Biocementation through Microbial Calcium Carbonate Precipitation

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Abstract

Biocementation through microbial carbonate precipitation is a new branch of microbial geotechnology that deals with the applications of microbiological methods to produce cemented materials used in engineering. The primary consideration of these applications is to improve the geophysical properties of soil so that it will be suitable for construction and environmental purposes. The applications of biocementation would require an interdisciplinary research at the confluence of microbiology, ecology, geochemistry, civil and environmental engineering. This new field has the potential to meet society's expanding needs for innovative treatment processes that improve soil engineering properties. This paper presents an overview of biocementation, particularly through microbial calcium carbonate ($CaCO_3$) precipitation, and non-destructive geophysical techniques for real-time monitoring of soil engineering properties. Focus is then narrowed to an example of laboratory-scale test of biocementation of sandy soil and measurement of strength development by shear wave velocity (V_s). Other analytical results included microscopic imaging by scanning electron microscope (SEM) and identification of $CaCO_3$ precipitation presented in biocemented sand by X-ray diffactometer (XRD) were discussed. Potential advantages and envisioned applications of biocemented soil improvement are identified.

Keywords : Ground improvement, Biocementation, $CaCO_3$ precipitation, Shear wave velocity, Non-destructive test

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1. Introduction

Current soil improvement techniques (i.e. the usage of cement and chemical additions) use large amount of energy, man-made materials and create environmental concerns. Recently, the new technique using microbial biotechnology for producing biocement is being interest due to environmentally friendly, low-energy input and also microorganisms used in the process are non-pathogen. Furthermore, unlike the use of cement, soils in the fields can even be treated or improved without disturbing the ground or environment as microorganisms can penetrate and reproduce themselves in the soil naturally. It has been reviewed that some microorganisms i.e. *Bacillus* sp. and *Sporosarcina* sp. in the medium contained urea and calcium ion can induce precipitation of calcite [1-3]. Thus, new exciting opportunities for utilizing biological processes to improve soil properties have recently emerged. These opportunities have been enabled through interdisciplinary research at variety of fields including microbiology, geochemistry, and civil engineering.

Calcium carbonate ($CaCO_3$) precipitation is a common natural phenomenon found in the environments that are oversaturated in carbonate ions, i.e. underground water, sea water, and soils. It has a great importance in many environmental and civil engineering applications. Generally, this process can occur via abiotic and biotic pathways. Biologically induced $CaCO_3$ precipitation by ureolytic bacteria has been proposed for several biotechnological applications. This article paid attention to research background on biological mechanisms of $CaCO_3$ precipitation and illustration of biocementation technique for improving the engineering properties of soils. Example of this technique was applied in sandy soil. The source of urea degrading bacteria was originated from natural water (Chaophraya River, Thailand), where the bacterial community is mixed cultures. Non-destructive test using shear wave technique was applied to measure strength development in biocementing process. The potential use of microbially induced $CaCO_3$ precipitation as a soil biocement is discussed.

2. Biological process induced calcium carbonate precipitation

Currently, techniques for ground improvement are being explored which aim at enhancing soil properties on demand by stimulating natural bio-chemical processes in- situ [1-6]. One of these technologies is biocementation (or biocalcification), an in-situ soil strengthening technique involving microbial-induced $CaCO_3$ precipitation. Several studies have shown that this process can be used to improve the mechanical properties of porous materials [1-3, 5-9]. In most of these studies, $CaCO_3$ precipitation was induced by hydrolysis of urea in a solution with calcium chloride ($CaCl_2$). Purified urease enzymes or whole bacterial cells, containing the enzyme in high concentrations, were used to catalyse the hydrolysis of urea and produce ammonium and carbonate ions leading to an increase of pH level and precipitation of $CaCO_3$.

Biocementation (or biocalcification) through ureolysis presents several advantages over the other carbonate generating pathways, as it can be easily controlled and it has the potential to produce large quantity of carbonate within a short period of time [10]. Fig. 1 illustrates bio-chemical reactions involving the induction of $CaCO_3$ precipitation. Bacteria produce enzyme urease that hydrolyzes urea ($CO(NH_2)_2$ or NH_2-CO-(NH_2) to ammonium (NH_4^+) and carbonate (CO_3^{2-}) ions.

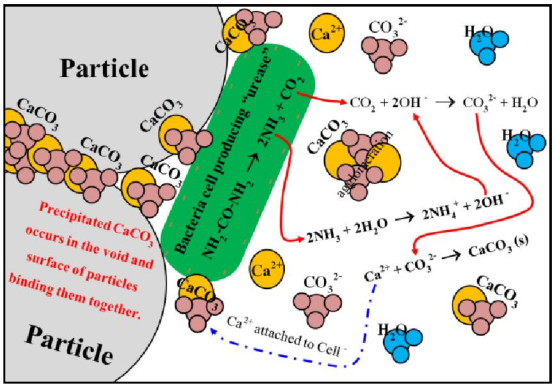


Fig. 1. Overview of biological and chemical processes via ureolysis inducing calcium carbonate precipitation adapted from [2].

The reaction is initiated by 1 mole of $CO(NH_2)_2$ hydrolyzing intracellularly to 1 mole of ammonia (NH_3) and 1 mole of carbamate (H_2COOH) as described in Eq. (1), which spontaneously hydrolyzes to 1 mole of NH_3 and carbonic acid (H_2CO_3) in Eq. (2). These products subsequently equilibrate in water to form CO_3^{2-} and two moles of NH_4^+ and hydroxide (OH) ions in Eqs. (3) and (4). These chemical sequences can be summarized and rewritten by Eq. (5). Production of NH_4^+ results in increase of pH. In the presence of sufficient Ca^{2+} and CO_3^{2-} ions, this will subsequently result in formation of $CaCO_3$ precipitation in Eq. (6). Some Ca^{2+} ions are bound to the cell wall of bacteria can result in the formation of $CaCO_3$ crystals on the bacterial cell and some Ca^{2+} ions are bound to soil particles can also result in the formation of crystals on the surface of particles. In addition, precipitation of $CaCO_3$ can also occur in the bulk phase of the liquid (see also Fig. 1). Those presences of $CaCO_3$ can fill the soil pores, bind soil particles together and increase solid content in soil [2, 10-12].

$$CO(NH_2)_2 + H_2O \rightarrow H_2COOH + NH_3 \quad (1)$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad (2)$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4 + 2OH^- \quad (3)$$

$$2OH^- + H_2CO_3 \rightarrow CO_3^{2-} + 2H_2O \quad (4)$$

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad (5)$$

$$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3 \quad : K_{sp} = [Ca^{2+}]\,[CO_3^{2-}] \quad (6)$$

Five key factors are involving precipitation of $CaCO_3$ including (1) the Ca^{2+} concentration, (2) the concentration of dissolved inorganic carbon (DIC), (3) microorganisms, (4) the medium pH and (5) the availability of nucleation sites [10, 12, 13]. It is noted that by biological $CaCO_3$ precipitation requires sufficient Ca^{2+} and CO_3^{2-} ions so that the ion activity product exceeds the solubility constant (described by Eq. (6), $K_{sp,\ calcite}$ at 25 °C = 4.8×10^{-9}) [10]. However, some researchers reported that increasing urea and Ca^{2+} concentration more than 90 g L^{-1} do not increase the amount of $CaCO_3$ obtained by this process [7].

The concentration of CO_3^{2-} ions is related to the pH and concentration of DIC of a given aquatic or water saturated terrestrial systems. A pH increase is an indication of urea hydrolysis; and at any media pH, NH_3 and NH_4^+ exist at different concentrations. Higher concentrations of NH_3 and NH_4^+ provide favorable conditions $CaCO_3$ formation [14]. Additionally, the DIC concentration depends on several environmental factors i.e. temperature (T), atmospheric pressure (P) and the concentration of soluble carbon dioxide (CO_2). The equilibrium reactions and constants governing the dissolution of CO_2 in aqueous media, for example at T = 25 °C and P = 1 atm, are given in Eqs. (3) to (6) [10, 15].

$$CO_2(g) \leftrightarrow CO_2(aq.) \quad : (pK_H = 1.468) \quad (7)$$

$$CO_2(aq.) + H_2O \leftrightarrow H_2CO_3^* \quad : (pK = 2.84) \quad (8)$$

$$H_2CO_3^* \leftrightarrow H^+ + HCO_3^- : (pK_1 = 6.352) \quad (9)$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ : (pK_2 = 10.329) \quad (10)$$

with $H_2CO_3^* = CO_2(aq.) + H_2CO_3$

Selection of suitable microorganisms used for biocementation process is the important step affecting the effectiveness of $CaCO_3$ formation. The bacteria should possess high ureolytic activity and non-pathogenic [14]. The bacteria should also have a high negative zeta-potential [10, 13] to promote adhesion and surface colonization, and produce large quantity of urease enzyme in the presence of high concentrations of ammonium [14] to enhance both the rate of ureolysis and biological $CaCO_3$ precipitation [7]. Typically, bacteria can isolate from natural carbonate producing environments and screened for their carbonatogenic yield [16]. Ureolytic bacteria especially *Bacillus* species have generated a lot of interest in this area, and have been studied extensively [10, 14, 15]. The highest ureolysis performance has been reported for *B. cereus*, which showed a carbonatogenic yield of 0.6 g $CaCO_3$ per g organic matter input [16].

3. Non-destructive tests in geophysical process

Geophysical measurements are useful tool in biological soil improvement treatments as they can monitor real-time biological and chemical components 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 [2]. Table 1 describes the overview of three techniques of non-destructive test used for biological soil improvement treatments.

Table. 1. Overview of geophysical monitoring methods for non-destructive test of soil improvements adapted from [2].

Geophysical technique	Soil properties affecting measurement	Measurement methods	
		Laboratory	Field
Shear wave velocity $V_s^2 = G/\rho$	Particle-particle contact stiffness, particle stiffness, soil density, confining stress, degree of saturation	Piezo-ceramic bender elements, resonant column	Seismic CPT, cross-hole, downhole
Compression wave velocity $V_p^2 = (B + 4/3G)/\rho$	Bulk modulus of the pore fluid, degree of saturation, porosity, bulk modulus of material comprising grains	Ultrasonic transducers, piezo-ceramics	Seismic reflection/refraction, surface analysis of spectral waves
Resistivity $\Omega = \varepsilon / J$	Particle volume fractions & voids, particle mineral composition, pore fluid chemical composition, soil particle specific surface area, degree of saturation, soil fabric anisotropy	Wenner and Schlumberger arrays deployed on surface or within in-situ probes (e.g. CPT)	

Note: V_s = Shear wave velocity, V_p = Compression wave velocity, Ω = Resistivity
G = Shear modulus, ρ = Density, B = Bulk modulus, ε = Electric field, J = Current density

3.1 Resistivity

Resistivity (or conductivity) measures the voltage potential gradient through a soil matrix when an electrical current is applied across a soil specimen. It has been applied to detect variations in soil density, displacements and deformations within soil [17], soil compression, altering of pore fluid composition [18], biological activity [19], and extent of soil improvement i.e. the migration of contaminant/chemical plumes, and the progress of passive bio-remediation methods [20]. However, this technique was found in biocementation (biocalcification) monitoring minimally due to the changes of many factors (Table 2) influencing soil resistivity making the difficulty to clarify the benefits actually realized in the soil matrix [2].

3.2 Compression wave velocity

Compression waves (P-waves) is the first mode of elastic wave propagation through soil occurring at very small stain levels [21] This wave travels effectively through solids and fluids, and are dependent on the bulk stiffness (B) and the shear stiffness (G) as described in Table 2. P-wave velocity is primarily dependent on the porosity, the fluid bulk stiffness, and the material comprising the soil particles. It is not sensitive to the shear stiffness of the soil matrix. It is excellent for monitoring changes in pore fluid compressibility, especially in single phase materials (i.e. rock, concrete) and biocemented soils having sufficient cementation quantity ($V_p > 1500$ m s^{-1}) [2]. However, measurement of 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 [2].

3.3 Shear wave velocity

Many studies reported the non-destructive test for measuring shear wave velocity and shear modulus [22-28] but few researches are found in biological soil improvement applications. In this article, we described the example of shear wave velocity for strength measurement in loose sand before and after biocementation process. This example is presented and discussed in Section 4. Shear waves (S-waves), the second mode of propagation, in which the direction of particle motion is perpendicular to the direction of propagation [21] and it is recently used for measuring the relative strength in soil [22-28]. Advantages of this application are a non-destructive examination and are capable to measure the soil strength in function of time. Thus, it can be applied in the field for measuring the changes of ground improvement conditions in the long period.

3.3.1 Piezo-ceramic bender element and operating sensors

Element tests involve sending elastic waves through a specimen to cause transient perturbation to the particles, of which the resistance encountered by the induced vibration is translated as stiffness of the material. The elastic waves can be compression or shear waves depending on the direction of the wave movement [29]. Elements generating S-waves are called bender elements (BEs) because of their shape of movement. The principle of BEs is based on the properties of piezoelectric materials as they distort or bend when subjected to a change in voltage and generate a voltage when are distorted or bent.

BEs consist of two thin piezo-ceramic plates that are mounted together, separated by an electrode surface and bounded by two further electrode surfaces. The two piezoceramic sheets may be polarized in the same or opposite directions by wiring either in parallel or series, depending on whether an electrical signal is to be transmitted or received. In a series connected element (Fig. 2a) the wires are connected to the outer electrode surfaces and the two piezoceramic plates are polarized in opposite directions. In a parallel connected element (Fig. 2b) the wires are connected to both the outer electrode surfaces and the centre electrode (by careful grinding away of a small portion of the element). The polarization of the two outer electrodes is the same, either positive or negative, while the centre electrode is the other pole [30]. *Leong et al.* [31] demonstrated that the quality of the transmitted and received signals is improved when a parallel connection is adopted for transmission and a series connection for the receiver bender element.

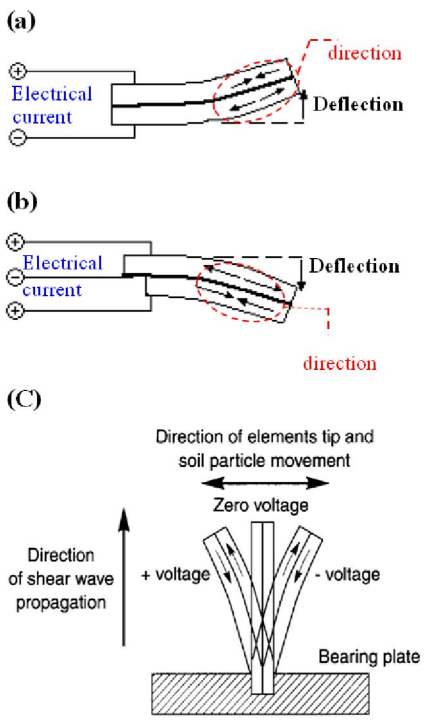


Fig. 2. Overview of bender elements (BE): (a) Connection of BEs by series type, (b) Connection of BEs by parallel type and (c) Mechanism of BEs generating S-wave by supplying electrical current adapted from [25, 30, 32].

For BE testing of soil specimens, a personal computer generates a signal through a sound card with 5V peak to peak as suggested by *Mohsin et al.* [29]. This signal is amplified to 40V peak to peak. An oscilloscope is used to measure the arrival time between a sending signal and a receiving signal. A voltage pulse is applied to the sending sensor for generating a shear wave (when excited by a small voltage created using a function generator the 'transmitter' distorts and generates a bending motion as depicted in Fig. 2c). When the shear wave reaches the other end of the soil sample, distortion of the receiving sensor produces another voltage pulse. The receiving sensor is directly connected to the oscilloscope to compare the difference in travel time between the sending and the receiving signals. Thus, measurement of time delay between sending and receiving of the shear wave will provide the shear wave velocity [24, 25, 30, 33]. The shear wave velocity measurements are usually performed with frequencies ranging between 2 to 12 kHz, at strains estimated to be less than 0.0001 % [25]. The schematic diagram describing an example of experimental setup of BEs for V_s measurement of biocemented sand is illustrated in Fig. 3.

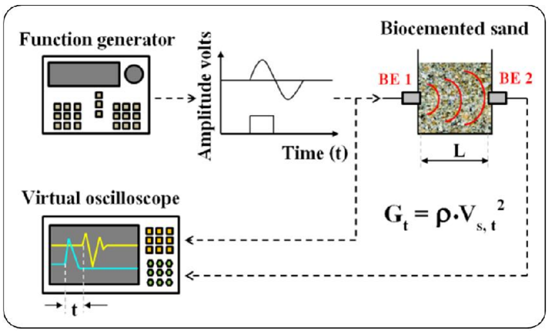


Fig. 3. Example of experimental setup of bender elements technique (BE) for real-time monitoring of strength development in biocemented sand adapted from [24, 33].

The shear wave velocity is calculated from the tip to tip distance between the two transducers and the time required by the shear wave to cover this distance and time as shown in Eqs. (11) and (12). In addition, the initial shear modulus (G_0) can be calculated by Eq. (13).

$$V_s = L/t \qquad (11)$$

$$t = t_t - t_c \qquad (12)$$

$$G_0 = \rho.Vs^2 \qquad (13)$$

where V_s is the shear wave velocity, L is the tip to tip distance between two sensors, t is the required time to cover this distance, t_t is the total travel time and t_c is the offset time, and ρ is the soil density [24, 25].

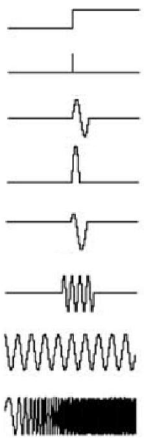
In the laboratory or filed tests, a transmitter and receiver element can be placed in various soil testing devices (i.e. conventional triaxial devices, oedometers and direct or simple shear devices), as in the example by this study (Section 4), in the sideways of laboratory constructed reactors. Although parallel connected element is effective element for transmitter and series connected element is effective element for a receiver, the example of BE experiment in this study in the latter section was performed by using the BE series type for both transmitter and receiver transducers due to the advantage in measurement of receiving signal [24, 25].

3.3.2 Wave signal and interpretation

Table 2 shows an example of input wave shape. In the past, many studies using BEs used a single square-wave pulse [34]. However, sine-wave pulses have become more popular, as these have been shown to give more reliable time measurements [35].

While the setup and operation of BE transducers is relatively simple, the convenience of BE tests is limited by subjectivity associated with identifying wave travel time arrivals. Fig. 4 shows an example of typical set of transmitted and received oscilloscope signals.

Table. 2. Possible input wave shape adapted from [34].



Input wave shape
Square or step signal
Impulse signal
Sine wave
Sine pulse (90° phase shift)
Distorted sine wave (typically 30° phase shift)
Forced oscillation (sine wave cycles at resonance frequency)
Continuous sine wave of constant frequency
Sine sweep of frequencies (typically 100Hz to 20kHz)

For interpretation of the received signals, diverse methodologies have been proposed ranging from the simplest method based on the immediate observation of the wave traces and measurement of the time interval between starting points, to more elaborate techniques, supported by signal processing and spectrum analyses tools [30, 36-40]. Alternative options for the selection of the input wave configuration have also been proposed, not only in terms of its shape (Table 2), but also in its frequency, with obvious impact in terms of output clarity and ease of interpretation.

Piriyakul [24] reviewed the signal interpretation method for BE interpretation involving visually picking the arrival position from the received trace within the time domain record directly from an oscilloscope. In addition, *da Fonseca et al.* [34] reviewed a number of potential sources of error involved in BE testing and interpretation including near-field effects, wave interferences at the rigid boundaries, specimen geometry, transducer resonance and overshooting, and electrical noise and grounding/shielding issues.

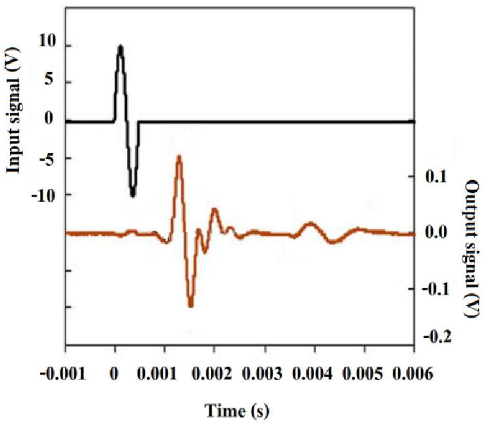


Fig. 4. Example of transmitted and received oscilloscope signals from BE technique adapted from [34].

In order to avoid some of these errors, a number of technical requirements and boundary conditions were suggested by *da Fonseca et al.* [34], these requirements include good electronic equipment, good shielding and grounding, properly connected and encased transducers, leak-free connections, and a noise-free environment. Other factors also play a part, especially spatial conditions, such as alignment of the BE, reflections of the wave from the edges and sides of the specimen, relative distance between transmitter and receiver; poor contact between the BEs and the soil resulting in poor coupling especially at low confining pressures; and overshooting, since at high frequencies the BE changes its predominant mode shape and the response becomes complex.

4. Biocementation technique in laboratory scale

In this section, the authors gave an example of laboratory experiment of biocementation process in sand. BE technique was applied to measure the S-wave velocity (V_s) in sand before and after biocemented treatment. Although the question of which microorganism types are the most effective at biocementation has not yet been thoroughly studied and utilizing different types of microorganisms may also result in different rates of calcite formation in soil as reviewed in Section 2. We believe that there should be plenty of natural ureolytic microorganisms growing in the natural environment. In this research, the source of urea degrading bacteria was originated from natural water (Chaophraya River, Thailand), where the bacterial community is mixed cultures.

Biocemented sand reactors (SRs) were made of plastic containers with dimensions of 80mm x 80mm x 80mm (width x length x height). The reactors were placed with free-drop 250 g of sieved sand (sand sample passed no. 100 and retained no. 200) with an approximate depth of 40 mm and filled with 300 mL of nutrient solution contained 250mM of urea, 250mM of calcium ion (by $CaCl_2$), and glucose ($C_6H_{12}O_6$) of 1.5mM. Source of water used for preparing the solution was collected from Northern part of Chaophraya River. Control reactor was made of sand sample mixed with only water. The experiment was performed in ambient with average temperature of 25 ± 2 °C. The water level of each reactor was remarked. An addition of deionized water to each BSR was sometimes needed to maintain the constant level of water table and prevent the level falling due to water loss by evaporation.

Treated sands (SR1) and control (SR2) were measured for strength development by bender element test. Dried sand samples were collected for observing the morphology by SEM and analyzed by XRD for identifying the presence of $CaCO_3$ precipitation in treated sand. Effluent water was sampled and analyzed for NH_4^+ production and water pH. These parameters were conducted through the period of the experiment and the analyses were based on the procedures of Standard Methods for the Examination of Water and Wastewater [41].

4.1 X-ray diffraction

Fig. 5 illustrates the different patterns of X-ray diffractogram between treated sand (SR1) and control sand (SR2). The different peaks between the typical sand and the treated sand in comparison with precipitated $CaCO_3$ powder obtained from the water evaporation of solution are marked by "symbol ▲" while the "symbol ●" marked the increment of chemical compositions in sand samples from SR1 and SR2.

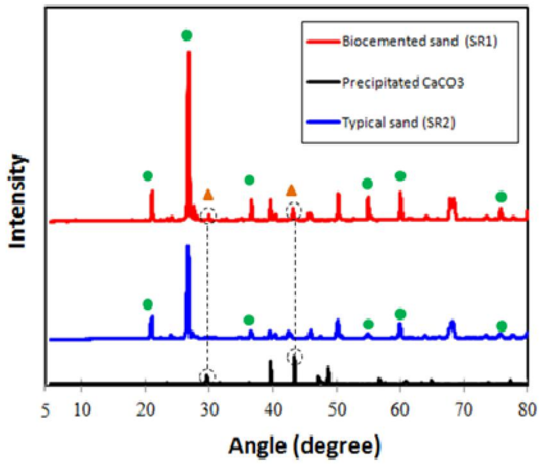
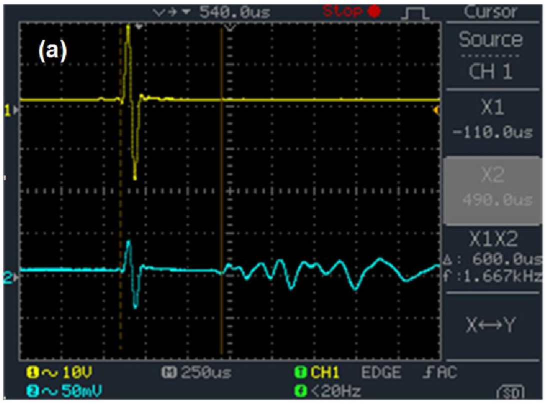


Fig. 5. Pattern differences of X-ray diffraction between typical sand and treated sand after biocementation

("▲" indicates the precipitated $CaCO_3$ in sand and "●" indicates the increment of further chemical compositions).

4.2 Bender element test

Fig. 6a shows the shear wave velocity of typical sand sample. Our research found the occurrence of cross talk during measurement. However, applying of high frequency input signal (12000 Hz) could be used to identify arriving signal without cross-talk interference.



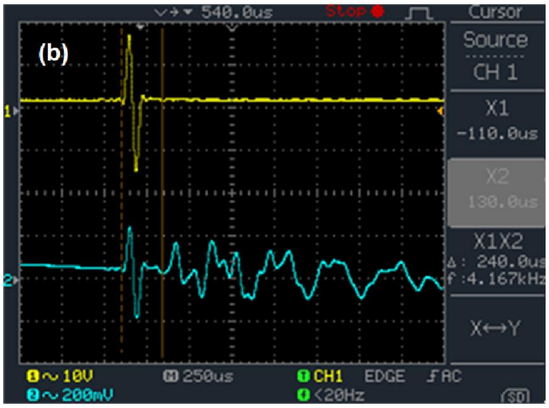


Fig. 6. Measurement of shear wave velocity: (a) Typical sand sample (control sand from SR2) and (b) Biocemented sand sample (sand from SR1).

The total travel time, t_t, is 600 µs and the offset time, t_c, is 4 µs. So, the required time, t, is 596 µs according to Eq. (12). The tip to tip distance between the transducers is 82.5 mm. Therefore, the shear wave velocity, V_s, of 138.4 m/s is obtained by using Eq. (11). In the similar way, Fig. 6b shows the shear wave velocity of biocemented sand sample. The total travel time, t_t, is 240 µs and the offset time, t_c, is 4 µs. So, the required time, t, is 236 µs according to Eq. (12). The tip to tip distance between the transducers is 79.5 mm. The shear wave velocity, V_s, of 336.9 m/s is obtained. From the results, the shear wave velocity was increased about 143 percent after the treatment process. It is noted that direct mapping of the cementation quality with V_s would be useful for identifying the spatial uniformity of biological induced $CaCO_3$ in soil where the technique is applied to the field (large area) and also the stability of the treatment over time.

4.3 Scanning electron microscopy

The effectiveness of a biocemented treatment is directly dependent on the spatial distribution of the $CaCO_3$ precipitate that contributes to the binding of sand particles together. Images from SEM, as shown in Fig. 7, provide clear images of sand particles collected from SR1 (typical sand reactor, Fig. 7a to 7c) and SR2 (biocemented sand reactor, Fig. 7d to 7f). The results show the phenomenon of precipitated $CaCO_3$ occurred in SR2 coated the exposed surfaces of sand particles resulting in the decrease in the pore space and the increase in the solid content, consequently the increase in V_s. *Dejong et al.* [2] reported that biocemented treatment resulted in reduction of 6-17% of the initial void space and increased the relative density correspondingly of 63-100% due to the pore being filled with precipitated $CaCO_3$. Thus, the effective densification of the soil (due to an increase of $CaCO_3$ solid content) provides significant improvement to soil engineering properties in terms of increased shear strength, increased stiffness, reduced compressibility and also reduced permeability.

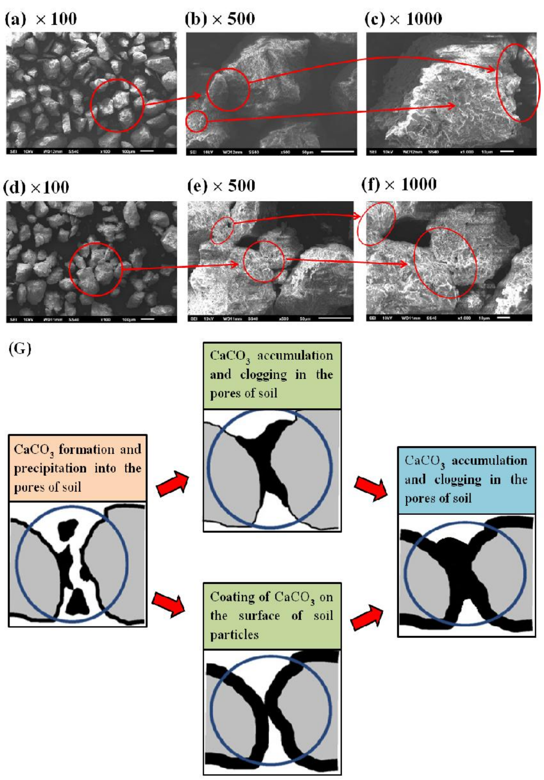


Fig. 7. Geophysical monitoring of sand particles by scanning electron microscope (SEM); typical sand (a, b and c) [33], treated sand after biocemented treatment (d, e and f) [33], and model of calcium carbonate ($CaCO_3$) distribution alternatives in pore spaces of sandy soil (g) after [2].

4.4 Ammonification rate and change of pH

When urea is added to the soil, it is firstly hydrolyzed to NH_4^+ by urease enzyme as depicted in Eqs. (1) to (3), and in a next step NH_4^+ may be oxidized to nitrate (NO_3^-) by nitrification process and/or reduced to oxide of nitrogen and nitrogen gas (N_2) by denitrification process [42-44].

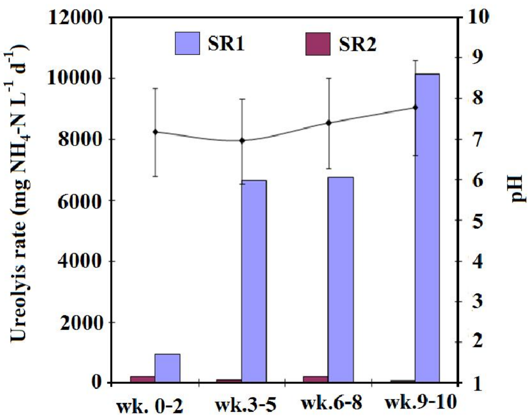


Fig. 8. Ureolysis rate (as NH_4^+ production) and change of water pH in biocemented treatment (SR1) and control (SR2) treatment.

Fig. 8 illustrates the formations of NH_4^+ produced by biocemented (SR1) and control (SR2) treatments. The production rate of NH_4^+ distinctly increased in SR1 after five weeks of experiment while there was no significant change of NH_4^+ production rate in SR2. The NH_4^+ production rate in SR1 was rapidly increased from 947 at week 2 to 6742 mg N L^{-1} d^{-1} at week 5 and still increased to 10127 mg N L^{-1} d^{-1} at week 11, while the NH_4^+ production rate in SR2 was only found between 50 and 148 mg N L^{-1} d^{-1} during the experiment.

The high NH_4^+ concentrations were found in SR1 (biocemented treatment) due to the degradation of urea presented in the solution of SR1 by ureolysis process, while small NH_4^+ production by control treatment might result from the degradation of residual organic nitrogen (Org. N) matter of only sand origin. Line graph presented in Fig. 8 illustrates the change of water pH in SR1. Relatively high production of NH_4^+ in SR1 in comparison with SR2 resulted on high alkalinity and rapid increasing of pH in SR1 as demonstrated in Eqs. (1) to (10). It was noted that change of pH in SR2 was insignificant and pH value only varied between 6.7 and 7.8.

5. Technology visibility and applications

5.1 Technological advantages

Improvement of the geophysical properties of soil can be achieved using mechanical compaction, chemical grouting, or biocementation. For shallow and deep mechanical compaction methods, most of their techniques (e.g. rolling or vibrating and vibro-compaction or dynamic compaction) are only effective or economically viable to a depth less than 10 m and some methods are not applicable for clayed soils and recent municipal landfills [6].

For chemical grouting by injection methods, some chemical reagents must be added to diminish soil permeability and increase its mechanical strength. Disadvantages are chemical costs, handling, and negative impact to environment. There are several ways of chemical injection including low pressure grouting, high pressure grouting, stage-down or stage up methods, and other grouting methods including grout port, vibrating beam and horizontal grout curtain methods [45, 46].

Table 3 Technology benefits of biocementation process to industry after [2]

Characteristics	Envisioned advantages
Reduction in costs	- Use of natural materials; - Reduce treatment injection.
Reduction in environmental impact	- Use of natural materials that do not permanently alter subsurface conditions and can be labeled as green technology.
Improvement of treatment uniformity	- Microbes are small and self mobile. They can readily penetrate into soils having potential to enhance spatial uniformity of treatment.
Optimal treatment condition	- Degree of treatment can be controlled and monitored.
Flexible and adaptable duration	- Treatments can be removed if only temporary support needed.

Biocementation method could be similar to those used in chemical grouting. Advantage of biological grouting over chemical one is that the microbial grouts may accessibility and large diversity of microorganisms, which can be used for soil biological treatment. In the soil environment, it was found that more than 10^9 microbial cells per gram of soil existed in the top few meters of soil. Although the population concentrations generally decrease with depth, at 30 m of depth, where is the lower limit of most soil improvement engineering applications, microbial population of about 10^6 cells per gram of soil can exist [47]. Thus, the numbers of microorganisms that can be used for biological ground improvement are numerous, although individually they are very small. Table 3 summarized several characteristics of soil improvement technique by biocementation through microbial $CaCO_3$ precipitation that may prove advantageous relative to industrial application.

For economic consideration, the cost of biocemented treatment schemes will be dependent on the process used, and on details of the specific field project. With very limited field applications, the actual costs of the various improvement processes are largely unknown. Table 4 gives an overview of the approximated cost related to the application of biocemented treatment to soil improvement. Basically, the cost of the biocemented treatment are attributable both to the price of the grouting medium and the number of applications required. The price of the bio-grouting medium depends on the price of the microorganisms and the price of the nutrients.

Disadvantages of soil biocementation in comparison with chemical grouting are usually slower (essential time requirement for carbonate production) and more complex than the chemical one because the microbial activity depends on many environmental factors such as temperature, pH, soil oxidation-reduction stages, concentrations and diffusion rates of nutrients and metabolites.

Table 4 Approximate cost of raw materials after [6]

Materials for biocemented medium	Cost of additives ($ / m^3 of soil)
$CaCl_2$ + urea + μ.org.	4.0–9.0
Iron ore + organic wastes + μ.org.	1.0–4.0
Molasses + μ.org.	0.5–4.0
Food-processing wastes + μ.org.	0.5–2.0

(Note: μ.org. = microorganisms)

Process design of biocementation must take into account not only soil conditions and grouting medium contents but also microbiological (growth and specific enzymatic activities; biosynthesis; biodegradation; biochemical reactions accompanied with formation of insoluble compounds; and physico-chemical processes such as precipitation, crystallization, and adhesion), and geotechnical engineering aspects (specific geotechnical parameters of soil can be used as process optimization criteria) [6].

5.2 Research activity and technological applications

Research has provided insights into biocementation technique through microbial induced $CaCO_3$ precipitation from micrometre- to metre-length scales (Fig. 9). At microscopic scale, formation of $CaCO_3$ varies with treatment options [2], precipitation of $CaCO_3$ occurs directly on the surface and in the pore space and of soil particles or around microbial cells and their aggregates and cementation of soil particles (bridging and agglomeration) occurs preferentially at particle contacts [2, 3, 10]. At laboratory scale tests, many studies have shown substantial increases in soil strength after biocemented treatment [1-16, 52-55, 58, 59]. In our research study at CIT-KMUTNB's laboratory (Fig. 3), rectangular-shaped column reactors were setup and explored the efficacy of biocementation and linkages between microbially ureolysis activity, V_s and $CaCO_3$ precipitation as described in section 4 [33]. Result found that V_s of experimented sand was increased from 138.4 to 336.9 m s^{-1} after biocemented treatment. From Eq. (13), estimated G value of biocemented sand was about six folds higher than typical sand before biocemented treatment. Further studies also reported an increasing of small strain stiffness by three orders of magnitude [1, 8, 55] and decreasing of hydraulic conductivity greater than two orders of magnitude [58, 59].

At pilot or field scale tests, only a few trials have been performed up to date [55, 60]. In *van Paassen* [55], the biocemented treatment was applied to 1000 m^3 of soil at depths varying between 3 and 20 m below the surface for gravel stabilisation and enabling horizontal directional drilling for a gas pipeline in the Netherlands. The treatment involved injection of a 200 m^3 bacterial suspension, two injections of 300 - 600 m^3 of reagent solution containing urea and $CaCl_2$, and extraction of groundwater. Overall, the treatment was a success, as horizontal directional drilling for a gas pipeline was possible without instability in the loose gravel deposit. In *Fugita et al.* [60], the research target was difference with *van Paassen*'s work [55]. They applied biocementation to form co-precipitation with heavy metal (strontium-90) immobilize, and prevent metal leakage to environment. Although the rate of $CaCO_3$ precipitation was slower than in the biocementation application in the Netherlands, but it was sufficient rate for the project requirement. Other scale model tests have demonstrated the effectiveness of biocementation through microbial induced $CaCO_3$ precipitation in reducing soil weathering [61], improving resistance to liquefaction [62], creating impermeable crusts for catchment facilities [63], healing/stabilising cracks in concrete and masonry [64, 65] , treating waste [66], and performing shallow carbon sequestration [67-69]. Additionally, not only bacterial species were reported for inducing $CaCO_3$ precipitation, other organisms including plants [67] and microalgae [70] were also reported for effective producing $CaCO_3$ precipitation.

The above attributes readily lend the treatment technique to civil and environmental applications, especially infrastructure applications, and possible broader applications for national and international security, energy storage, and global warming. Table 5 gives an overview of engineering applications by biocementation process.

Table 5 Overview of technological applications of biocementation in civil and environmental engineering after [2, 6]

Engineering issues	Technical applications
Liquefaction prevention	- Cementation of subsurface to prevent liquefaction and its damage.
Building settlement reduction	- Reduce settlement and increase bearing capacity for foundations; - Increasing the bearing capacity of piled or nonpiled foundations; - Treating pavement surface.
Dam and levee safety	- Upstream injection of technique would plug erosive piping; - Enhancing stability for retaining walls, embankments, and dams.
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; - Controlling erosion in coastal area and rivers.
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; - Immobilising bacterial cells into a cemented active biofilter.
Groundwater protection	- Treatment to immobilize materials before contamination of aquifers; - Creating water filters and bore hole filters.
Emergency immobilization	- Rapidly secure contaminants from hazards (e.g. terrorist activities); - Stabilising pollutants from soil by the binding.
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; - Increasing the resistance to petroleum borehole degradation during drilling and extraction.
Carbon sequestration	- Used to create subsurface facilities for storage of CO_2.

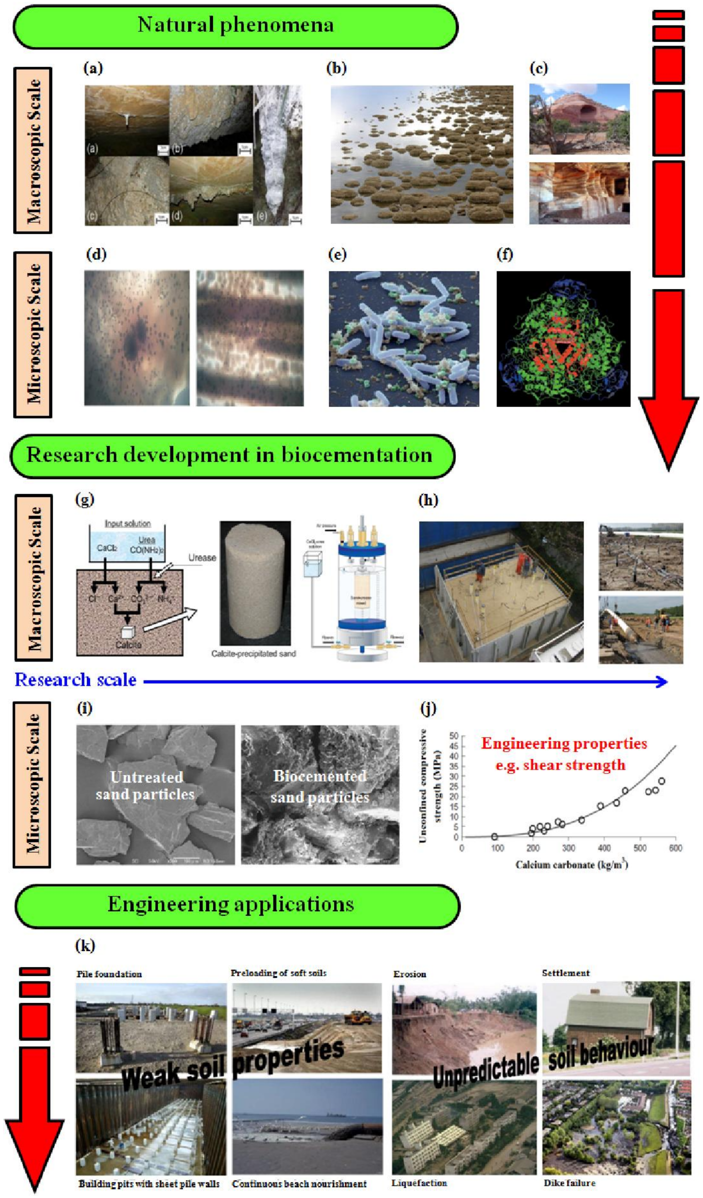


Fig. 9 Overview of upscaling of biocementation through microbial carbonate precipitation: (a) Limestone cave [48]; (b) Limestone at seashore [49]; (c) Natural sandstone [50]; (d) Optical microscopic images of colonies formed by bacteria presented in limestone [48]; (e) Example of ureolytic bacteria *B. pumilus* [51]; (f) Urease enzyme structure [52]; (g) Laboratory scale of biocementation research for soil improvement [53]; (h) Pilot scale and field test of biocement research [54, 55]; (i) Geophysical images of sand particles before and after biocemented treatment [53]; (j) Improvement of soil engineering improvement properties [56]; and (k) Examples of land engineering issues [57] that biocementation technique may apply for soil improvement.

6. Conclusions

The knowledge about the microbial origin of limestone has resulted in research concerning microbial induced calcium carbonate ($CaCO_3$) precipitation. We provided an in-depth overview of biocementation technique, its prospective technological applications, and example of biocementation research with real-time instrument for monitoring strength improvement at CIT-KMUTNB Laboratory. Although strengthening of porous soil using cement or chemical techniques is often used in geotechnical engineering works, usage of biological treatment is being interest due to technology viability of cost (economic and inexpensive) and environmentally friendly. In biological approach, a new technique based on the microbially induced calcium carbonate ($CaCO_3$) precipitation (sometimes called microbially induced calcite precipi-tation or MICP, biocalcification, biocementation) is being worldwide studied and applied in the large scale of soil improvement projects. In this technical process, the enzymatic reaction of urea hydrolysis (ureolysis) is used to control the pH and $CaCO_3$ precipitation according to biochemical reactions of ureolysis and carbonate production. The reaction is catalyzed by enzyme urease produced from different microorganisms. The urease activity controls the rate of $CaCO_3$ formation inside the pores of soil due to an increase of alkalinity.

Generally, there are several mechanisms that control the soil biocementation including (1) filling (or clogging) the voids between soil particles by $CaCO_3$ formed by consequence products of ureolysis; (2) filling (or clogging) the voids between soil particles by growth of bacterial biomass and biofilms; (3) coating and binding (or bridging) the soil particles with $CaCO_3$; and (4) coating and binding (or bridging) the soil particles with bacterial biomass and biofilms. Several researchers including this study have proposed the use of ureolytic bacteria for $CaCO_3$ forming, precipitating and strengthening of sandy soil. However not only bacteria, but also other ureolytic producing organisms (i.e. microalgae, plant) or extraction of enzyme urease products can be used for soil biocementation process.

Overall, the laboratory scale tests of biocementation of sandy soil using ureolytic bacteria presented in natural water of Chao Phraya River was success. Progress of the research showed that natural ureolytic bacteria in mixed cultures performed well its activity for biocemntation. Geophysical method by shear wave velocity (V_s) measurement is effective for real-time monitoring of the biocementation process that links how the bio-mediated chemical processes are influencing the soil matrix. For most biological 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. In test result, the strength (shear modulus, G value) of treated sand after biocemention was substantially increased, approximately six folds higher than control treatment. Analytical results from XRD ensured the occurrence of $CaCO_3$ in treated sand under biocemented process. The microscopic scale observing from SEM technique illustrated the coating of $CaCO_3$ on the surface of the loosed sand particles, resulting in more solid content and less pore space, hence an increase of V_s value. These results suggested that substantial cementation in loose sand structures can be engineered through harnessing and controlling natural biological processes. With the maturation of this technology in future studies, multiple new opportunities for engineering soil improvement implementation can be envisioned, i.e. treatment of liquefiable sand deposits; pretreatment of the subsurface prior to tunneling; building settlement reduction; soil weathering and erosion; and dam, levee, and slope stabilization. However, It is clear that the successive works done by several researchers in literatures, focusing on laboratory and field trials, can only improve our understanding on the possibilities and limitations of this technique. However, the challenge for the immediate future is to translate some of the promising results obtained in the laboratory and field tests into practical applications. Furthermore, the uniform treatment of a large zone of soil requires advanced system modeling and the development of real-time field- or practical- scale monitoring techniques to ensure spatially uniform treatment is necessary.

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