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Evaluating the Effectiveness of Soil-Native Bacteria in Precipitating Calcite to Stabilize Expansive Soils

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Abstract

The use of chemical additives to stabilize expansive soils is a common practice. However, the environmental concerns associated with the greenhouse gas generation during the production of these chemicals have launched engineers in search of sustainable stabilization alternatives. Microbial Induced Calcite Precipitation (MICP) is a bio-cementation technique that could be a potential solution to this problem. Typically, MICP is achieved via bio-augmentation; however, bio-stimulation was argued to be a more realistic alternative due to its field implementation potential. Hence, in this research study, two expansive soils with varying plasticity characteristics were examined to understand the potential of MICP in treating expansive soils. These two soils were subjected to MICP treatments using enrichment and cementation solutions. The treatment effectiveness was studied via response measures such as Atterberg limits, unconfined compressive strengths, one-dimensional swell test and Calcium Carbonate precipitation. The results indicate that MICP has potential in stabilizing expansive soils and further research is warranted to explore this idea.

Keywords: MICP, expansive soils, soil stabilization, bio-stimulation, substrate solution

Introduction

Clayey soils in general present major geotechnical challenges to engineering and construction firms at significant costs. Engineering properties of clays span extreme ranges, exhibiting high shear strengths when dry, to being very soft under wet conditions. Expansive clays have been a major concern since they swell and shrink as moisture fluctuates. As a result, structures built on expansive soils tend to undergo moderate to severe cracking problems (Mackenzie and Mitchell 1966) (A. J. Puppala, E. Wattanasanticharoen 2003). Lightly loaded structures such as one or two story residential and industrial structures and pavements have experienced severe damage (Petry and Little, 2002), often associated with substantive repair and mitigation costs. In their study of U.S. construction, (Jones and Holtz 1973) show losses associated with the repairs of damaged structures constructed on expansive soils as close to \$9 billion per year.

Over the years, researchers have developed a variety of methods to address construction problems resulting from the expansive soil. Petry and Little (2002) present a historical perspective on expansive soil treatment dating back to the late 1950s. In their work, several stabilization methods including mechanical compaction, chemical stabilization, pre-wetting and moisture barriers, lime injections, and deep soil mixing were described. Altering the physicochemical behavior of these soils by mixing with chemicals such as lime and cement is a widely-used approach both in the United States and around the world (Sherwood 1993). However, doing so raises environmental concerns because of: (1) greenhouse gases generated to produce these chemicals; and (2) negative impacts on plant growth that come from elevated pH levels in the soils after treatment. The elevated pH levels (often >12.4) become a major problem where

soil erosion is a concern and plant growth is necessary to protect soils against erosion. Environmentally safe techniques such as pre-wetting and moisture barriers are only possible for small confined spaces and are not suitable for larger construction projects such as highways and railways which spread for miles especially in the case of high swelling soils where the active zone can extend several meters into the ground.

Therefore, an environmentally-friendly alternative that is sustainable and cost-effective is needed. Turning soils into a cement-like material utilizing bacteria known as biocementation is one such method that can be a viable alternative to treat expansive soils. The most successful biocementation process to date is microbial induced calcite precipitation (MICP) using *Sporosarcina pasteurii*. In this method, microorganism hydrolyzes urea and facilitates the formation of calcium carbonate (or calcite) in the presence of calcium source (Al Qabany and Soga 2013). MICP had successful implication on sandy soil according to the previous studies (Chu et al. 2012) (DeJong et al. 2006). It has become a subject of research in recent years (Chu et al. 2012)(DeMuynd et al. 2010).

Despite advances in the understanding of MICP and few field trials, the necessity of cultivation and injection of bacterial strain hinders this technology to become a cost-effective approach. From the environmental perspective, uncertainty regarding the ecological consequences of introducing non-native bacterial culture into natural soil ecosystem has become a challenge. Therefore, the role of indigenous bacteria in the bio-cementation process must be considered to determine the feasibility of MICP as a field-scale implementation (Gomez et al. 2015). Biostimulation is the process of modification of environmental conditions such as substrates, nutrients, electron acceptors to improve indigenous microorganism with desirable metabolic capabilities (Snoeyenbos-West et al. 2000).

Previous results proved that soil improvement through the bio-stimulation process has potential to improve soil properties in situ for sandy and silty soils ((Burbank et al. 2011). In this research study, an attempt is made to broaden the horizons of this technique by applications into expansive soil treatment. Laboratory experiments were performed where indigenous microbes in natural expansive soils were stimulated to hydrolyze urea in the presence of divalent calcium ions and thereby causing the precipitation of calcite within the pores of soil. This paper presents the details of this study and the findings thereof.

Background

Microorganisms that are capable of hydrolyzing urea to carbon dioxide and ammonia are common in soils (Burbank et al. 2011). (Lloyd and Sheaffe 1973) showed that 17-30% microorganisms from cultivable aerophilic, micro-aerophilic and anaerobic microorganisms are capable of hydrolyzing urea. In MICP, one mole of urea, $(\text{NH}_2)_2\text{CO}$, is hydrolyzed into two moles of NH_4^+ and one mole of CO_3^{2-} by the microbial enzyme urease: $\text{CO}(\text{NH}_2)_2 + 2\text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + \text{CO}_3^{2-}$. In the presence of calcium ions, CO_3^{2-} spontaneously precipitates as calcium carbonate: $\text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3$. The generation of NH_4^+ increases local pH (~8.5), and importantly further increases the rate of calcium carbonate precipitation (Hammes and Verstraete 2002). Microbial-induced calcite creates a bridge between soil grains which cements soil grains together (DeJong et al. 2006).

There are two approaches to apply MICP: bio-stimulation and bio-augmentation. In bio-stimulation, indigenous bacteria are stimulated with the nutrient and carbon source to increase in number and calcite precipitation (Burbank et al. 2011). It depends on the availability of calcifying bacteria and also on spatial distribution. In the case of bio-augmentation, exogenous bacteria are provided to the soil system. Augmented culture to survive and work effectively in a new environment is difficult because of the presence of native microorganism which affects their survival rate and metabolic potential (Wenderoth et al. 2003). Several research studies have injected solutions containing a model ureolytic bacterium, *Sporosarcina pasteurii*, into soil followed by passing nutrient solution which induces the calcite precipitation. Problems such as uneven distribution of bacteria and clogging near the inlet due to calcite precipitation were reported in the case of bio-augmentation (Stocks-Fischer et al. 1999). Also, it was observed that survivability of exogenous microorganisms, after introducing into a new environment tend to decline rapidly and rarely propagate (van Veen et al. 1997). In one study, it was shown that a bacterium strain which was isolated from a coastal marsh in Louisiana and grown in the laboratory could be reintroduced to their environment but failed to survive into another similar coastal marsh environment (LaRock and Donovan, 2001).

On the other hand, in bio-stimulation elimination of non-native bacterial cultivation and injection into the soil can be avoided. (Burbank et al. 2011) discussed the feasibility of biostimulation of MICP treatment based on the soil sample collected from Snake River through laboratory and field testing. Also, in one-dimensional centimeter scale column

experiments, calcite precipitation through bio-stimulation was possible in a variety of granular soils from the depositional environment (Gomez et al. 2015). There are investigations needed to address the possibilities and limitation related to biostimulation process. Further, MICP through bio-stimulation to stabilize expansive soil is still a hypothesis. This research is an initial step to check the feasibility of this hypothesis and understand the challenges associated with stabilizing expansive soil.

Materials and Methods

To evaluate the effectiveness of MICP in mitigating expansive soil swelling, two natural soils with varying plasticity characteristics were selected. Both soils were obtained from Marsing, Idaho along highway US-95 that runs north-south along Idaho/Oregon border. The soils are denoted as S1 and S2. Soil S1 has a liquid limit of 111 and a plasticity index of 70.6 while soil S2 has a LL of 62 and PI of 40.7. Both soils are considered to have high swelling potential. The soils were obtained in their natural form without much disturbance to the microorganism population. The soils were first tested for various geotechnical engineering properties such as maximum dry unit weight (MDUW) and optimum moisture content (OMC); unconfined compression strength (UCS) along with one-Dimensional (1-D) swell strain and swell pressure as per the corresponding ASTM standards provided in Table 1.

Table 1: Baseline data for the two natural soils tested in this research

Soil Type	LL	PI	MDUW	OMC	UCS	1-D Swell	Swell
	(ASTM D4318)		(ASTM D698)	(%)	(ASTM D2166)	Strain (%)	Pressure (kPa)
S-1	111	71	10.95	32.6	156.36	17.9	287
S-2	62	41	13.98	28.5	76.49	9.14	179

Note: LL-Liquid limit; PI-Plasticity Index; MDUW-Maximum Dry Unit Weight; OMC-Optimum Moisture Content; UCS-Unconfined Compression Strength

The soil samples were subjected to MICP using bio-stimulation process. The bio-stimulation process requires that the ureolytic bacteria present in the soil be stimulated by providing the necessary nutrients and ensure urea hydrolysis. Once the bacteria start to hydrolyze urea calcium is introduced into the soil system so that calcium carbonate is precipitated. The solution containing the nutrients and urea is called *enrichment solution* while the solution with calcium source is termed *cementation solution*. Since expansive clays have very low permeability gravity feeding these solutions into the soil microcosm is very time taking. Hence a new device is developed that can deliver the treatment solutions at a faster pace.

Treat Solution Delivery System (TSDS)

The TSDS was designed and developed to deliver treatment solutions to the microorganism in soil samples at different pressures. Trial runs were performed for ensuring no leakage while doing the final test run. Four chambers have been constructed where two sources for enrichment solution and cementation solution have been separately connected. Solutions were able to provide at specified flow pressure.

In this setup, a schedule 80 clear Poly Vinyl Chloride (PVC) chamber houses the soil sample on a 5 cm thick PVC base pedestal. Latex membranes wrap around the soil sample to protect it from unwanted surface erosion and soil samples with latex membrane were shown in Figure 1. Both top cap and bottom pedestal had grooves that are capable of holding O-rings that hold the latex membrane tightly in place and also restrict water from percolating through the gap between soil sample and membrane. Holes in the top cap allows water and treatment solution to flow through them. The bottom pedestal was glued to the base plate and includes holes with a puddle arrangement to collect effluent from the sample. Once the soil sample was ready, we placed the PVC chamber to the base plate. We selected a scheduled PVC clear tube to accommodate threaded connections. Soil samples in the treatment delivery system were shown below in Figure 1. After adjusting all the connections, the chamber is usually filled with treatment solution through a pipe arrangement from a pressure-regulated water reservoir above the base plate.

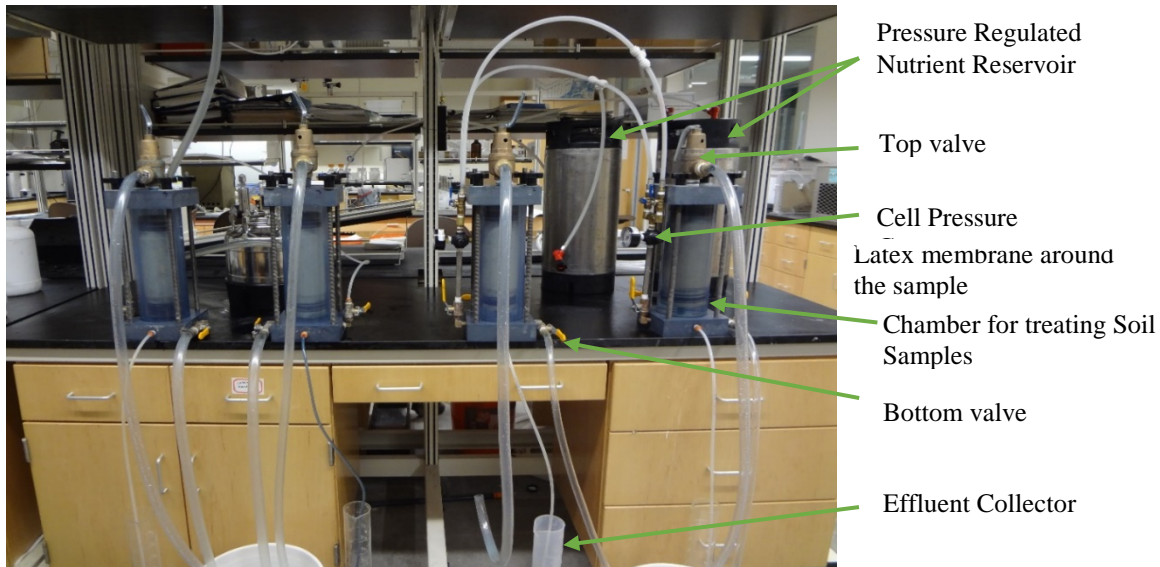


Figure 1: Soil samples in treatment solution delivery system

Treatment Solutions

As discussed earlier, two types of treatment solutions were used in this research. The enrichment solution consisted of 100 mM of Sodium Acetate, 333 mM of Urea, 0.5 g/L of Corn Steep Liquor (CSL). The cementation solution consisted 100 mM of Sodium Acetate, 333 mM of Urea, 0.5 g/L of Corn Steep Liquor (CSL) along with 250 mM of Calcium Chloride. Corn steep liquor consists of amino acids, vitamins, and minerals necessary for microorganism survival. Hence, it is congenial to grow bacteria, it was provided in both enrichment solution and cementation solution. The enrichment solution stimulates the growth of bacteria which use acetate as a carbon source and urea or ammonia as a nitrogen source. The increase in the pH results from the production of ammonia from urea hydrolysis which creates an environment that is favorable for bacteria. With the microbe population becomes more ureolytic, more hydrolysis happens and more calcite is precipitated (Burbank et al. 2011).

Test Protocol

The S-1 and S-2 soil samples were prepared using their respective OMC and MDUW. Static compaction was used to compact the specimens in order to ensure continuous pore connectivity within the sample which will ease the flow of water. The prepared soil samples were wrapped using latex membranes and were placed inside the PVC chamber. The chamber is then closed and enrichment solution is allowed into the chamber. Using the top and bottom valves it is ensured that there are no air bubbles at top of the chamber. After checking all the connections, the enrichment solution was allowed to pass through the soil specimen under 20 psi pressure. It was decided to collect one pore volume of the effluent after which the effluent is tested for a pH. When the pH reached 8.0 or higher the enrichment solution is stopped and cementation solution was started to initiate the precipitation of calcite within the soil mass. The pH of 8~9 was achieved throughout the processes of enrichment and cementation.

Results and Discussion

Several geotechnical tests including Atterberg limits, Unconfined Compression Strength, and 1-D Swell tests were conducted after the treatment process was complete to evaluate the plasticity, strength and swelling behavior of expansive soil. The amount of calcium carbonate present in the soil before and after treatments was also determined. Table 2 presents a summary of these test results and the following sections discuss these data.

Table 2: Treated test results of two natural soils tested in this research

Soil Type	LL	PI	UCS after Treatment (kPa)	UCS before Treatment* (kPa)	1-D Swell Strain (%)	Swell Pressure (kPa)
S-1	139	101	77.22	17.47	13.13	191
S-2	76	57	42.12	21.54	5.96	95

Note: LL-Liquid limit; PI-Plasticity Index; UCS-Unconfined Compression Strength;
*Samples tested at same moisture content as treated sample

Atterberg Limits

Figure 2 presents the variation of LL and PI for both soils before and after treatments. It can be observed that the liquid limit for S1 and S2 soil increased after treatment. The LL increased by 28% and 14% for S-1 and S-2 while the PI increased by 29.5% and 16.1%. Similar results were observed by Neupane (2016). Possible reasons for this increase could be the presence of extracellular polymer substance (EPS) secreted by microbes during the formation of biofilm. EPS can work as a sponge which can absorb water from the environment. In EPS matrix surface water can be attracted by osmotic and capillary forces (Or et al. 2007).

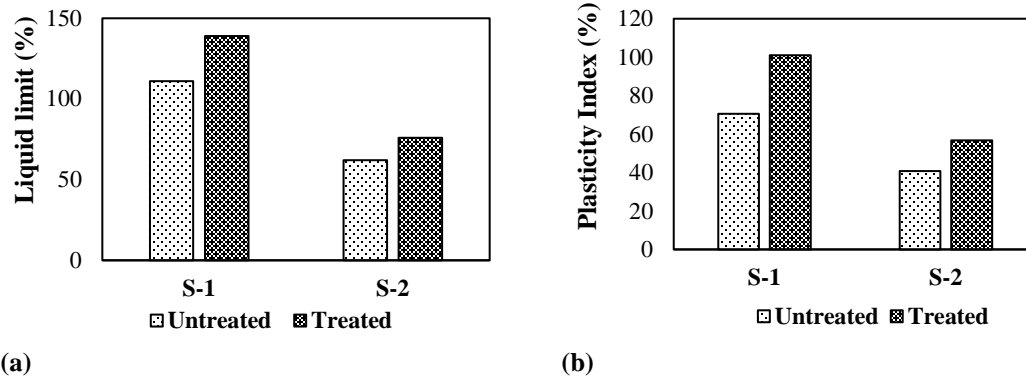


Figure 2: Comparison of test results of treated soil with untreated soil (a) Liquid Limit and (b) Plastic Limit

Unconfined Compressive Strength

Figure 3(a) presents the UCS test results obtained before and after MICP treatments for both soils S1 and S2. The UCS values shown for treated soils were tested immediately after one pore volume of cementation solution was collected. Hence the moisture content at which these samples were tested were different from the control soil samples which were tested at OMC. In order to be able to compare the UCS values before and after treatments control soil samples were re-compacted at the same moisture content at which the treated soils were tested and UCS values were determined. After treatment, the moisture content for S1 and S2 soils was determined to be 70% and 59% respectively. Comparing these values, UCS increased by 77% and 49% for S-1 and S-2 respectively.

Figure 3(b) presents the variation in initial tangent modulus for both soils before and after treatments. This modulus is obtained from the stress-strain curves generated during the UCS testing. It can be noted that the stiffness of the treated samples increased with treatment and this could be due to the higher stiffness of the calcium precipitated.

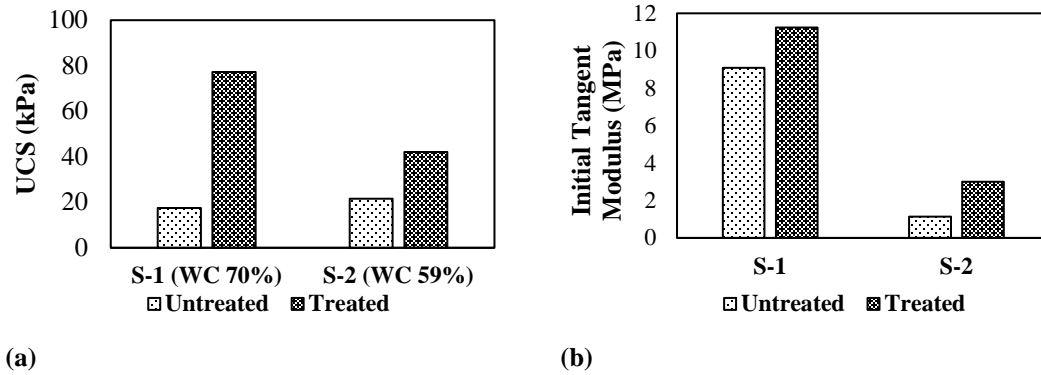


Figure 3 Comparison of test results of treated soil with untreated soil (a) UCS and (b) Initial Tangent Modulus

One-Dimensional Swell Strain and Swell Pressure

1-D Swells tests been performed on treated soils on re-compacted oven dried samples. Similar swelling and loading sequences to control soil samples were followed. Test results presented in Figure 4(a) show that the swell strain decreased by 27% and 35% for soils S1 and S2 respectively. Similarly, the swell pressures were also observed to decrease by 33% and 47% for S1 and S2 soils respectively (Figure 4b). This reduced swell strain and stress could be due to the precipitation of calcite which binds soil particles. Hence, this study shows that MICP could be used for expansive soil treatments and further studies are underway to establish threshold levels where MICP could be effectively used in expansive soil treatments.

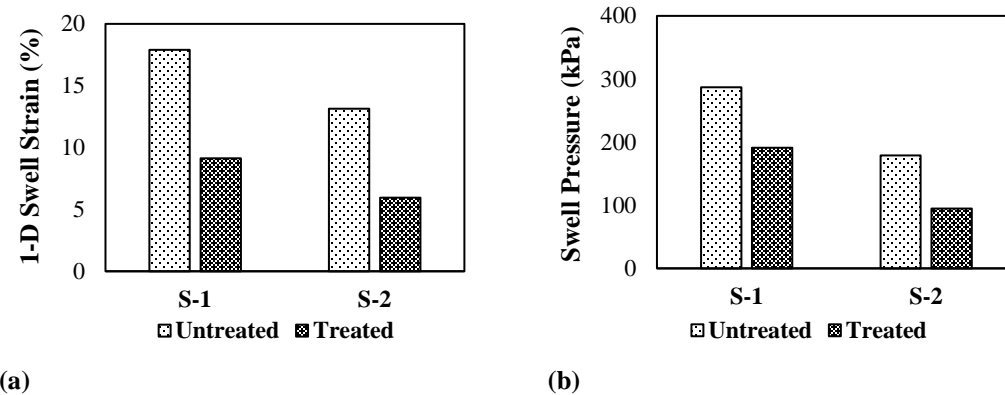


Figure 4: Comparison of test results of treated soil with untreated soil (a) 1-D Swell Strain (b) Swell Pressure

Calcium Carbonate Content

In addition to the UCS and 1-D Swell tests percentage calcium carbonate was also determined on untreated and treated soil samples. Precipitated calcium carbonate was detected using Rapid Carbonate Analyzer. Test results show that the control soil samples did not contain any calcium carbonate while the treated soils contained 1.56 % and 0.88% of calcium carbonate (by dry weight of the soil) for S-1 and S-2 soils respectively as shown in Figure 5(a). This amount of precipitation was obtained after one MICP treatment and resulted in strength increase and swell reduction. The incorporation of more treatment cycles could increase more calcite precipitation. The challenge right now is the permeability of the soil samples. Due to the precipitation of calcite and other microbial activity within the pore spaces of the soil sample the permeability is further reducing which means that the treatments could take longer. The permeability changes before and after treatments are presented in Figure 5(b).

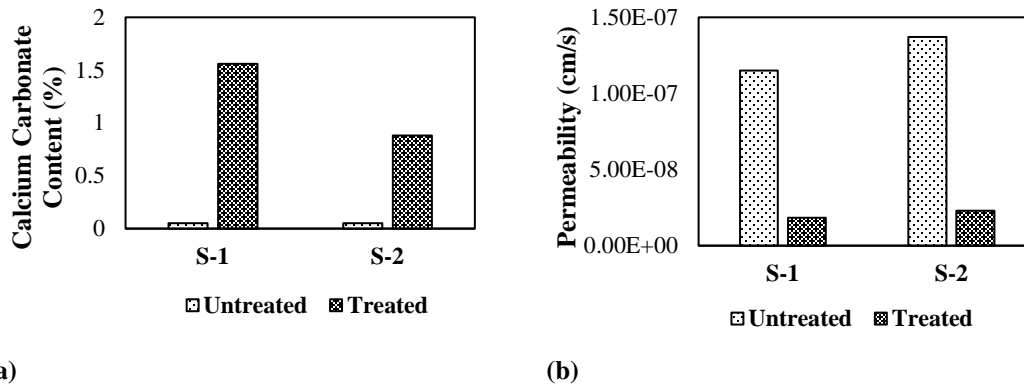


Figure 5: Comparison of test results of treated soil with untreated soil (a) Calcium Carbonate Content (b) Permeability

Summary and Conclusions

Experiments were conducted to demonstrate that indigenous bacteria can be induced to precipitate calcite and stabilize expansive soils. It has been realized that induced calcite precipitation can significantly change soil-engineering properties. Strengths varied significantly and it could be more than 40%. The variation in swelling could be more interesting as 27% and 35% reduction was observed in S-1 and S-2 soils respectively. Calcium carbonate tests were performed to determine the calcite content in soil and 0.8~1.6% calcite precipitation were found. The increase in unconfined compressive strength, change in liquid limit and plastic limit, reduction in swell strain, permeability were the outcomes of calcite precipitation. So, from this research study, it can be concluded that to make biomodification of soils more economically feasible, use of indigenous bacteria to precipitate calcite can be a viable way. Although the reduced swell strains and the swell pressures may not within the allowable limits depending the depth of treatment. However, this study shows that MICP could be used for expansive soil treatments and further studies are underway to establish threshold levels where MICP could be effectively used in expansive soil treatments.

Acknowledgements

The authors acknowledge the support provided by the IDEA program of National Co-operative Highway Research program. Special thanks are due to Dr. Inam Jawed for his continued support throughout the course of this research. Thanks are also due to John Arambarri and Keith Nottingham of Idaho Transportation Department (District 3) for their help with sample collection and delivery.

References

- A. J. Puppala, E. Wattanasanticharoen, and K. P. (2003). "Proceedings of the Institution of Civil Engineers - Ground Improvement." *ICE Ground Improvement*, 7(1), 25–35.
- Burbank, M. B., Weaver, T. J., Green, T. L., Williams, B. C., and Crawford, R. L. (2011). "Precipitation of Calcite by Indigenous Microorganisms to Strengthen Liquefiable Soils." *Geomicrobiology Journal*, 28(February), 301–312.
- Chu, J., Stabnikov, V., and Ivanov, V. (2012). "Microbially Induced Calcium Carbonate Precipitation on Surface or in the Bulk of Soil." *Geomicrobiology Journal*, 29(February), 544–549.
- DeJong, J. T., Fritzsche, M. B., and Nüsslein, K. (2006). "Microbially induced cementation to control sand response to undrained shear." *Journal of Geotechnical and Geoenvironmental Engineering*, American Society of Civil Engineers, 132(11), 1381–1392.
- DeMuynck, W., DeBelie, N., and Verstraete, W. (2010). "Microbial carbonate precipitation in construction materials: A review." *Ecological Engineering*.
- Gat, D., Ronen, Z., and Tsesarsky, M. (2016). "Soil Bacteria Population Dynamics Following Stimulation for Ureolytic Microbial-Induced CaCO₃ Precipitation." *Environmental Science and Technology*, 50(2), 616–624.

- Gomez, M. G., Martinez, B. C., DeJong, J. T., Hunt, C. E., DeVlaming, L. a., Major, D. W., Dworatzek, S. M., Gomez, M. G., Martinez, B. C., DeVlaming, L. a., DeJong, J. T., Hunt, C. E., and Major, D. W. (2015). "Field-scale bio-cementation tests to improve sands." *Proceedings of the Institution of Civil Engineers - Ground Improvement*, 168, 1–11.
- Hammes, F., and Verstraete, W. (2002). "Key roles of pH and calcium metabolism in microbial carbonate precipitation." *Reviews in Environmental Science and Biotechnology*, 1(1), 3–7.
- Jones Jr, D. E., and Holts, W. G. (1973). "Expansive Soils - The Hidden Disaster." *American Society of Civil Engineers*, 43(8), 49–51.
- Lloyd, A. B., and Sheaffe, M. J. (1973). "Urease activity in soils." *Plant and Soil*, 39(1), 71–80.
- Mackenzie, R. C., and Mitchell, B. D. (1966). "Clay mineralogy." *Earth-Science Reviews*, 2, 47–91.
- Neupane, S. (2016). "Evaluating the Suitability of Microbial Induced Calcite Precipitation Technique for Stabilizing Expansive Soils." Boise State University.
- Or, D., Phutane, S., and Dechesne, A. (2007). "Extracellular Polymeric Substances Affecting Pore-Scale Hydrologic Conditions for Bacterial Activity in Unsaturated Soils." *Vadose Zone Journal*, 6(2), 298.
- Petry, T. M., and Little, D. N. (2002). "Review of Stabilization of Clays and Expansive Soils in Pavements and Lightly Loaded Structures—History, Practice, and Future." *Journal of Materials in Civil Engineering*, 14(6), 447–460.
- Al Qabany, A., and Soga, K. (2013). "Effect of chemical treatment used in MICP on engineering properties of cemented soils." *Geotechnique*, 63(4), 331–339.
- Sherwood, P. T. (1993). *Soil Stabilization with Cement and Lime: State-of-the-Art Review. TRL STATE OF THE ART REVIEW*.
- Snoeyenbos-West, O. L., Nevin, K. P., Anderson, R. T., and Lovley, D. R. (2000). "Enrichment of Geobacter species in response to stimulation of Fe(III) reduction in sandy aquifer sediments." *Microbial Ecology*, 39(2), 153–167.
- Stocks-Fischer, S., Galinat, J. K., and Bang, S. S. (1999). "Microbiological precipitation of CaCO₃." *Soil Biology and Biochemistry*, 31(11), 1563–1571.
- van Veen, J. A., van Overbeek, L. S., and van Elsas, J. D. (1997). "Fate and Activity of Microorganisms Introduced into Soil." *Microbiology and Molecular Biology Reviews*, 61(2), 121–135.
- Wenderoth, D. F., Rosenbrock, P., Abraham, W. R., Pieper, D. H., and H??fle, M. G. (2003). "Bacterial community dynamics during biostimulation and bioaugmentation experiments aiming at chlorobenzene degradation in groundwater." *Microbial Ecology*, 46(2), 161–176.