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Advancement In Microbial Soil Treatment For Enhancing Geotechnical Properties: A Review

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Abstract. The concept of utilizing metabolic pathway (ureolysis) of non-pathogenic bacteria to enhance the geotechnical properties of soil is comparatively new. Uerolytic bacteria precipitate calcium carbonate ($CaCO_3$) or calcite in an alkaline environment as a binding material and bind soil particles together to enhance its strength and other geotechnical properties. This has been claimed as sustainable and environment friendly ground improvement technique and also termed as microbial induced calcite precipitation (MICP). Sizeable numbers of small and large scale laboratory experiments have been demonstrated on MICP since 2004. Laboratory investigations established that MICP can be effectively used to improve the geotechnical properties of both granular as well as fine-grained cohesive soils. Some of the laboratory investigations have been carried out to evaluate the effectiveness of different bacterial strain while some investigations have reported the effects of environmental factors on MICP. The aim of this review paper is to appraise the advancement in microbial soil treatment process towards field implementation by addressing factors affecting its efficiency and effectiveness of different bacterial strains.

Keywords: Soil treatment, MICP, Ureolytic bacteria, Biocementation.

1 Introduction

Urban land cover is expected to increase at higher rate during the first three decades of 21st century (Intergovernmental Panel on Climate Change 2014). Hence, scarcity of suitable land for foundations becomes prominent. This has become a formidable task for geotechnical engineers to develop and strengthen the week soils as construction materials. Current methods of soil improvement that have been practicing worldwide are either energy consuming or harmful to the environment. The demand for a new and sustainable ground improvement technique has turned into a challenge for the researchers worldwide due to ever increasing demand of infrastructure, unavailability of suitable soils for its construction, and particularly due to the environmental concerns in the use of conventional ground improvement technique such as cement & lime stabilizer, chemical jet grouting etc. (Karol 2003). Meanwhile, an interdisciplinary approach utilizing the knowledge of civil engineering, chemistry and microbiology has emerged to modify the soil engineering properties by the use of calcite producing bacteria. With the increasing environmental awareness, this new interdisciplinary ground improvement technique using bacteria known as bio-mediated ground improvement has been gaining more focus among the researchers in the field of soil stabilization and ground improvement technique (DeJong et al. 2010).

This has been motivated by the successful demonstration of microbial treatment in other field like stabilization of metals, development of biological shields for zonal remediation, environmental stabilization of contaminated soils, encapsulation of hazardous and other contaminants in natural soils and acid mine tailings, to increase the efficiency of pumping and production in lower yield oil reservoirs, mineral plugging to reduce the permeability of granular media, to remediate cracks in concrete structures etc. (DeJong et al. 2006). The concept of soil bio-cementation using microbially induced calcite precipitation (MICP) via urea hydrolysis was first studied systematically by Whiffin (2004) in his Doctoral Program. A wide range of in-situ applications has been envisioned of this soil improvement technique (Whiffin 2004; DeJong et al. 2006; DeJong et al. 2010). Literatures have reported that the effectiveness of this technique was demonstrated by researcher through their state-of-the-art experimental studies later on.

Microbial soil treatment is an innovative bio-mediated soil improvement technique which is capable of enhancing the strength and stiffness of soil. It is a biological process that uses the microorganisms either supplied externally or naturally present in subsurface soil and nutrients to improve their engineering properties by producing calcium carbonate or calcite ($CaCO_3$) inside void spaces of the soil matrix (DeJong et al. 2006; van Paassen et al. 2009). The process of producing void-filling material is termed as bioclogging that reduces the porosity and hydraulic conductivity of soil. On the other hand, the process which generates particle-binding materials in situ through microbial process that enhances shear strength of soil is termed as bio-cementation (Ivanov and Chu 2008). This can be achieved by two different approaches, either by bio-augmentation or by bio-stimulation.

Bio-augmentation. In this approach a large quantity of bacterial culture solution is supplied into the treated soil along with a growth and precipitation medium. Therefore, calcite producing pure-cultured ureolytic bacteria is required (e.g., *Sporosarcina pasteurii*) to inject along with growth media into the soil system. In this method, pure-culture of ureolytic bacteria is purchased or isolated from urea rich soil and cultivated upto certain count of bacteria into nutrient broth in the laboratory then injected into soil matrix. After uniform distribution of bacterial solution (bacteria+urea+nutrient), it is allowed to set for some times. Then, calcium rich solution is supplied at a certain interval. Finally, calcite is precipitated within the soil matrix.

Bio-stimulation. In bio-stimulation, indigenous bacteria are stimulated with the nutrient and carbon source to increase the number and thereby calcite precipitation. Bio stimulation encourages indigenous urea-hydrolyzing bacteria by providing appropriate enrichment and precipitation media. It relies on the natural ubiquity of ureolytic soil bacteria and bacterial spatial distribution. Microbial Induced Calcite Precipitation (MICP) via bio-stimulation could be a green technology and potential solution to the problem where indigenous ureolytic bacteria present in the soil.

2 General Review of Urease Activity

Ureolysis or urea hydrolysis is the metabolic pathway of ureolytic bacteria that can precipitate calcium carbonate ($CaCO_3$) or calcite in presence of excess calcium ions. This biological process of calcite precipitation under enrichment environment is also termed as Microbial Induced Calcite Precipitation (MICP). Three types of metabolic activities of micro-bio-organism can induce calcite precipitation are urea hydrolysis, iron and sulphate reduction and denitrification. However, ureas hydrolysis is the most robust and commonly explored mechanism. Several microorganisms use urea as a source of nitrogen by importing it into the cell's cytoplasm. *Sporosarcina pasteurii* (also known as *Bacilus pasteurii*) is a highly active most and commonly using ureolytic bacterium which uses urea as an energy source and produce ATP through urea hydrolysis and there by produce ammonia which is responsible for pH gradient inside and outside the cells. The generation of ATP coupled with ammonium during urea hydrolysis in *S. pasteurii* (Al-Thawadi 2011) is shown in Fig.1. In this process, urea is hydrolysed to ammonia and carbamate (Eq.1). Again, carbamate spontaneously hydrolysed to ammonia and carbonic acid (Eq. 2). Further, this ammonia molecules and carbonic acid equilibrate in water (Eq. 3 – Eq. 4) and resulting in increase the pH gradient (Mobley and Hausinger 1989). *S. pasteurii* is alkalophilic microorganism that grows in alkaline (high pH) medium. On the other hand some microorganisms that prefer to grow in a neutral medium are known neutrophilic organisms. The mechanisms of neutrophiles are differing from alkalophiles.

$$NH_2\text{-}CO\text{-}NH_2 + H_2O \xrightarrow{Urease} NH_3 + CO(NH_2)OH \quad (1)$$

$$CO\,(NH_2)OH + H2O \rightarrow NH_3 + H_2CO_3 \quad (2)$$

$$H_2CO_3 \leftrightarrow HCO_3 + H^+ \quad (3)$$

$$2NH_3 + 2\,H2O \rightarrow 2NH_4^+ + 2OH^- \quad (4)$$

3 Calcite Precipitation by Ureolytic Bacteria

The bio-chemical process of Microbial Induced Calcite Precipitation (MICP) is mainly controlled by the calcium ion concentration, concentration of dissolved inorganic carbon, pH and availability of nucleation sites (Castanier et al. 1999; Hammes and Verstraete 2002). The reasons for commonly adopted this method of calcite precipitation are (i) This process is straightforward and easily controlled (Dhami et al. 2013) and (ii) It has maximum chemical conversion efficiency (Al-Thawadi 2011). The result of urease activity creates alkaline environment and generate carbonate. This free carbonate in presence of excess calcium ion precipitated as $CaCO_3$. The Eq. 5 – Eq. 6 shows the possible bio-chemical reaction of $CaCO_3$ precipitation in urea-$CaCl_2$ medium (Stock-Fischer et al. 1999). Literature based schematic diagram to describe the role of uerolytic bacteria on $CaCO_3$ precipitation is shown in Fig. 2 (Al-Thawadi 2011).

$$Ca^{2+} + Cell \rightarrow Cell\text{-}Ca^{2+} \tag{5}$$

$$Cl^{-} + HCO_3^{-} + NH_3 \leftrightarrow NH_4Cl + CO_3^{2-} \tag{6}$$

$$Cell\text{-}Ca^{2+} + CO_3^{2-} \rightarrow Cell\text{-}CaCO_3 \downarrow \tag{7}$$

In this process the ureolytic bacteria serve as nucleation site for calcite crystal. Once the bacterial cell becomes saturated by heterogeneous nucleation on its wall, it starts precipitation of carbonate crystals inside the pore space of soil matrix. Fig. 3 shows a scanning electron microscopy (SEM) image of a bridging phenomenon inside the sand particles.

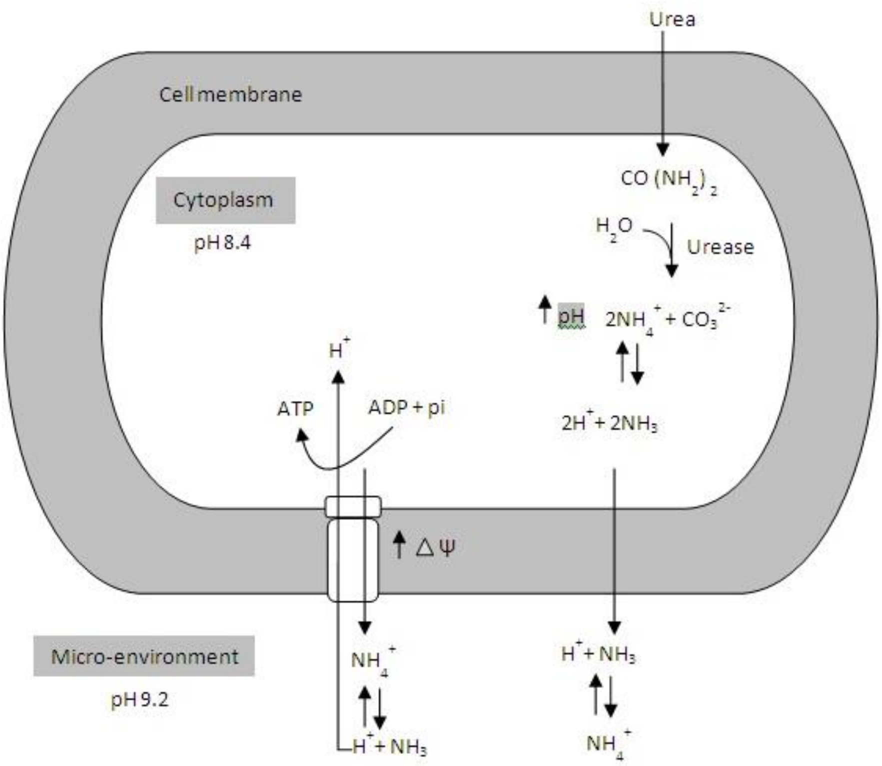

Fig. 1. Generation of ATP coupled with ammonium during urea hydrolysis in S. pasteurii (Al-Thawadi, 2011).

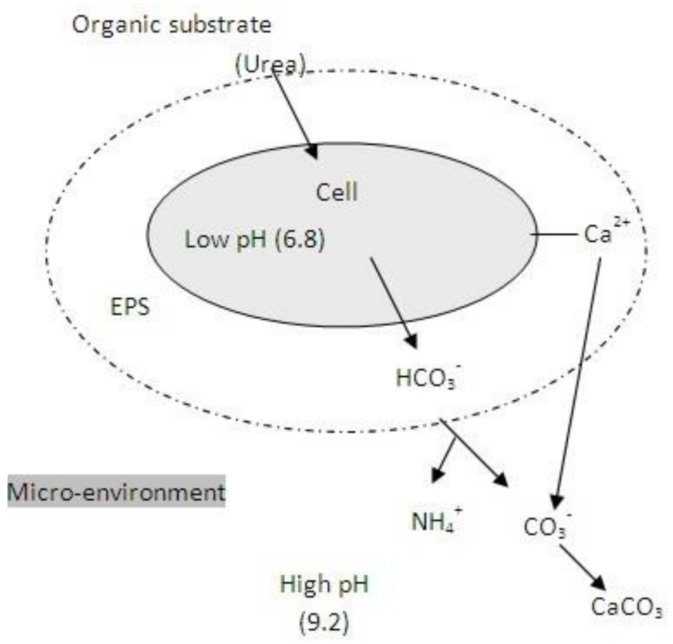

Fig.2. Schematic diagram to describe the role of ureolytic bacteria on $CaCO_3$ precipitation (Thawadi 2011).

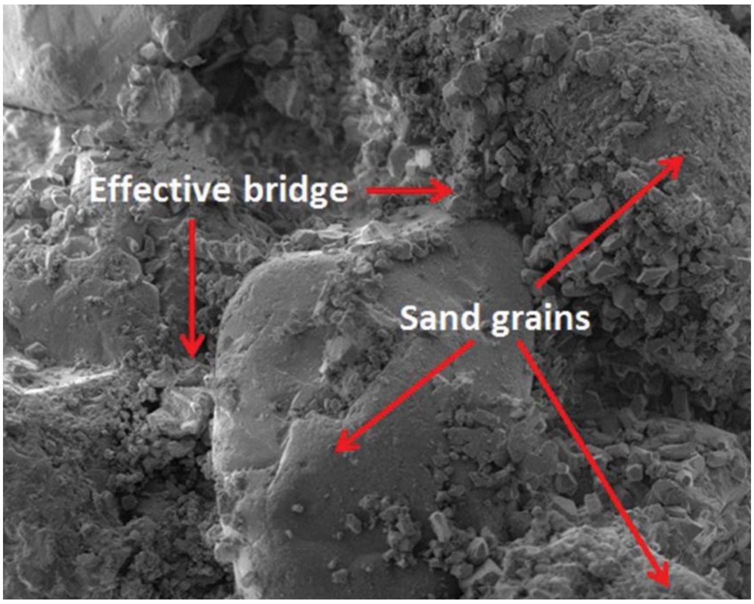

Fig. 3. Scanning electron microscopy (SEM) image of $CaCO_3$ precipitation (Mujah et al., 2017)

4 Factors Affecting Ureolytic Soil Treatment

Among several factors that affect the microbiological treatment of soil are mainly temperature, pH level, concentration of cementation solution (urea+$CaCl_2$), treatment formula and injection rate, retention time, soil type and size etc. The effect of temperature on ureolytic activity is very complex in nature. It was demonstrated that an increase in temperature from 20^0 C to 50^0 C enhance the rate of $CaCO_3$ precipitation (Nemati and Voordouw 2003). Ureolysis activity starts working in aqueous solution and sand column at a temperature range from 10^0 C to 30^0 C and rate of $CaCO_3$ precipitation is high at higher temperature within early 20 hrs, after that it decreases more quickly (Peng and Liu 2019).

Ureolytic bacteria create an alkaline environment which is suitable for calcite precipitation (DeJong et al. 2010). Precipitation starts at a pH level of 8.3 and increases up to pH value of 9.0 (Stocks-Fischer et al. 1999). Experimental investigation shows that when (urea+$CaCl_2$) solution injection rate is less than 0.042 mol/L/h in sandy soil with bacterial concentration density (OD_{600}) between 0.8 – 1.2 (bacterial concentration of 10^7 cells/Ml), the rate of $CaCO_3$ precipitation remains very high irrespective of liquid medium concentration. But, precipitation pattern is affected by liquid medium concentration (Al-Qabany 2012).

The granular behavior of soil influences the microbial activity by allowing the microbes to freely move throughout the pore space. It requires a balanced relationship between soil particle size distributions (D_{10}) and individual microbe cell size. Fig. 4 shows a compatibility relationship between soil particle and microbe cell size.

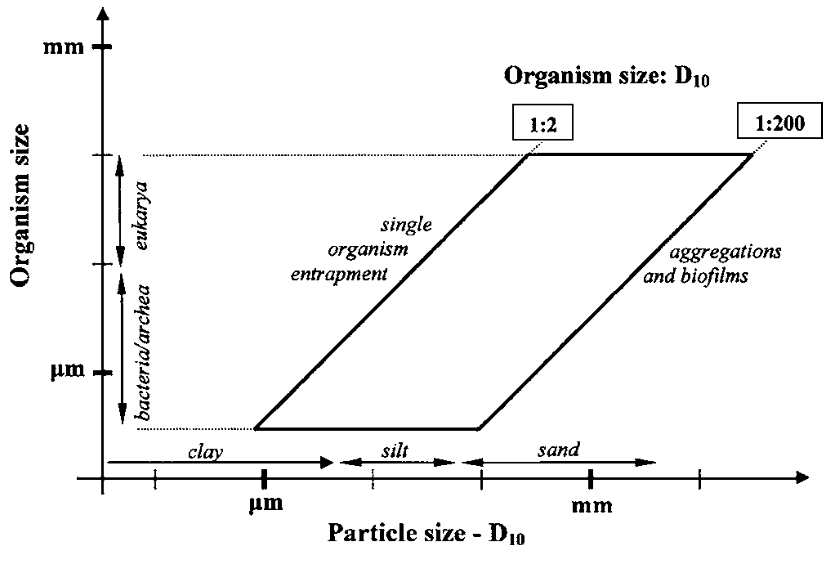

Fig. 4. Size compatibility relationship between individual microbe cell and soil particle (Mitchell and Santamarina 2005)

5 Possible Application of Ureolytic Soil Treatment

Successful and convincing small scale laboratory results have encouraged the researchers to go for upscale experiments and field trials. The large scale experiments have not been fully optimized and field applications are under trial. But, the researchers are hopeful for practical implementation of this "green" technology (DeJong, 2006). Ureolytic soil treatment can improve variety of engineering properties of soil including permeability, stiffness, compressibility, shear strength and volumetric behaviour (DeJong et al., 2010). Different researcher reported different results from their individual laboratory experiments. For example, Ferris et al. (1996) found 15% to 20% reduction of the initial permeability after treatment while Whiffen et al. (2007) observed 22% to 75% reduction. DeJong el al. (2010) envisioned some possible boarder applications of this soil improvement technique which include, but not limited to: treatment of liquefiable soil, settlement reduction of foundation, dam and levee erosive piping plugging, soil stabilization prior to tunneling, erosion prevention of river embankment, slope stabilization, impermeable barrier by reducing permeability of soil etc. Table 1 summarizes the use of MICP technique in different geotechnical applications.

Table 1. MICP process for different geotechnical engineering applications

Application	Soil type	Microorganism	Microbial process / Reference
Soil stabilization/ Settlement reduction	Sand	S. pasteurii	Biocementation DeJong et al. (2006) ; Al-Qabany & Soga (2013); Zhao et al (2014)
	Expansive soil	B. megaterium	Biocementation Li et al. (2018)
Permeability reduction	Sand	Basillus sp. VS1	Bioclogging Chu et al. (2012, 2013)
	Sand	B. sphaericus	Biocementation Cheng et al. (2012, 2014)
	Lateritic soil	B. megaterium	Smith et al. (2017); Soon et al. (2014)
Erosion control	Sand	S. pasteurii	Biocementation Whiffin et al. (2007)
	Granular soil	S. pasteurii	Biocementation Haouzi et al. (2019)
Liquefaction protection	Liquefiable Sand	S. pasteurii	Biocementation Monotoya et al. (2013); Han et al. (2016)
		Soil indigenous bacteria	Biocementation Burbank et al. (2013)

6 Conclusions

This paper provides a review of ureolytic soil improvement technique. The metabolic pathway of ureolytic bacteria, possible $CaCO_3$ precipitation mechanism, factors affecting ureolytic activity and potential applications of this technique has been discussed. Based on the review, the following observations have been made:

1. Most of studies have been carried out on granular or sandy soil and positive results have been found. There is a scope of through study in other soil also.
2. The large scale experiments are not fully optimized and it is under trial.
3. Temperature effect on ureolytic activity for calcite precipitation may not be identical for soil native bacteria.
4. The drawback of this technique is that the by-product of urea hydrolysis is ammonia, which is harmful for aquatic ecosystem.
5. Cost effective analysis of this technique need to be carried out alongside.

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