Research Article

Effects of Biostabilization on Engineering Properties of Geomaterials

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Biostabilization is a newly proposed method to improve the strength and durability of geomaterials, and it can serve as an alternative to chemical and mechanical stabilization. The objectives of this study are to perform biostabilization treatments for selected roadway construction geomaterials and to evaluate the biostabilization effects on engineering properties of the geomaterials. Three types of geomaterials were selected, and two of them were compacted soil from unpaved road surface. *Bacillus pasteurii*, the biostabilization bacterium, was used to induce mineral precipitates within the geomaterial pore spaces, where the biostabilization effects were performed. Two types of liquid incubation media, containing NH_4Cl or $(\text{NH}_4)_2\text{SO}_4$, were applied for bacteria culturing. Unconfined compression, scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), and X-ray diffraction (XRD) measurements were conducted to evaluate the biostabilization results. From unconfined compression, sample strength performance was improved by the biostabilization treatments; the benefits of biostabilization were pronounced by a relatively long culturing time and an oven-dry procedure; the liquid culturing medium containing NH_4Cl performed better than the medium containing $(\text{NH}_4)_2\text{SO}_4$. After biostabilization, SEM photographs provided direct evidence for the precipitates induced by bacteria within the geomaterial pore space. The precipitates either connected the adjoined particles or partially covered the particle surface, which increased the surface roughness. EDS and XRD results indicated that calcite, dolomite, and albite were the major precipitates produced during biostabilization treatments. In conclusion, biostabilization ameliorated the microstructures of the geomaterials and improved their strength. Future research topics should include the applications of biostabilization for in situ road construction.

1. Introduction

Geomaterials, such as soils and aggregates, are widely applied in roadway infrastructure systems. Soils are used for embankment fills and subgrades, while aggregates are used for pavement bases and in Portland cement concrete (PCC) or asphalt concrete. The quality of geomaterials directly

influences the longevity of infrastructure systems; hence, enhancing the stability of geomaterials is a prominent issue considered during construction. For example, at sites with marginal or weak soils, mechanical and chemical stabilization should be applied [1]; when aggregates with high porosity are used in PCC or asphalt concrete [2, 3], chemical treatments with sodium silicate solutions are usually

performed to seal the pores, improve the strength and durability of PCC or asphalt concrete, and prevent concrete deterioration [4]. However, the applicability of those operations was limited by the cost, suitability, availability, constructability, and environmental concerns.

Biostabilization has been proposed as an alternative to improve the mechanical properties of geomaterials in a sustainable and environment friendly way [5, 6]. In biostabilization, naturally occurring or engineered bacteria are injected into the geomaterials, and precipitates of inorganic cementing materials, induced by microbe-produced polymers, serve as binding agents to bond soil particles and plug pores among coarse aggregates [7].

Previous studies involving biostabilization were mostly directed to laboratory studies of carbonate precipitates as the cementation material, with emphasizing on the selection of materials and procedures, the morphology of carbonate precipitates, as well as the evaluation of biostabilization effects. For example, DeJong et al. [5] recommended to use *Bacillus pasteurii* as the bacteria and considered the stabilization procedures as two steps, i.e., bacteria incubation and cementation treatments. Whiffin et al. [8] reported the use of *Sporosarcina pasteurii* as an alternative bacterium in biostabilization. Ramakrishnan et al. [6] reported the use of water, urea, and phosphate buffer as three growth media and concluded that *Bacillus pasteurii* grew in phosphate buffer provided optimal biostabilization results in concrete based on the evaluation of precipitate sizes and concrete strength against adverse environments, e.g., sulfate, alkaline, and freeze-thaw cycles. Bachmeier et al. [9] reported that immobilization of bacteria-produced urease, an enzyme inducing urea decomposition and carbonate precipitates, could result in smaller and less organized crystals comparing with free urease. Jonkers et al. [10] monitored the change of cement pore size under biostabilization and reported a shift of the pore size distribution from a range of 0.1–1.0 μm to a range of 0.01–0.1 μm , demonstrating the shrinkage of pore size after biostabilization treatments. Burbank et al. [11] and Meyer et al. [12] presented the effects of biostabilization on soil resistance to seismic-induced liquefaction and dust control through wind tunnel testing, and both effects implied that biostabilization enhanced the binding strength among soil particles.

Evaluations of biostabilization results fall into two scales. The microstructures and chemical compositions of biostabilized and untreated geomaterials are characterized by scanning electron microscopy (SEM) and X-ray diffraction (XRD) measurements. For example, DeJong et al. [7] applied SEM and observed calcite precipitates in the pore spaces of biostabilized silica sand. XRD is used to quantitatively analyze the mineral constituents of precipitates. For example, Bang and Ramakrishnan [13] measured the weight fraction of the calcite precipitates for samples under biostabilization or control treatments and verified the causal relation between bacteria activity and amount of calcite precipitates. For macroscale, pore size distribution is one of the critical characteristics, which was measured with mercury porosimetry [10], and the decreasing of porosity implies positive effects of biostabilization.

Biostabilization is pollution-free [6]; and the non-pathogenic bacteria used in biostabilization are native to subsurface soil environment [5]. Applications of biostabilization can substantially reduce the use of nonrenewable resources and energy, as well as decrease the production of nonbiodegradable wastes. Hence, biostabilization mitigates environmental burden of geo- and civil engineering operations [10].

The current designs and tests of biostabilization are mostly based on laboratory experiments under controlled environments. To assist implementations, an experiment based on samples from roadway sites should be appreciated. For example, some of the county roads are remained unpaved in China, where compact soils (gravel) are applied on road surface. Rainfall, strong wind, drought, and freezing-thawing could cause road surface damages, while biostabilization could be one of the potential ways to alleviate such damages. Thus, the objectives are to perform biostabilization treatments for selected geomaterials and (2) evaluate the biostabilization effects on engineering properties of geomaterials.

2. Materials and Methods

2.1. The Procedures of Biostabilization. Calcite (calcium carbonate, CaCO_3) precipitate in biostabilization treatments is defined as “microbiologically induced calcite precipitation (MICP),” which belongs to a broader category of science called biomineralization. MICP process can be presented with a simplified reaction as follows [11]:

where NH_4^+ increases the pH values in the liquid phase and CO_3^{2-} can react with calcium ions (Ca^{2+}) and produce CaCO_3 precipitates as follows:

A bacterial species named *Bacillus pasteurii* was adopted in this study following the suggestion by DeJong et al. [7] because (1) *Bacillus pasteurii* is an aerobic and urease production bacterium, naturally occurring in soil; (2) *Bacillus pasteurii* cells do not aggregate, ensuring a relatively high cell surface to volume ratio and free urease redistribution during biostabilization treatments; and (3) *Bacillus pasteurii* provides two sources of CO_2 , cellular respiration and urea decomposition [5].

In this study, the freeze-dried bacteria were procured from America Type Culture Collection (ATCC). Following the ATCC instructions, 1.0 L liquid culturing medium was made with 20.0 g yeast extract, 10.0 g $(\text{NH}_4)_2\text{SO}_4$ or NH_4Cl , and 0.13 M Tris buffer (pH=9.0). Yeast extract provides nutrients and Vitamin B for bacteria reproduction, where Vitamin B also promotes the growing speed of *Bacillus pasteurii*. $(\text{NH}_4)_2\text{SO}_4$ or NH_4Cl serves as nitrogen sources for amine groups, enzyme, and other proteins of bacteria. ATCC recommends the used of $(\text{NH}_4)_2\text{SO}_4$ during incubation of bacteria; however, SO_4^{2-} can be harmful to some civil engineering materials, so the culturing medium with

NH_4Cl is proposed. In this study, both types of NH_4^+ , i.e., $(\text{NH}_4)_2\text{SO}_4$ and NH_4Cl , were considered. Before culturing, *Bacillus pasteurii* was first rehydrated using the culturing medium (5–6 ml) under a $30^\circ\text{C} \pm 2^\circ\text{C}$ and aerobic environment, and one single colony was obtained from the rehydrated bacteria via the streak plate method. Then, the selected colony was transferred into a flask and mixed with the culture medium. Such a mixture was placed in a shaking incubator under a $30^\circ\text{C} \pm 2^\circ\text{C}$ and aerobic environment for 2 days to culture enough bacteria.

After the 2-day culturing, the concentration of *Bacillus pasteurii* was increased. Before application, the concentration of bacteria was adjusted to 1.6×10^8 cells mL^{-1} by adding new culturing liquid. The final concentration of *Bacillus pasteurii* was calibrated by optical density (OD) via a UV-visible spectrophotometer. Culturing liquid under the target bacteria concentration was then injected into geomaterial samples, and those samples were stored under a $30^\circ\text{C} \pm 2^\circ\text{C}$ and aerobic environment for 5 days to finish a single-round of biostabilization treatment. In this study, we also allowed a duplication of the 5-day biostabilization treatment. Therefore, samples under single-round and double-round of culturing were obtained. Before the performance evaluations, the biostabilized geomaterials were saturated with water and air-dried or oven-dried at 110°C to simulate a range of ambient (weather) conditions occurred on gravel roads.

2.2. Geomaterial Samples. Three types of geomaterials were tested in this study: (1) the standard silica sand was the clean, dry, and free-flowing uncemented sand, with a porosity of 40%, a specific gravity of 2.65 Mg m^{-3} , and a uniformity coefficient of 1.4; (2) surface materials from an unpaved road were sampled, with classification as poorly graded and silty sand (SP-SM soil); and (3) soils from a highway construction site were obtained and classified as silty sand (SM soil). DeJong et al. [5] and DeJong et al. [7] adopted the silica sand to demonstrate the positive effects of biostabilization. The SM soil samples in this study were subjected to frequent freeze-thawing cycles, which may lead to damages such as frost boils, rutting, and potholes. Photographs for the three types of geomaterials are shown in Figure 1.

PVC molds were used to hold the geomaterial samples. The mold was a column with 7.3 cm in height and 3.5 cm in cross-sectional diameter. Each mold included two pieces, and stainless-steel clamps were used to join the tops of those two pieces and rubber bands were used to bind the bottoms of those two pieces together. The two-piece design of PVC molds allowed the geomaterial samples to be easily transported out of the molds after biostabilization, and the interior surface of PVC molds was covered by the aluminum foil to minimize the sample lost during transportation. PVC molds were filled with the geomaterial samples and stored within glass beakers. Before injecting the bacteria liquid, aluminum foil was also wrapped around the bottom of PVC molds to reduce liquid leaking, and the glass beakers and PVC molds were placed into an autoclave for a 25-minute sterilization, with temperature equal to 120°C .

2.3. Testing Methods. After the biostabilization treatment, geomaterial samples were transported out of the PVC molds. Since the two-piece design of PVC molds was easy to split, after releasing the stainless-steel clamps, the undisturbed geomaterial samples could be demolded easily. Multiple tests were performed to evaluate the biostabilization effects on the engineering properties of the geomaterials.

Unconfined compression test is a simple method to assess the undrained strength and the stress-strain characteristics of the geomaterials, and it can verify the biostabilization effects on bonding geomaterial particles. A Geotest Instrument Corporation model S2010 device with 907.2 kg capacity was used in this study, and the procedures followed the ASTM D2166–16 Standard Test Method for Unconfined Compressive Strength of Cohesive Soil [14]. The load applied in the measurement could be read from the device in an increment of 4.5 kg. The compression strength (σ_c , kPa) under a given load can be determined with the following equation:

$$\sigma_c = \frac{P}{A}, \quad (3)$$

where P (g) represents the load and A (cm^2) is the mean cross-sectional area of the sample. Noted that the device provided values when gauge readings ≥ 22.5 kg, and any gauge readings < 22.5 kg would be marked as $\sigma_c = 0$, indicating negligible compressive strength.

The scanning electron microscope (SEM) provides a nondestructive way to measure the surface morphology of geomaterial samples. Accelerated electrons in SEM carrying certain amount of kinetic energy are dissipated as a variety of signals due to the interactions between electrons and samples. These signals include secondary electrons, backscattered electrons, diffracted backscattered electrons, photons, visible light, and heat. SEM images are produced by the secondary electrons and backscattered electrons [15]. Diffracted backscattered electrons can be used to determine microstructures and mineral constituents.

Energy-dispersive X-ray spectroscopy (EDS) is a technique for analyzing the element abundance of the geomaterial samples with or without biostabilization treatments. EDS measurements are based on the X-ray emission spectrums from the samples, and each chemical element corresponds to a specific set of peaks in the observed spectrums, depending on its atomic structure [15, 16]. At ground state, an atom has electrons in quantized energy levels forming layered electron shells bound to the nucleus. To obtain the characteristic X-ray emissions, a high-energy beam of charged particles or a beam of X-ray strikes the geomaterial samples, excites electrons in inner (low-energy) shells, ejects them from inner shells, and creates electron vacancies. Electrons from outer (high-energy) shells can migrate to inner shells and fill those vacancies. Energy differences between high-energy and low-energy shells can be released as X-ray emissions. The energy of such X-ray emissions is measured by an energy-dispersive spectrometer, and the measured results correspond to the element abundance of the geomaterial samples.

FIGURE 1: Three types of geomaterials included in this study: standard silica sand (a), surface material (SP-SM soil) (b) from an unpaved road, and surface material (SM soil) (c) from a highway construction sites.

X-ray diffraction (XRD) was used to determine the chemical compositions of the geomaterial samples with and without biostabilization. XRD characterizes chemical components based on the crystalline species [17, 18]. Crystals are composed of regularly aligned atoms, and planes of atoms in crystals are separated by a constant distance. Such a constant distance is the characteristic factor of the crystalline species. When monochromatic X-rays with finite energy and wavelength strike the crystal in a specific direction, presented by the angle (θ) between the X-rays and the normal direction of the crystal surface, the X-rays scattered by crystal planes may be superposed and reinforced based on θ , expressed via Bragg's law. Because minerals have their unique θ when diffraction occurs, depending on their interatomic distances within a mineral crystal, unique arrays of diffraction maxima can be obtained from XRD. Although the geomaterials contain particles or fine powders, there are still sufficient crystals properly oriented so that every set of crystal plane will be capable of diffraction, which ensured the

operability of XRD measurements in determining the mineral species.

3. Result and Discussion

3.1. Unconfined Compression Tests. Geomaterial samples were treated using the liquid medium with NH_4Cl or $(\text{NH}_4)_2\text{SO}_4$ for single-round or double-round of culturing and then saturated, air-dried, or oven-dried before the unconfined compression tests. Because the untreated, loose silica sand had no cohesion, therefore, no unconfined compressive strength, comparing with the SP-SM soil and SM soil, it was used as a reference to represent the “absolute effect” of the biostabilization. The results of unconfined compression tests are presented in Figure 2; log-scale was used in the y -axis due to the data range. Because the unconfined compressive strength (σ_c) is drawn in log-scale, $\sigma_c = 0$ cannot be directly presented; hence, negligible unconfined compression ($\sigma_c = 0$) is placed at the level of x -axis. In additional, experiments on

the silica sand treated in the liquid medium without the bacteria were performed to present the cohesion induced by the culturing procedures, and only two positive compression strength were observed from oven-dried samples, with values equal to 46.1 kPa and 19.6 kPa for NH_4Cl and $(\text{NH}_4)_2\text{SO}_4$ media, respectively.

In Figure 2, the saturated samples did not present any strength. For air-dried and oven-dried samples, besides the coherence due to the drying procedures, biostabilization contributed to the increase of σ_c values. In general, the double-round culturing performed better than the single-round culturing, which is as expected because the longer the culturing period was, the stronger the MICP reaction were performed.

Oven-dried samples tended to produce larger σ_c values comparing to the air-dried samples. One possible reason is when the water evaporated quickly under relatively high temperature, the water films between soil particles were shrunk and particles were pushed together, which enhanced the particle connections and made the soil matrix dense, while in air-dried samples, soil structures as well as the water films on particle surface tended to be retained. However, because of biostabilization, σ_c values of oven-dried silica sand samples were 2–40 times larger comparing to the values from the oven-dried silica sand samples treated without bacteria, while no effective σ_c values were observed for air-dried silica sand samples treated without bacteria. Thus, positive effects of biostabilization can be demonstrated.

Comparing the liquid media with NH_4Cl or $(\text{NH}_4)_2\text{SO}_4$, the trends of σ_c values in saturated, air-dried, and oven-dried samples among the three geomaterials were similar; measurements under the saturated condition did not report effective σ_c , and σ_c achieved maximum values under oven-dried conditions. However, since sulfates promote the formation of sulfuric acid, which could damage concrete, σ_c values for samples with $(\text{NH}_4)_2\text{SO}_4$ in the culturing medium tended to be lower than ones with NH_4Cl . Thus, NH_4Cl would be a better choice for the biostabilization culturing medium.

3.2. SEM Photograph. Among all the samples, the oven-dried silica sand treated with the NH_4Cl liquid medium (Figure 2(a)) showed a large increase in unconfined compressive strength, especially for the samples with double-round culturing. Therefore, it would be interesting to perform analyses for their microstructure and chemical compositions. Another benefit of using silica sand in these measurements is the original microscale shape of silica sand is relatively

uniform, and the chemical compositions of silica sand are relatively simple, comparing to SP-SM soil and SM soil. Since all the three types of geomaterials were processed with the same biostabilization process, the consequences obtained from silica sand can be easily generated to the SP-SM soils and the SM soils.

Figure 3(a) shows the uniform and regular-shaped silica sand particles and the pore spaces between particles, which provides a reference for the morphology of silica sand. The surface of individual particle is relatively smooth with no additional substances. Some cracks, embossing, and cavities existed but can only be observed in $3000\times$ SEM photographs, which is much smaller than the size of biostabilization-induced precipitates. Thus, we omit such photographs for simplicity.

For the silica sand after biostabilization treatments, SEM photographs were taken at multiple magnifications, and $100\times$, $300\times$, and $600\times$ pictures are presented in Figures 3(b)–3(d), respectively. When sand particles are closed to each other, the pore spaces between them were partially filled and some particle-particle bridges were formed by precipitates induced by bacteria. However, most of the pore spaces were too large and the separation of particles was too wide, where filling or connections cannot be formed directly. In those cases, precipitates were observed on particle surface, which increased the particle surface roughness. This provided an explanation for the increasing unconfined compressive strength in silica sand after biostabilization, especially when silica sands were oven-dried.

3.3. EDS and XRD Tests. Figure 4 presents a ($1500\times$) SEM picture that focused on a portion of particle surface. The shape and texture of the precipitates can be observed, including round, flat, clubbed, and irregular shapes. Four possible substances in Figure 4 are labelled, with the corresponding EDS results presented in Table 1. The element analysis for the untreated silica sand is also presented. Untreated silica sand consists of silicon, oxygen, and aluminum, while silica sand after biostabilization showed relatively complicated chemical compositions.

To examine the chemical compositions within the precipitates, XRD tests (Figure 5) were performed. Four main minerals were detected, including calcite (CaCO_3), dolomite [$\text{CaMg}(\text{CO}_3)_2$], quartz (SiO_2), and albite [$(\text{Na}, \text{Ca})(\text{Si}, \text{Al})_4\text{O}_8$]. Combining with the EDS results, calcite, dolomite, and albite were produced from the biostabilization. As shown in Figure 4, Label 1 indicated calcite and Label 3 indicated albite, while more than one mineral constituent existed at the other two locations.

FIGURE 2: The results of unconfined compression tests for silica sand (a), SP-SM soil (b), and SM soil (c) after single-round of culturing (S) and double-round of culturing (ID). Red diamonds mark the measured σ_c results. Because the unconfined compressive strength (σ_c) is drawn in log-scale, zero values (negligible unconfined compression) are placed at the level of horizontal axis.

FIGURE 3: Continued.

FIGURE 3: The SEM photograph of original silica sand and silica sand after biostabilization: untreated silica sand (50 $\times$) (a); silica sands after biostabilization (100 $\times$) (b), (300 $\times$) (c), and (600 $\times$) (d). The white squares in (b) and (c) identify the magnified regions for (c) and (d), respectively.

FIGURE 4: The SEM photograph of silica sand after biostabilization (1500×). Four red labels present 4 possible precipitation substances produced in biostabilization.

TABLE 1: Element abundance analyses with EDS for the 4 labelled substances in Figure 4 and the untreated silica sand.

[illegible]

FIGURE 5: XRD results for untreated silica sand (a) and silica sand after biostabilization (b).

4. Summary and Conclusions

Biostabilization was reported as a novel method to enhance unconfined compressive strength and improve other engineering properties of geomaterials. Biostabilization utilized certain types of bacteria to induce precipitates on geomaterials, which bonded the particles and partially filled the pore spaces. In this study, biostabilization was applied on three types of geomaterials, i.e., silica sand, SP-SM soils, and SM soils, where the two soil samples were from roadway surface. Biostabilization was performed using *Bacillus pasteurii*, and the selected geomaterial samples after biostabilization were tested for unconfined compressive strength, surface morphology, and chemical compositions.

Based on the results, biostabilization increased the averaged unconfined compressive strength by 3–6 times. Additional culturing period (double-round culturing) and drying with relatively high temperature (i.e., oven-dried samples) could magnify the biostabilization effects. By SEM and chemical analysis, the precipitates induced by bacteria included calcite, dolomite, and albite, and the precipitates adhered to particle surface to provide connections between adjoint particles and partially filled the pore spaces and increased the particle surface roughness, which explained the increasing of unconfined compressive strength from microscale perspectives.

Biostabilization, as a new geomaterials improvement technology, was studied to develop a series of standard procedures for bacteria culturing, biostabilization treatments for geomaterials, and performance evaluation. Results from this study verified the effectiveness of the biostabilization method in improving the geomaterial stability, quantified by unconfined compressive strength.

Comparing to literature results, this study adopted the soil and highway embankment and the subgrade filling-material as the geomaterial samples. New mineral composites, such as dolomite, calcium phosphate, and albite, were detected after the biostabilization treatments. The findings in this study provided evidence for the effects of biostabilization and reinforced our understanding to the biostabilization method. Future research could include the

investigation of in situ biostabilization applications for soils, especially for gravels utilized in roadway infrastructure systems or the roadside soils received alkaline substances during road maintenance [19, 20].

Data Availability

The original data used to support the findings of this study are available from the first author upon request.

Additional Points

Highlights/Core-Ideas.(1) Biostabilization on three types of geomaterials was performed.(2) Precipitates produced by biostabilization included calcite, dolomite, and albite.(3) Pore space of samples was partially filled by precipitates after biostabilization.(4) Particle bonding and surface roughness were improved by biostabilization.(5) Unconfined compressive strength of samples after biostabilization was increased.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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