Biological process of soil improvement in civil engineering: A review

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Abstract
The concept of using biological process in soil improvement which is known as bio-mediated soil improvement technique has shown greater potential in geotechnical engineering applications in terms of performance and environmental sustainability. This paper presents a review on the soil microorganisms responsible for this process, and factors that affect their metabolic activities and geometric compatibility with the soil particle sizes. Two mechanisms of biomineralization, i.e. biologically controlled and biologically induced mineralization, were also discussed. Environmental and other factors that may be encountered in situ during microbially induced calcite precipitation (MICP) and their influences on the process were identified and presented. Improvements in the engineering properties of soil such as strength/stiffness and permeability as evaluated in some studies were explored. Potential applications of the process in geotechnical engineering and the challenges of field application of the process were identified.

Keywords geotechnical engineering, biomineralization, microbially induced calcite precipitation (MICP)

1. Introduction

Recent studies on applications of bio-mediated soil improvement method have proved the viability of the approach for effective performance and environmental sustainability. The promising outcomes of these studies have shown greater potential of exploring a wider application of the technique in geotechnical engineering. Bio-mediated method of soil improvement has been considered as an inventive and new approach in geotechnical engineering that can be utilized to prevent liquefaction and landslide in loose sand which usually results in foundation deformation and/or failure (Alvarado, 2009). The great promise of the use of biological treatments has been demonstrated in many applications, such as improving the shear strength and decreasing the permeability of soils (Whiffin et al., 2007; Ivanov and Chu, 2008; Harkes et al., 2010; van Paassen, 2011), improvement in strength and durability of concrete and mortar, remediation of cracks in buildings (Qian et al., 2010; Achal et al., 2013), improvement in engineering properties of soil, and cementation of sand column (Achal et al., 2009a; Dhami et al., 2013).

Bio-mediated method of soil improvement generally refers to the biochemical reaction that takes place within a soil mass to produce calcite precipitate to modify some engineering properties of the soil (DeJong et al., 2010). Meanwhile, utilizing the interdisciplinary knowledge of civil engineering, chemistry and microbiology to alter the soil engineering properties in the subsurface has emerged recently (Whiffin et al., 2007; Ivanov and Chu, 2008; Mitchell and Santamarina, 2005; DeJong et al., 2010). The technique utilizes soil microbial processes, which is technically referred to as microbially induced calcite precipitation (MICP), to precipitate calcium carbonate into the soil matrix. The calcium carbonate produced binds the soil particles together (thereby cementing and clogging the soils), and hence improves the strength and reduces the hydraulic conductivity of the soils. MICP can be a practicable alternative for improving soil-supporting both new and existing structures and has been used in many civil engineering applications such as liquefiable sand deposits, slope stabilization, and subgrade reinforcement (DeJong et al., 2006; Cheng et al., 2013). It was revealed that microorganisms influence the formation of fine-grained soils and change the behavior of coarse-grained soils such as strength and hydraulic conductivity. They also facilitate chemical reactions within a soil mass, promote weathering and change the chemical and mechanical properties of specimens after sampling. Hence, the effects of these microorganisms on mechanical properties of soils are still not fully discovered in geotechnical engineering field (Mitchell and Santamarina, 2005). Though it was understood that there are more microorganisms in the subsurface than on the ground, and studies of many years have proved the relevance of biological activities in influencing soil behavior, less work has been done in exploring the importance, relevance, usefulness and application of biology in geotechnical engineering. Meanwhile, it is expected that a clear understanding of the impact of microorganisms and biological activity on soil behavior can lead to proper soil characterization and/or classification and even alternative geotechnical engineering solutions. This paper reviews the concept of biomineralization and its applications in improving the engineering properties of soils.

2. Soil microorganisms

Soil contains more genera and species of microorganisms than other microbial habitats. This may be due to the fact that it contains a lot of nutrients and usually retains some liquid within its pore spaces. Some species of these microorganisms are present in large numbers while some are otherwise not, probably because the factors necessary for the survival and growth of these microorganisms are not evenly distributed naturally across the depth of the lithosphere. Microorganisms are highly adaptable to varying conditions both genetically and physiologically, because they have been in existence for over 3.5 billion years (Stotzky, 1997). There are approximately 10^9-10^{12} organisms per kilogram of a soil mass close to the ground surface. Among the microorganisms present in soil are bacteria, archaea and eukarya. Some of the important characteristics of bacteria and archaea include simple cell structure without membrane-enclosed nucleus, more than one chromosome and distinct chemical composition which are more pronounced than structure. Identification, characterization and classification of microorganisms are usually achieved using the type of cell wall, shape, nutrients, type of biochemical transformation, and DNA and RNA sequences (Woese et al., 1990; Ehrlich, 1998; Chapelle, 2001).

According to Mitchell and Santamarina (2005), the most abundant microorganisms in soils are bacteria. In order to withstand adverse environmental conditions, some bacteria make spores. They have a cell diameter ranging from 0.5 mm to 3 mm and shape of nearly round, rod like or spiral. Madigan *et al.*,(2008) revealed that bacteria can survive in an environment of low to high acidity and/or salinity. They can also survive at very low to high temperatures ranging from below freezing to above boiling points and withstand very high pressures. Majority of bacterial cells have a negative surface charge for groundwater pH values between 5 and 7, which is typical for near surface soils; and the negative surface charge decreases with increasing concentration and valence of ions in the pore fluid (Chapelle, 2001). Because the bacteria are native to the earth, they may not likely cause any environmental hazard in future (Fritzges, 2005). A number of bacteria species are capable of producing urease enzyme and are used in bio-mediated soil improvement technique, including genera Bacillus, Sporosarcina, Spoloactobacilus, Clostridium and Desulfotomaculum (Kucharski et al., 2006).

The activity of urease-producing microorganisms can be divided into two different classes based on their response to high presence of ammonium. The first group includes the bacteria whose urease activity is not repressed due to high ammonium concentration, as indicated in Table 1. While the second group includes *Bacillus megaterium, Alcaligenes eutrophus, Klebsiella aerogenes* and *Pseudomonas aeruginosa* (Kaltwasser et al., 1972; Friedrich and Magasanik, 1977), whose urease activity is repressed by high ammonium concentrations. Therefore, microorganisms whose urease activity are not repressed by the high content of ammonium are preferred in bio-mediated soil improvement since high concentrations of urea are hydrolyzed in the process (Whiffin, 2004).

Table 1
Microorganisms whose urease activity is not repressed by NH_4^+ (Whiffin, 2004).

Microorganisms	High activity	Not repressed by NH_4^+	Not pathogenic or genetically modified
Sporosarcina pasteurii	Yes	Yes	Yes
Proteus vulgaris	Unknown	Yes	Moderately
Proteus mirabilis	Unknown	Yes	No
Helicobacter pylori	Yes	Yes	No
Ureplasmas (*Mocllicutes*)	Yes	Yes	No

Hence, all microorganisms are found to be good for biomineralization applications because of their urease activity; they must also be safe for the environment during and after the treatment process. Therefore, urease-producing bacteria for bio-mediated applications should not be pathogenic, genetically being modified or enclosing any exchangeable elements that may enhance the pathogenicity of environmental microbes. According to Burne and Chen (2000), urea hydrolysis generally follows a series of chemical reactions that lead to the formation of ammonia (NH_3) and carbon dioxide (CO_2). The chemical reaction is presented in Eq. (1). The hydroxyl ions (OH^-) generated from the conversion of ammonia to ammonium result in the increase in local pH value that leads to the decomposition of bicarbonate to carbonate ions (Eq. (2)). The carbon dioxide quickly decomposes in the presence of water into bicarbonate (HCO^{-3}) and it reacts with the hydroxyl ions to form carbonate ions (Eqs. (3) and (4)). Hence, in the presence of calcium ions (Ca^{2+}), the calcite ($CaCO_3$) is precipitated (Eq. (5)) (Castanier et al., 1999; Burne and Chen, 2000). The overall process of urea hydrolysis and formation of calcium carbonate are presented in Eq. (6). Fig. 1 shows the details of urea hydrolysis reactions for the precipitation of calcium carbonate by Sporosarcina pasteurii.

$$CO(NH_2)_2 + H_2O \rightarrow 2NH_3 + CO_2 \quad (1)$$

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

$$CO_2 + H_2O \rightarrow HCO_3^- + H^+ \quad (3)$$

$$HCO_3^- + H^+ + 2OH^- \leftrightarrow CO_3^{2-} + 2H_2O \quad (4)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \downarrow \quad (5)$$

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} \rightarrow 2NH_4^+ + CaCO_3 \downarrow \quad (6)$$

Environmental factors such as temperature and humidity affect metabolic reactions inside the cells and some physical properties such as viscosity and diffusion. Other factors such as availability of other microorganisms may restrict the available space for bacterial growth and activity, and limit the population of the bacteria. The soil pH value which generally increases the salinity of an environment affects adsorption, surface charge and dissolution of some minerals in the soil (Degens and Harris,1997). Though microbes are viable to move freely within the voids of the soil aggregates, their movements are restricted by the narrow pore sizes formed by fine grained soils. Bacteria sizes range between 0.5 mm and 3 mm, as such they are not likely to pass through pore spaces smaller than 0.4 µm. Likewise, fungi and protozoa require pore sizes greater than 6 µm to pass (Castanier et al., 2000). Fig. 2 shows comparison of soil particle sizes and microorganisms. Meanwhile, in coarse-grained soils, bacteria can freely move between the soil mineral particles and may stick on the mineral surfaces and form microcolonies or biofilms.

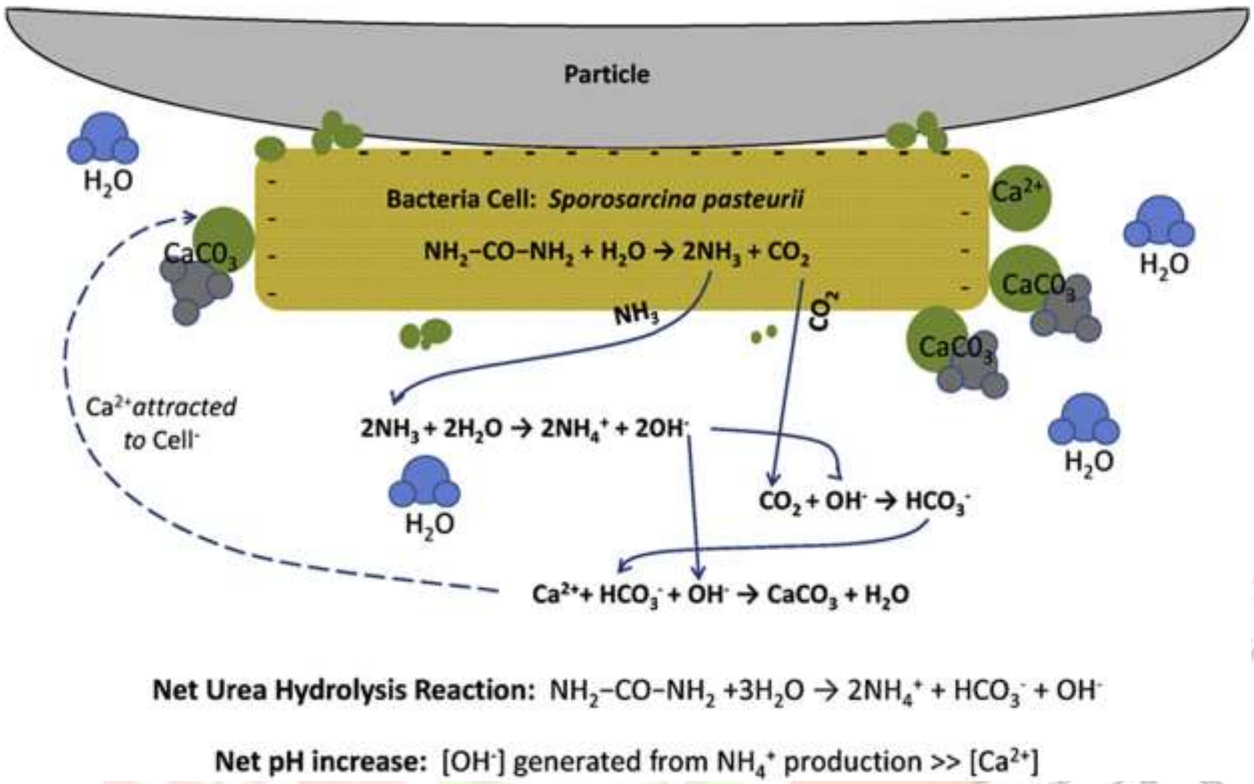

Fig. 1. Microbial calcite precipitation process by urea hydrolysis (DeJong et al., 2010).

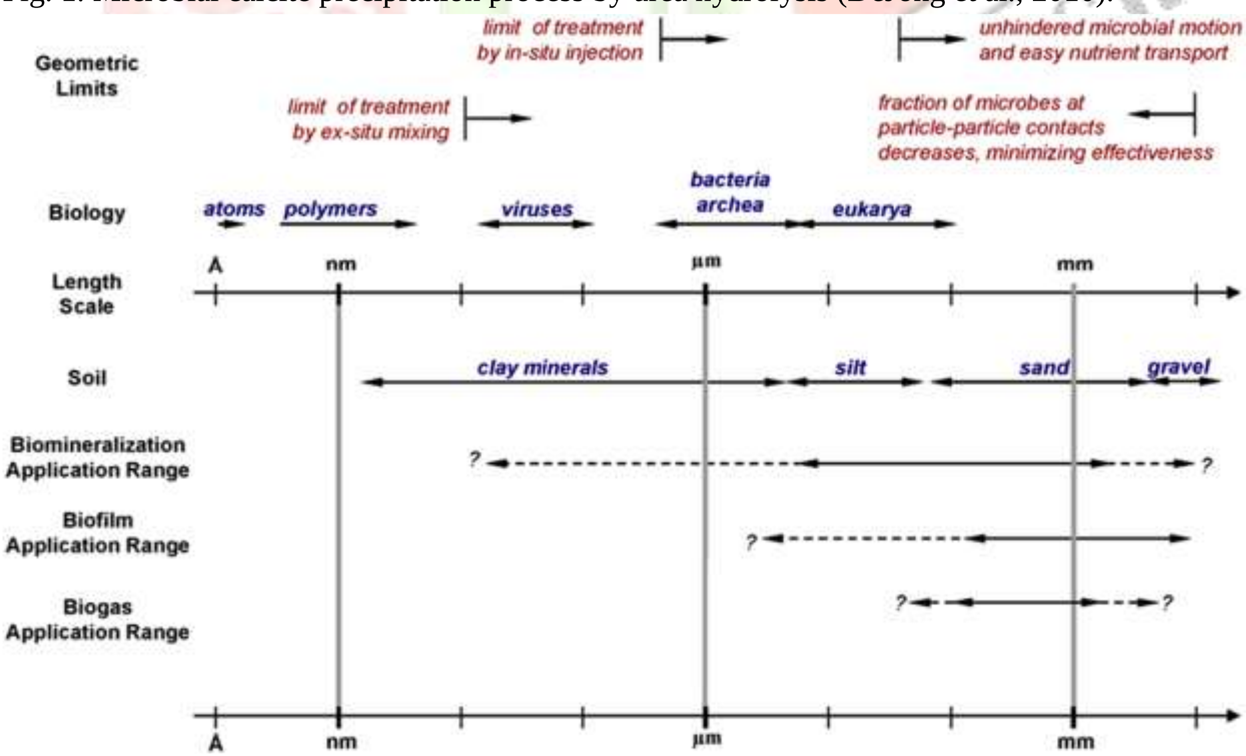

Fig. 2. Comparison of soil particles sizes, geometric limitations and microorganisms extended from Mitchell and Santamarina (2005) by DeJong *et al.*,(2010).

3. Biomineralization

The process that living organisms produce minerals is referred to as biomineralization. These minerals may possibly be silicates in algae and diatoms, carbonates in invertebrates organisms and phosphates, calcium and carbonate in vertebrates organisms (Lowenstam and Weiner, 1989). The minerals are synthesized under two mechanisms: biologically controlled and biologically induced mechanisms. In biologically controlled mineralization, the organisms control the process independent of the environmental conditions. The processes of nucleation and growth of the minerals are to a larger extent controlled within or on the cell of the organisms. While in the biologically induced mineralization process, extracellular metabolic activities of the microorganisms which depend substantially on the environmental conditions result in the formation of the minerals (Dhami et al., 2013). Thus, bacterial precipitation of calcium carbonate is generally regarded as the biologically induced process which depends largely on the type of bacteria involved, abiotic factors such as salinity and composition of medium, and other environmental conditions (Knorre and Krumbein, 2000; Rivadeneyra et al., 2004). Biomineralization processes as documented in many studies reported by Lian *et al.*,(2006) are found to be active in almost every environment on Earth, with much of the microbial activity resulting in the carbonate minerals formation near the surface of the Earth. The microbial activity has been considered to play an essential role in the carbonate formations as sediments and soil carbonate deposits. Thus microbes from soils and some aqueous media are predominantly responsible for the inducement of calcium carbonate precipitates in both natural and laboratory settings (Peckmann et al., 1999). The most evident minerals resulting from biomineralization process are carbonates. Microbially induced calcium carbonates are mainly considered for their relative applications in the fields of biotechnology, geotechnology and civil engineering (Dhami et al., 2013).

Therefore, four factors are generally considered to mainly govern the chemical process that leads to the precipitation of calcium carbonate: calcium concentration, concentration of dissolved inorganic carbon (DIC), the pH value and availability of nucleation sites (Hammes and Verstraete, 2002). Many bacterial species have earlier been identified and suspected to be connected with natural carbonate precipitates from different environments. The main function of the bacteria in the precipitation process has consequently been attributed to their capability of creating an alkaline environment through the increase in pH value and dissolved inorganic carbon during their physiological activities (Hammes and Verstraete, 2002).

Microbial carbonate precipitation (MCP) has been extensively studied under natural environments and controlled laboratory conditions, but the precise mechanisms of the carbonate precipitation and the role of the precipitating organisms in this process within the microbial ecology remain contentious. Thus, the process seems to be recognized in three different related mechanisms. First, biomineralization takes place as unwanted and accidental by-product of microbial metabolism (Knorre and Krumbein, 2000). This is the most widely accepted mechanism. The process is depicted in Fig. 3. Then carbonate nucleation occurs on the cell wall of the microorganisms due to ion exchange through the cell membrane, though the mechanisms are still poorly known (Castanier et al., 2000). The third mechanism involves the extracellular macromolecules which are known to be capable of trapping calcium ions or sometimes serve as growth modifiers to control crystallization (Braissant et al., 2003). Therefore, recent understanding of the concept of bacterially mediated carbonate precipitation relies on the fact that the carbonate precipitate produced does not have any specific biological functions which may be genetically related to the microorganisms involved in the process. This confirms that microbially induced mineralization to produced carbonate is the most prevailing process (Mann, 2001). However, the existence of different possible mechanisms with respect to the role of the microorganisms in the carbonate precipitation describes the complexity of the biomineralization process and the need to explore more into the process.

4. Factors affecting MICP process

Microbially induced calcium carbonate precipitation as a natural process that involves metabolic activities of the microorganisms and some chemical reactions is generally governed by some environmental conditions. Mortensen *et al.*,(2011) assessed the effects of some factors that may be encountered in field during MICP treatment and are likely to affect bacterial growth, metabolism and the precipitation induced by the bacteria using the bacterium *Sporosarcina pasteurii*. Soil column and batch tests were used to assess the likely subsurface environmental factors in the treatment process. Microbial growth and carbonate precipitation were evaluated in fresh and sea water as the possible aqueous environments in situ. Ammonium concentration, oxygen availability, mineralogy and soil particle sizes are part of the conditions that may influence ureolytic activity of the bacteria that are assessed. The authors revealed that MICP treatment can be achieved over a wide range of soil types, particle sizes, concentrations of ammonium chloride and range of salinities.

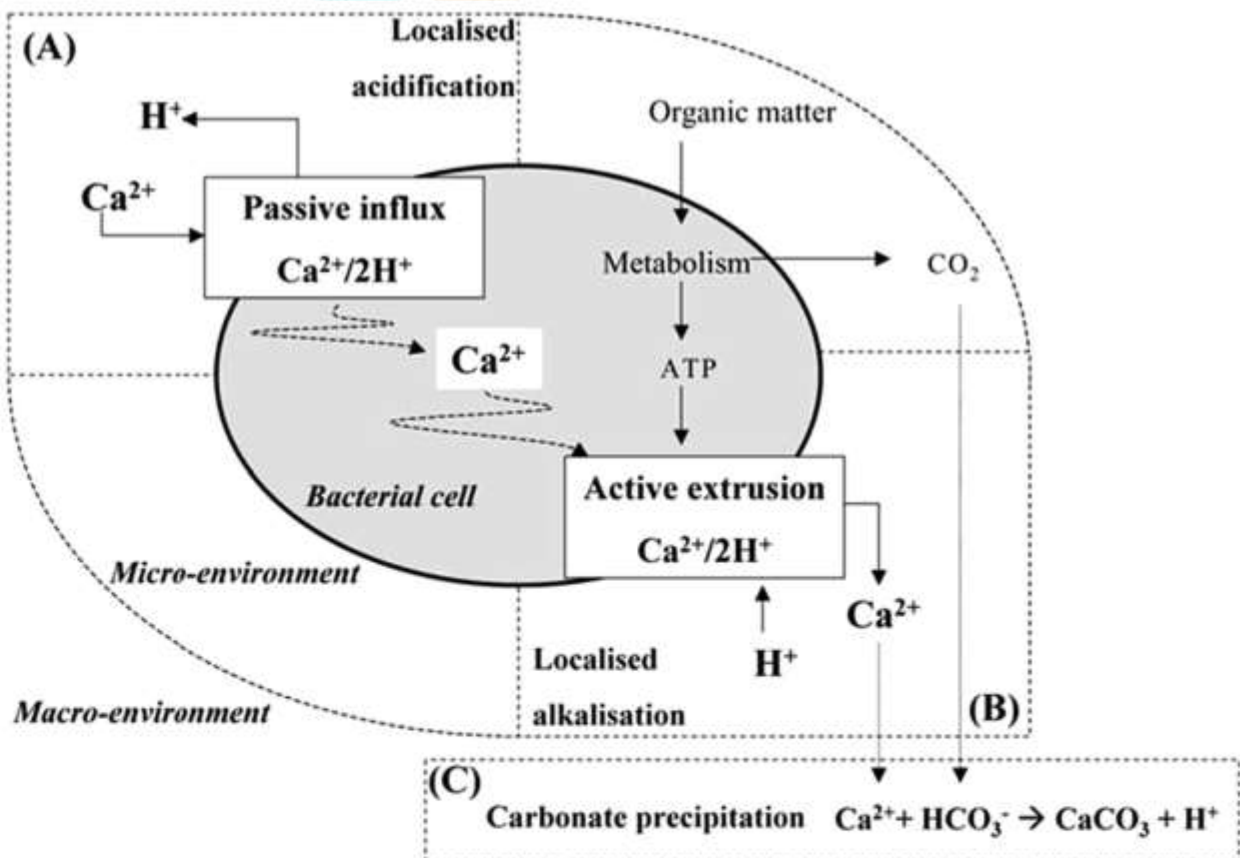

Fig. 3. Schematic diagram of bacterial metabolism and subsequent $CaCO_3$ precipitation under high pH and high Ca^{2+} extracellular conditions (Hammes and Verstraete, 2002).

Okwadha and Li (2010) used *Sporosarcina pasteurii* at a constant temperature to evaluate the effects of other environmental conditions such as bacterial cell concentration, urea and calcium ions concentration. The study revealed that the rate of urea hydrolysis increases with the increase in bacterial cell concentrations and a tremendous increase in calcium carbonate precipitates of 100% was recorded when the calcium ions were increased by ten times. The authors also reported that urease-catalyzed ureolysis is temperature-dependant like any other enzymatic reaction, as such a temperature range of 20ºC-37ºC provided efficient MCP depending on environmental conditions and concentrations of other reactants in the system. Nemati and Voordouw (2003) and Ferris *et al.*,(2004) reported that the rate of ureolysis increases by twice and five times when the temperature was increased from 10ºC to 15ºC and 15ºC to 20ºC, respectively. This clearly indicated that the increase in temperature within the optimum range accelerates the rate of ureolysis depending on the other conditions. It was also reported by Whiffin (2004) that the urease activity increases proportionally with the increasing temperature up to 70ºC. Soon *et al.*,(2014) investigated the effects of some factors on the performance of *Bacillus megaterium* species in inducing calcium carbonate precipitates in residual soils. The factors considered in the study were concentration of bacteria, concentration of cementation reagent, treatment duration, and flow pressure of the cementation reagents. The study revealed that substantial increase in shear strength and reduction in hydraulic conductivity (69% and 90%, respectively) were recorded after 48 h treatment period of 0.5 M cementation reagents and 1x108 cfu/mL bacteria concentrations. Though many factors influence the ureolysis of the bacteria and the subsequent calcium carbonate precipitates such as temperature, bacterial cell concentrations, type of bacteria, salinity and pH value of the medium, concentration of calcium ions, availability of nucleation sites, mineralogy and particles sizes of the soil and many more, only some of these factors were evaluated in relation to bacterial carbonate precipitates mostly in coarse grained soil, i.e. sand. Therefore, more studies are needed to assess the effects of these factors particularly in residual soil for field implementation of the process. Likewise, studies on the effect of different degrees of saturation on the geotechnical properties of bio-cemented sands were conducted by Cheng *et al.*,(2013). Scanning electron microscope (SEM) was used for the tested sand samples. It was revealed that the distribution of calcite precipitates depends on the degree of saturation of the samples, with fully saturated samples forming scattered crystals on the grain surface, while samples with lower degree of saturations forming strong calcite coating on the sand grains which bonds them together. A mathematical model also confirmed the findings of this study, indicating a positive relationship between degree of saturation and particle size of the soil with the crystallization efficiency. The findings from Cheng *et al.*,(2013) revealed that higher strength can be obtained at lower degree of saturation with less chemicals, which is in compliance with the result of Horn and Meike (1995). Meanwhile, it was found that aerobic microbial activity is optimum at 60%e80% degree of saturation. Thus conducting the MICP treatment at lower degree of saturation would make it more economical by using smaller quantity of cementation reagents, contrary to the other MICP treatments conducted under fully saturated conditions.

5. Improvement in soil engineering properties

Many studies have been conducted to evaluate the strength/stiffness and permeability of different soils using calcite precipitation induced by microbes. The changes in strength, stiffness, compressibility and permeability of the treated soil depend on many environmental and other factors that govern the microbial reaction with the required reagents to induce calcite precipitates. Hence, improvement of soil properties is always governed by some physical properties of soil. The degree of saturation of the soil has a considerable impact on the resulting strength and stiffness of the treated soil. It was reported by Cheng *et al.,*(2013) that particle size distribution, mineralogy, shape, density and texture of the mineral aggregates affect the cementation process in bio-mediated treatment process.

However, excellent results demonstrated by this technique in sealing leakages in water retaining structures and reducing the permeability of some soils by means of bioclogging have led to many interesting researches and applications of biosealing in many civil engineering works (Whiffin et al., 2007; Ivanov and Chu, 2008; van Paassen, 2011). The technique of using microorganisms to improve the strength of granular soil which is referred to as biocementation started in 2001 in Australia. As reported by Kucharski *et al.*,(2006), the technique was widely accepted by civil engineers after a bag of sandwas turned into columns of calcareous sandstone when treated by Australian research group. Biocementation can be defined as the soil improvement process through the production of particle-binding materials via microbial means. It is mainly used in geotechnical engineering applications

for strengthening, plugging and improving soils (Ferris *et al.,* 1997; Nemati and Voordouw, 2003; Whiffin *et al.,* 2007). Recent studies by Soon *et al.*,(2013) revealed the effectiveness of microbial induced calcite precipitation in improving the shear strength and reducing the permeability of tropical residual soil and sand. The results proved an excellent improvement in shear strength of 96% at 0.5 M concentration of the cementation reagents. However, the strength improvement was retarded at higher concentration of the reagent (i.e. 1 M) due to high salinity that resulted in inhibitory effects on the microbial activities. The findings are in agreement with those of De Muynck *et al.*,(2010) who found that higher concentration of cementation reagents usually increases the salinity of the medium thereby retarding the microbial activity due to inhibitory effects, though the activity of some microorganisms is not really affected by the high salinity of the environment (Whiffin, 2004). Some of these organisms are presented in Table 1. Bioclogging can be defined as the reduction of hydraulic conductivity of soils or porous rocks by pore-filling materials generated by microbial processes. The carbonate precipitate generated microbially is responsible for clogging the soil pore spaces, thereby restricting flowofwater and decreasing the permeability of the soil. Whiffin *et al.*,(2007) reported a reduction in permeability from 22% to 75% of the initial permeability of the treated soil. Yasuhara *et al.*, (2012) similarly revealed a decrease in permeability of 60%e70% of a soil sample when an extract of urease enzyme was used directly to calcite precipitations induced. Meanwhile, Soon *et al.*,(2014) presented a decrease in hydraulic conductivity of 90% in residual soil after a species of bacillus, *Bacillus megaterium*, was used to trigger calcite precipitation in the soil. Hence, considerable increase in unconfined compressive strength and limited reduction in permeability of treated samples are the basic qualities that make biocementation treatment attractive (Harkes *et al.*, 2010; Cheng and Cord-Ruwisch, 2012; Soon *et al.*, 2014). Meanwhile, preservation of permeability allows for multiple treatments, use of low injection pressure and possibility of treating large volume of soil. Therefore, biocementation can be used for in situ treatment underneath without disturbing the existing buildings (Karol, 2003). Microbial calcite precipitate induced in sands was studied and various microscopy techniques were used to assess how the pore space volume was altered by calcite precipitation. The calcite precipitate was distributed spatially within the pore spaces of the sand, thereby reducing the permeability and increasing the stiffness of the sand samples (DeJong et al., 2010). Table 2 shows some reaction conditions reported in literature for the production of calcium carbonate via microbial urea hydrolysis for biocementation, bioclogging and other applications.

Table 2
Reaction conditions for the production of calcium carbonate using microbial urea hydrolysis.

Application	Urea (mM)	Ca^{2+} (mM)	Urease activity (mM urea/min)	Microorganisms	Soil type	References
Biocementation	1500	1500	4–18	*Sporosarcina pasteurii*	Sand	Whiffin (2004)
Biocementation	500	500	n/s	*Bacillus megaterium*	Sand/silt	Soon et al. (2014)
Biocementation	500	500	5–20	*Sporosarcina pasteurii*	Silica sand	Al Qabany et al. (2012)
Biocementation	1000	1000	10	*Bacillus sphaericus*	Silica sand	Cheng et al. (2013)
Biodeposition	330	25	0.65	*Sporosarcina pasteurii*	Sand	Achal et al. (2009a)
Biocementation	500	500	0.67–1.33	*Sporosarcina pasteurii*	Sand	van Paassen (2009)
CO_2 sequestration	670	250	n/s	*Sporosarcina pasteurii*	–	Okwadha and Li (2010)
Biodeposition	25	25	n/s	*Sporosarcina pasteurii*	Sand	Achal et al. (2009b)
Bioclogging	1500	750	6.2	*Bacillus* sp.	Sand	Chu et al. (2012)
Removal of Ca^{2+} from wastewater	16	14	0.293	Isolates closed to *Bacillus sphaericus*	–	Hammes et al. (2003)

Though different methods of injecting the cementation reagents into the soils were used in the treatment processes, substantial improvement in strength and reduction in hydraulic conductivity of treated soils were reported. The major concern regarding the usually adopted methods of injecting the cementation reagent from surface downward is the differential distribution of the calcite, with many being deposited at the surface compared to that at the bottom of the specimen, as reported by van Paassen (2009). Chu *et al.*,(2012) observed substantial reduction in hydraulic conductivity and shear strength improvement both on the surface and within the bulk of soil upon application of ureolytic bacterial *Sporosarcina pasteurii* isolated from tropical beach sand. They also revealed that the modulus of rupture of the thin layer of calcium carbonate formed at the surface of the soil was 35.9 MPa, which is comparable with that of limestone. Likewise, Filet *et al.*,(2012) described MICP as a competitive treatment method for consolidation of fine-grained soil. The findings of the study revealed that volume of soil treated turned like calcareous sandstone in few days without considerable modification of the initial permeability of the soil. Hence, an up scaling work for site application that led to the validation of industrial concept was also presented. A 5m sand column was treated using MICP under the condition that reflects field application process. After treatment, the sand column shows considerable improvement in strength, stiffness and load bearing capacity without making the soil impermeable (Whiffin *et al.*, 2007). Biomineralized calcium carbonate has proved its efficiency in both bioclogging and biocementation of soils and could be used in geotechnical engineering to improve the engineering properties of soil in situ (Ivanov and Chu, 2008). The authors further emphasized that these methods could be used as a replacement of the traditional energy demanding mechanical compaction and chemical grouting methods that are expensive and sometimes harmful to the environment. However, collaborative studies of civil engineering, ecology and micro-biology are very fundamental for effective microbial treatment method. Nemati and Voordouw (2003) considered the effects of varying concentration of urease enzyme, cementation reagents and temperature on the permeability of unconsolidated porous media. They found that the increase in the concentration of urease enzyme from 0.01 g/L to 0.1 g/L increases the production of CaCO3, while increases in urea and calcium chloride beyond 36g/L and 90 g/L, respectively, do not increase the amount of calcite obtained by MCP. Hence, the temperature range of 20^oC-50^oC enhanced the production of $CaCO_3$ at low concentration of enzyme. Although the temperature effect has been evaluated by some authors in relation to microbial carbonate precipitation, other environmental factors such as humidity, dissolved cations, degree of saturation of the soil and many more need to be evaluated. Yasuhara *et al.*,(2012) reported potentials of using urease enzyme from other sources different from bacteria to catalyze the hydrolysis of urea in the presence of calcium chloride to precipitate calcium carbonate for the improvement of engineering properties of sand samples. Findings from this study indicated that unconfined compressive strength of the treated samples increases considerably, with the initial hydraulic conductivity of the treated samples being decreased by 60%-70%. Hence, since the urease enzyme can be obtained directly from some plants such as sword beans, exploring into this alternative would be of immense contributions particularly in field applications of this technique. This is because that handling of bacteria in terms of cultivation and storage needs some technical expertise, and microbial metabolism which is a key factor in MICP may not be straightforward enough to be controlled. Therefore, it may be impossible to constrain the extinction and/or the generation of living bacteria in natural environments.

6. Conclusions and suggestion for further studies

MICP can be considered as a practicable technique that can improves soil-supporting new and existing structures and can be used in many geotechnical engineering applications, such as slope stabilization and subgrade reinforcement. The process has shown greater potential in many engineering applications, but much work has to be done to bring this convenient technology to field applications. Comparative studies need to be conducted to assess the feasibility of MICP with that of traditional grouting, particularly with regard to environmental implications and economy. Though the technique has been recommended as an alternative method of soil improvement, degrading of the calcite precipitate during loading as reported by DeJong *et al.*,(2010) poses a question on the durability of strength induced by calcite precipitation. Likewise, using higher concentration of cementation reagents would result in higher strength but may make the environment more salty and affect some bacterial growth. Therefore, more studies on the feasibility of combining this technique with other methods that would provide long-term strength and durability such as lime are recommended. Soil microorganisms play an important role in formation of fine grained soils and change of behaviors of coarse-grained soils. Hence, despite their relevance in influencing the properties and behaviors of soil, less work has been done in exploring the importance, relevance, usefulness and application of these microorganisms in geotechnical engineering.

Although MCP has been investigated extensively both in natural environments and under controlled laboratory conditions, the exact mechanism of precipitation and the function of this process within the microbial ecology of the precipitating organism remain unresolved. Thus, the existence of different possible mechanisms with regard to the role of microorganisms in the carbonate precipitation describes the complexity of the biomineralization process and the need to explore more into the process. Many factors influence bacterial ureolysis and the subsequent calcium carbonate precipitation. These factors include temperature, bacterial cell concentrations, type of bacteria, salinity, humidity, pH value of the medium, concentration of calcium ions, availability of nucleation sites, mineralogy and particles sizes of the soil and many more. Only some of these factors were evaluated in relation to bacterial carbonate precipitations mostly in coarse-grained soil, i.e. sand. Therefore, more studies need to be carried out to assess the effects of these factors particularly in residual soil for field implementation of the process. Although studies have been conducted to evaluate the strength/stiffness and permeability of different soils using calcite precipitation induced by microbes, a lot of work has to be done to evaluate the compressibility and settlement properties of soils in their natural state. The main challenge in the success of this approach is to overcome the mass transfer limitations and effectively transport the cementation reagents to deeper parts of the area to be treated.

Since most of the studies conducted used injection methods to pump the reagents into the soil vertically either in continuous or stepped applications, studies conducted revealed that more calcite is precipitated at the upper part of the specimen than that at the lower part, thereby causing disparity in the calcite formation within a soil mass. Though measures were suggested by some authors with regard to the pumping pressure/rate based on the soil types in order to minimize clogging at the inlet and allow for more penetration of the reagents downward, less work has been done for lateral flow of the reagents which may likely be the case in field application for treating large volume of soil. Although the potential advantages and application of the process have been identified in the study, optimization and up scaling of the process, education/training of researchers/practitioners were identified as the challenges ahead. Hence, it is needed to evaluate the long-term durability of strength induced by the process. Conflict of interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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