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## Bio-mediated soil improvement

## Jason T. DeJong<sup>a,\*</sup>, Brina M. Mortensen<sup>b</sup>, Brian C. Martinez<sup>b</sup>, Douglas C. Nelson<sup>c</sup>

<sup>a</sup> Department of Civil and Environmental Engineering, University of California, Davis, CA 95616, 530 754 8995, United States <sup>b</sup> Department of Civil and Environmental Engineering, University of California, Davis, CA 95616, United States

<sup>c</sup> Department of Microbiology, University of California, Davis, CA 95616, 530 752 6183, United States

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#### ABSTRACT

New, exciting opportunities for utilizing biological processes to modify the engineering properties of the subsurface (e.g. strength, stiffness, permeability) have recently emerged. Enabled by interdisciplinary research at the confluence of microbiology, geochemistry, and civil engineering, this new field has the potential to meet society's ever-expanding needs for innovative treatment processes that improve soil supporting new and existing infrastructure. This paper first presents an overview of bio-mediated improvement systems, identifying the primary components and interplay between different disciplines. Geometric compatibility between soil and microbes that restricts the utility of different systems is identified. Focus is then narrowed to a specific system, namely bio-mediated calcite precipitation of sands. Following an overview of the process, alternative biological processes for inducing calcite precipitation are identified and various microscopy techniques are used to assess how the pore space volume is altered by calcite precipitation, the calcite precipitation is distributed spatially within the pore space, and the precipitated calcite degrades during loading. Non-destructive geophysical process monitoring techniques are described and their utility explored. Next, the extent to which various soil engineering properties is identified through experimental examples. Potential advantages and envisioned applications of bio-mediated soil improvement are identified. Finally, the primary challenges that lie ahead, namely optimization and upscaling of the processes and the education/training of researchers/practitioners are briefly discussed. © 2009 Elsevier B.V. All rights reserved.

## 1. Introduction

#### 1.1. Societal context

Population and civil infrastructure continue to expand at unprecedented rates. The United States population of 304 million is growing at an annual rate of 0.9% and the American Society of Civil Engineers estimates that a US\$1.6 trillion investment in civil infrastructure is necessary (ASCE, 2006). Infrastructure demands are even more severe in other countries, particularly in India and China. Infrastructure is insufficient in countries such as China, where 10 million people immigrate to major cities each year. Population growth is particularly acute for historic cities and regions where expansion is limited by geographical boundaries and inadequate soil conditions (e.g. Boston, New York, Los Angeles, Mumbai, Tokyo, Taiwan, Istanbul, Holland, Japan). Rehabilitation and expansion of civil infrastructure is required to meet ever-growing societal needs

URL: http://www.sil.ucdavis.edu (J.T. DeJong).

and is directly limited by the availability of competent soils upon which they can be constructed.

Simultaneously, environmental conditions necessary to support life are degrading, and are forecasted to continue to degrade at increasing rates into the future. This is clearly highlighted by the estimated sea level rise due to global warming (Cayan et al., 2008), which can be attributed in large part to the release of carbon through the burning of fossil fuels. A large contributor to this problem is the manufacturing of cement, a material used pervasively in construction processes including ground improvement. As a result, there is a clear societal need for the technologies developed to improve soil to be sustainable.

## 1.2. Current soil improvement practice

The demand for new, sustainable methods to improve soil continues to increase, with more than 40,000 soil improvement projects being performed per year at a total cost exceeding US\$6 billion/year worldwide.

The majority of these soil improvement techniques utilize mechanical energy and/or man-made materials, both of which required substantial energy for material production and/or installation. A common approach is to inject synthetic man-made



<sup>\*</sup> Corresponding author. Tel.: +1 530 754 8995.

*E-mail addresses:* jdejong@ucdavis.edu (J.T. DeJong), dcnelson@ucdavis.edu (D.C. Nelson).

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materials, such as micro-fine cement, epoxy, acrylmide, phenoplasts, silicates, and polyurethane (Xanthakos et al., 1994; Karol, 2003) into the pore space to bind soil particles together. This is accomplished using a variety of chemical, jetting, and permeation grouting techniques (e.g. http://www.haywardbaker.com). These approaches create environmental concerns and are increasingly under the scrutiny of public policy and opinion: all chemical grouts except sodium silicate are toxic and/or hazardous (Karol, 2003). In 1974, acrylamide grout was associated with five cases of water poisoning in Japan, resulting in the ban of nearly all chemical grouts (Karol, 2003). This reverberated in the US, with pending federal regulations forcing the withdrawal of most products on the market. Recent initiatives in certain countries propose to ban all synthetic man-made grouting materials.

Furthermore, all current grouting injection approaches suffer from low "certainty of execution", i.e., the ability to create the conditions specified in the original engineering design in-situ. In general, grouting treatment methods are only effective up to 1-2 m from the injection point, yet, quality control during construction is primarily limited to monitoring the injection volume and pressure; no realtime measurements are made of the changes that actually occur in the subsurface. The uncertainty in the final constructed condition forces conservative over-design, resulting in unnecessary project costs and excess quantities of grout consumed.

#### 1.3. Alternative solutions needed

The confluence of these factors necessitates the exploration and development of new alternative soil improvement methods and associated reliable monitoring techniques. New exciting opportunities for utilizing biological processes to mediate the improvement of soil properties have recently emerged. These opportunities have been enabled through interdisciplinary research at the confluence of microbiology, geochemistry, and civil engineering. The field is young and many decades of exciting research lie ahead if current ideas are to be fully realized in practice and the needs of society met.

With this context, the paper sets forth to present an overview of bio-mediated soil improvement. First, a conceptual framework is presented to provide an overview of the primary components of bio-mediated soil improvement systems and to emphasize the interdisciplinary nature of this field. Second, the geometric compatibility required between soil and microbes is considered. The different aspects of bio-mediated soil improvement systems are then examined in greater detail. Focus is placed on bio-mediated calcite precipitation of sands since research for this process is currently more advanced than the alternatives. Following an overview of the bio-chemical process, alternative biological processes for inducing calcite precipitation are identified and various microscopy techniques are used to assess how the pore space volume is altered by calcite precipitation, how the calcite precipitation is distributed spatially within the pore space, and how the precipitated calcite degrades during loading. Non-destructive geophysical process monitoring techniques are then described and their utility explored. Next, the extent to which various soil engineering properties change is identified through experimental examples. The potential advantages and envisioned applications of bio-mediated soil improvement are then identified. Finally, the primary challenges that lie ahead, namely optimization and upscaling of the processes and the education/training of researchers/practitioners are briefly discussed.

#### 2. Overview of bio-mediated soil improvement systems

A bio-mediated soil improvement system broadly refers to a chemical reaction network that is managed and controlled within soil through biological activity and whose byproducts alter the engineering properties of soil. An overview of these types of systems is presented schematically in Fig. 1. It is noted that similar processes are being explored for in-situ immobilization of metal contaminants (e.g. Fujita et al., 2008) and restoration of masonry structures (de Muynck et al., 2008; Mastromei et al., 2008; Shen and Cheng, 2008).



Fig. 1. Overview of bio-mediated soil improvement systems. ([-] = chemical concentration, Ω = resistivity, Vp = compression wave velocity, Vs = shear wave velocity).

#### 2.1. Underlying chemical reaction networks

Central to these systems is a network of chemical reactions whose byproducts have the potential to alter the engineering properties of soil. A majority of the chemical reactions identified to date can occur in solution in the absence of any biological activity. Examples of the byproducts generated can be generally categorized into inorganic precipitation, organic precipitation, and gas generation. The byproducts produced by these chemical reactions are those required to improve soil properties. However, they are produced en masse nearly instantaneously and remain primarily in the pore fluid. The chemical reaction occurs, but improvement of soil properties is not realized.

#### 2.2. Role of biological processes

Biological activity provides an ability to control and manage the timing, rate, and spatial distribution of the chemical network reaction, and hence the byproducts which improve soil properties. When bio-mediated, the chemical processes of inorganic precipitation, organic precipitation, and gas generation can be considered as biomineralization, biofilm formation, and biogas generation, respectively. Biomineralization processes identified in the literature include production of magnetite, greigite, amorphous silica, and calcite (Kohnhauser, 2007).

The use of microbes to control and manage the chemical processes is attractive given their pervasive presence in the subsurface and the millennia over which they have been active. More than 10<sup>9</sup> cells per gram of soil exist in the top meter of soil (Whitman et al., 1998), and the population concentrations generally decrease with depth. At 30 m depth, the lower limit of most soil improvement engineering applications, microbe concentrations of about 10<sup>6</sup> cells per gram of soil can exist (Whitman et al., 1998). The numbers of microbes that can be utilized for bio-mediation are numerous, although individually they are very small.

Microbial organisms that have already been utilized in biomediated soil improvement research are estimated to be more than 1.5 billion years old (Madigan and Martinko, 2003), and the processes that are being controlled and managed have been active for much of this time (Kuo and Bolton, 2008).

In bio-mediated soil improvement, the bio-mediated chemical reaction network is regulated to control the timing of the reaction. This is enabled by the amendment of chemicals into the subsurface. The microbial population in-situ is typically either stimulated (bio-stimulation) through the injection of nutrients or augmented (bio-augmentation) by the injection of additional microbes. In either case, the goal is to increase activity levels and/or concentrations of the microbial population to the level required to initiate and sustain a chemical reaction. Control of timing enables transport and spatial distribution of the microbes and chemicals throughout the soil. Microbial activity gradually alters the environmental conditions - often in the form of increasing the pH - until the environmental conditions required to initiate the chemical reaction are reached. Once the chemical reaction network is triggered, the desired rate of byproduct production (e.g. calcite precipitation) is governed by the rate of microbial metabolic processes and/or the available chemicals.

The improvement to soil properties is not realized, however, unless the byproducts are spatially located in the specific region of the soil matrix required to affect soil behavior. In the case of bio-mineralization, the inorganic precipitates that form at particleparticle contacts are the most important. The precipitates formed in solution or on exposed particle surfaces do not (or negligibly) contribute to changes in strength, stiffness, and permeability. This is also generally true for bio-films through organic precipitates in solution and on particle surfaces, which have a larger contribution to changes in permeability. Finally, for biogas generation the bubbles formed must be within a specific size range and uniformly distributed within the pore fluid in order to significantly reduce pore fluid compressibility.

#### 2.3. Process monitoring

Control and management of bio-mediated soil improvement processes require real-time, nondestructive monitoring of chemical, biological, and geotechnical components (indicated in Fig. 1 in *italics* beneath the respective header). With improvement of soil properties being the primary objective, methods are needed to determine how the byproducts of a given bio-mediated chemical process are altering the soil properties. The three primary methods of geophysical measurements that can be utilized are the shear wave velocity, compression wave velocity, and resistivity (the inverse of conductivity). Since these methods induce very small (Santamarina et al., 2001) or no strain (the case for resistivity), the soil and treatment process is undisturbed by the measurements (discussed subsequently in this paper).

The state of the biological and chemical components is intimately linked. The biological component can primarily be captured by monitoring the microbial concentration, activity state, activity potential, biomass, and nutrient concentrations. The chemical component can primarily be captured by monitoring the pH, chemical concentrations, and conductivity. The experimental methods used to assess these components are generally well established in the respective fields (Madigan and Martinko, 2003) but do require discrete (often pore fluid) samples to be obtained and subsequent tests performed on them. Consequently, real-time information is not attainable, labor is intensive, and potentially destructive invasive sampling is required.

Process monitoring of select chemical, biological, and geotechnical parameters is necessary to develop a full understanding of a given bio-mediated process. Of these measurements, the geophysical methods are arguably the most important as only they provide a direct indication of how the properties of the soil are being altered.

#### 2.4. Improvement of engineering properties of soils

The improvement of engineering soil properties may vary widely between different bio-mediated treatment methods. The primary properties that can realize a 10-fold change or more include permeability, stiffness, compressibility, shear strength, and volumetric behavior. Changes in these properties of this order provide numerous possibilities for application.

In all cases, the potential extent of how a given bio-mediated treatment method may improve the engineering soil properties can be reasonably estimated through analytical analysis and/or examination of previously researched non-biomediated treatment methods. As an example, the potential increase in shear strength of sands from bio-mediated calcite precipitation will be similar to previous studies examining the use of gypsum, cement, CIPS (calcite in-situ precipitation system, Ismail et al., 1999a), and epoxy to improve soils (Neelands and James, 1963; Acar and El-Tahir, 1986; Dvorkin and Nur, 1996; Ismail et al., 1999a,b, 2001; Avseth et al., 2000; Sharma and Fahey, 2003). Consequently, the primary question for bio-mediated research is not whether a soil laboratory sample can be improved. Instead the primary questions lie at the fundamental science and micron length scale level-How does this improvement occur?-and at the field performance level-How can a large zone of soil be treated uniformly? How long will the treatment last?



Fig. 2. Comparison of typical sizes of soil particles and bacteria, geometric limitations, and approximate limits of various treatment methods. (extended from Mitchell and Santamarina, 2005).

#### 2.5. Upscaling for field treatment

Upscaling a bio-mediated soil improvement method from the bench scale to the field scale requires an understanding of science and engineering from the fundamental level (process at a single particle-particle contact) through the system performance level (field application). Development of a system model based on first principles is critical to successful upscaling.

The two primary concerns for upscaling of a ground improvement method are the ability to uniformly treat a large zone of soil and ensuring the permanence of the treated soil. These respectively link to the "certainty of execution" during treatment and "health monitoring" throughout the service life of the treated soil. Fundamental science and engineering can enable optimization of a treatment process for attaining maximum uniformity and understanding of possible degradation mechanisms that may shorten the service life of the treated soil. However, soil deposits as well as their chemical and biological constituents are highly complex living systems that cannot be fully captured in any model. As a result, monitoring during treatment and throughout the service life of a treated soil zone is necessary. Secondary concerns and challenges include, but are not limited to, reducing treatment duration, recirculation of injection media, stimulation of indigenous microbial population, maximizing nutrient transport distance, and minimizing undesired residual byproducts from microbial metabolism (i.e., ammonium).

#### 3. Microbe-soil size compatibility

Central to the issue of treatment uniformity is the geometric compatibility between the microbes (either native or augmented species) and the soil in which they are injected. The relatively small



Net Urea Hydrolysis Reaction:  $NH_2$ -CO- $NH_2$  +3 $H_2O \rightarrow 2NH_4^+$  +  $HCO_3^-$  + OH

**Net pH increase:**  $[OH^-]$  generated from  $NH_4^+$  production >>  $[Ca^{2+}]$ 

Fig. 3. Overview of bio-mediated calcite precipitation using ureolysis.



Fig. 4. Possible alternative bio-mediated processes to create supersaturated conditions required for calcite precipitation. Gibbs free energy computed for standard conditions.

size of microbes, typically between 0.5 and  $3 \mu m$  (Madigan and Martinko, 2003), is advantageous. Soil particles cover a broad range in size, with a primary differentiation being made between "coarse" and "fine" grained soils at 75  $\mu m$  particle size (Fig. 2). As a result, microbes are capable of travelling through many soil types.

The primary restriction on microbial transport is the size of pore throats within the soil matrix through which the microbes must pass as they move from one pore space to another (Mitchell and Santamarina, 2005). The size of the pore throat is dependent on the smaller fraction of particles in soil, and can be estimated as 20% of the soil particle size that corresponds to 10% passing in a mechanical sieve analysis (Holtz and Kovacs, 1981). This provides an approximate lower bound limit on treatment by in-situ injection which depends on the particle size relative to the microbe size (Fig. 2). However, ex-situ mixing of microbes and nutrients with soil may extend the range of soils amenable to treatment into pure clays. The upper bound for effective treatment depends primarily on the fraction of microbes acting at particle–particle contacts.

In addition to considering the geometric compatibility before the desired chemical reaction is triggered and byproducts are produced, it is also necessary to consider how an aggregation of byproduct (inorganic precipitate), potentially including the facilitating microbes, may be able to migrate through treated soil. This requires estimation of the aggregate size as well as the reduction in pore throat size due to accumulation of precipitation around the pore throat and growth/degradation of microbial communities.

Based on these geometric limitations and published research, approximate application limits have been observed (solid lines in Fig. 2) and a broader range is possible. As evident, biomineralization appears applicable to the broadest range of soils, but this may be due in part to these systems being the primary focus of research studies to date. Biofilms have successfully formed in sand and in coarse gravels (Perkins et al., 2000; Rowe, 2005) in engineered systems.

#### 4. Bio-mediated calcite precipitation

The precipitation of calcite uniformly within soils through a process in which biological activity is used to elevate the pH in order to create supersaturated conditions is the treatment process which to date has been the focus of most studies (DeJong et al., 2006; Whiffin et al., 2007; Harkes et al., 2008). The process in which ureolysis is the chemical reaction used to increase the pH level is shown schematically in Fig. 3.

#### 4.1. Bio-geo-chem reaction network

Metabolic activity of Sporosarcina pasteurrii, an alkalophilic soil bacterium with a highly active urease enzyme (Ferris et al., 1996), consumes urea within the microbe, decomposing it into ammonia  $(NH_3)$  and carbon dioxide  $(CO_2)$ . These chemicals diffuse through the cell wall of the gram positive microbe and into the surrounding solution. Two reactions spontaneously occur in the presence of water; ammonia is converted to ammonium (NH<sub>4</sub><sup>+</sup>) and carbon dioxide equilibrates in a pH-dependent manner with carbonic acid, carbonate and bicarbonate ions. The net increase in pH is due to hydroxyl ions (OH<sup>-</sup>) generated from the production of NH<sub>4</sub><sup>+</sup> which exceeds the available Ca<sup>2+</sup> for calcite precipitation (Fig. 3). This provides the alkaline environment and carbonate required for the precipitation of calcite (CaCO<sub>3</sub>). The negatively charged bacterial cell is attracted to the soil particle surface due to a higher concentration of nutrients adjacent to surfaces (Hall-Stoodley et al., 2004) in addition to the physicochemical properties of both the bacterial cell and soil particle (Falk and Wuertz, 2007; Oliveira et al., 2003; Bouwer et al., 2000).

# 4.2. Alternative biological processes for inducing calcite precipitation

Identification of alternative biological processes that can increase the pH and create the supersaturated conditions necessary for calcite precipitation may be desirable due to the potential of natural soil microbial communities' efficiency for alternative reaction networks.

The primary alternative processes, namely denitrification, iron reduction, and sulfate reduction, are outlined in Fig. 4. Each reaction is configured for the consumption of acetate. The Gibbs free energy for each reaction is computed for standard conditions ( $T = 25 \degree C$ , P = 1 atm, [C] = 1 M). The equations are arranged in the hierarchy of subsequent electron acceptors used in nature, the same sequence in which these various mechanisms would engage given that all electron acceptors are present.

Predominance of these various mechanisms depends on their associated reaction's propensity to occur in the environment. This is quantified by the change in free energy of the overlying reaction (Morel and Hering, 1993). Ureolysis will be predominant as long as urea exists in the system. As seen in Fig. 4, the computed change in free energy at standard conditions for urea hydrolysis is relatively low as compared to other processes. Ureolysis is predominant in a manipulated soil amongst others because the reaction





Fig. 6. SEM elemental mapping of silica sand particles, precipitated calcite, and pore space (highlighted by carbon due to pore space being infilled with expoy). (*Note*: Lighter gray scale in each image denotes respective element concentration) (adapted from DeJong et al., 2006).

changes the environmental conditions of a system (i.e. increase in pH), which inhibits other competitive processes (Pikuta et al., 2007). Furthermore, introduction of the Sporosarcina-urea mixture does not include an oxidizable organic carbon source (e.g. acetate) required to drive the alternative processes. The challenge lies in the production of ammonia gas and how it might affect the cycling of nitrogen. If oxygen is present outside the zone of maximum biocementation, it will contact ammonia as it diffuses away from the area of urea depletion and result in substantial production of nitrate, i.e. nitrification. If an oxidizable carbon source is also introduced, denitrification would then be expected to dominate due to its highly negative free energy change (Fig. 4). This process is largely anaerobic and thus could possibly be advantageous for inducing this reaction at greater depths. One challenge would be controlling the output of carbon dioxide since for every mole of acetate this reaction produces twice as much  $CO_2(g)$ . Denitrification would dominate until nitrate was depleted (i.e. with greater depths in environmental systems). At this point, iron may be the predominant electron acceptor for microbial anaerobic respiration. Because solid iron oxyhydroxides are expected to be the dominant form of oxidized iron in soils, the remarkable ability of this bacterial group to employ a solid as a biological oxidant necessitates contact between bacteria and particles, which is attractive for initiating biocementation. Once iron(III) is depleted, sulfate reducing systems may prevail. Sulfate reduction is the least energetically favorable of these processes, but the electron uptake capacity based on sulfate can be substantially greater than for Fe(III) if soils are substantially influenced by seawater (Schink, 1999).

Researchers are beginning to examine these alternative mechanisms (e.g. Karatas et al., 2008; van Paassen et al., 2008a). The primary disadvantage/challenges of these alternative processes are

Change in phase relationship properties due to calcite precipitation.

the slower rate at which supersaturated conditions can be created and sustained. However, these issues may still be outweighed by the relatively innocuous byproducts produced. It is also noted that secondary benefits may be realized. As an example, a fortunate byproduct of denitrification may be gas generation, which could reduce the saturation level (and reduce liquefaction potential, for example).

#### 5. Spatial distribution of calcite within treated soil

The effectiveness of a bio-mediated treatment technique is directly dependent on the spatial distribution of the byproduct (i.e. calcite). Of particular interest is the percentage of byproduct produced that contributes to the binding of sand particles together and how the binding of particles degrades with loading.

#### 5.1. SEM microscopy

Results from scanning electron microscopy (SEM), as shown in Fig. 5, provide clear images of two particles that remain bonded after dissection of a laboratory specimen. Examining the image sequence in order of increasing magnification, the precipitated calcite coats the open/exposed surfaces of particles relatively uniformly and there is a greater concentration of calcite at the particle–particle contacts. Examination of the highest resolution image reveals impressions of the microbes within the precipitated calcite. Elemental SEM scanning of treated sand specimens prepared by epoxy impregnation and surface polishing clearly reveal the silica particles and the precipitated calcite phases (Fig. 6).

Treatment conditionInitial void ratio $V_{calcite}$ $V_{v-initial}$ (%)Final void ratioRelative density (%)Shear wave velocity @ 100 Kpa (mUntreated - $e_{max}$ 0.870160Untreated-40% Dr0.74-40180Lightly cemented0.7460.6763~350Heavily cemented0.74170.55100~1000Untreated - $e_{min}$ 0.55100210						
Untreated - $e_{max}$ 0.870160Untreated-40% Dr0.7440180Lightly cemented0.7460.6763 $\sim350$ Heavily cemented0.74170.55100 $\sim1000$ Untreated - $e_{min}$ 0.55100210	Treatment condition	Initial void ratio	$V_{\text{calcite}} V_{\text{v-initial}}$ (%)	Final void ratio	Relative density (%)	Shear wave velocity @ 100 Kpa (m/s)
Untreated-40% Dr 0.74 - 40 180   Lightly cemented 0.74 6 0.67 63 ~350   Heavily cemented 0.74 17 0.55 100 ~1000   Untreated -e <sub>min</sub> 0.55 - - 100 210	Untreated – e <sub>max</sub>	0.87	-	-	0	160
Lightly cemented 0.74 6 0.67 63 ~350   Heavily cemented 0.74 17 0.55 100 ~1000   Untreated -e_min 0.55 - - 100 210	Untreated-40% Dr	0.74	-	-	40	180
Heavily cemented 0.74 17 0.55 100 ~1000   Untreated -e_min 0.55 - - 100 210	Lightly cemented	0.74	6	0.67	63	~350
Untreated - <i>e</i> <sub>min</sub> 0.55 100 210	Heavily cemented	0.74	17	0.55	100	$\sim \! 1000$
	Untreated $-e_{\min}$	0.55	-	-	100	210



**Distribution Alternatives** 

Fig. 7. Illustration of calcite distribution alternatives within pore space.

#### 5.2. Alteration of pore space

The precipitation of calcite results in a decrease in the pore space and an increase in the solids content. The alteration of these phases can be captured through quantitative analysis of SEM images similar to those presented in Fig. 6a, but at a lower magnification such that more than 200 particles are captured in a single image. Images of untreated specimens, specimens treated to a moderate level of cementation, and specimens treated to a higher level of cementation were analyzed. All specimens are of Ottawa 50–70 sand ( $D_{50} = 0.12$  mm,  $C_u = 1.6$ ,  $C_c = 0.8$ ,  $G_s = 2.65$ ) prepared to a "loose" initial state at a void ratio of 0.74 (Table 1). This corresponds to an initial relative density of 40% given a minimum void ratio of 0.87 (0% relative density).

Bio-treatment resulted in 6% and 17% of the initial void space (prior to treatment) being filled with precipitated calcite for the moderately and heavily treated specimens (Table 1). This effectively reduced the void ratio (porosity, n = e/1 + e) from 0.74 down to 0.67 and 0.55, respectively. The relative density correspondingly increased to 63% and 100% with respect to the untreated minimum and maximum void ratios. The effective densification of the soil alone (ignoring all the additional benefits of particle–particle cementation, increased particle angularity, etc.) provides significant improvement to soil properties in terms of increased shear strength, reduced compressibility, reduced permeability, and a change from contractive to dilative volumetric behavior (at the test stress level of 100 kPa).

#### 5.3. Distribution of calcite within pore space

The reduction in void space (porosity) provides insight into the change in global properties, but as mentioned before, the calcite distribution within the pore space at the particle level (mm scale) is critical. Fig. 7 provides schematics of the two extreme possibilities of how calcite may be distributed around soil particles. "Uniform" distribution indicates the calcite precipitated at an equal thickness around soil particles. Consequently, the bonding between the two particles by calcite is relatively small, and as a result insignificant changes to soil properties (aside from those discussed in the previous section regarding soil densification) may be expected. "Preferential" distribution indicates the calcite precipitated only at particle-particle contacts. This is the desired engineered spatial distribution as all calcite directly contributes to the improvement in soil properties.

Bio-geo-chemical processes do not naturally optimize for soil engineering properties. Therefore the "preferential" distribution is unrealistic. Fortunately, the "uniform" distribution (highly inefficient from an engineering perspective) is also unrealistic. Analysis of SEM (Figs. 5 and 6) and X-ray computed tomography images consistently reveal that the "actual" distribution of precipitated calcite (Fig. 7) is a balance of these two extreme conditions. Importantly from the soil improvement perspective is that a significant portion of the calcite is in the vicinity of the particle-particle contacts.

The spatial distribution of calcite is governed by biological behavior and filtering processes. Microbes have a general preference to remain away from exposed particle surfaces and instead prefer positioning themselves in smaller surface features, such as near particle-particle contacts. This preference is due to reduced shear stresses and a greater availability of nutrients at the grain contacts. A greater concentration of microbes near the particleparticle contacts directly results in increased calcite precipitation in the region. In addition to these biological preferences, filtering (straining) processes may also contribute. Calcite precipitated in the pore fluid or precipitated elsewhere on particle surfaces but subsequently released and suspended within the pore fluid will have a tendency to become (re-) attached near particle-particle contacts as the pore fluid flows through the surrounding pore throat (i.e. the smallest pore space that connects two larger pore cavities). Filtering processes are highly dependent on the relative size of the suspended calcite and the pore space (Valdés and Santamarina, 2005), and therefore this effect will become more significant as the extent of precipitation increases (the pore throat space decreases).

#### 5.4. Failure mechanisms of calcite due to shearing/loading

After calcite binds particles together at their contacts during the initial treatment phase, the performance of the binding under loading (i.e. shear, compression, and tension loading during the construction and service life of a project) is of interest.

Two simplified alternatives of how the calcite binding silica particles may fail are shown schematically in Fig. 8. Under shear, tension, and compression loading a fracture may occur within the precipitated calcite ("calcite-calcite" in Fig. 8) or between the precipitated calcite and silica sand ("calcite-silica" in Fig. 8). A series of SEM and X-ray computed tomography images of the fracture planes of specimens subjected to one of the three modes of loading were examined. Images consistently revealed that a layer of calcite remained on both surfaces of silica sand particles where a particle contact once was. The generation of small particles (fines content) in the form of precipitated calcite was also noticeable (and gualitatively appeared to be greater in compression and shear loading modes). These observations maybe expected (based on the middle images of Fig. 5 for example) which indicates that the calcite aggregates form a rather heterogeneous structure that is weak relative to the silica particles.



Fig. 8. Illustration of calcite failure mechanism alternatives of calcite due to compression and/or shearing.

Table 2

Overview of process monitoring methods for non-destructive detection of improvements to soil matr from bio-mediated processes.

Monitoring technique	Fundamental relationships	Primary soil properties affecting measurement	Primary measurement methods
Shear wave velocity (Vs)	$Vs = (G/\rho)^{1/2} G$ = shear modulus, $\rho$ = density	Particle-particle contact stiffness, particle stiffness, soil density, confining stress, degree of saturation (especially as fines content increases)	Laboratory: piezoceramic bender elements, resonant column field: seismic CPT, cross-hole, downhole
Compression wave velocity (Vp)	Vp = $((B + 4/3G)/\rho)^{1/2} G$ = shear modulus, $\rho$ = density, B = bulk modulus	Bulk modulus of the pore fluid, degree of saturation, porosity, bulk modulus of material comprising grains	Laboratory: ultrasonic transducers, piezoceramics Field: Seismic reflection/refraction, Surface analysis of spectral waves
Resistivity ( $\Omega$ )	Ω = ε J ε = electric field, J = current density	Volume fractions of particles and voids, particle mineral composition, pore fluid chemical composition, soil particle specific surface area, degree of saturation, soil fabric anisotropy	Laboratory & Field: Wenner and Schlumberger arrays deployed on surface or within in-situ probes (e.g. CPT)

#### 6. Non-destructive geophysical process monitoring

Geophysical measurements are invaluable in bio-mediated soil improvement treatments as they serve as the "interdisciplinary language" capturing in real-time how the biological and chemical components are altering the soil engineering properties. These processes measure the treatment impact on the soil matrix and correlate reasonably with other engineering properties. Shear wave velocity, compression wave velocity, and resistivity (the inverse of conductivity) are the three primary geophysical methods of use.

#### 6.1. Resistivity

Resistivity (or conductivity) provides a measure of the voltage potential gradient through a soil matrix when an electrical current is induced across a soil specimen. Soil resistivity is dependent on the volume fractions comprised of particles and voids, the mineral composition of the particles, and the chemical composition of the pore fluid as well as soil particle specific surface area, degree of saturation, and soil fabric anisotropy (Table 2). In the case of bio-treatment, the volume fraction and composition of the third soil phase (e.g. precipitated calcite) also influences the resistivity as it differs from the particle and fluid characteristics and coats the particles. Resistivity measurements have been utilized to detect variations in soil density (Li et al., 2005), soil compression, changes in pore fluid composition (Klein and Santamarina, 2003), and biological activity (Snieder et al., 2005). When implemented with two and three dimensional sensor arrays, electrical resistivity tomography (ERT) has been used to track displacements and deformations within soil (Li et al., 2005), the extent of soil improvement, the migration of contaminant/chemical plumes, and the progress of passive bio-remediation methods (Williams et al., 2005). The use of resistivity for monitoring bio-mediated soil improvement (e.g. calcite precipitation) has been studied minimally, in part because changes to all (or nearly all) of the variables stated above during treatment and therefore it is difficult to separate out the benefits actually realized in the soil matrix (the engineering objective) from the different stages of the chemical and biological systems (the process).

#### 6.2. Compression waves

The propagation of elastic waves through soil occurs at very small stain levels (Santamarina et al., 2001) in two primary propagation modes. The first mode, the compression wave, corresponds to compression and dilation of the material parallel to the direction of propagation. Compression waves (P-waves) travel effectively through solids (e.g. rock, soil particles) and fluids (e.g. pore fluid), and are dependent on the bulk stiffness (*B*) and the shear stiffness (G) (Table 2). In single phase materials (including bonded multi-phase materials that can be reasonably modeled as a continuum), such as rock, concrete, etc., the P-wave velocity is generally larger than 2500 m/s and can be up to 6000 m/s. In multi-phase (particulate-pore space) systems (including unbounded and lightly bonded multi-phase materials), such as cohesionless and lightly cemented soil - the materials that would be candidates for biomediated soil improvement - the P-wave velocity is primarily dependent on the porosity, the fluid bulk stiffness, and the material comprising the soil particles. However, it is not sensitive to the shear stiffness of the soil matrix. The P-wave velocity of water is about 1500 m/s. When a soil is 100% saturated (i.e. no air bubbles) the P-wave velocity through soils is nearly equal to that of water. As the degree of saturation decreases (from bubbles forming from air injection or gas generation from a chemical or biological process) the P-wave velocity decreases rapidly down to about 500 m/s as the saturation is decreased to 99% and then continues degrading to a value of about 200 m/s dry sand. This is because the presence of air/gas bubbles in the pore fluid has substantially increased the soil compressibility. The properties of the particle matrix (i.e. density, particle coordination number, stress level, etc. which all affect the particle matrix compressibility) have a secondary influence on the P-wave velocity. As a result, the use of P-wave velocity is excellent for detecting changes in pore fluid compressibility such as due to bio-mediated gas generation for reduction in liquefaction potential (Ishihara et al., 1998) and improvements due to cementation when sufficient cementation exists such that the compression velocity through the cemented soil matrix is greater than 1500 m/s. However, P-wave velocity in unbounded and lightly cemented soils does not correlate directly with strength unless the soil matrix maintains a constant saturation level and/or until sufficient cementation has occurred such that the particle matrix compressibility significantly exceeds that of water.

#### 6.3. Shear waves

Shear waves (S-waves), the second mode of propagation, in which the direction of particle motion is perpendicular to the direction of propagation requires the material to have shear stiffness (G>0) (Santamarina et al., 2001). Therefore shear waves do not propagate through fluids. This is advantageous when monitoring changes to the particle soil matrix since the measured shear wave velocity is largely unaffected by the pore fluid composition (the exception to this case is when large matrix suction forces generated by very low saturation increase the effective confining stress at particle-particle contacts). The shear wave velocity of unbounded materials (cohesionless soils such as sands) is then directly dependent on the void ratio, coordination number (average number of surrounding particles a given particle is in contact



Fig. 9. Schematic (a) and experimental data (b) exemplifying the stages of discrete bioaugmentation treatment injections into sand and the associated changes in shear wave velocity as measured by bender elements.

with), and confining stress. Cementation, or binding, between particles also directly influences the shear stiffness. Shear wave velocity has broad utility for monitoring bio-mediated methods in which the soil is improved through cementation between particles (Bian et al., 2008; Brandenberg, 2008). S-waves can accurately capture the initial untreated soil conditions as well as treatment impact on the actual soil. For the bio-mediated calcite precipitation process the shear wave velocity only captures the precipitated calcite active in binding soil particles together. It is not influenced by calcite suspended in the pore fluid or attached to the open particle surfaces.

#### 6.4. Measurements with shear and compression waves

Measurement of shear and compression waves can be easily made in the laboratory and in the field, lending themselves to use during upscaling of a bio-treatment process from the laboratory to the field. Technologies have already been developed across all relevant length scales. In the laboratory piezoceramic bender elements can be installed for S- or P- wave measurements in most devices (e.g. Pennington et al., 1997; Santamarina et al., 2001) while other devices are designed specifically for S- and P- wave measurements (e.g. resonant column). A pair of bender elements, separated across a soil specimen at a known spacing, is used to generate and sense an elastic wave as it travels through the soil. The time delay between wave generation and sensing along with the known spacing is used to directly measure the shear wave velocity. Ultrasonic transducers can also be used for P-wave measurements (Lee and Santamarina, 2005). At the field scale, downhole (ASTM D7400 2007) and crosshole (ASTM D4428 2007) systems as well as the seismic cone penetration test (SCPTu, ASTM D5778 2007) can be employed in the field for direct measurement. All of these systems, both laboratory and field, can be configured in two- and threedimensional arrays for spatial mapping of changes in soil properties (Lee et al., 2005).

# 6.5. Example of shear wave velocity monitoring during bio-treatment

An example of utilizing shear wave velocity measurements in the laboratory using bender elements is presented in Fig. 9. As described in further detail by DeJong et al. (2006), a shear wave (sine wave with 10V amplitude and frequency of 10kHz) is generated by a parallel poled transmitting bender element. This wave travels through the soil specimen across a known distance to the series poled receiving bender element, and the time delay between the sending and received wave is determined using an oscilloscope. The shear wave velocity is then determined as the separation distance divided by the time delay. The measurement is immediate and performed as desired throughout testing.

A generalized trend of how shear wave velocity will detect the cementation of soil particles using bio-mediated calcite precipitation is shown schematically in Fig. 9a. The process shown is for a sequence of discrete treatment injections (as opposed to continuous pumping). For each injection the shear wave velocity remains constant as the pore fluid is replaced with fresh medium. After injection the biological activity gradually raises the pH to a level where calcite precipitation occurs. The initiation of calcite precipitation at particle-particle contacts is detected with an initial increase in shear wave velocity. The velocity continues to increase until some limiting condition is reached, such as no additional calcite is available in solution. The velocity then remains constant until a subsequent injection occurs and the process is repeated. Experimental results of this process are shown in Fig. 9b, with each vertical arrow indicating an additional discrete injection.

The direct mapping of the cementation quality with shear wave velocity would be equally effective in a continuous injection process. Furthermore, it is also useful for mapping the spatial uniformity of bio-treatment, the stability of the treatment over time, and the degradation of the treatment due to treated soil failing upon loading (shown subsequently).

#### 7. Improvement of engineering properties of soils

Bio-mediated treatment techniques can result in the improvement of a variety of soil properties including permeability, stiffness, compressibility, shear strength, and volumetric behavior. As mentioned, the potential improvement a given treatment method could have on engineering properties can often be reasonably estimated based on data in existing literature of conventional (non-biomediated) treatment methods that induce similar changes to the soil matrix. For the bio-mediated calcite precipitation treatment



Fig. 10. Illustration of impact of calcite precipitation on shear response of loose sand.

method, prior studies on cementation agents (e.g. epoxies, cement, gypsum, CIPS) provide clear insight.

#### 7.1. Permeability

The formation of calcite precipitation near particle-particle contacts reduces the pore throats and restricts water flow. Experimentally, Ferris et al. (1996) observed a reduction in permeability from 15% to 20% of the initial permeability and Whiffin et al. (2007) similarly observed a reduction in permeability from 22% to 75% of the initial permeability. However, it is conceivable that the permeability could be reduced further with additional treatment. In experimentation it should be recognized that permeability may not be uniform across treated specimens, with a general tendency of greater reduction in permeability occurring closer to the injection source.

#### 7.2. Stiffness

The stiffness increase induced by bio-mediated calcite precipitation can effectively be captured throughout treatment using bender elements as shown previously in Fig. 9. Subsequent experiments have shown increases in the shear wave velocity to more than 1200 m/s. For context, this corresponds to "rock" according to the National Earthquake Hazards Reduction Program (NEHRP, 2003) site classification index based on shear wave velocity that is used in the United States to screen for potentially liquefiable soils. This general range of increase is consistent with those observed for nonbio-mediated treatment methods as documented by Santamarina et al. (2001) and Brandenberg (2008).

## 7.3. Shear strength and volumetric response

The improved response of loose sand treated with bio-mediated calcite precipitation to undrained shearing occurs from two effects – densification and cementation – as outlined schematically in Fig. 10. The behavior of untreated loose sand subjected to undrained

monotonic triaxial shearing begins with an initial void ratio ( $e_{initial}$ ) and mean effective stress acting on the particle matrix (p') that exists above the critical state line (CSL; which corresponds to unique combinations of void ratio and mean stress at which soil can shear without changing in volume) (Wood, 1996). As a result, during undrained shear (no volume change allowed and void ratio must stay constant) the pore fluid will carry an increasing portion of the applied load (q) and the mean effective stress will decrease until the specimen reaches the critical state line (CSL). This decrease in mean stress effectively results in sand collapse and relatively small mobilized shear strength (correlated to the applied deviator stress, q) when the failure envelope (with slope of M where  $M = (6sin\varphi)/(3 - sin\varphi)$  and  $\phi = tan^{-1}\mu$  where  $\mu$  is the coefficient of friction) is reached.

The densification of soil alone (not considering any of the binding/cementation effects created by the calcite) can significantly alter soil behavior. As documented in Table 1, precipitated calcite reduces the void ratio (from  $e_{\text{iniital}}$  to  $e_{\text{treated}}$  in Fig. 10). This densification can result in the soil conditions being below the critical state line. Upon undrained shearing the mean stress will increase until the critical state line is reached due to the propensity of soil to dilate. It is noted that the critical state line itself is shifted (from CSL to CSL\* in Fig. 10) due to the calcite changing the particle properties (e.g. shape) and gradation. The extent and direction the CSL shift is unknown, but may be slightly upward (Cho et al., 2006). Due to localization (bifurcation) during shearing, the critical state line is often temporarily exceeded before stabilizing back on the critical state line. This results in a substantial increase in strength (as evidenced in *p*'-*q* space) to a peak resistance before the resistance then decreases until the failure envelope is reached (Fig. 10). The failure envelope reached (line with slope M<sup>\*</sup>) is greater than that for the untreated soil (line with slope M) since the increased density and particle angularity (from precipitated calcite) increases the resistance to shearing.

Cementation of particles together by calcite precipitation further increases soil strength. The cementation increases the initial stiffness of soil at small strains and the maximum deviatoric stress



Fig. 11. Experimental data from undrained triaxial shear response of untreated and bio-treated specimens.

(q) that can be applied before the specimen begins to yield. This is revealed in a more rapid increase in shear resistance (q) as the mean stress (p') increases, and a greater overall shear resistance (q) (Fig. 10). As the maximum shear resistance is reached, localized shearing and breakage of calcite cementation at particle-particle contacts initiates (usually in the form of a failure plane or shear band). As bonds continue to break the shear resistance continues to decrease until the soil has completely failed and the benefit of cementation is lost. However, at this failed state the benefits of densification are still realized (i.e. higher failure envelope of M\* due to densification still applies).

Experimental results capturing this behavior are shown in Fig. 11. The details of the experimental program, including soil type, specimen size, treatment program, etc. is presented in DeJong et al. (2006). The untreated loose sand specimen exhibits monotonic collapse while the bio-treated specimen increases substantially with shear to a strength above the failure surface (Fig. 11a). During the shearing progression (Fig. 11b) the untreated specimen generates



Fig. 12. Experimental results from bio-mediated improvement of soil support a shallow footing foundation.



**Fig. 13.** Variables in optimization of calcite precipitation bio-treatment process. (a) Biogeochemical variables exemplified by increase in precipitation rate (capture indirectly through increases in soil stiffness detected by shear wave velocity) due to increase in CaCl<sub>2</sub> concentration. (b) Geotechnical variables exemplified by differences in precipitation rate in different soil gradations.

no initial peak strength and the strength gradually increases (to q/p' = 1.2) as the specimen moves along the failure surface (Fig. 11a). In contrast, the bio-treated specimen mobilizes a larger peak strength (q/p' = 1.7) and then degrades towards a higher-strength envelope (with slope M\*).

The shear wave velocity measurements obtained provide clear insight into the degradation of calcite cementation when specimen failure occurs during shearing. The shear wave velocity for the untreated specimen decreases slightly initially due to a decrease in p as the critical state line is approach and then gradually increases. The bio-treated specimen velocity begins at about 540 m/s (the final velocity shown for the treatment phase in Fig. 9b) and then decreases rapidly at small strains as the peak resistance is mobilized. The shear wave velocity continues to capture the degradation of the cemented sand matrix as the shear failure continues. Since the shear wave velocity is an average measurement over the specimen length and localized shearing occurs at larger strains in cemented soils, the S-wave values at these larger strains are averages across zones of intact and failed cemented soil.

#### 8. Envisioned advantages

The development of bio-mediated processes for soil improvement has several characteristics that may prove advantageous relative to industry standard soil improvement techniques. These include:

- Reduced costs use of natural materials, reduced treatment injections, etc.
- Reduced impact to the environment use of natural materials that do not permanently alter subsurface conditions
- Improved treatment uniformity biological processes have potential to enhance spatial uniformity
- Optimal treatment concentration degree of treatment can be controlled and monitored
- Adaptable duration treatments can be removed if only temporary support needed (e.g. by reversal of chemical processes)
- Hydraulic and mechanical control degree of treatment can be adjusted
- Flexible implementation methods can be used in new and retrofit construction
- $\bullet$  Penetration into soils w/ fines cells are typically 1–3  $\mu m$  in size and self-mobile.

## 9. Envisioned applications

The above attributes readily lend the treatment technique to civil infrastructure applications and possible broader applications for national and international security, energy storage, and global warming. These include, but are not limited to:

- *Liquefaction prevention* cementation of subsurface to prevent liquefaction and its damage
- Building settlement reduction reduce settlement and increase bearing capacity for foundations
- *Dam and levee safety* upstream injection of technique would "plug" erosive piping
- Tunneling soil stabilization prior to tunneling would reduce disruption and increase efficiency
- Scour/erosion prevention treatment would increase resistance to erosive forces of water flow
- *Bluff and slope stabilization* treatment could provide additional stability needed to prevent failures
- Impermeable barriers barriers to stop/divert subsurface transport of contaminants
- *Reactive barriers* opportunity for creation of barriers that treat/clean groundwater as it flows
- *Groundwater protection* treatment to immobilize materials before contamination of aquifers
- *Emergency immobilization* rapidly secure contaminants from hazards (e.g. terrorist activities)
- Aquifer storage and recovery treatment to enhance storage and reduce losses in aquifers
- Energy (fuel) storage used to create subsurface facilities for storage of liquefied natural gas
- Carbon sequestration used to create subsurface facilities for storage of CO<sub>2</sub>.

A simple example of the improved application performance that can be realized is shown in Fig. 12. A zone of soil beneath a model footing was treated with bio-mediated calcite precipitation. The settlement induced by loading of the footing was decreased by five times at a footing stress of about 30 kPa. Details of the model shallow foundation test are presented in Martinez and DeJong (in press).

#### 10. Primary challenges

Realization of the above applications using bio-mediated soil improvement techniques requires step improvements in aspects of science, engineering, and education. Many of these issues were identified and discussed at an international workshop as reported by DeJong et al. (2007).

#### 10.1. Research

Bio-mediated soil improvement requires significant advancement in fundamental science and engineering regarding system heterogeneity, soil structure and pore space distribution, fluid movement and transport, and biodiversity. Each of these items requires further research in their respective disciplines, let alone integration of each item across disciplines (e.g. characterization of nutrient transport through and biological distribution within a realistic pore structure).

Successful field implementation requires upscaling of biological, chemical, and geotechnical (soil) systems that is not empirical, but instead based on fundamental principles. This effort will integrate analytical work, numerical modeling (van Paassen et al., 2008b), and experimentation. The two primary issues regarding field application are the optimization of the treatment process given site specific conditions and monitoring of the treated soil during treatment and periodically throughout the service life of the system. An example of two variables relevant to treatment optimization is exemplified in Fig. 13. The concentration of available calcium is one factor that will influence the treatment duration assuming a shear wave velocity criterion is specified for the end of treatment (Fig. 13a). Similarly, results of bio-treatment in different soils indicate an inability to treat soils with no sand or fines content (Fig. 13b). The actual variables are more numerous (e.g. biostimulation versus bioaugmentation, natural groundwater chemistry, available oxygen/electron acceptor, in-situ temperature, microbial competition) and an appropriate balance of modeling/analysis based on fundamental principles and applied field engineering with performance monitoring (e.g shear waves) must ultimately be developed.

## 10.2. Education

Overcoming the above challenges will require decades of work, and currently there is no formal education or training system to develop competent individuals equipped to lead this effort. Education - and graduate education in particular - in the latter part of the 20th century focused on individuals developing expertise in a single niche area. This is insufficient for an interdisciplinary field such as bio-mediated soil improvement where an individual must have expertise (core competency) in a certain area(s) but must be knowledgeable and able to communicate across several different disciplines. Efforts must be made in education institutions as well as in government and industry to foster this broader, renaissance type, education. Examples of what may be done include interdisciplinary seminar series, interdisciplinary (co-taught) courses, research internships, paper discussion groups, interdisciplinary graduate programs, science/engineering/business combined Master's degrees, and doctoral training degrees (interdisciplinary coursework only degree) (DeJong et al., 2007).

## 11. Conclusions

The field of bio-mediated soil improvement is rapidly emerging, the range of opportunities continues to expand, and challenges lie ahead. The following summarizes the primary observations made herein:

- The \$6B/yr, 40,000 projects/yr ground improvement industry continues to expand due to the ever increasing need for the improvement of soils to support new infrastructure and existing infrastructure that must be further stabilized.
- The development and management of bio-mediated systems are uniquely interdisciplinary since biological processes are used to control the rate, timing, and distribution of how a chemical process occurs within soil.
- The geometric compatibility between the soil matrix (most effectively characterized with particle size and pore throat parameters) and the microbial size (both before treatment when the actual microbial size is relevant and after treatment when microbes may be attached/bound/encased by precipitate) is the primary factor dictating the range of soils that can be treated by a given microbe via in-situ injection. Ex-situ mixing of microbes with soils provides a broader range of possibilities.
- Bio-mediated calcite precipitation in supersaturated fluids by pH control through the management of microbial ureolysis has strong potential. Alternative methods for pH control, namely through denitrification, iron reduction, and sulfate reduction, while less efficient should also be explored due to more benign byproducts being produced.
- Microscopy techniques have established calcite precipitation reduces the pore space, effectively resulting in densification of the soil.
- Microscopy techniques have established that calcite precipitation occurs preferentially near particle-particle contacts within the soil matrix.
- Fracturing of treated soil due to shear and tension loading results in breakage with the precipitated calcite and not between the precipitated calcite and the silica soil particles.
- Non-destructive geophysical process monitoring techniques provide real-time information that links how the bio-mediated chemical processes are influencing the soil matrix. For most biomediated improvement systems the shear and compression wave velocities are the two best indicators, while resistivity primarily provides insight into the change in pore fluid composition.
- The geophysical methods provide a promising method to monitor a site during treatment and throughout the service life, enabling assessment of treatment uniformity and permanence, respectively. It is noted that supplemental sampling and laboratory tests may be required for verification and calibration of geophysical data.
- A range of soil properties can be significantly altered with bio-mediated improvement processes. Results indicate that the permeability can be reduced by 10<sup>-3</sup>, the stiffness can be increased by 10<sup>2</sup>, the compressibility deceased by 10<sup>-2</sup>, the shear strength increased by 10<sup>2</sup> and the volumetric response to be changed from being contractive to being dilative.
- Bio-mediated soil improvement processes offer a number of potential advantages over current techniques and the range of envisioned applications is far-reaching.
- Upscaling of bio-mediated improvement techniques to the field scale provides a significant challenge. The uniform treatment of a large zone of soil require system modeling based on first principles and the development of real-time field-scale monitoring techniques to ensure spatially uniform treatment.
- Institutional reform is required in order to provide a renaissance type education in which individuals are developed with expertise in a specific area as well as knowledge of and the ability to communicate with different fields.

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