

Microbially Induced Cementation to Control Sand Response to Undrained Shear

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Abstract: Current methods to improve the engineering properties of sands are diverse with respect to methodology, treatment uniformity, cost, environmental impact, site accessibility requirements, etc. All of these methods have benefits and drawbacks, and there continues to be a need to explore new possibilities of soil improvement, particularly as suitable land for development becomes more scarce. This paper presents the results of a study in which natural microbial biological processes were used to engineer a cemented soil matrix within initially loose, collapsible sand. Microbially induced calcite precipitation (MICP) was achieved using the microorganism *Bacillus pasteurii*, an aerobic bacterium pervasive in natural soil deposits. The microbes were introduced to the sand specimens in a liquid growth medium amended with urea and a dissolved calcium source. Subsequent cementation treatments were passed through the specimen to increase the cementation level of the sand particle matrix. The results of both MICP- and gypsum-cemented specimens were assessed nondestructively by measuring the shear wave velocity with bender elements. A series of isotropically consolidated undrained compression (CIUC) triaxial tests indicate that the MICP-treated specimens exhibit a noncollapse strain softening shear behavior, with a higher initial shear stiffness and ultimate shear capacity than untreated loose specimens. This behavior is similar to that of the gypsum-cemented specimens, which represent typical cemented sand behavior. SEM microscopy verified formation of a cemented sand matrix with a concentration of precipitated calcite forming bonds at particle-particle contacts. X-ray compositional mapping confirmed that the observed cement bonds were comprised of calcite.

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Introduction

The improved engineering behavior and performance of cemented soil over its uncemented state contributed to the development of artificial cementation treatment methods that could effectively cement large in situ deposits. Over the last century, various methods of treating soil with a solution/grout have been developed, and today they are used widely in geotechnical projects. The injection formulas utilized in these projects have been man-made, and when injected into the subsurface, they often alter the subsurface pH level and in a few cases have been toxic.

An innovative alternative approach lies in the combined use of microorganisms, nutrients, and biological processes naturally present in subsurface soils to effectively improve their engineering properties. By temporarily regulating the concentration of microorganisms and nutrients in the soil, a cement component could

be added to the soil matrix of an initially uncemented deposit. Such a natural process would use nonpathogenic organisms that are native to the subsurface environment. This type of process is more environmentally friendly than conventional treatment methods.

The great promise of the use of biological treatments has been demonstrated in other fields. Microbes have been used in environmental applications, including the stabilization of metals (Etemadi et al. 2003), development of biological shields for zonal remediation (Yang et al. 1993), environmental stabilization of contaminated soils (Khachatoorian et al. 2003), and encapsulation of hazardous and other contaminants in natural soils and acid mine tailings. Microbially enhanced oil recovery (MEOR) uses microbes to increase the efficiency of pumping and production in lower yield oil reservoirs. Bacteriogenic mineral plugging is a patented process that uses calcite precipitating bacteria to reduce the permeability of granular media (U.S. Patent No. 5,143,155). Researchers have even reported the use of microbes to remediate cracks in concrete structures (Ramakrishnan et al. 1998; Ramachandran et al. 2001).

The focus of this study was to examine the effect of biological treatment on the stress-strain-strength properties of cohesionless soils assessed using monotonic undrained triaxial tests (Fritzges 2005). Following a review of cementation in sands and its effect on soil properties, biological processes in soils relevant to the research herein are presented. Details of specimen preparation, process monitoring, and undrained triaxial shear facilitate in-depth examination of the degree to which the strength of sand can be improved by the microbially induced calcite cementation treatment process. These behavioral observations are confirmed with

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microscopy analysis of the treatment method at different levels of resolution as well as X-ray compositional mapping.

Cementation in Sands

Natural Cementation

In the natural environment, cement is created through chemical deposition and chemical processes associated with weathering. Naturally cemented sands exist at various places in the earth's crust. The formation of sandstones in particular is primarily attributed to the precipitation of calcite cement (Saxena and Lastrico 1978). Cementation in sands is also facilitated by small amounts of other agents such as silica, hydrous silicates, and hydrous iron oxides (Clough et al. 1981). Cementation from calcite, or calcium carbonate (CaCO_3), will be the primary focus herein. Natural cementation usually varies not only on a small scale but also on a larger scale over the entire stratigraphic interval of a particular formation (Saxena and Lastrico 1978). The degree of cementation within a deposit can vary widely and is controlled by the characteristics of the environmental conditions that facilitate cementation as well as the degree and mode of weathering, which gradually degrades the cement formed.

Calcite precipitates in situ as a cementing agent through two different mechanisms. First, it precipitates by deposition from water saturated with calcium carbonate. Second, calcite can be formed from chemical exchanges at the water-soil grain surface interface (Ismail et al. 1999b). A number of factors either inhibit or facilitate the process of cementation, contributing to its variability. These factors include the pore-water chemistry (degree of supersaturation), the ability to transport Ca^{2+} and/or HCO_3^- to the precipitation site, the presence of pre-existing carbonate substrate, and the permeability as well as the texture, composition, and stabilization of the sediment itself (Molenaar and Venmans 1993, Hall et al. 2004, Mozley and Davis 2005). In addition there is growing evidence that microbial activity plays an important role in calcite precipitation (Mozley and Davis 2005).

Synthetic Cementation Methods

Traditionally, artificial soil cementation has been used either to improve the properties of an uncemented soil deposit (e.g., reduce liquefaction potential, densification, etc.) or to replicate light cementation and/or aging effects that exist in natural sand deposits but have been compromised by the sampling process.

When artificial cementation of the subsurface is the objective, treatment options are generally limited to nondisplacement grouting techniques. An important property of nondisplacement (permeation) grouting is its additive nature because the method should not destroy the in situ state of a soil deposit (i.e., pre-existing light cementation is not destroyed during treatment). Chemical grouting is typically used to treat granular soils (Karol 2003; Leonard and Moller 1963) with a permeability range from 10^{-4} to 10^{-1} cm/s (Neelands and James 1963). Once a chemical grout is injected, the materials set, or gel, by precipitation of sedimenting particles or the tangling of long crystals that fill voids. The time it takes materials to gel, or gel time, depends on the concentration of activator, inhibitor, and catalyst and can range from 1 to 300 min (Karol 2003). Filling the voids with a polymerized gel results in increased shear strength, which increases the bearing capacity and stability of the soil, reduces settlements, and immobilizes particles in the granular mass.

As noted, artificial cementation has also been used to restore natural cementation conditions in samples that have been compromised during sampling. Undisturbed sampling of sands is extremely difficult and costly, and the strains the sample undergoes during sampling can destroy light cementation, distorting lab-measured properties. Cementing agents commonly used to study in situ cementation include calcium carbonate, gypsum, and Portland cement. The use of calcium carbonate as a laboratory cementation agent is attractive because it is often found in naturally cemented deposits. One particular system available for this purpose is Calcite In Situ Precipitation System, or CIPS, which involves injecting a proprietary chemical solution that causes the precipitation of calcite crystals within the pore fluid and on the surfaces of constituent sand grains (Ismail et al. 1999a). The level and rate of cementation can be altered by using multiple solution flushes or different chemical formulations. A series of successful studies using CIPS has been performed by researchers at the University of Western Australia (Ismail et al. 1999b; Ismail et al. 2001). Samples prepared using gypsum or Portland cement are typically prepared by dry mixing the cement and sand according to the soil-cement ratio required (Ismail et al. 2001). The soil-cement mixture is then pluviated, or funneled, into the specimen to achieve a desired density. A seating load is applied, and the specimen is saturated with water to initiate cementation.

Influence of Cementation on Specimen State and Response

The formation of cementation within a previously uncemented specimen lowers the effective void ratio, which in turn induces minor decreases in the porosity and increases in the density. These changes occur through cementation, adding substance to the particulate mass forming around individual particles and at particle-particle contacts.

Cementation mechanisms that coat or bridge individual soil particles, such as natural cementation, CIPS, and biologically induced cementation, gradually reduce the pore throat size within the soil fabric and thereby lower the hydraulic conductivity. Ferris et al. (1996) reported a permeability reduction from 13 and 5.5 Darcy (1.1×10^{-2} and 4.7×10^{-3} cm/s) to 2.8 and 0.9 Darcy (2.4×10^{-3} and 7.8×10^{-4} cm/s), respectively, for microbiologically induced calcite cemented specimens. Nemati and Voordouw (2003) created calcite cementation within a Berea sandstone core enzymatically and reduced the permeability by 98%.

The formation of cement at particle-particle contacts serves to increase the stiffness of the soil matrix, thereby influencing the small strain dynamic properties, which can be effectively characterized by shear modulus and damping ratio. Acar and El-Tahir (1986) performed resonant column testing of Monterey No. 0 sand specimens prepared at 50% relative density and cemented with varying percentages of Portland cement (0% to 4% by weight). They observed an increase of G_{max} with increases in confining pressure and cement content. They also determined that the damping ratio at any given strain ratio decreases with increasing cementation. Sharma and Fahey (2003) made similar observations and cited higher G_{max} values for calcareous soil specimens that were treated with CIPS. Through cyclic simple shear testing, they quantified trends in stiffness degradation with strain amplitude, noting that the degradation of stiffness depends on cycle number and amplitude, with the most rapid decreases after the point of initial cementation yield. It is noted that parallel observations of cementation causing an increase in the shear and com-

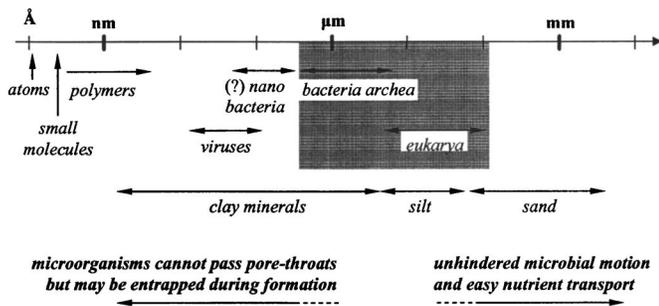


Fig. 1. Microorganism-pore throat size relationship (Mitchell and Santamarina 2005, ASCE)

pression moduli have been observed in laboratory tests on in situ samples of sand in which cementation formed naturally over time (Dvorkin and Nur 1996; Avseth et al. 2000).

Cementation also influences sand response to monotonic and cyclic shearing. The response of sand specimens cemented with gypsum, Portland cement, and CIPS to isotropically consolidated undrained triaxial compression (CIUC) tests was examined in an investigation by Ismail et al. (2001). The gypsum and calcite cemented specimens both exhibited brittle failure at low axial strain (<0.5%) followed by strain softening to about 5% axial strain. Specimens cemented with Portland cement exhibited a ductile yield and strain hardening response up through about 10% axial strain. The Portland cement specimen exhibited the highest yield strength in the test series, followed by the specimens treated with CIPS (calcite) and gypsum. The uncemented loose specimen yielded the lowest capacity and remained lower through strain hardening. Through these and other tests, Ismail et al. (2001) concluded that the CIPS method, with its high initial strength followed by brittle collapse and strain softening, is more representative of the behavior of in situ sand deposits with natural calcite cementation than alternative methods.

Biological Processes in Sands

When considering the relationship between microbial life and the subsurface environment, a number of factors of both a geotechnical and microbiological nature must be considered. The following sections weigh these considerations in relation to the use of microbes to improve the characteristics of cohesionless soils.

Geotechnical Factors

Bacteria are the dominant microorganisms in soils (Janssen 2006; Schloss and Handelsman 2004). Soil harbors an enormous diversity of microbial species ($>10^4 \text{ g}^{-1}$), and top soil contains 10^8 to 10^9 bacterial cells per gram. Bacteria vary in shape and may be nearly round, rod-like, or spiral. The cell diameter is usually in the range of 0.5 to 3 μm , and bacterial spores, stress resistant resting stages of some species, may be as small as 0.2 μm (Madigan and Martinko 2003). Microorganisms are capable of moving freely in the pore spaces of coarse-grained materials, either by self-propelled movement or by passive diffusion; however, the smaller pore throats offered by finer grained soils prohibit their entry and free passage. Therefore, bacteria are not expected to enter through pore throats smaller than approximately 0.4 μm , while fungi and protozoa require pore throat sizes greater than 6 μm for entry (Mitchell and Santamarina 2005). Fig. 1

provides a view of microorganism size in relation to their ability to pass through soil pore throats.

Bacteria may be referred to as colloids, since their effective diameter is less than 10 μm . Colloids are affected by many of the same physical and chemical processes that influence solute transport, i.e., advection, diffusion, dispersion, and sorption (Bradford et al. 2002). Bacterial transport and retardation within the interconnected porous network of a soil mass is controlled by relative size (pore throat relative to a single bacterium or bacterial aggregations), electrical interactions, surface roughness, and cell shape (Mitchell and Santamarina 2005). Bacteria adsorption to mineral surfaces depends on the pore fluid chemistry, including the pH, ion type and concentration, and dissolved organic carbon (Mueller 1996).

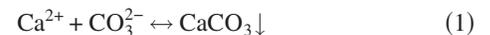
Biological Factors

Minerals that form from fluid ions in situ by precipitation are defined to be authigenic, i.e., they derive from nucleation and subsequent crystal growth. For insoluble precipitates to form, the concentration of mineral ions in solution must reach a certain degree of supersaturation to attain the maximum free energy, thereby achieving nucleation. Once the critical nucleus is formed, crystal growth occurs with further contact with mineral ions in solution (Ferris et al. 1991). Nucleation may occur homogeneously, by random collisions of ions or atoms in solution, or heterogeneously, by formation of critical nuclei on surfaces of foreign solids that enhance nucleation (Ferris et al. 1991). Heterogeneous nucleation can also occur on the surface of individual bacterial cells. The presence of millions of cells in a given solution presents an abundance of nucleation sites for crystallization.

Bacterial mineral precipitation efficiency is affected by the porous media properties, the number of bacteria present, the metabolic activity of bacterial cells, and the total volume of bioavailable nutrients injected (Kantzas et al. 1992). The pH of the environment is also a critical factor in microbiologically induced cementation. Stocks-Fischer et al. (1999) determined that microbiologically induced calcium carbonate mineral precipitation begins at a pH level of 8.3 and occurs at an increasing rate up to a pH value of 9.0.

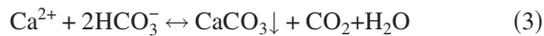
The creation of calcium carbonate (calcite) cement occurs as a consequence of bacterial metabolic activity that raises the pH of the proximal environment. The local pH rise may be achieved by the production of ammonia resulting from the enzymatic hydrolysis of urea, known as urease activity. Urease activity is found in a wide range of microorganisms and plants, some of which produce the enzyme in large quantities (Bachmeier et al. 2002). *Bacillus pasteurii* is a common alkalophilic soil bacterium with a highly active urease enzyme (Ferris et al. 1996). *Bacillus pasteurii* uses urea as an energy source and produces ammonia, which increases pH in the proximal environment, causing Ca^{2+} and CO_3^{2-} to precipitate as CaCO_3 (Kroll 1990). The local rise in pH often causes the microbes themselves to serve as nucleation sites for crystallization.

In calcite precipitation, the overall equilibrium reaction is

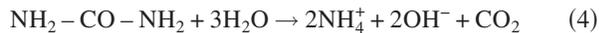


Microbiologically induced calcite precipitation occurs according to the reactions (Ramakrishnan et al. 2001)

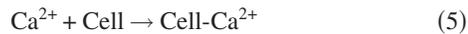




Eq. (2) is triggered by the pH changes induced by bacterial metabolic activity. The high pH environment is provided by the decomposition of urea according to the reaction



In addition to calcite precipitation from the above mechanism, calcium ions deposit on the surface of microorganisms with a net cell surface charge that is negative. The equations for the precipitation of calcite at the cell surface serving as the nucleation site are as follows (Kantzas et al. 1992):



Previous Research on Microbially Induced Calcite Treatments

Previous research involving microbially induced treatments have not included the treatments' influence on the mechanical strength of sand and instead have primarily been focused on reducing the permeability of porous media. Microorganisms and microbially derived products have been in use for over 50 years to aid in the recovery of reservoir oil or provide wellbore cleanout (Nelson and Launt 1991). Microbially enhanced oil recovery (MEOR) is a process that uses bacteria to plug highly permeable soil deposits in an effort to seal off water-bearing zones and improve the yield of reservoir oil.

Ferris et al. (1996) introduced the use of bacteria to actively precipitate calcium carbonate as a mineral plugging agent. This research, termed bacteriogenic mineral plugging, involved the injection of indigenous microorganisms to precipitate authigenic minerals in high-permeability water channels. The mineral precipitation could be accomplished by using bacteria as passive nucleation sites while injecting an appropriate solution to oversaturate the formation water with respect to a certain mineral phase and/or to stimulate bacteria with enough metabolic activity to bring about a mineral oversaturation (Ferris et al. 1996).

A study performed by Kantzas et al. (1992) that attempted to reproduce the results of Ferris et al. (1991) used columns of glass beads and sand, which were flooded first with bacteria and growth medium and then with a solution containing dissolved CaCl_2 and NaHCO_3 . Plugging occurred in both glass bead and sand columns, with observed porosity reductions of up to 50% and permeability reductions of up to 90%. The amount of mineral plugging was roughly proportional to the amount of nutrients flushed continuously/episodically through the columns.

In addition to reducing the permeability of porous media, microbiological processes have been considered for remediation of structural cracks in concrete. Ramakrishnan et al. (1998) constructed mortar beams using a mix of standard proportions of cement and sand combined with suspensions of bacteria (*Bacillus pasteurii*) and growth medium. An artificial crack was then treated with an alternative material (e.g., sand, lime dust, silica fume) and bacteria mixture, cured for 28 days, and tested. The cracks treated with the silica fume-bacteria mixture produced a 36% increase in stiffness. SEM investigation of the cracks confirmed the presence of calcite crystals. Supplemental studies performed by Ramakrishnan et al. (2001) and Ramachandran et al. (2001) expanded on the above research, varying bacterial concen-

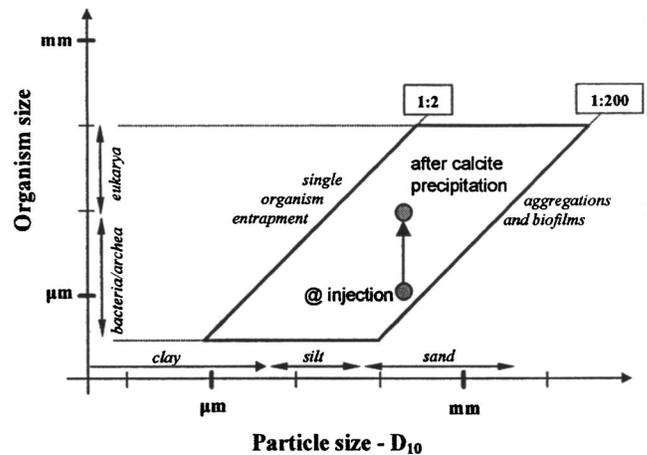


Fig. 2. Particle-organism size compatibility relationships for microbes in soils (adapted from Mitchell and Santamarina 2005). Note that particle-organism size at time of injection is indicated for individual bacterial cells as well as for their volumetric increase after calcite precipitation occurs on cell surface.

tration and crack depth to refine the understanding of microbial behavior applied to concrete crack remediation.

Materials and Equipment

The following sections describe the materials, equipment, and testing techniques used to prepare specimens, induce and monitor cementation, shear specimens, and perform microscopic analyses.

Screening of Microbes

For microbially induced calcite precipitation (MICP) to be effective, a microorganism must be selected that is capable of CO_2 production paralleled by a pH rise in the surrounding environment to an alkaline level that induces precipitation of calcium carbonate. Aerobic microorganisms capable of consuming urea as an energy source are particularly good candidates because they provide two sources of CO_2 : respiration by the cell and decomposition of urea. *Bacillus pasteurii*, a common bacterium naturally occurring in the subsurface, is such an aerobic microorganism. In addition, cells of *Bacillus pasteurii* do not aggregate; this ensures a high cell surface to volume ratio, a condition that is essential for efficient cementation initiation.

Selection of Sand and Microbe Type

The influence of microbial cementation on granular behavior is dependent on the ability of microbes to freely move (either by locomotion or injection) throughout the pore space and on sufficient particle-particle contacts per unit volume at which cementation will occur. These conditions require a balanced relationship between the microbe size and the pore structure characteristics, namely the pore throats. A particle-organism size compatibility relationship that indicates the relative dimensional boundaries of compatibility is presented in Fig. 2 (Mitchell and Santamarina 2005).

Compatibility for this study was achieved using Ottawa 50–70 sand and *Bacillus pasteurii* (American Type Culture Collection 6453). Ottawa 50–70 (Table 1) was believed to provide a suffi-

Table 1. Characteristics of Ottawa 50–70 Sand

Soil type	D_{50} (mm)	C_u	C_c	G_s	e_{min}	e_{max}	Mineralogy	Shape	Hardness
Ottawa 50–70	0.12	1.6	0.8	2.65	0.55	0.87	Quartz	Round	7

cient concentration of particle-particle contacts per unit volume so that, when cemented, the global shear behavior would be notably altered. *Bacillus pasteurii* at the time of injection is about 1–3 μm , enabling free passage through the Ottawa 50–70 pore structure (Fig. 2 at injection). When extracellular calcite precipitation occurs, the microbe size effectively expands to approximately 20 times its original (Fig. 2 after calcite precipitation), this reduces transport of the precipitated calcite cement if it detaches from the soil particle.

Cementation Methods

Three separate specimen cementation conditions were used to evaluate the proposed microbial induced cementation method. These included untreated (uncemented) sand tests, gypsum-cemented tests, and microbially induced calcite precipitation (MICP) cementation tests. To provide a control condition reflective of a loose uncemented natural deposit, tests were also conducted using untreated heat sterilized (180°C for 24 hours) Ottawa 50–70 sand prepared at 35% relative density using air pluviation.

Gypsum Cementation

Gypsum was chosen as the reference cementing material for this study because it facilitates cementation behavior most similar to calcite cementation (Ismail et al. 2001) and because it cures rapidly (<2 h). The gypsum treatment is not intended as a direct inorganic control of the microbial method presented subsequently, but rather is designed to enable evaluation of the microbially treated sand behavior against the behavior of a sand cemented by a method well established in the literature.

Dry heat sterilized gypsum powder was mixed with heat sterilized Ottawa 50–70 sand at 5.0% proportion by weight. The two constituents were thoroughly mixed and dry-pluviated into the specimen, and the gypsum was activated during gradual specimen saturation with de-aired water. It is noted that this method of preparation—in which gypsum is mixed with sand during specimen formation—was used to create uniformly cemented specimens in the lab and cannot be reproduced in situ because the gypsum powder cannot be injected without hydration (and hence cannot cement).

Microbial Cementation

Microbial cementation was achieved by preparing a triaxial specimen using the same procedure as that performed for the uncemented tests. Once the specimen was mounted in the triaxial apparatus, placed under cell pressure and vented, the microbial treatment process was initiated.

The *Bacillus pasteurii* (American Type Culture Collection 6453) cells required for a treatment were initially grown on solid nutrient medium and were transferred twice onto fresh medium to ensure purity of the culture. A single colony was then transferred to liquid medium, and after 19 h at 37°C under agitation, the cells were spun down in a centrifuge at 1,000 g and 4°C for 10 min. At the conclusion of centrifuging, the supernatant was removed. The cells were resuspended in fresh liquid growth medium, centrifuged a second time to remove extracellular materi-

als, and resuspended in a final volume of 20 mL urea growth medium. The microbial injection solution and precooled urea growth medium (Table 2) were prepared simultaneously. The pH was adjusted by aerating and stirring 400 mL of the urea growth medium, until the initial value of approximately 6.5 increased to approximately 7.5. To achieve a final concentration of 25.2 mM Ca^{2+} , 10 mg/L calcium chloride solution was then added to the aerated urea. The concentrated *Bacillus pasteurii* cell suspension was added, and the solution was gently agitated to ensure suspension of all cells. It is noted that sterility during treatment solution preparation was ensured by the use of sterilized solutions and glassware.

The introduction of the microbial treatment solution to the triaxial specimen was accomplished using a peristaltic pump. The 400 mL solution consisting of urea, calcium chloride, and *Bacillus pasteurii* cells (Table 2) was aerated with filtered air while it was pumped into the base of the specimen over a period of approximately twenty minutes (~20 mL/min). This initial “biological treatment” then set within the specimen for 4 h to allow the microbes to attach to the particle matrix.

Once the microbes bonded to the particle matrix subsequent “nutrient treatments” were passed through the specimen. Every subsequent treatment was identical and consisted of an aerated urea growth medium solution amended with CaCl_2 (Table 2). The urea solution was aerated while stirring to raise the pH to approximately 7.5 so that the solution would support alkalophilic bacterial activity. Filtered air was injected along with the cementation medium to supply the bacteria with oxygen required for respiration. The nutrient treatments were pumped through the specimen more slowly than the initial flush (~4 mL/min). The effluent pH was monitored periodically as it exited the specimen to ensure that sufficiently alkaline conditions existed within the specimen (>pH 8.2). Nutrient treatments were periodically flushed through the specimen until the desired level of cementation was attained.

It is noted that the parallel inorganic control of the above system (identical formulation and procedures without *Bacillus pasteurii*) was performed during treatment formulation development. Duplicate parallel tests with the inorganic control in test-tube

Table 2. Summary of Microbial Induced Cementation Treatment Formulations

Solution	Constituents
	Contains per liter of double distilled water
Urea	3 g Bacto nutrient broth (Difco, Detroit, MI)
Medium	20 g Urea $\text{NH}_2(\text{CO})\text{NH}_2$
(used in	10 g NH_4Cl
treatments	2.12 g NaHCO_3
below)	Adjust pH of the medium to 6.0 with 5 N HCl prior to sterile filtration
Initial	2×10^6 cells/mL <i>Bacillus pasteurii</i>
biological	400 mL Urea medium
treatment	8 mL of CaCl_2 stock solution (140 g/L)
Cementation	400 mL Urea medium
treatments	8 mL of CaCl_2 stock solution (140 g/L)

sized cylinders did not produce any detectable cementation between particles while the microbially mediated treatment created easily detectable levels of cementation over a period of a few days. These observations are consistent with previous research that has shown the inorganic system to generate substantially less cementation at a much slower rate (Buczynski and Chafetz 1991; Neumeier 1999).

Experimental Methods to Assess Cementation Effects

The improvement of triaxial soil specimens resulting from the cementation treatments was evaluated during treatment with bender elements and after treatments with isotropically consolidated undrained (CIUC) triaxial tests. After testing, microscopy analysis was performed to assess the cementation structure at the microscale.

Specimen Preparation

Triaxial specimens 72 mm in diameter with aspect ratios of both 2:1 and 1:1 were used as differences in boundary conditions force different modes of shearing. In the 2:1 specimens cementation degradation occurs primarily within a localized shear band, while in the 1:1 specimen localization is prohibited by end effects and as a result cementation degradation is more uniform.

Specimens were prepared by dry pluviation of autoclaved Ottawa 50-70 sand to a target relative density of 35% for all “loose” and cemented specimens and to 70% for one “dense” specimen using a calibrated pluviator that can routinely create specimens within $\pm 5\%$ of the target density (Fritzges 2005). After pluviation the specimen was sealed, vacuum was applied, and the actual specimen relative density was determined. Once seating cell pressure was applied, the specimen was vented to allow treatments.

Process Monitoring

The cementation process, as described previously, alters the particle-particle contact conditions within the specimen. This change of condition is reflected in the shear wave velocity, which can be readily measured with bender elements. A bender element design (Landon 2004) was integrated into custom triaxial end platens designed for treatment injections. A 20 V sine wave was applied at 10 kHz frequency to a parallel transmitting element and the received signal was detected by a series bender element. Signal stacking was used to clarify the received signal. The shear wave velocity was determined using the peak-to-peak interpretation method (Landon 2004) after verifying that other interpretation methods provided similar velocity values.

A baseline bender element test was performed on the vented dry specimens prior to saturation with de-aired water in the case of the inert and gypsum-cemented tests, or with microbe-urea- CaCl_2 solution in the case of the MICP tests. Once the specimens were saturated, bender element tests were performed at time intervals to capture the increase of shear wave velocity as the cementation develops within the specimen. Bender element tests were continued for a given treatment flush until the shear wave velocity reached a maximum value.

The timeframe for property changes in the biologically cemented specimens depended upon a number of factors, including frequency of cementation medium flushes and CaCl_2 concentration. It is convenient to consider each cementation medium flush as a “treatment unit,” which is simply one increment of an additive process. Each treatment unit consisted of three durations, which were the injection time, set or “equivalent gel” time, and time to next injection. The shear wave velocity began to increase

at the onset of injection as the calcite precipitates out of solution. Once injection of the cementation medium is complete, the shear wave velocity continues to increase while the calcite continues to precipitate from the solution. The extent of shear wave velocity increase appeared to occur when the supply of Ca^{2+} within the solution was exhausted, corresponding to the set time. The time to next injection was simply the duration between each treatment unit. The incremental increase in V_s was expected to decrease as the number of treatment units increased, as the bacteria became more encapsulated with calcite and less capable of metabolizing urea and providing the environment necessary for calcite precipitation. One notable advantage of biologically induced cementation is that the degree of cementation can be closely regulated by controlling the number of flushes and the formulation of the cementation treatment medium.

Specimen Shearing

At the completion of cementation, the specimens were back pressure saturated. Back pressures were kept low early in the 2:1 specimen test series to reflect the in situ condition at 10 m below ground surface. It was later determined that this back-pressure level was insufficient to remove the gas microbes produced during metabolic activity. To remove any effect of partial saturation on shear response, a higher back pressure of 1200 kPa, which the microbes could withstand, was used for the 1:1 specimen tests. The 2:1 and 1:1 specimens were back-pressured with effective confining stresses of 100 kPa and 50 kPa, respectively. The shear wave velocity was also measured after back pressure, and given V_s changes of less than 50 m/s for all tests, it was determined that the degree of partial saturation was not sufficient to substantially alter the shear wave velocity. The CIUC tests were sheared at a rate of 2.5%/h, continuing to a maximum axial shear strain of 10%. The shear wave velocity was periodically monitored throughout this process to detect the degradation of cementation during shearing. Bender element tests were performed on 2 to 5 min intervals for the first 1% of axial strain, increasing to 20 min intervals as the test progressed to higher axial strains.

Scanning Electron Microscopy and X-Ray Compositional Mapping

The combined analysis of the scanning electron microscopy (SEM) and electron probe microanalysis allowed the form and function of the surface modifications to be assessed. The untreated and MICP specimens were embedded in low viscosity epoxy resin (Buehler, Ltd., Lake Bluff, Illinois), while the gypsum specimen was embedded in acrylic resin (London Resin Company, Ltd., Theale, UK). All specimens were prepared by sequential polishing with diamond paste (6, 3, and 1 μm) and 0.3 μm Al_2O_3 paste. Polished specimens were carbon coated (20 nm thickness under vacuum evaporation), then observed by backscattered electron (BSE) imaging. SEM and X-Ray compositional mapping was performed using the Cameca SX-Ultrachron Electron Microprobe (Cameca, Paris, France). A beam of 15 kV and 20 nA, focused to ~ 0.4 μm diameter was used for BSE imaging. X-Ray compositional mapping was performed at 15 kV with a beam current of 200 nA.

Microbial Growth: Initiation and Process Monitoring

Gypsum Cementation

Cementation for the gypsum tests is initiated as soon as water enters the pore space. The shear wave velocity versus time for

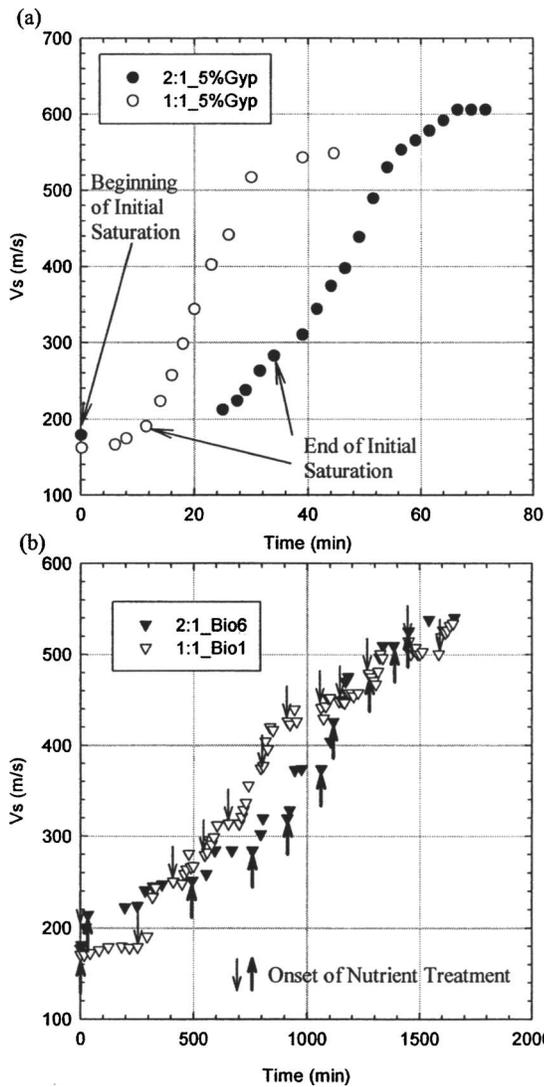


Fig. 3. Shear wave velocity versus time for (a) gypsum cemented; (b) microbially induced cemented specimens

the 2:1_5%Gyp and the 1:1_5%Gyp specimens are plotted on Fig. 3(a). Inspection of 2:1_5%Gyp indicates an initially slow increase of shear wave velocity for the first 25 min, followed by a rapid increase in V_s until approximately 60 min after initial water injection, when the V_s increase slows considerably, reaching a plateau value of approximately 600 m/s. The shear wave velocity versus time for 1:1_5%Gyp indicates similar trends over about one-half the time duration, reflecting the 50% smaller specimen height. The V_s for 1:1_5%Gyp eventually plateaus at a value of approximately 550 m/s. This slightly lower stable value relative to 2:1_5%Gyp is attributed to partial hydration of the gypsum prior to sample preparation and was confirmed based on complementary tests at different gypsum concentrations. It is noted that the relatively rapid cementation time of 60 min or less is the result of a very rapid reaction time for cementation following hydration.

Microbially Induced Cementation

Cementation of the microbially induced calcite precipitation (MICP) tests occurred shortly after the initial biological treatment. A plot of V_s versus corrected time (overnight downtime

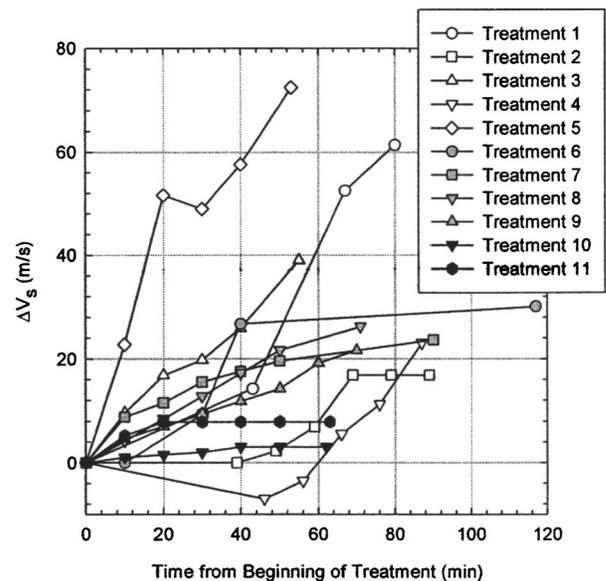


Fig. 4. Change in shear wave velocity versus time from initial injection for 1:1_Bio1 specimen to indicate relative amount of cementation that resulted from each cementation treatment

removed) for the 2:1_Bio6 and 1:1_Bio1 specimens reveals similar trends (Fig. 3(b)), with any differences attributable to varying injection frequency and cementation medium content. Bender element tests were continued for a given flush until the shear wave velocity reached a maximum value characterized by no further increase with time. Data collected from 2:1_Bio6 and 1:1_Bio1 reached a similar maximum V_s value of approximately 540 m/s after approximately the same treatment time of 1,700 min. It is noted that the treatment time is dependent on numerous factors such as the microbial concentration, reaction kinetics, soil characteristics, and length scales, and as a result, the time required for in situ treatment will vary.

The relative amount of cementation that resulted from each cementation treatment is presented for 1:1_Bio1 in Fig. 4 as a plot of ΔV_s versus the time from the onset of injection. Inspection reveals the general trend conceptually discussed previously, albeit with scatter between subsequent treatments. The initial treatment with microbes provided a strong initial increase in V_s caused by initial binding of the microbes to the soil matrix. The degree of additional improvement (incremental increase in V_s) generally remained high (e.g., flushes 3 and 5) in earlier treatments. The smallest increase in V_s was observed near the end of the test in the last treatments.

Normalized Cementation Behavior

To account for different cementation methods (microbial versus gypsum), treatment methods (single versus multiple treatments), and height to diameter specimen ratios (2:1 and 1:1), normalized shear wave velocity versus normalized time plots were developed (Fig. 5). The normalized shear wave velocity is calculated as

$$V_n = V_s / V_{sMAX} \quad (7)$$

where V_n =normalized shear wave velocity (-); V_s =measured shear wave velocity (m/s); and V_{sMAX} =maximum obtained shear wave velocity (m/s). The normalized time is calculated as

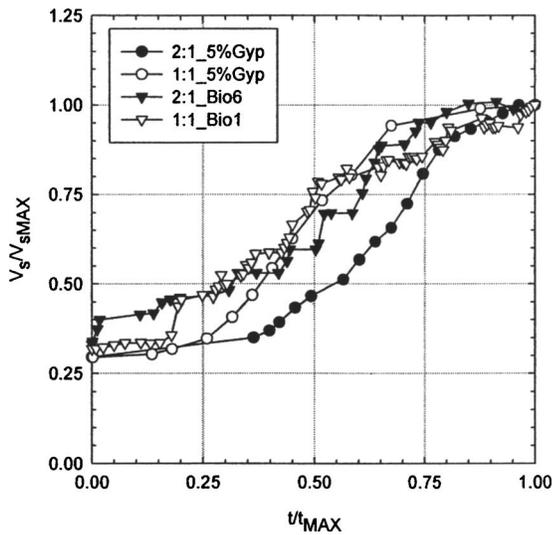


Fig. 5. Normalized shear wave velocity versus time relationship for gypsum and microbial induced cemented specimens

$$t_n = t/t_{MAX} \quad (8)$$

where t_n = normalized time (-); t = time at shear wave velocity measurement (min); t_{MAX} = maximum time for test (min).

The normalized V_s behavior for all tests was similar and can be characterized by a general behavior as evident in Fig. 5. This behavior is highlighted by an initial slowly increasing slope reflecting initiation of the cementation processes, which then transitions to a higher rate of productivity reflecting active cementation. Finally the cementation rate begins to decrease, eventually stabilizing at zero slope and maximum V_n .

Response to Undrained Monotonic Loading

The shear wave velocity was also monitored during the shearing phase of the CIUC tests to detect the onset and rate of cementation degradation. The response of the 2:1 and 1:1 specimens to monotonic loading is presented in Figs. 6 and 7, respectively.

Untreated Specimens

The untreated specimen responses to monotonic loading served as a benchmark from which the improvement in undrained shear response by the artificial cementation methods could be assessed. Two loose (35% relative density) specimens (2:1_Loose, 1:1_Loose) as well as one dense (70% relative density) 1:1 specimen (1:1_Dense) were tested.

The 2:1_Loose specimen exhibited the conventional undrained monotonic collapse type of behavior in p' - q space (Fig. 6), exhibiting a barreling-type deformation without localization and beginning to fail (beginning of nonlinear behavior) after only 0.15% axial strain. Gradual strain hardening occurred at a decreasing rate through the remainder of the test. The V_s remained relatively constant but increased as the effective stress gradually increased because of the negative pore pressure generated at higher strain levels.

The 1:1_Loose exhibited a similar barreling-type deformation, failing at a higher q/p' ratio of about 0.4 at 0.15% axial strain (Fig. 7). It did not exhibit the p' - q collapse behavior as the 2:1_Loose specimen did owing to end platen effects. Mild strain

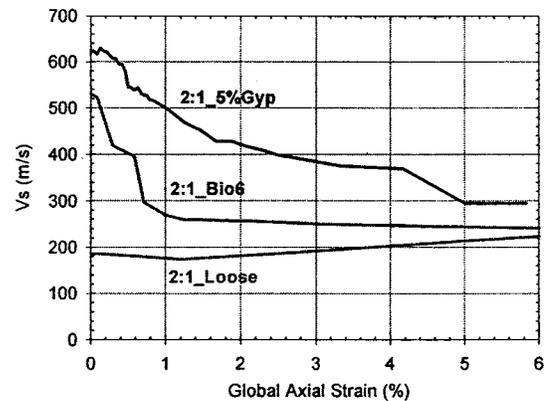
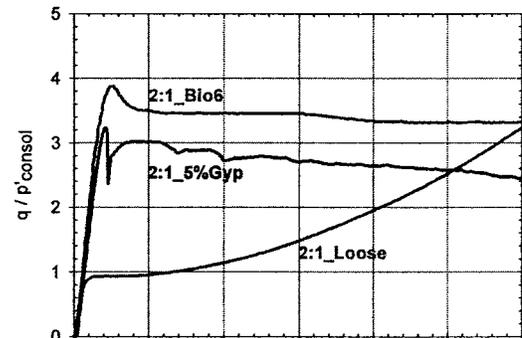
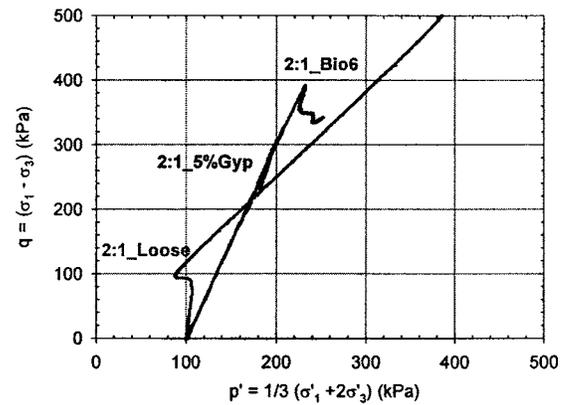


Fig. 6. Response to undrained monotonic triaxial shear for 2:1 specimens

hardening steadily occurred until the end of shear. Again V_s increased slightly with strain because induced pore pressure increased the effective stress.

The 1:1_Dense specimen was tested to provide an upper bound of the improvement in shear resistance caused by density and to assess the influence of the 1:1 specimen boundary conditions on a specimen condition with normally favorable shear localization. As expected, the 1:1_Dense specimen did not fail in a localized manner but exhibited a barreling-type deformation. At about 0.18% axial strain, the dense specimen yielded at a similar q/p' ratio to the 1:1_Loose, after which it exhibited substantial strain hardening. V_s again remained relatively stable, increasing slightly as the effective stress increased.

From previous research on specimen geometry (Yang 2002), it is known that decreasing the height to diameter ratio from 2:1 to 1:1 results in an increase in shear resistance. This higher resistance is induced by increased constraint on the particles from end platen effects, which effectively force specimen barreling and prohibit the development of shear bands (localizations). This ef-

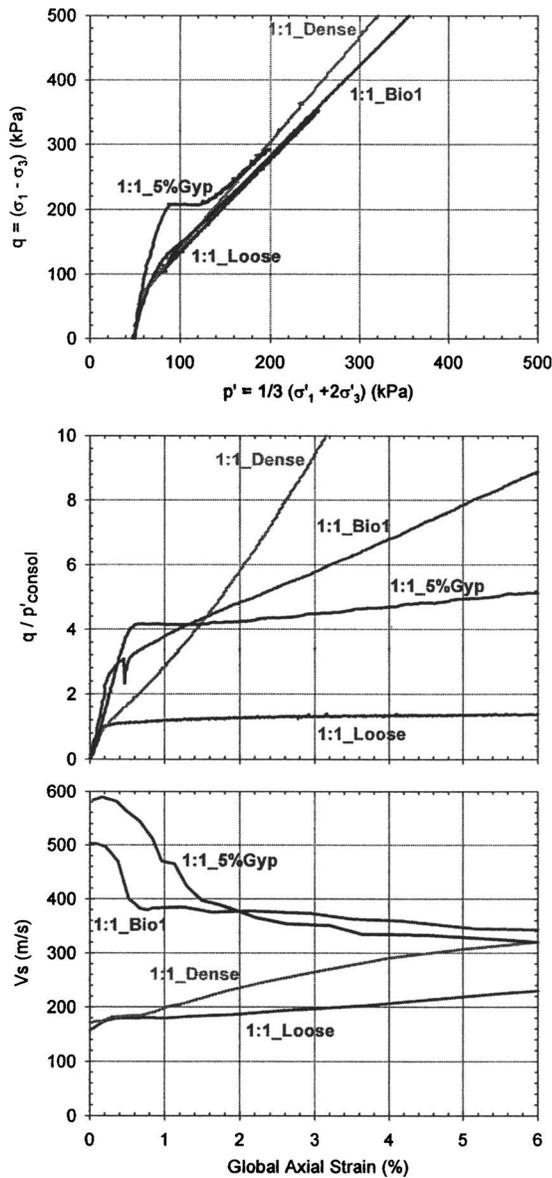


Fig. 7. Response to undrained monotonic triaxial shear for 1:1 specimens

fact becomes important where localization would normally occur because it results in increased deformation of the soil matrix and damage of cementation at particle-particle contacts.

Gypsum Cementation

The effect of cementation on shear behavior was captured by using gypsum as the cementing agent. The 2:1_5%Gyp test, as expected, exhibited a stiffer and higher shear resistance (up to $q/p'_{consol}=3.2$ ($q/p'=1.8$) at 0.4% strain) response relative to its uncemented counterpart (Fig. 6). Once this peak strength was achieved, brief collapse followed by a gradual decrease toward a residual q/p'_{consol} ratio of 2.4 ($q/p'=1.3$) occurred. This brief collapse, followed by strain softening, reflects the initiation of cementation degradation along a well-defined failure plane, which was visually confirmed during post-test inspection.

The initiation of cementation degradation, which caused the rapid strain softening response observed in the shear behavior, was readily detected in the V_s measurements. The value of V_s

decreased rapidly within the first 0.5% of strain and then continued to degrade at a decreasing rate with strain. The most rapid decrease of V_s appeared to occur immediately after the brittle peaks of the 2:1_5%Gyp and 2:1_Bio6 capacities. It should be noted that V_s is an average measurement on the center axis of the specimen over the entire height. When cementation degradation occurs in a localized manner (e.g., along a shear band), the velocity through the upper and lower portions of the specimen, which remain intact, changes minimally or remains constant. As a result, V_s becomes an average measurement, and even if all calcite cement bonds break within a shear band, the V_s value will not reflect the velocity of a fully uncemented specimen.

The parallel gypsum specimen prepared at a 1:1 specimen ratio (1:1_5%Gyp, Fig. 7) exhibited increased shear resistance relative to its uncemented counterpart, though this is primarily the result of increased boundary effects. The 1:1_5%Gyp achieved the highest q/p'_{consol} ratio of the 1:1 specimens (2.3 at 0.6% axial strain) and then yielded and approached a residual q/p' of 1.4 with continued shearing. As with the 2:1_5%Gyp, the shear wave velocity decreased from its initial value, with calcite cement bond breakdown initiating at the strain level corresponding to the peak resistance. It is noted that the V_s value at large strains is similar to the uncemented dense specimen (1:1_Dense), indicating that the entire specimen may have undergone cement bond breakdown when the boundary conditions prevented localized failure (confirmed in post-test specimen examination).

Microbially Induced Cementation

With the noncemented and gypsum cemented specimens providing reference behavior modes and the 2:1 and 1:1 providing difference failure modes, the effectiveness of microbially induced cementation on undrained shear response can be readily assessed. For both specimens (2:1_Bio6, 1:1_Bio1), increased strength relative to the uncemented specimen was observed, with values being roughly comparable to the gypsum tests. The shear wave velocity clearly indicates the initiation of cementation bond breakage at peak capacity and continued degradation with shearing.

The microbially induced cementation 2:1 specimen (2:1_Bio6, Fig. 6) generated the highest shear ratio ($q/p'_{consol}=3.9$ ($q/p'=1.7$) at 0.5% axial strain) and exhibited distinct strain softening toward a q/p' value of 1.3, similar to the 2:1_5%Gyp softening trend. A well-defined failure plane was evident during testing and was confirmed during post-test analysis. It is noted that the relatively low back pressure of 100 kPa applied, in combination with the gases produced by the microbial metabolic activity, resulted in a specimen that was not fully saturated. As a result, the shear response of 2:1_Bio6 may be partly influenced by partial saturation. Nonetheless, the degradation of the microbially induced cemented structure is again clearly evident in the trend of the shear wave velocity with strain. The shear wave velocity remains relatively constant (520 m/s) until the peak strength is attained. After the peak resistance, V_s decreases rapidly and approaches a stable value of about 240 m/s.

The 1:1 specimen treated with the microbial technique (1:1_Bio1, Fig. 7) also provided increased shear resistance relative to the uncemented specimen, reaching a q/p'_{consol} ratio of about 3.1 ($q/p'=1.5$) at 0.24% axial strain. After reaching peak capacity, the specimen strain gradually softens and approaches a residual q/p' of 1.3. The decrease in V_s again was initiated as peak strength was achieved and cement bond breakdown initiated. At large strains, V_s stabilized at a value similar to that of the

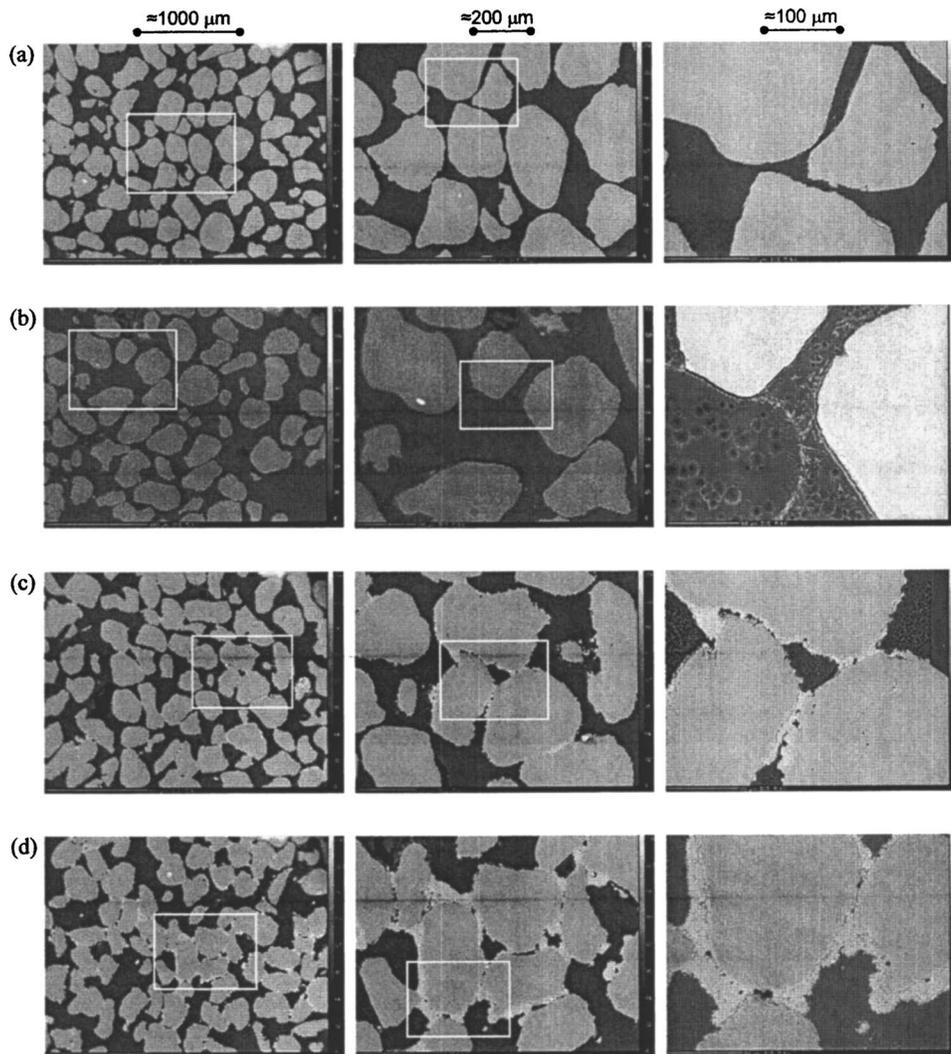


Fig. 8. Scanning electron microscopy images for (a) uncemented sand; (b) gypsum cemented sand; (c) lightly; and (d) heavily microbially cemented sand.

dense specimen (1:1_Dense), perhaps indicating that the calcite precipitated mass within the loose specimen resulted in a denser specimen after cementation degradation.

Microscale Examination of Microbial Growth

A microscale investigation was performed to directly observe the characteristics and degree of bonding between particles as well as the compositional nature of the cementing agents. Transmission light optical microscopy, scanning electron microscopy, and X-Ray compositional mapping using electron probe microanalysis techniques were used to perform the analyses; results from the latter two methods are presented herein. Four subspecimen samples were prepared and analyzed. These included noncemented sand, a gypsum-cemented sample from the 2:1_5%Gyp specimen, and lightly and heavily biologically cemented samples obtained from the middle portion of the 2:1_Bio6 specimen and the lower portion of the 2:1_Bio9 triaxial specimen, respectively.

Untreated Sand

Images of untreated Ottawa 50–70 sand are provided in Fig. 8(a).

The subrounded nature of the sand particles is evident from inspection of these images. The presence of small asperities and other microfeatures, which serve as potential nucleation sites for gypsum and calcite cementation, is also visible at this image scale.

Gypsum Cementation

Images of gypsum cemented sand are shown in Fig. 8(b). Close inspection reveals the presence of needle-shaped crystals, which are characteristic of gypsum cementation. The crystal shape is visible in the array of gypsum-cemented sand. Cementation is apparent both on the surface of sand grains and at particle contacts. Only some of the densely grouped gypsum needles appear in the backscatter electron imaging, but the border between solid gypsum and the acrylic resin filling the remaining pore spaces is defined.

Microbially Induced Cementation

The lightly and heavily biologically cemented specimens are presented in Figs. 8(c and d), respectively. The images show calcite cement in a lighter shade of grey on the surface of the sand

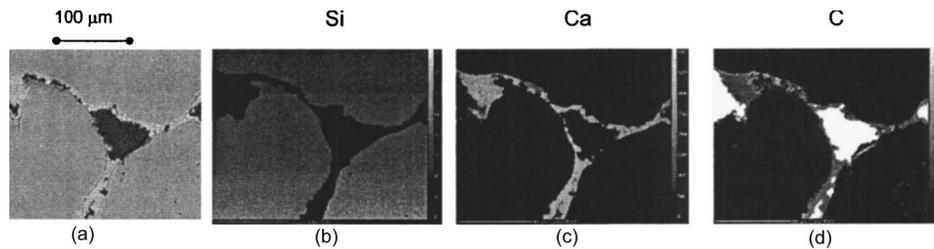


Fig. 9. X-ray compositional mapping of (b) silica (Si); (c) calcium (Ca); and (d) carbon (C) elements of subspecimens of lightly microbially cemented sand. As a reference a subsection of the SEM image in Figure 8(d) is added (a).

grains, with the cementation more evident in the heavily cemented specimen. Both the lightly and heavily biologically cemented specimens show evidence of cementation on the particle surface and at particle contacts. The shape of the cemented structures adhering to and joining the sand particles appears grainy in nature, in contrast to the needle-shaped gypsum cement. Keeping in mind the limitations of inferring soil matrix and pore connectivity from 2-D images, it is apparent in the 2-D images that many of the particles are connected via cementation even though the actual particle-particle contacts are few. In addition, the calcite crystal faces in the highly magnified images show well-distributed, hollow, sphere- to rod-like impressions of *Bacillus pasteurii*, indicating positions where microorganisms were encased in the calcite crystal growth initiated by them.

The presence of calcite on the silicate grains was confirmed by x-ray compositional mapping of the same heavily biologically cemented specimen used for SEM images in Fig. 8(d) (for reference see closeup in Fig. 9(a)). The compositional maps, obtained by integrating rastered x-ray diffraction with an electron microprobe at a high resolution of 0.01 μm , indicate a clear distinction between the silicate of the sand (Figure 9(b)) and the carbon and calcium of the surrounding calcite (Figs. 9(c) and (d)). The whiteness in the image is proportional to the abundance of the element under consideration and clearly indicates the dense layer of calcite around the silicate grains. Similarly, the extremely carbon-rich epoxy resin embedding material accentuates the remaining pore space in Fig. 9(d).

Further confirmation that the cement bonding produced by the MICP tests is indeed calcite was provided by visual validation using a low-powered optical light microscope and a simple chemical test. A calcite-cemented sand-grain cluster (conceptually similar to Fig. 8(d)) was exposed to 1N hydrochloric acid. The grain cluster immediately effervesced, signaling the dissolution of the calcite cement. The reaction continued until all of the calcite was dissolved from the surface of the sand grains. Optical microscopy revealed that the sand-grain surfaces, which were coated with white calcite, now appeared clear and identical to untreated sand grains.

Conclusions

The successful development of a treatment procedure to beneficially alter the behavior of uncemented cohesionless soil using natural microbial processes has been presented. Microbially induced calcite cementation was achieved using the common soil microorganism *Bacillus pasteurii*. Factors determined critical to the success of the microbial treatment include pH, oxygen supply, metabolic status, and concentrations of microbes, and ionic cal-

cium in the biological and nutrient treatment flushes, as well as the timed sequence of injections.

Specimens cemented with gypsum and microbially induced calcite both exhibited similar behavior in terms of the observed and normalized shear wave velocity (V_s). The rate of change in the observed V_s was also detectable. Initially, the rate of ΔV_s was low, and it gradually increased to a maximum at approximately mid-test, when ΔV_s then began to decrease, approaching zero at the conclusion of cementation. This trend corresponded with the saturation and hydration of cement in the gypsum-cemented specimens and with the microbial activity, treatment injection formulation, and pH environment within the microbially treated specimens.

The microbially induced cemented specimens exhibited an increase in axial capacity under undrained monotonic shearing conditions. The q/p' response of the specimens indicated a non-collapse behavior. They exhibited increased initial shear stiffness and higher elastic capacity compared to untreated loose specimens, and behavior similar to the gypsum-cemented control specimens. The degradation of cementation of both gypsum and microbially treated cemented specimens was detectable using bender element V_s measurements. Rapid cementation degradation was observed in the initial 1% of axial strain, followed by more gradual reduction at larger strains. Uncemented loose and dense specimens exhibited an increase in V_s with increasing axial strain, with this effect more pronounced in the dense specimen.

The nature of the bonding in the artificially cemented tests was investigated using a suite of optical and electron microscopy techniques, and elemental distribution was confirmed using an X-ray electron microprobe. Both gypsum and MICP cementation were observed on the sand-particle surfaces as well as at particle contacts. The gypsum cement was characterized by well-formed, needle-shaped crystals, while the microbially induced calcite cement exhibited a more grainy texture with little structure at the investigation magnification.

The results presented have established that substantial cementation in loose sand structures can be engineered through harnessing and controlling natural biological processes. With the maturation of this "green" technology in future studies, multiple new opportunities for engineering soil improvement implementation can be envisioned, including treatment of liquefiable sand deposits, pretreatment of the subsurface prior to tunneling, building settlement reduction, and dam, levee, and slope stabilization.

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