deionized water are: 20 g of yeast extract; 10 g of $(NH_4)_2SO_4$; and 0.13 M Tris buffer (pH 9.0).
   1. Autoclave ingredients separately.
   2. Dissolve 20 g of yeast extract, and 10 g of $(NH_4)_2SO_4$ in 1 L of deionized water containing 0.13 M Tris buffer.
   3. Mix the components together using magnetic stirrer post-sterilization.

2. Propagation procedure of *Sporosarcina pasteurii*
   NOTE: Use 50 mL centrifuge tubes in this experiment.
   1. Thaw the frozen bacteria in a vial.
   2. Open the vial.
   3. Transfer 0.1 mL of bacteria suspension to a centrifuge tube with 10 mL of fresh growth medium. Mix well by hand (inoculation rate is 1:100). Repeat 5 more bacterial suspensions with growth medium. Prepare a control tube only with 10 of fresh growth medium inside.
      NOTE: The cryoprotectant used in the freeze/drying procedure may inhibit growth in the primary tube. The lids of tubes were tightened loosely in order to maintain the aerobic condition.
   4. Incubate all tubes in a shaker at 200 rpm at 30 °C for 48 to 72 hours. Stop the incubation if the growth medium becomes turbid after 48 h. Otherwise, extend the incubation to the maximum of 72 h.
   5. Centrifuge the tubes with bacteria and growth medium at 4,000 x *g* for 20 min.
   6. Remove the supernatant, replace with 25 mL of fresh growth medium, and mix well using a vortex machine.
   7. Repeat steps 1.2.3-1.2.6 twice to fully stimulate the activity of bacteria.
   8. Use the suspension from the tubes in step 1.2.7 to inoculate more tubes with 25 mL of growth medium to enhance the culture of bacteria (inoculation rate is 1:100).
   9. Incubate all tubes in a shaker at 200 rpm at 30 °C for 48 hours.
   10. Centrifuge the tubes with bacteria and growth medium at 4,000 x *g* for 20 min.
   11. Remove the supernatant, replace with fresh growth medium, and mix well using a vortex machine.
   12. Adjust the bacteria concentration using fresh growth medium before the MICP experiments. Calculate the bacteria concentration by optical density of the suspension at 600 nm, which was measured using a spectrophotometer. The OD_{600} in this experiment was 0.6.

2. Preparation of cementation media

NOTE: Cementation media is used to provide chemicals to induce the calcite precipitation during the MICP treatment. The urea-Ca^{2+} molar ratio is 1:1. The chemical components of cementation media is shown in **Table 1**. The following procedure is for 20 L of cementation media with 0.5 M Ca.

1. Prepare 20 L of water in a plastic box.
2. Dissolve 200 g of NH_4Cl, 60 g of nutrient broth, 42.4 g of $NaHCO_3$, 600 g of urea, and 1470 g of $CaCl_2.2H_2O$ in the 20 L of distilled water. Mix well using stirring rod.

3. Preparation of molds

1. Preparation of full contact flexible mold (FCFM)
   NOTE: The full contact flexible mold is made of geotextile. The geotextile has a grab tensile strength of 1,689 N, a trapezoidal tear strength of 667 N, an apparent opening size of 0.15 mm, a water flow rate of 34 mm/s, a thickness of 1.51 mm, and a unit mass of 200 g/m^2. The size of mold can be varied to prepare different sample sizes (for example, unconfined compression test sample or direct shear test sample).
   1. As the FCFM consists of an annular part, a bottom, and a cover, cut the geotextile into constituent parts of FCFM.
   2. Sew the three parts of FCFM together as shown in **Figure 1**.

2. Preparation of rigid full contact mold (RFCM) for bio-bricks
   NOTE: The rigid full contact mold consists of a flexible layer and a rigid holder. The flexible layer is made of the same geotextile as the FCFM. The rigid holder is made of a polypropylene perforated sheet with 6.35 mm diameter staggered holes distributed on the polypropylene perforated sheet and the clearance distance between adjacent holes is 9.53 mm. One mold consist of three chambers and the size of each chamber is 177.8 mm in length, 76.2 mm in width and 38.1 mm in height. The size of RFCM can be varied to prepare different sample size. The holes in the rigid holder allow cementation media flow through the flexible layer freely.
   1. Prepare the polypropylene perforated sheet for constituent pieces of the rigid holder.
   2. Assemble the pieces of rigid holder using plastic screws and nuts.
   3. Prepare the constituent parts of the geotextile flexible layer. The flexible layer consists of a bottom and a cover.
   4. Enclose the bottom of the flexible layer in the rigid holder.

5. Once the sand is added into the mold, place the cover of flexible layer and fix by sewing on the top of the sand sample as shown in **Figure 2**.

3. Preparation of hollow brick mold
NOTE: The hollow brick mold includes a rigid holder, a flexible layer, and cardboard tubes. The size of the cardboard tube is 60 mm x 140 mm x 60 mm. Three chambers are included in one mold and the size of each mold chamber is 177.8 mm in length, 76.2 mm in width and 38.1 mm in height in this procedure.
    1. Prepare the polypropylene perforated sheet for the constituent pieces of the rigid holder.
    2. Drill holes on the bottom of the rigid holder piece. The size of the holes is 61 mm in diameter. The location of holes in each chamber is shown in **Figure 3a**.
    3. Assemble the pieces of the rigid holder using plastic screws and nuts.
    4. Assemble the cardboard tubes in the drilled holes on the bottom of the rigid holder.
    5. Prepare the constituent parts of the geotextile flexible layer. The flexible layer consists of a bottom and a cover. Holes are also needed on the flexible layer at the same location of cardboard tubes.
    6. Once the sand is added into the mold, place the cover of flexible layer and fix by sewing on the top of the sand sample as shown in **Figure 3b**.

4. Preparation of the batch reactor

NOTE: The reactor shown in **Figure 4** consists of a plastic box, cementation media, a sample supported shelf, and air pumps. The soil samples can fully immerse into the cementation media while the cementation media can freely diffuse into the soil samples by this method. The air pump in the reactor provides oxygen for bacteria. To determine the effects of different oxygen supply on MICP treatment catalyzed by *Sporosarcina pasteurii*, Li et al. 2017[18] conducted contrast tests under three different conditions: an aerated condition, an air restricted condition, and an open-air condition. They found that a well-oxygenated condition is essential to improve MICP processes catalyzed by aerobic bacteria.

1. Connect the air pump with air supply using a plastic hose.
2. Place the air pump in the plastic box.
3. Pour cementation media into the plastic box.

5. Preparation of soil samples

1. Preparation of MICP-treated soil sample
NOTE: Ottawa sand (99.7% quartz) is used in the experiments. The sand is uniform with a median particle size of 0.46 mm and no fines are included. It is classified as poorly graded sand based on the Unified Soil Classified System (USCS).
    1. Add dry sand into molds by the air pluviation method (FCFM, RFCM, hollow brick mold) to reach a median dense condition (D_r in the range of approximately 42–55%, and dry density of sand in the range of 1.58–1.64 g/cm^3).
    NOTE: The weight of sand varies according to different kinds of molds: 145 ± 5 g sand for the UCS test sample, which is 38.6 mm in diameter and 76.2 mm in height.
    2. Place the cover on the top of samples and fix it by sewing.
    3. Pour the bacteria solution with a fixed optical density value through the permeable geotextile cover into the samples and make sure that they are saturated.
    NOTE: The amount of bacteria solution varied according to different samples: 50 mL of bacteria solution for a UCS test sample, which is 38.6 mm in diameter and 76.2 mm in height.
    4. Place samples on the sample supported shelf as shown in **Figure 5a**.
    5. Immerse the entire shelf into the batch reactor filled with cementation media.
    6. Turn on the air supply and adjust the air output to keep 100% air saturation. Wait for 7 days of MICP reaction.
    7. Take out the samples from the reactor as shown in **Figure 5b**.
    8. Remove the samples by cutting the full contact flexible mold or demolding the rigid holder and then cutting the flexible layer.
    9. Wash the samples with water to remove the residual solution in the pore space.
    10. Place the samples in the 105 °C oven for 48 h until their weights remain constant. The samples can be tested or treated additionally after the oven drying.

2. Preparation of fiber reinforced MICP-treated soil sample
NOTE: Synthetic fiber (see **Table of Materials**) and natural palm fiber as shown in **Figure 6** are used in these procedures.
    1. For the synthetic fiber, mix the proposed content of fibers and 900 g of dry sand in small increments by hand to obtain a uniform mixture. The fiber content in this experiment is fixed as 0.3% by weight of the dry sand.
    2. For the natural palm fiber, distribute 760 g of sand into four equal parts. Add these four parts of sand and three layers of fiber in RFCM at intervals.
    3. Repeat the same procedure as steps 5.1.2—5.1.10 to get the MICP-treated sample.

3. Preparation of cement-treated bricks with bio-surface treatment
NOTE: Portland cement (TYPE I/II) with specific gravity of 3.15 is used as the cementing agent for the cement-treated samples in this experiment. The early strength gain of this cement allowed the various curing times ranged from 7 to 21 days. The proportion of added cement in this procedure is 10% by weight of dry sand.
    1. Mix 900 g of sand, 90 g of cement, and 200 mL of water to achieve a uniform mixture.
    2. Add the mixture to the rigid mold. The size of rigid mold is 177.8 mm in length, 76.2 mm in width and 38.1 mm in height.
    3. Cure for 7 days at a constant humidity of 100% and constant temperature of 25 °C.
    4. Place samples in the 105 °C oven for 48 hours until their weights remain constant.

5. Repeat the same procedure as steps 5.1.3—5.1.8.
6. Place samples in the 105 °C oven for 48 hours until their weights remain constant. The samples can be tested or treated additionally after the oven drying.

Representative Results

Figure 7 shows the distribution of precipitated $CaCO_3$ throughout the MICP-treated sample. The MICP-treated sample was divided into three different areas. The $CaCO_3$ content in each area was tested by the acid washing method. To dissolve precipitated carbonates, the dry MICP-treated samples were washed in a HCl solution (0.1 M), then rinsed, drained, and oven-dried for 48 hours. The difference value between the masses of samples before and after acid washing was considered to be the mass of the carbonates precipitated in the MICP-treated samples. The $CaCO_3$ content is indicated as percentage of sample weight. The $CaCO_3$ content of the MICP-treated sample by the immersion method ranged from 9.0% to 9.5%. The results indicated that the precipitated $CaCO_3$ was distributed uniformly throughout the soil sample. While Martinez et al. 2013[19] conducted experiments on 50 cm long sand columns by an injection method in the laboratory, they found that the calcite distributed nonuniformly along the MICP-treated sand column. Most of the calcite precipitated near the influent column and hindered the cementation reaction in the deeper section of the column.

The stress-strain curves of bio-brick reinforced with three layers of palm fiber and unreinforced bio-brick obtained using a four-point test is shown in **Figure 8**. The flexure strength of unreinforced bio-brick was 1,150 kPa, while that of reinforced bio-brick was 980 kPa. Their flexure strengths were similar, but the flexure strain was improved significantly by addition of the palm fiber. These results indicate that palm fiber can contribute to the improvement of ductility.

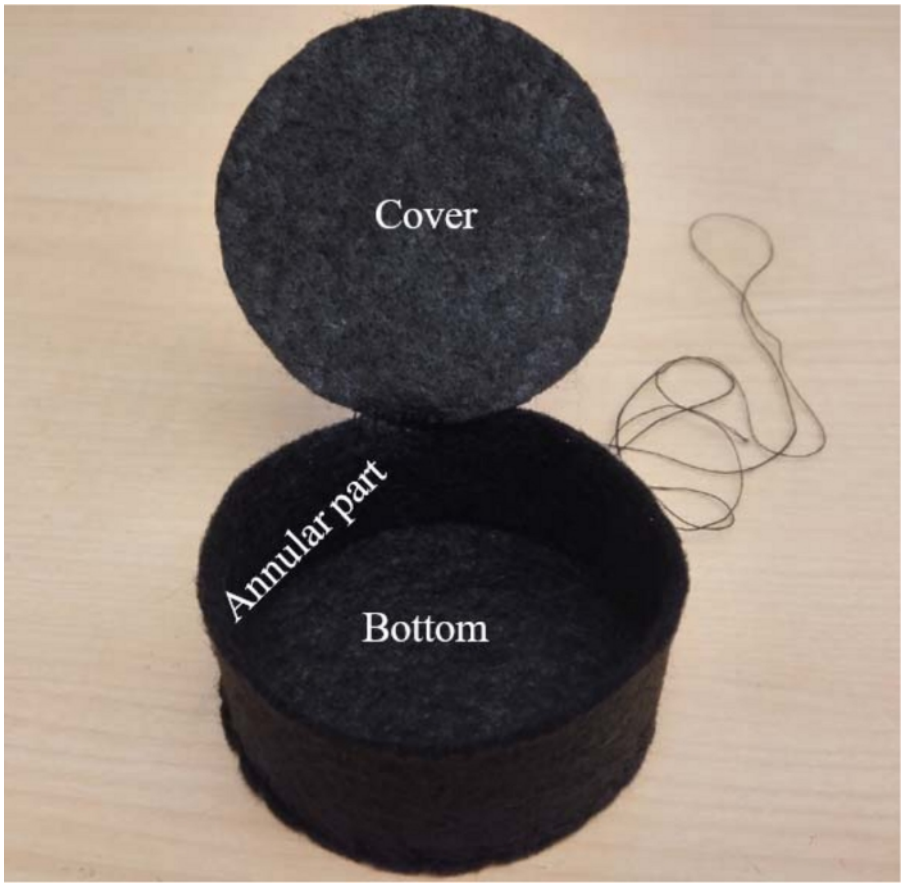

Figure 1: Full contact flexible mold for direct shear tests.
The full contact flexible molds were made of geotextile. The geotextile was a polypropylene, staple fiber and needle punched nonwoven material. The cylinder-shaped mold had a diameter of 62 mm and a height of 26 mm. Please click here to view a larger version of this figure.

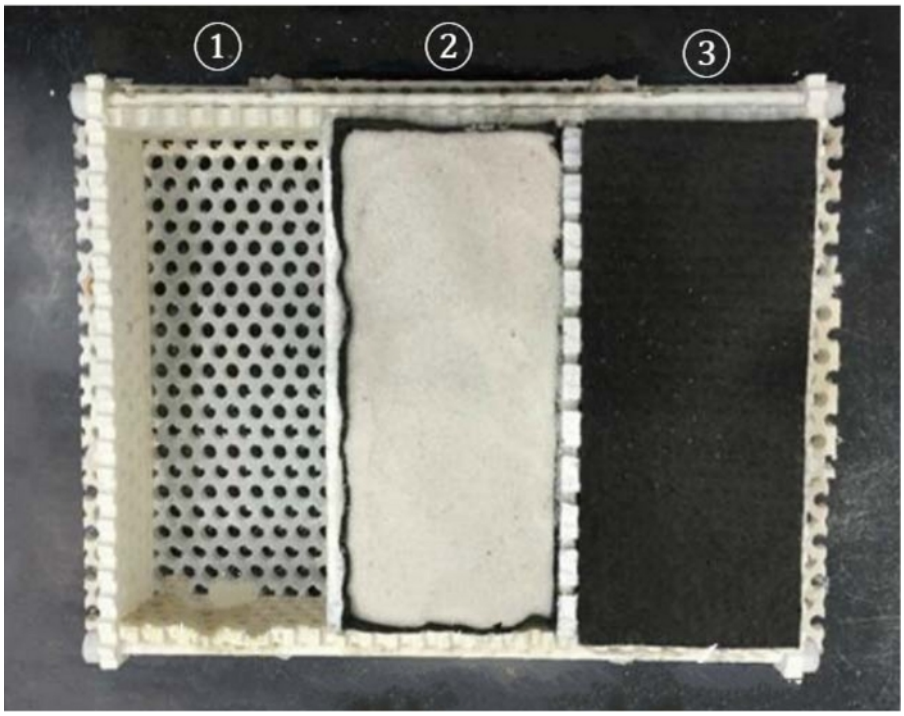

Figure 2: Sample preparation of bio-bricks.
(1) Assembled mold for brick; (2) Sand added into the mold; (3) Flexible cover added on the top of sand sample. The rigid full contact mold consists of a flexible layer and a rigid holder. The flexible layer was made of geotextile, and the rigid holder was made of a polypropylene perforated sheet. The mold consisted of three chambers and the size of each chamber was 177.8 mm in length, 76.2 mm in width and 38.1 mm in height. Please click here to view a larger version of this figure.

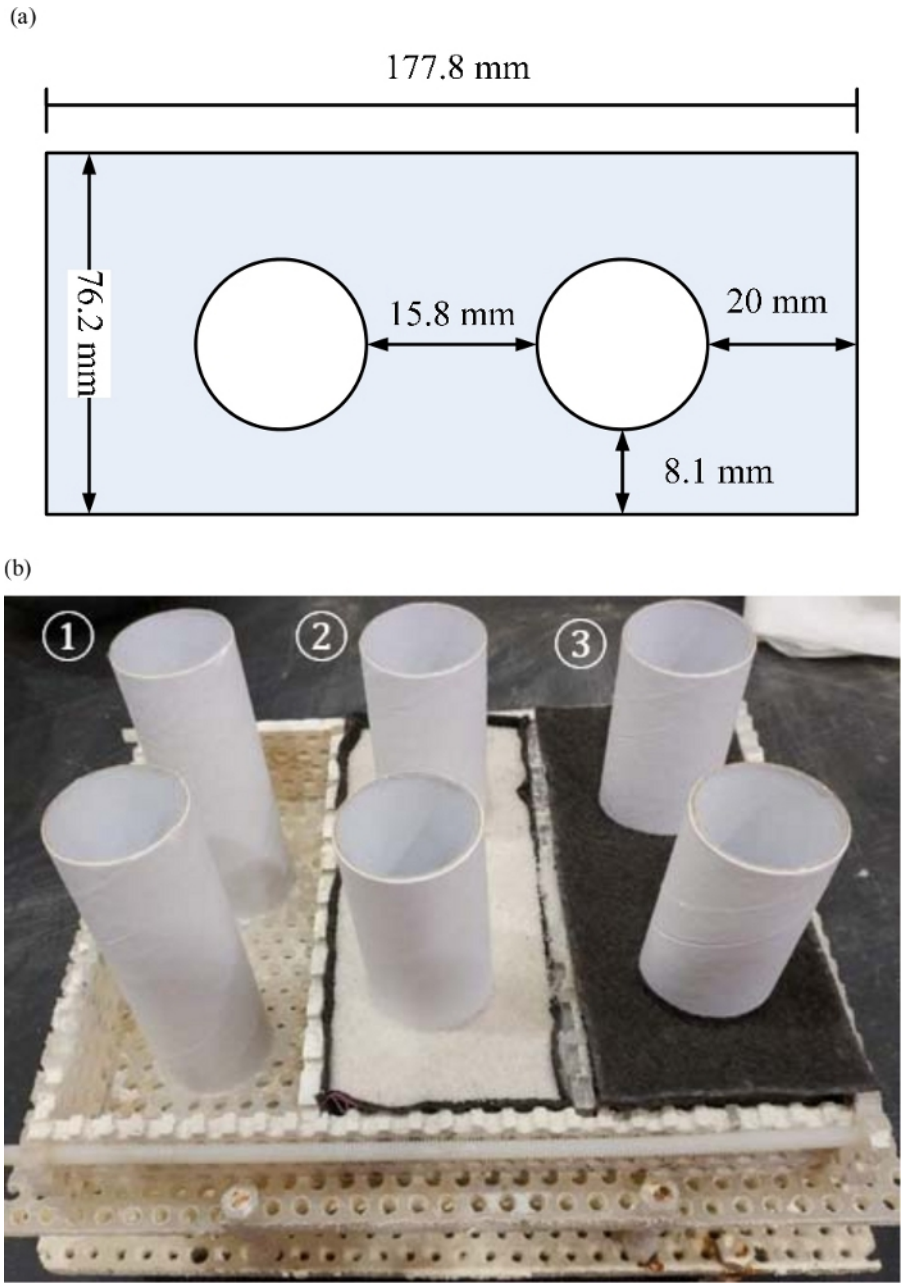

Figure 3: Cored brick molds.
(**a**) Holes distribution on one chamber of mold; (**b**) Sample preparation of bio-cored bricks ① Assembled mold for cored brick; ② Sand added into the mold; ③ Flexible cover added on the top of sand sample. The cored brick mold included a rigid holder, a flexible layer, and cardboard tubes. The size of cardboard tube was 60 mm x 140 mm x 60 mm. Three chambers were included in one mold and the size of each chamber of mold was 177.8 mm in length, 76.2 mm in width and 38.1 mm in height. Please click here to view a larger version of this figure.

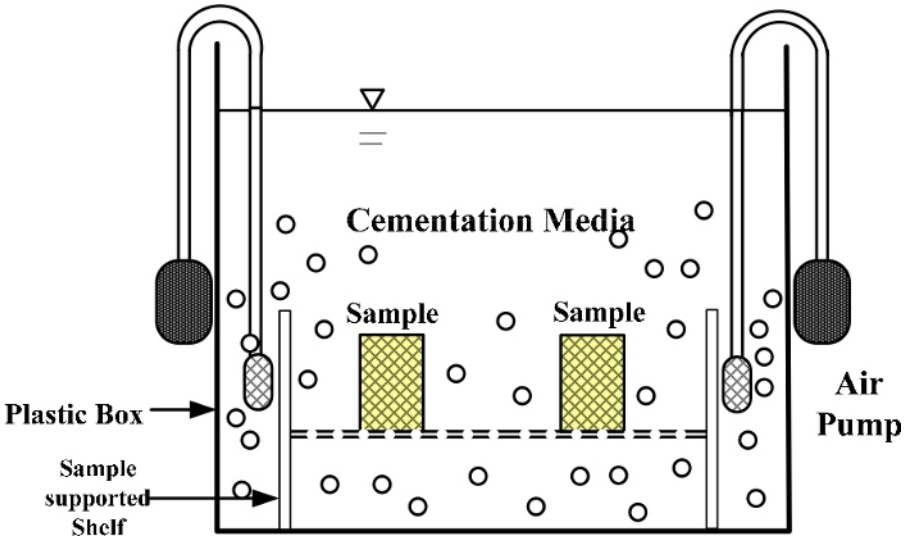

Figure 4: Sketch of batch reactor for MICP.
All samples were prepared in a completely stirred tank reactor. The batch reactor included a plastic box to contain soil samples and cementation media, a magnetic mixer to keep the solution uniform, and an air pump to provide oxygen for bacteria. A major feature of this method is to allow soil samples fully immerse into the cementation media and to allow the cementation media to freely penetrate into the soil samples. Please click here to view a larger version of this figure.

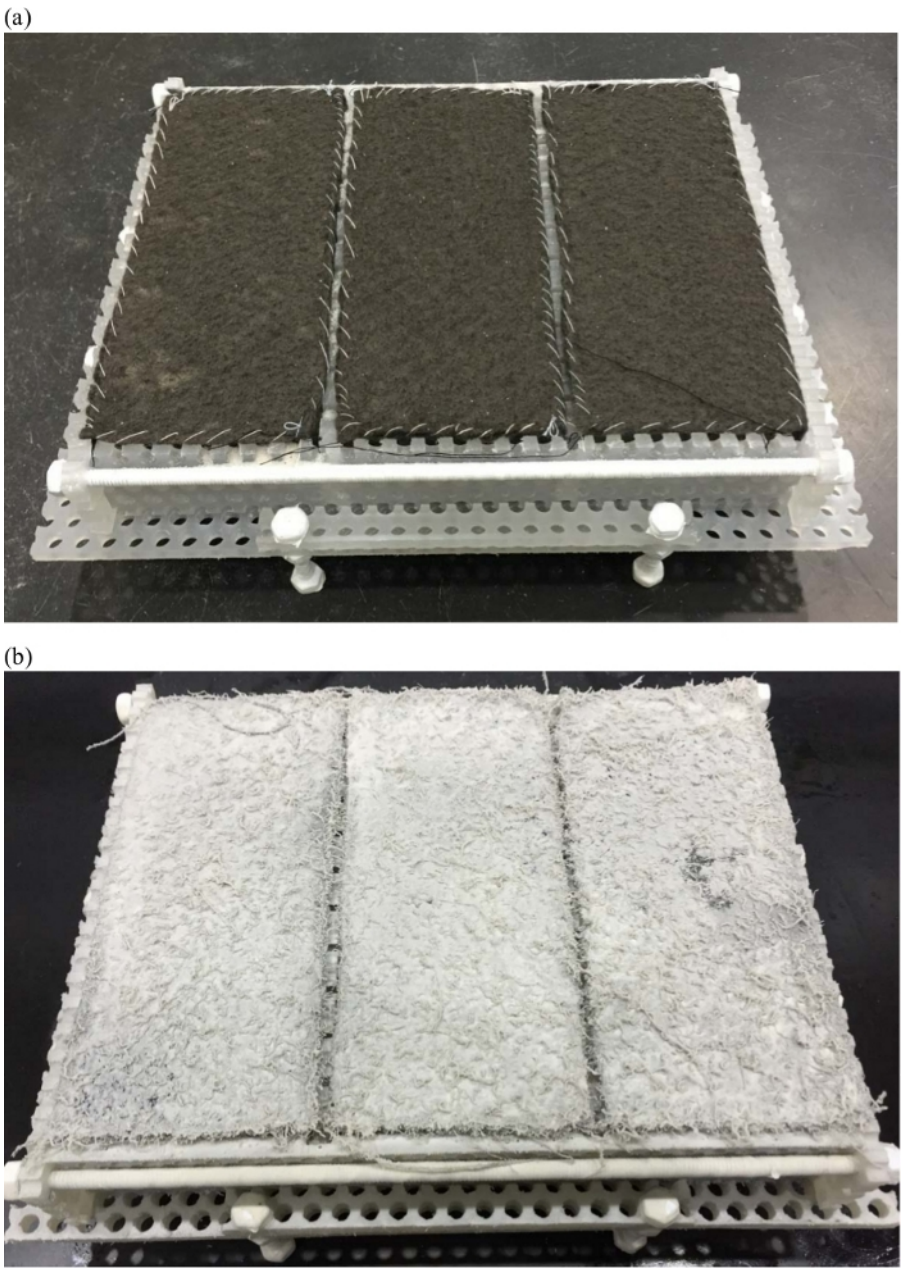

Figure 5: Soil samples placed on the supported shelf.
(**a**) before the MICP reactions; (**b**) after the MICP reactions. The bio-brick samples were prepared with the full contact mold. A geotextile cover was applied on the top of the mold. Each bio-brick had a size of 177.8 mm in length, 76.2 mm in width and 38.1 mm in height. Please click here to view a larger version of this figure.

(a)

(b)

Figure 6: (a) Synthetic fiber; (b) natural palm fiber.
The synthetic fiber was a homopolymer polypropylene multifilament fiber with a specific gravity of 0.91. It is chemically inert with high acid salt resistance. The length and thickness of the fibers used in this study were 12 and 0.1 mm, respectively, with an aspect ratio of 120 between the length and thickness of the fiber. Please click here to view a larger version of this figure.

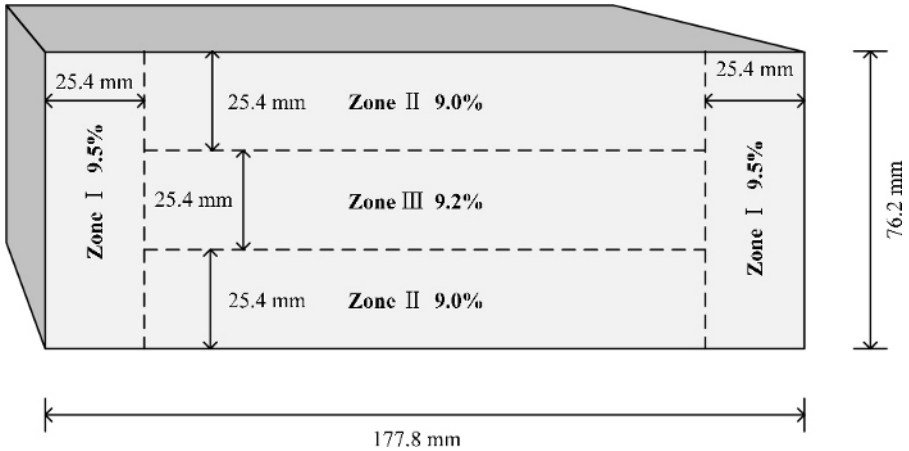

Figure 7: $CaCO_3$ distribution in three areas of MICP-treated sample.
Three zones were divided in the sample. In each zone, the amount of precipitated $CaCO_3$ was measured and calculated as a percentage by weight. Please click here to view a larger version of this figure.

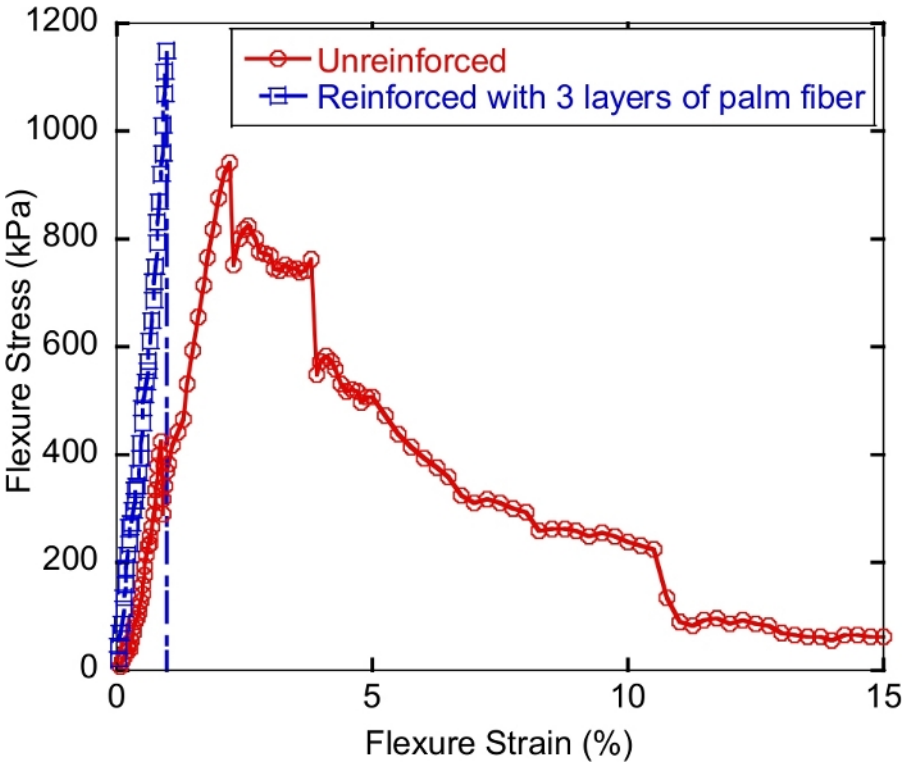

Figure 8: Flexure stress as a function of flexure strain for unreinforced bio-brick and palm fiber reinforced bio-brick with MICP treatment.
The flexure strength of unreinforced bio-brick was 1,150 kPa, while that of reinforced bio-brick was 980 kPa. The flexure strain was improved significantly by addition of palm fiber. These results indicate that palm fiber can contribute to the improvement of ductility. Please click here to view a larger version of this figure.

Chemical	Concentration of cementation media (g/L)			
	0.25 M Ca	0.5 M Ca	1 M Ca	1.5 M Ca
NH_4Cl	10	10	10	10
Nutrient broth	3	3	3	3
$NaHCO_3$	2.12	2.12	2.12	2.12
Urea	15	30	60	90
$CaCl_2{\cdot}2H_2O$	36.8	73.5	147	220.5

Table 1: Chemical components of cementation media. The chemicals were used to prepare four concentrations of cementation media in 0.25 M Ca, 0.5 M Ca, 1 M Ca, and 1.5 M Ca. The urea-Ca^{2+} molar ratio was fixed as 1:1.

Discussion

The MICP technique by immersion was presented in this paper. Soil samples were immersed into the batch reactor to get fully penetrated by cementation media in the MICP process. In this method, a full contact flexible mold, a rigid full contact mold, and a cored brick mold were applied to prepare MICP-treated samples.

Different molds can be designed for different geometry requirements. The fibrous structure of geotextile increased the contact area between sand and cementation media, which effectively increased the penetration of cementation media into soil samples. The large amounts of pores of geotextile also allowed more precipitation occurring inside the mold to improve the strength of MICP-treated samples. The soil properties of MICP-treated samples, such as strength and calcite content, were greatly improved by using these molds in the immersion method. The immersion method showed an advantage in preparing prefabricated building materials, such as bio-bricks and bio hollow bricks. Synthetic fiber and natural fiber can be added in the soil to enhance the MICP-treated samples. Fiber addition is an appropriate way to improve prefabricated MICP-treated materials. MICP technique with immersion method can be applied to perform surface treatment for cement-treated bricks to improve their properties, such as enhancing the durability of cement-treated materials by reducing their permeability. However, this immersion method is difficult to implement in the field due to the limitation of its operation, future research on how to use this method on site is needed to apply this method in the field.

Disclosures

We have nothing to disclose.

Acknowledgments

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