Engineering Behaviour of Microbially Cemented Soils using *Sporosarcina Pasteurii*

THESIS

Submitted to the Delhi Technological University for the award of the degree of

DOCTOR OF PHILOSOPHY

By

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DECLARATION

I hereby declare that the work embodied in this thesis entitled ***'Engineering Behaviour of Microbially Cemented Soils using Sporosarcina Pasteurii'*** has been carried out by me in the Department of Civil Engineering, Delhi Technological University, Delhi, India, for the Degree of Philosophy under the supervision of Professor Ashutosh Trivedi.

In keeping with the scientific tradition, due acknowledgments have been made wherever the work described is based on the findings of other investigators.

For the present thesis, I am submitting to the University, no degree or diploma has been conferred on me before, either in this or any other University.

Place: Delhi
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CERTIFICATE

This is to certify that the thesis entitled ***'Engineering Behaviour of Microbially Cemented Soils using Sporosarcina Pasteurii'*** being submitted by ***Sangeeta Shougrakpam*** to the Delhi Technological University, Delhi, for the award of the degree of Doctor of Philosophy is a record of bonafide research work carried out by her under my supervision and has fulfilled the requirements for the submission of this thesis, which is to my knowledge has reached requisite standards under the regulations of the University.

The results in this thesis are original and have not been submitted to any other university or institute to award any degree or diploma.

Place: Delhi
Date: 22-04-2022

(**Prof. Ashutosh Trivedi**)
Supervisor
Department of Civil Engineering
Delhi Technological University
Delhi, India

Dedicated

to

my loving mother

Leishangthem Pramodini Devi

&

in the loving memory of

my father

Shougrakpam Debendra Singh

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Place: Delhi

Sangeeta Shougrakpam

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Nomenclature

°F	Degree Fahrenheit
µM	Micrometer
ACC	Amorphous calcium carbonate
BCM	Biologically controlled mineralization
BIM	Biologically induced mineralization
BS	Bacterial solution
Ca^{2+}	Calcium ions
$CaCl_2$	Calcium chloride
$CaCO_3$	Calcium carbonate
$CaCO_3 \cdot 6H_2O$	Hexahydrocalcite or ikaite
$CaCO_3 \cdot H_2O$	Monohydrocalcite
CFU	Colony forming units
$CO(NH_2)_2$	Urea
CO_2	Carbon dioxide
CRS	Cementation reagent solution
DIC	Dissolved inorganic carbonates
e_{max}	Maximum void ratio of coarse grained soil in its loosest state
e_{min}	Void ratio of coarse grained soil in its densest state
EPS	Extracellular polymeric substances
GHG	Greenhouse gas
IPCC	Intergovernmental Panel on Climate Change
kPa	Kilo Pascal
M	Molarity

MICP	Microbially induced calcite precipitation
MPa	Mega Pascal
MTCC	Microbial type culture collection and gene bank
NB	Nutrient broth
NCR	National capital region
NO_3^-	Nitrate ion
OD	Optical density
OMC	Optimum moisture content
OPC	Ordinary Portland cement
ppm	Parts per million
PVC	Polyvinyl chloride
rpm	Rotations per minute
S. pasteurii	*Sporosarcina pasteurii*
SEM	Scanning electron microscope
SO_4	Sulphate
Type-A sand	The clean sand specimen
Type-B sand	The clean sand mixed with 10% fine-grain content
UCS	Unconfined compressive strength
UPB	Urease-producing bacteria
USEPA	United States Environmental Protection Agency

DELHI TECHNOLOGICAL UNIVERSITY, DELHI
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ABSTRACT

Engineering Behaviour of Microbially Cemented soils using *Sporosarcina Pasteurii*

Microbial induced calcite precipitation (MICP) is a novel biomediated ground improvement method that can bind the sand grains together and improve the engineering properties of soil in a sustainable and environmentally-friendly method. The present study investigated the feasibility of using the MICP method in soil treatment of the Yamuna river basin soil to increase soil shear strength and stiffness while reducing permeability by the induced calcium carbonate precipitates as calcites. *Sporosarcina pasteurii*, a common alkalophilic soil bacterium with a high urease activity, was used to facilitate the biochemical reaction that induced $CaCO_3$ precipitation. The soil treatment was carried out using a bacterial solution (BS) containing bacterial cells and a cementation reagent solution (CRS) containing urea and $CaCl_2$ as nitrogen and calcium sources.

Treatment was carried out with different molar concentrations of 0.25 M, 0.5 M, 0.66 M, and 0.75 M CRS. The optimum concentration for effective soil treatment was 0.66 M CRS for cost-effectiveness and to avoid wastage of chemical reagents.

The improvement in soil strength of the biocemented sand was determined by unconfined compressive strength and direct shear tests and the effect of clogging by

permeability tests. In addition, a significant increase in the grain sizes was observed in the biocemented sand.

Higher $CaCO_3$ content with more mineral precipitation and higher strength was observed in the various biocemented sand. The improvements in UCS of the various biocemented sand varied from 0.55–2.2 MPa with approximately 4–11% $CaCO_3$ content. The $CaCO_3$ content in the MICP-treated specimens is comparable to 5–15% $CaCO_3$ content in the natural cave soils, stones, and rock masses. The 14 days MICP-treated biocemented sand have achieved more calcite content and higher strength than those treated for prolonged durations due to washing away of detached calcites and decementation.

The strength improvement in the biocemented sand treated with sterile and non-sterile treatment solutions was also determined. The UCS achieved was 835 kPa in biocemented sand treated with non-sterile treatment solution prepared with tap water has indicated its use for typical field conditions by skipping the sterilization process. The cohesion and angle of internal friction of the various biocemented specimens varied from 28–34 kPa and 9–10°, respectively, confirming the strength development in the treated biocemented specimens.

The effectiveness of fine content in the biocemented sand was confirmed by the higher calcite content of 14-15% and UCS of ~ 850 kPa than those without fine contents. The permeability tests confirmed the bioclogging of sand pores by the calcite crystals, which were reduced to three order-of-magnitude from the initial day at 0.6×10^{-4} m/s to 1.1×10^{-7} m/s on the 7th day of treatment which is acceptable to control leakage for aquaculture ponds. In addition, the formation of thin water-impermeable biocemented crust layers of all the biocemented sands may benefit in mitigation of surface erosion and seepage from water bodies.

The scanning electron microscope (SEM) images confirmed the presence of microbial beds in the biocemented sands from the imprints left by the bacteria as hollow spaces on the calcite crystals surfaces. In addition, the presence of biocementation and bioclogging between the sand grains by the calcite crystals and the variation in calcite distribution on the bacteria and sand surfaces were viewed in the images. Lastly, the permeability retention in the biocemented sand, unlike the cement grouting, is advantageous for applying more treatment solutions to control further strength enhancement.

CHAPTER 1

Introduction

1.1 MOTIVATION

Due to rapid urbanization and limited space for infrastructure development, designing sustainable civil engineering structures with low maintenance costs has always been challenging for geotechnical engineers. Concrete consumption has been increasing in the last few decades. Cement is the primary binding material of concrete, but conventional cement production releases significant amounts of carbon dioxide (CO_2), a greenhouse gas (GHG), through its production processes, disturbing the ecosystem. If greenhouse gas levels continue to increase, climate models predict that the average temperature at the earth's surface could increase from 3.2 to 7.2°F by the end of this century, as reported by the United States Environmental Protection Act (USEPA, 2009). This finding has suggested that every possible way to reduce GHG emissions associated with construction technologies should identify and

evaluate them to reduce the human-induced hazards. Timely identification and implementation of green construction practices will reduce not only CO_2 emissions but also the consumption of global energy, as reported by the Intergovernmental Panel on Climate Change (IPCC), 2013. According to IPCC (2013) report, Ordinary Portland cement (OPC) production currently contributes approximately 6% to global anthropogenic CO_2 emissions. Building construction and operation in the current global setting results in 50% of all CO_2 emissions worldwide. The industrial process involved in cement production from lime (precursor of concrete) consumes between 2% and 3% of the global energy demand, generating 0.73–0.99 t CO_2/t of cement produced, which accounts for about 8–10% of the global anthropogenic emissions of CO_2 and 3.4% of the total CO_2 global emissions (Achal et al., 2016; Aprianti, 2017).

From the above discussions, it can be concluded that the present building materials require vast quantities of energy and produce high volumes of CO_2. Therefore, improving the engineering properties of loose sandy soils using the biomineralized calcite precipitates in the soil may consume less energy which may be considered an environmentally friendly method of soil treatment. In addition, the large quantities of CO_2 released by cement production processes (Park et al., 2014) and concrete mix preparation is absorbed and react with Ca^{2+} present in the soil to form the bio-induced calcium carbonate ($CaCO_3$), referred to as calcite from here on. Thus, the rate of CO_2 sequestration is directly proportionate to the amount of calcite precipitates during the MICP process. Thus, the precipitation of $CaCO_3$ enhances the carbon sequestration and storage through solubility and mineral trapping of CO_2 induced by bacterial ureolysis and carbonate formation to form $CaCO_3$.

Many researchers have initiated a multidisciplinary approach across chemical and biological sciences and engineering techniques to solve critical geotechnical

engineering-related problems. In the last two decades, they have been incorporating the microbially induced calcite precipitation (MICP) process in concrete, known as the bio-concrete, based on the bacterial activity in the cement matrix for inducing mineral formation such as $CaCO_3$. This process facilitates the ingress of water, CO_2, and other chemical substances such as SO_4 and NO_3 (De Muynck et al., 2008; Jonkers et al., 2010; Dhami et al., 2014; Achal and Mukherjee, 2015). Therefore, MICP will improve the physical and mechanical properties of the concrete and soil structure if applied in soil improvement, which is a pathway for CO_2 sequestration. Here, the soil is referred to loose and collapsible sandy soils in the present study. During the MICP process, the precipitated calcites bridge the sand at particle to particle contacts. This process is known as biocementation, which increases the strength and stiffness of loose and collapsible sandy soils. In addition, the calcites formed in the pore fluids may either clog the pores or coat the individual particles that can reduce the pore spaces, and this process is known as bioclogging. Reduction in pore spaces will reduce the soil permeability and pore pressure. The reduction in pore pressure in liquefiable prone areas may mitigate the collapsible potential of sandy soil areas. Therefore, it can conclude that the precipitated calcites biocemented and clogged the soil matrix during MICP treatment. Such soil treatment can be considered an environmentally friendly alternative to the harmful conventional techniques such as applying cement or chemical grouts to improve soil strength and permeability. However, these can lead to permanent soil and water contamination or air pollution.

1.2 BACKGROUND OF THE STUDY

MICP has been investigated as a sustainable soil treatment method to improve the engineering properties of soil, such as shear strength and permeability. The sand treatment is to increase the bacterial adhesion to sand before MICP treatment, reduce porosity, and improve the strength of the soil. Pore pressure decreases as porosity decreases and helps mitigate liquefaction, prevent excessive ground movements, reduce permeability, and seepage from storage ponds and other hydraulic structures. At the same time, earthquakes can cause liquefaction in loose sandy soils and damage structures built in earthquake-prone areas. Therefore, soil treatment will mitigate the earthquake-induced liquefaction leading to ground failures. Liquefaction is a geotechnical phenomenon that primarily occurs in saturated sand pores and loses the shear strength in the soil subjected to cyclic loading during an earthquake. Liquefaction may be due to increased pore water pressure caused by the cyclic undrained loading, leading to a decrease in the confining pressure.

In recent years, chemical grouting has been increasingly popular to treat low-strength soil formations, which is cost-effective. In chemical grouting, various additives are used, including Portland cement, lime, asphalt, sodium silicate, acrylate, lignin, urethane, and resins. Many researchers have proven the successful application of additives in the soil (Anagnostopoulos and Hadjispyrou, 2004; Basha et al., 2005; Karol, 2003). But, various chemicals often modify the soil pH and contaminate the soil and groundwater (Dejong et al., 2006; Karol, 2003), thus destroying the ecosystem. Moreover, all chemical grouts except sodium silicate are toxic and hazardous and may cause human-induced hazards during treatment for soil improvement (Dejong et al., 2010). Therefore, a new soil improvement technique termed MICP is introduced by utilizing the physical, mechanical, and biological

processes of soil microorganisms. As a result, it has emerged as an eco-friendly soil treatment method, an alternative to the conventionally used chemical grouting that uses harmful admixtures in soil.

In India, limited studies have been done on MICP to improve the engineering properties of soil. It is a multidisciplinary research approach to harness the metabolic process of microorganisms to precipitate calcium carbonate ($CaCO_3$). $CaCO_3$ is used as a cementing agent to improve the engineering properties of soil (Dejong et al., 2006; Mortensen et al., 2011). $CaCO_3$ is a sustainable and a naturally occurring cementing agent in the form of rocks, stalactites, and caves (Baskar et al., 2006) through different metabolic and biomineralization activities of microorganisms present in the soil, oceans, saline lakes, and other water bodies (Stocks-Fischer et al., 1999). The microorganisms include bacteria, fungi, and protists. The microbes exist above 10^{12} microbes per kilogram of soil near the subsurface (Mitchell and Santamariana, 2005; Dejong et al., 2006). However, the microbial population decreases to about 10^{11} to 10^{6} microorganisms per kilogram, at depths ranging from 2 to 30 m in geotechnical systems. Biomineralization activities occur from time immemorial, from shells, bone, and teeth to limestone caves in vast sedimentary rock masses, as limestone marble and calcareous sandstone in marine, freshwater, and terrestrial environments. $CaCO_3$ is abundantly found on earth, constituting 4% of the earth's crust (Abo-El-Enein et al., 2013). Therefore, MICP is an innovative and eco-friendly cementing mineral that bacteria deposit on the cell and sand surfaces within a soil matrix.

The present research will investigate the feasibility of using MICP as a biocement for soil treatment by using microorganisms. The selected bacterial starin was the urease-producing bacteria known as *Sporosarcina* (*S.*) *pasteurii* (MTCC

1761). It was collected from the microbial type culture collection and gene bank (MTCC), Chandigarh in India. *S. pasteurii* is a spore-former (Madigan et al., 2009) and was reclassified from the *Bacillus* to the *Sporosarcina* genus (i.e., form spores) by Yoon et al., 2001. The bacteria is a common alkalophilic, gram-positive, nonpathogenic, aerobic bacteria found abundantly in natural soil. The bacteria releases urease enzymes that can hydrolyze urea into precipitate carbonates and then react with free calcium ions present in the soil to precipitate $CaCO_3$ as a biocement. In addition, it can produce extracellular polymeric substances (EPS). Based on thermal stability, calcium carbonate can exist in different polymorphs, such as calcite, aragonite, vaterite, and amorphous calcium carbonate (ACC). Among them, calcite is the most stable, with low solubility. Therefore, the calcite precipitates can play a vital role in the biocementation and bioclogging process in biotreated soils.

The surface soil formation in Delhi mostly has Yamuna sand with percentages of silt varying between 0-20% from location to location and significantly from a point in the ground to another depending upon its typical geological deposits (Ojha, 2015), land uses, biological changes, man-made depositions, and disposal of wastes. Therefore, there is a need to investigate the soil type found along the Yamuna river basin and adopt the MICP method for soil improvement. Such a method will benefit infrastructure development where most of the buildings are constructed in the National Capital Region (NCR) of Delhi. Hence, the Yamuna river basin soil was selected for treatment using the MICP method. There should be some ideal size range of the soil to be treated (Rebata-Landa, 2007) that prefers free bacterial movement (Maier et al., 2009).

Moreover, the penetration of microbial cells into the soil is limited when the soil pore size is less than 0.5 to 2 μm. Therefore, MICP treatment is limited to soil

types such as sand with sufficiently high hydraulic conductivity for the free movement of bacteria and nutrients. The technology used for chemical grouting is also applicable for microbial grouting. However, the penetration of the grout will also depend on the size of microorganisms to penetrate freely into the soil. Therefore, in the present study, the poorly graded sand of the Yamuna river basin is selected for treatment.

Several pathways undergo the biomineralization process for mineral precipitation, such as urea hydrolysis, photosynthesis, denitrification, ammonification, sulfate reduction, iron reduction, and methane oxidation (Dejong et al., 2010; Anbu et al., 2016; Zhu et al., 2016). Chapter 2 will focus on the various biomineralization pathways for mineral precipitations. The main groups of microorganisms that can induce carbonate precipitation are photosynthetic microorganisms such as cyanobacteria and microalgae; sulfate-reducing bacteria; and somespecies of microorganisms involved in the nitrogen cycle.

The urea hydrolysis using urease-producing bacteria (UPB) can precipitate $CaCO_3$, improving the soil behaviour and solving many geotechnical engineering issues. The precipitated calcites bind the soil grains within the soil matrix at the particle-particle contacts, thus, increasing the strength and stiffness of the soil by using the biocementation mechanism (Harkes et al., 2010). In addition, the calcite mineral fills or clogs the soil pores, thus reducing the pore volume and hydraulic conductivity of the soil by the bioclogging mechanism (Ivanov and Chu, 2008; Chu et al., 2012). Both the biocementation and the bioclogging process of the MICP can alter the engineering behaviour of soil to increase its strength for construction purposes (DeJong et al., 2006; Whiffin et al., 2007; van Paassen et al., 2010).

Some researchers (Whiffin et al., 2007; Dejong et al., 2006; Ivanov and Chu, 2008) have reported injection of bacteria clogged near the injection points due to the rapid calcite production. In addition, there can be an uneven distribution of calcite spatially and throughout the desirable soil depth, even when clogging does not occur (Harkes et al., 2010; van Paassen et al., 2010). MICP is a very complex process. The successful applications of MICP for ground improvement have been only in small-scale studies (van Paassen et al., 2009; Montoya et al., 2013). Further, there is a compatibility issue between the size of microbes and the pore throats and the effectiveness of using either indigenous (native) or exogenous soil microbes. Natural environments, such as soil, contain numerous microbial species in a complex ecological framework. The injection of exogenous bacteria might disrupt the ecological equilibrium before injection (Whiffin et al., 2007). The indigenous microbes may compete and inhibit their activity resulting in a rapid decline in their numbers. Therefore, it is better to use indigenous microbes and stimulate them using appropriate nutrients to precipitate microbially induced calcite precipitates. In addition, the utilization of indigenous microbes will reduce the cost of application of microbes by obtaining a uniform and even distribution of precipitates in-situ at large scales.

Figure 1.2 shows an overview of the factors influencing the geometric compatibility between the microbes (either indigenous or exogenous species) and the soil in which they are suitable. The relatively small size of bacteria, typically between 0.5 and 3 μm (Madigan and Martinko, 2003), is advantageous and, as a result of their size, should be able to move through different sizes of pore throats as they move from one pore space to another (Mitchell and Santamarina, 2005). The pore throats are dependent on the smaller fraction of particles in the soil. Ex-situ

mixing of microbes and nutrients with soil can widen the range of soils amenable to treatment, including pure clays, as indicated in Figure 1.1.

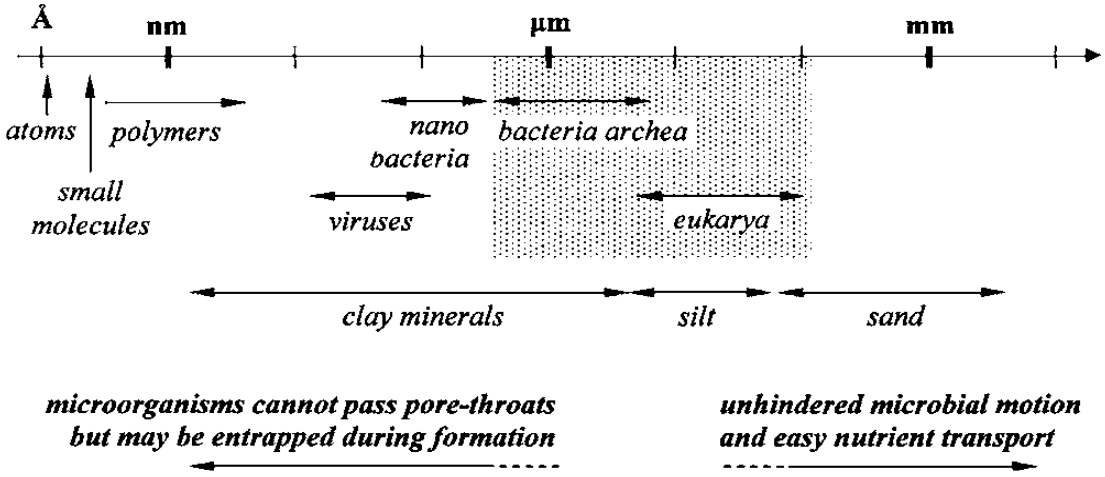


Figure 1.1 Typical size of soil particles and bacteria; and transport of bacteria and nutrients through pore throats (adapted from Mitchell and Santamarina, 2005)

Most of the researchers on liquefaction mitigation have emphasized the feasibility of precipitating calcite that binds the soil particles together rather than strength development in porous media required for ground improvement using chemical solutions. However, DeJong et al., (2006) have introduced a more sustainable alternative of calcite precipitation mediated by bacteria that avoids toxic chemicals in soils. As a result, the soil has increased strength and stiffness and mitigates seismic-induced liquefaction (DeJong et al., 2013; Al Qabany and Soga, 2013).

MICP has been used for other engineering applications, such as repairing concrete structures (Ramachandran et al., 2001). For example, many synthetic filler agents can be used extensively in concrete crack repair. However, the same function can be achieved by using bacteria to prevent the deterioration of concrete structures by deposition of minerals by the microbial activity (Gollapudi et al., 1995). Therefore, the present research could provide alternative solutions to the bacteria used by geotechnical engineers.

1.3 SCOPE OF RESEARCH

The present study is a multidisciplinary research approach across chemical and biological sciences and engineering techniques to investigate the potential of MICP in improving the engineering properties of loose sandy soil collected from the Yamuna river basin in Delhi, India. Efforts have been made to harness $CaCO_3$ using the microbial activity of *S. pasteurii* as a biocement or bioclogging material used for soil improvement through a multidisciplinary approach. Biocementation, bioclogging, and biosealing are different processes of MICP but occur in parallel. Biocementation controls the shear strength, bioclogging reduces the hydraulic conductivity, and biosealing reduces the porosity. In addition, biocementation binds the soil particles together, increasing the shear strength of the soil. The process of biocementation is very complex to understand. It requires microbiology, ecology, geochemistry, and geotechnical engineering to assess its viability to specific sites before applying the MICP method for ground improvement.

The research conducted and presented in this thesis has been planned from a geotechnical engineering perspective to explore creating in-situ mixed cemented columns toreinforce and strengthen loose sandy soils. Since cemented soil columns are an acceptable solution for ground improvement of liquefiable soil, replacing cement with biocement in making soil columns has been investigated. A significant effort has also been made to understand the biogeochemical process involved to facilitate the practical treatment approach. The steps of MICP treatment and the strength test of the MICP-treated soil for the present study are shown in Figure 1.2.

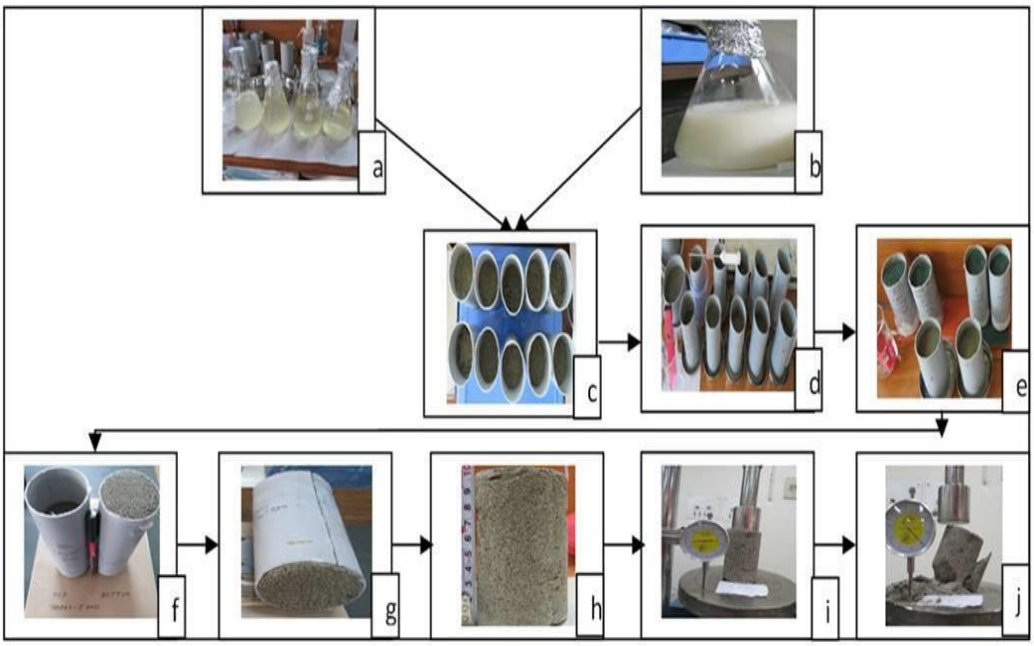


Figure 1.2 Steps of formation of biocemented sand columns using MICP method and its strength testing using unconfined compression strength tests (a) Preparation of bacterial solution; (b) Preparation of cementation reagent solution; (c) Applied the two solutions in the sand-column specimens; (d) Treatment process monitoring temperature and pH of the treatment solution; (e) Drying of specimens in the air; (f) showing top and bottom surfaces of the biotreated sand columns; (g) Specimen removal through vertical cuts of the molds; (h) The treated solid specimen; (i) Mounting of the dried-specimen for UCS tests; (j) Sheared specimen after loading

An attempt has been made in the present study to optimize the use of chemical reagents and nutrients during treatment using the MICP method for calcite precipitation. Calcites can bind the soil particles that increase soil strength and infill the soil pores, reducing permeability. Optimizing chemicals and nutrients will avoid wastage of nutrients during MICP, reducing the cost of treatment. It is necessary to use a suitable growth media to obtain the desired concentration of bacteria. The present study will use two treatment solutions: a bacterial solution (BS) and a cementation reagent solution (CRS), to undergo the treatment process. The cementation solution consists of urea as the nitrogen source and calcium chloride as the calcium source used during the biomineralization process to generate $CaCO_3$.

The urease activity of the bacteria exists if the pH level of the treatment solution is above 7 to maintain alkalinity. The biocementation process may validate by the increase in strength and stiffness of the biotreated specimens by using the unconfined compressive strength (UCS) tests and direct shear strength tests. In addition, the bioclogging process in the soil matrix may validate by reducing permeability. Reduction in permeability indicates the applicability of MICP treatment in embankment structures, channels, and aquaculture ponds built in sandy formations. Unconfined compressive strength (UCS) tests may confirm the increase in strength of the biotreated sand columns with the BS and CRS. The improvement in the biotreated sand specimens may vary on the amount of the effective calcite precipitates in different soil types at different treatment sites. Hence, MICP is a complex process whose end product depends on many environmental factors, such as soil microorganisms and nutrients.

1.4 RESEARCH OBJECTIVES

The main objective of the present study is to use the microbially induced calcite precipitates, technically known as the MICP technique, to improve the engineering properties of loose and collapsible sandy soils of the Yamuna river basin found in Delhi. To date, limited studies have been made in India to explore the effective use of MICP in soil improvement. So far, no studies have been done to treat the Yamuna river sand using the MICP technique. The collected sand is from the Yamuna river basin, where most of the buildings in the Delhi NCR stand. The MICP treatment undergoes the mechanism of biocementation through the binding of sand particles with calcites and bioclogging through the filling of the sand pores with the insoluble calcite precipitates to improve the engineering properties of the soil. The

biocementation process can reduce the soil susceptible to earthquake-induced liquefaction (van Paassen, 2009). Therefore, it has an advantage over existing ground improvement techniques such as stone columns, vibro-floatation, and dynamic compaction. In addition, MICP treatment can improve the ground without disturbing the surrounding area. Therefore, the MICP technique may be an innovative and alternative technique to conventional ground improvement techniques.

A multidisciplinary research approach with an integration of microbiology, ecology, geochemistry, and geotechnical engineering is applied here to increase the efficacy of MICP in soil treatment. An appropriate growth medium was used to culture the selected bacteria to prepare the bacterial solution (BS). In addition, different molar concentrations of the cementation reagent solution containing urea and $CaCl_2$ were prepared (CRS). BS and CRS are the two treatment solutions used in the MICP process to trigger calcite precipitates. DeJong et al., 2006 have focused on selecting bacteria and conditions to achieve different degrees of biocementation in small-scale column experiments. In the present study, the soil collected from the Yamuna river basin was used as the specimen for soil treatment using the MICP method to achieve the following objectives:

- To test the feasibility of using microbially induced calcite precipitates (MICP) to treat Yamuna river basin soil by using *S. pasteurii* to improve the engineering properties of soil in a sustainable and eco-friendly.
- To harvest the selected bacterial strain in the laboratory to prepare a bacterial solution (BS) and a cementation reagent solution (CRS) consisting of urea and $CaCl_2$ as treatment solutions.
- To examine the optimum treatment period to achieve a desirable

cementation level and the compressive strength of the MICP-treated specimens.

- To conduct experiments to understand the behaviour of sand-bacteria-calcite mixture as a biocemented soil to improve the engineering property of soil to make it suitable for construction or environmental purposes.
- To investigate the possibility of MICP treatment of soil surfaces to form water-impermeable biocemented crust layers.
- To examine the difference in the improvement of the engineering behaviour of MICP-treated soil by using a sterile and a non-sterile treatment solution
- To assess the improvement in the engineering properties of the MICP-treated soils by unconfined compressive strength tests, direct shear tests, permeability tests, determination of calcite content, checking if there is any increase in particle sizes due to binding of the soil particles by precipitated calcites.
- To investigate the effectiveness of the bioclogging process by the reduction in the rate of permeability.
- To investigate the calcite content in the naturally biocemented products like rocks and cave soils and compare it with the calcite content in the MICP-treated specimens.

1.5 GAPS IN LITERATURE

From the review of literature, so far, no studies have been found for soil treatment using the MICP method for soils collected from the Yamuna river basin located in the National Capital Region of Delhi. The soil treatment using the MICP method may be feasible to improve the engineering properties of loose sandy soil. Many

researchers have been confined primarily to studying the standard fine sands of Ottawa and Atlas river in Canada, Snake river and Mississippi river in the United States, etc. However, very little work has been taken up in India to treat the loose and sandy soils using the MICP method to increase the strength and reduce permeability. So far, no work has been done in India to investigate the effective use of MICP to treat the soil found along the Yamuna river basin. The present study on soil treatment using the MICP method was carried out with the soil collected from the Yamuna river basin. Such a treatment method is an initial research work that can extend to other parts of India for soil stabilization, where soil improvement is required. The MICP method requires collaboration with multidisciplinary research in biochemistry, microbiology, and geotechnical engineering to focus on the potential of microorganisms in the MICP process. The specimens may increase strength and stiffness by binding the soil particles at contact points during MICP-treatment. In addition, the soil permeability may reduce by either filling the pore spaces or coating the soil particles with the calcite precipitates.

The bioclogging and sealing of pores and fissures by the calcite precipitates may control the seepage and erosion problems along the canals in the Indo-Gangetic plain and other hydraulic structures constructed along the flood plains of the Yamuna river. The Yamuna river covers a total length of 1,376 km. The basin is 3,66,223 sq. km, so it is a critical stretch of land that requires proper soil investigation to apply a suitable and environmentally friendly soil treatment method. Therefore, the results from the present study may benefit the geotechnical engineers in introducing the MICP method in places where the space is limited to accommodate a high density of population in Delhi. The Yamuna river passes through Uttrakhand, Haryana, and Uttar Pradesh while passing Himachal Pradesh, and it later moves to Delhi. Hence,

the present findings will benefit the states where the Yamuna river flows. Efforts have been made to make optimum treatment solutions and the bacterial concentration to minimize the overall construction cost and adopt different treatment methods using the cementing solution for MICP treatment. The released calcite precipitates may be considered an eco-friendly geomaterial for binding and clogging soil matrix.

1.6 THESIS STRUCTURE

This thesis consists of six chapters:

Chapter 1 briefly introduces the present study with a clear motivation for how the research was moved forward for designing sustainable soil improvement techniques at a lower cost. Then, the background of the study presents a brief discussion on how the harmful effect of chemical grouting as a soil stabilization method may be replaced by an environmentally friendly method, technically known as MICP, used to improve the engineering properties of loose and collapsible sandy soil. The scope of the present study is a multidisciplinary research approach across chemical and biological sciences and engineering techniques to explore the potential of microorganisms in MICP. Further, the scope of the research and the main objectives are clearly described in this chapter and concluded by stating the research gap observed during the literature review of previous researchers for adopting the soil treatment using the MICP method.

Chapter 2 reviews the literature on the MICP technique used in improving the engineering properties of soil contributed by various researchers. Generally, this chapter can be divided into three main parts. First, this chapter primarily deals with the different soil improvement techniques, a section on polymorphs of $CaCO_3$, and different mechanisms of the biomineralization process for calcite precipitation.

Among the soil microorganisms, discussion on various types of urease-producing microorganisms, the different mechanisms and pathways involved in the biocementation process, the advantages of the processes, etc., are highlighted in this chapter. Further, a fundamental understanding of microbiological and biogeochemical principles in biocementation to improve the engineering properties of soil.

Chapter 3 covers the methodology used in designing and commissioning the laboratory experiments. This chapter explains the material used, such as the physical properties of the sand, the methods of preparation of sand-column, preparation of the bacterial solution, and the cementation reagent solution. The detailed soil treatment method using the MICP method is also included. Various experiments were conducted to find effective and efficient methods to precipitate sufficient calcite crystals. The tests were conducted to measure the strength, permeability, and other improvements in the soil properties of the biotreated specimens. Finally, the results and discussion section analyzed the effect of pH and CRS on MICP. In addition, a section was added to highlight the percentage gain in particle sizes in the treated sand particles and found that size is also a function of MICP. Finally, the scanning electron microscope images (SEM) of the treated specimens validated the presence of calcite. A list of results obtained from the various experiments of the study is discussed at the end of the chapter.

Chapter 4 is about harnessing MICP to improve the engineering properties of loose sandy soils of the Yamuna river in Delhi, India. The chapter includes materials and methods adopted for performing the experiments on sand specimens to increase strength and impermeability. The biotreated specimens were used for UCS tests, permeability tests, sieve analysis tests for biotreated sands to check the percentage

increase in particle sizes, and SEM analysis. The chapter ends with a list of observations from the results highlighting the soil properties obtained after treatment.

Chapter 5 analyzes the effectiveness of using sterile and non-sterile treatment solutions for soil treatment using the MICP method. Further, biotreated specimens will be tested for shear strength based on unconfined compressive strength and direct shear tests.

Chapter 6 describes the overall conclusion of the results and the discussion section of chapters 3, 4, and 5. Finally, based on the results, the various potential of MICP in other multidisciplinary applications will be highlighted. Some recommendations that can be taken up by the future researchers are also included.

CHAPTER 2

Review of Literature

2.1 INTRODUCTION

Microbially induced calcite precipitation (MICP) is a bio-geochemical process to harness the metabolic process of microorganisms to precipitate calcium carbonate ($CaCO_3$) as a cementing agent for use in soil improvement. $CaCO_3$ is a sustainable and a naturally occurring cementing agent in the form of rocks, stalactites, and caves (Baskar et al., 2006) through different metabolic and biomineralization activities of microorganisms present in the soil, oceans, saline lakes, and other water bodies (Stocks-Fischer et al., 1999). It takes place from time immemorial, from shells, bones, and teeth to limestone caves in vast sedimentary rock masses, as limestone marble and calcareous sandstone in marine, freshwater, and terrestrial environments. $CaCO_3$ is abundantly found on earth, constituting 4% of the earth's crust (Abo-El-

Enein et al., 2013). It is a well-known cementing mineral that bacteria deposit on the cell surface or within a soil matrix.

The microbial activities include ureolysis, photosynthesis, denitrification, ammonification, sulfate reduction, and methane oxidation (Dejong et al., 2010; Anbu et al., 2016; Zhu et al., 2016). Among the microbial activities, the biomineral precipitation of $CaCO_3$ (referred to as calcite) solves many geotechnical engineering issues. First, the calcite precipitates bind the soil grains within the soil matrix at the particle-particle contacts, increasing the strength and stiffness of the soil (Harkes et al., 2010). The process of bridging the soil particles with the calcite precipitates is known as biocementation. Second, the calcite mineral fills the pores to reduce soil pore volume and hydraulic conductivity (Ivanov and Chu, 2008; Chu et al., 2012). Filling the soil pores with the calcite precipitates is known as bioclogging. Finally, the MICP process can improve soil engineering and geomechanical properties for construction purposes (DeJong et al., 2006; Whiffin et al., 2007; van Paassen et al., 2010).

2.2 SOIL IMPROVEMENT TECHNIQUES

The conventional soil improvement techniques, such as chemical or cement grouting, compaction, blasting, vibroflotation, and deep soil mixing, are expensive, energy-intensive, and hazardous to the eco-environmental system. In addition, the compaction methods are limited to shallow depths and could affect settlement, cracking, and failure of nearby structures (Wang et al., 2019). Various advanced methods for ground improvement emphasize the need for multidisciplinary research, especially when using waste materials like fly ash, rice husk ash, blast furnace slag, silica fume, polypropylene fibers, and natural fibers, for chemical and mechanical

stabilization (Sharma et al., 2021; Tiwari and Satyam, 2019, 2020; Tiwari et al., 2020). However, these methods are expensive, and field applicability is challenging. In addition, the method of cement grouting releases carbon dioxide (CO_2), a harmful greenhouse gas (GHG), and other toxic pollutants during the manufacturing phase of cement or while applying chemicals in the field, which ultimately could contaminate the soil and groundwater. Due to its economic benefit, chemical grouting is becoming increasingly popular to treat low-strength soil deposits. However, various harmful additives are used in chemical grouting, including Portland cement, lime, asphalt, sodium silicate, acrylate, lignin, urethane, and resins. Many researchers have proven the successful application of additives in the soil (Anagnostopoulos and Hadjispyrou, 2004; Basha et al., 2005; Karol, 2003). But, the addition of various chemicals often modify the pH of soils and may contaminate the soils and groundwater (Dejong et al., 2006; Karol, 2003) and destroy the ecosystem. Furthermore, all chemical grouts except sodium silicate are toxic and hazardous and threaten their use for soil improvement (Dejong et al., 2010).

The recent collaborative advanced research of geotechnical engineers, chemical engineers, geologists, and microbiologists has led to utilizing the bacterial phase of soil for biocementation and overcoming the challenges of conventional soil improvement practices. This soil improvement technique utilizes biological processes, is technically microbially induced calcite precipitates (MICP), and has emerged as an eco-friendly alternative to conventionally used chemical grouting. MICP treatment used microorganisms to produce calcite precipitates to bridge the soil particles to increase the strength and reduce the hydraulic conductivity. In addition, MICP treatment can improve the soil without disturbing the surrounding soil because microorganisms can penetrate and reproduce in soil. Using cement, one

usually must wait for weeks to develop the full strength. On the contrary, in biocemented soils using bio-induced $CaCO_3$ precipitates, the reaction time to gain significant strength is within a short time. The $CaCO_3$ obtained in different polymorphs due to variation in the biomineralization process and its products resulting from the bacterial activity is reviewed in the following section.

2.3 POLYMORPHS OF $CaCO_3$

Biomineralization is the process of releasing minerals from living cells as a metabolism by-product of microbial activities in a favorable environment. However, it occurs naturally in very diverse environments, and it is stimulated artificially for soil improvement and other bioremediation purposes. It is a chemical alteration method by microbial activity that results in the precipitation of minerals (Stocks-Fischer et al., 1999), consisting of inorganic minerals and trace elements of organic compounds.

The biomineralization process can produce different phases of $CaCO_3$ anhydrous polymorphs such as calcite, aragonite, and vaterite, as well as hydrated crystalline phases such as monohydrocalcite ($CaCO_3 \cdot H_2O$) and hexahydrocalcite or ikaite ($CaCO_3 \cdot 6H_2O$) and amorphous calcium carbonate (ACC) (Dhami et al., 2013). The density of calcium carbonate is 2.71 g/cm^3. Aragonite will change to calcite at 380–470°C. Vaterite is a minor, metastable, and transitional phase during calcite formation. Calcite is the most thermodynamically stable polymorph of $CaCO_3$ and a primary product of $CaCO_3$ in many MICPs (Okwadha and Li, 2010; Stocks-Fischer et al., 1999). Besides, it is a constituent of limestone. It is insoluble in water but soluble in dilute acids (Dejong et al., 2006). However, calcite exhibits an unusual characteristic called retrograde solubility, in which it has

become less soluble in water as the temperature increases. ACC is with low stability and high solubility. The morphological differences in the crystal formation may be strain-specific, owing to differences in the urease activity. Alternatively, these differences could reflect the specific extracellular polymeric substances (EPS) produced by different bacterial types controlling calcite or aragonite polymorph selection. EPS proteins may specifically bind Ca^{2+} and promote carbonate precipitation (Dhami et al., 2014). The crystal morphology is also affected by the growth media or culture composition. The different bacterial species can precipitate different amounts, shapes, and carbonate crystals from the same growth medium.

2.4 BIOMINERALIZATION

Biomineralization is the chemical alteration of an environment by microbial activity that results in the precipitation of minerals (Stocks-Fischer et al., 1999). Biomineralization is a widespread phenomenon leading to more than 60 different biological minerals that exist as extracellularly inorganic crystals (Dhami et al., 2013) or intracellularly (Yoshida et al., 2010). Extracellular mineralization syntheses (e.g., carbonate precipitation) from all living organisms are widespread and well-known phenomena.

Geotechnical engineers explore the microbially induced calcite precipitation (MICP) process as a soil treatment method using the biomineralized byproducts of soil microorganisms. The process needs a bacterial solution (BS) and a cementation reagent solution (CRS) to trigger bio-induced $CaCO_3$ precipitates. The bacterial solution consists of bacterial cells that can release a high capacity of urease enzymes by the urease-producing bacteria (UPB). The CRS consists of urea ($CO(NH_2)_2$) as nitrogen and a carbon source, while calcium chloride ($CaCl_2$) as a calcium source.

The in-situ process of delivering the $CaCO_3$ as a biocement causing behavioral changes in the soil through biocementation and bioclogging is known as biogrouting. The $CaCO_3$ materials in the biogrouts bridge at interparticle contacts during biocementation and fill the pores during the bioclogging process. The viscosity of biogrout is very low, so it can enter into the ground without mixing with soil to a greater depth by gravity flow. This process enables the field constructions to improve the soil without any vibration or by installing equipment to operate heavy machinery for compaction.

The biochemical precipitation of minerals during the MICP process are the metabolic by-products of different soil microorganisms. The biomineralized products may consist of bio-induced calcium carbonate crystals, minerals, and slimes. They will act as cementing or clogging agents between interparticle soils to increase shear strength or reduce hydraulic conductivity. Most crystals formed through biomineralization consist of inorganic minerals, but they may also contain trace elements of organic compounds, regulating the biomineralization process (Yoshida et al., 2010). Different mechanisms of biomineralization for the chemical alteration of an environment due to microbial activity are:

i. Biologically controlled mineralization (BCM) consists of cellular activities that direct the formation of minerals (Phillips et al., 2013). In this process, organisms control nucleation and act as a nucleation site for the deposition and growth of minerals.

ii. Biologically influenced mineralization is the process by which passive mineral precipitation occurs due to cell surface organic matter such as extracellular polymeric substances (EPS) associated with biofilms (Phillips et al., 2013). Biofilm formation and the production of other extracellular

biopolymers can impact soil behaviour. These processes generate organic solids that occupy a portion of the pore space with a soft, ductile, elastomeric material that reduces pore size, rearrangement of particles during soil deformation, and increases elasticity. These changes can reduce hydraulic conductivity and perhaps reduce rapid strain-softening during undrained shearing. The difficulty is the nourishment of microorganisms continuously; otherwise, their engineering performance may become unreliable.

iii. Biologically induced mineralization (BIM) is the chemical modification of an environment by biological activity that results in supersaturation and the precipitation of minerals (Stocks-Fischer et al., 1999; De Muynck et al., 2010; Phillips et al., 2013). BIM is an uncontrolled metabolic consequence of microbial activity, and the production of $CaCO_3$ is dependent on some environmental conditions (De Muynck et al., 2010). Its effectiveness depends on essential factors such as the concentration of dissolved inorganic carbon, nucleation site, pH, temperature, and available nutrients as the energy source for microorganisms. Available nutrient types and their amount in the system are essential to survive the organisms. Some nutrients are ammonia acids, CO_2, N, P, K, Mg, Se, etc. (Mitchell and Santamariana, 2005). Nutrient transport, precipitation in chemical reactions, the type and amount of soluble materials, the environment pH, aeration control, and thermal stability are dependent on water availability in the micro-environment.

The degree of control of the biomineralization process is the main difference between BIM and BCM processes. In BCM, the organism controls the

biomineralization process for the nucleation and growth of the mineral particles and independently synthesizes minerals in a unique form regardless of the environmental conditions. This highly regulated mechanism produces more uniform particles with consistent mineral morphologies. The inorganic solids of calcium carbonate are one such biomineralized product of microorganisms. The mineral precipitates are deposited on or within the organic matrices or vesicles inside the cell (Bazylinski and Moskowitz, 2018; Berenjian et al., 2013). Well-defined mineral structures, such as bones, teeth, shells, and fish otoliths, are formed through the BCM process. The $CaCO_3$ precipitate may alter the properties of loose sandy soils (Shougrakpam and Trivedi, 2021). For example, the calcites bridge the soil at particle contacts to increase the strength and stiffness of the soil. In addition, the fine aggregates in the

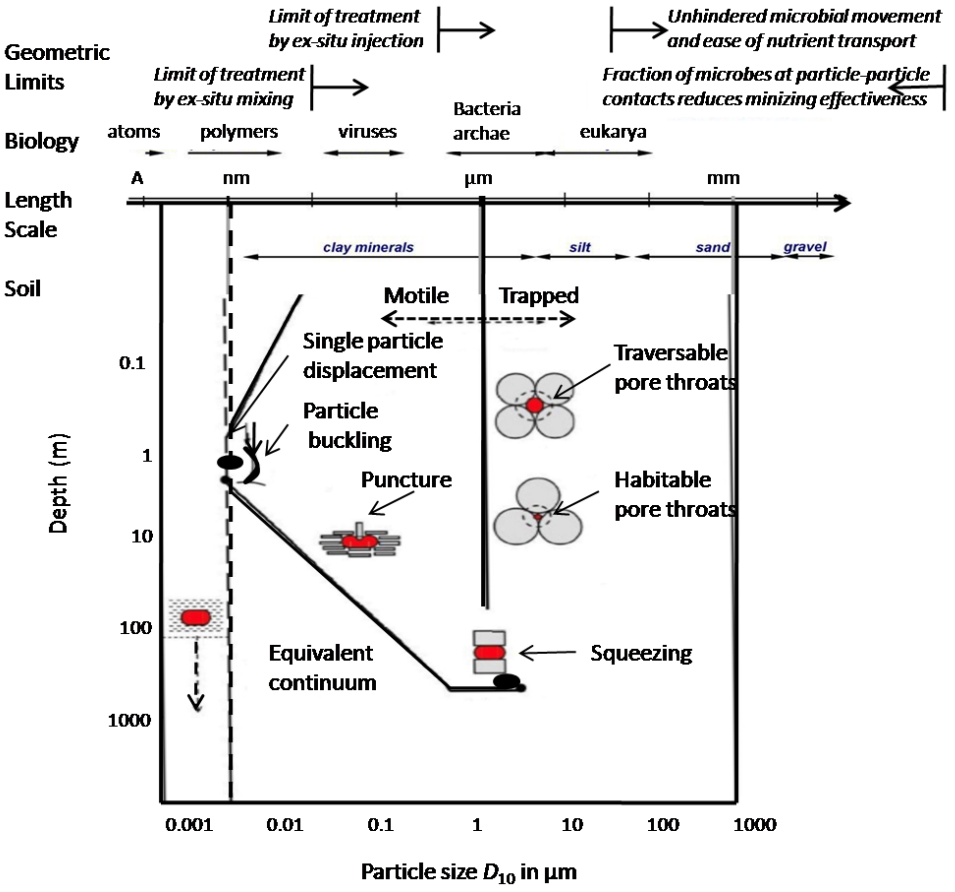


Figure 2.1 Overview of compatibility regimes considering particle size and ambient stress conditions (after Rebata Landa and Santamarina, 2006)

soil matrix will increase if decementation occurs. The fine particles will act as material to fill the pores and coat more surface area, decreasing the void spaces and pore throat sizes. These effects will predictably reduce hydraulic conductivity, increase small-strain stiffness, increase large-strain strength, and increase dilative behaviour. Biomineralization appears to apply to the broadest range of soil at various stress conditions. Figure 2.1 shows comparative sizes of microorganisms and pore sizes, and particle size boundary limits that govern microbial activities at respective depths for microorganisms.

The biogas released during the biomineralization reaction product of microbes cause biodesaturation of soil and decrease the liquefaction potential of loose sandy soils. The liquefaction resistance increases when the sand is slightly de-saturated with some voids displaced by nitrogen gas produced by denitrifying bacteria (Rebata-Landa and Santamarina, 2012). The biogas production in situ has three significant advantages over the other methods:

i. The distribution of gas is uniform because bacteria and reagents are in the liquid form and can be distributed evenly in the sand;
ii. The gas bubbles generated by bacteria are tiny, and thus the gas bubbles are relatively stable; and
iii. Nitrogen gas.

In addition, biogas generation from denitrification or other biogeochemical processes may enable a long-term reduction in soil saturation. Reduction in the degree of saturation increases pore space compressibility and may reduce excess pore pressure build-up during cyclic loading, mitigating earthquake-induced liquefaction potential in liquifiable soils.

2.5 SOIL MICROORGANISMS

Many microorganisms are present in the soil, including bacteria, fungi, and protists. The soil-borne microbes exist above 10^{12} microbes per kilogram of soil near the subsurface (Mitchell and Santamariana, 2005; Dejong et al., 2006). However, the microbial population decreases to about 10^{11} to 10^{6} microbes per kilogram (Whitman et al., 1998) at depths of 2 to 30 m in geotechnical systems. Therefore, the genera *S. pasteurii* is the preferred species for MICP and is selected as a viable microorganism in the present study to biocement the loose sandy soils via MICP. The following section reviewed this bacteria.

2.5.1 *Sporosarcina pasteurii*

The urease-producing bacteria, *S. pasteurii,* can precipitate calcite and consolidate the sand by applying calcium and a urea source through microbiologically induced calcite precipitation (MICP) or biological cementation. It is a gram-positive, aerobic, endospore-forming, and alkalophilic bacteria (DeJong et al., 2006; Zhao et al., 2014). The bacterial size ranges from 0.5 to 3.0 µm in length (Al Qabany et al., 2012, Madigan et al., 2003). Thus, the free passage of this bacteria is usually inhibited when the pore throat is smaller than 0.4 mm (DeJong et al., 2006). In addition, these bacteria can form nanoscale calcium carbonate crystals on the cell surface. It is also known as a ureolytic or urease-producing bacteria as it can release a high amount of urease enzymes (urea amidohydrolase: EC:3.5.1.5), as reported by Ferris et al., 1996. Furthermore, urease enzymes can hydrolyze urea to ammonium and carbonate ions (Dhami et al., 2014; Achal et al., 2009; Mortensen et al., 2011; Tobler, 2012; Whiffin et al., 2007; Shougrakpam and Trivedi, 2020). Moreover, such bacteria are present in almost all terrestrial and aquatic sites where urea is a final product of nitrogen

metabolism in mammals. In addition, this bacteria has high adaptability to the ambient environment with no pathogenicity, classified as risk group 1 that is unlikely to cause human disease (Venda Oliveira et al., 2015).

Production of urease enzyme from *S. pasteurii* is essential for the precipitation of $CaCO_3$, which can be used as a building material to improve the geotechnical properties of soils. Microbial urease catalyzes urea to ammonium ions, making a higher environmental pH shift. The calcium carbonate is accumulated by the activity of UPB such as *S. pasteurii* and *Bacillus* (*B.*) *sphaericus* (Boquet et al., 1973; Douglas and Beveride, 1998; Hammes and Verstraete, 2002, Sharma et al., 2020).

Other known urease-producing bacteria include genera *Spoloactobacilus, clostridium,* and *Desulfotomaculum.* Other physiologically similar species for biocementation are *B. megaterium* (Dhami et al., 2014), *B. sphaericus* (De Muynck et al., 2008), *B. pseudofirmus* (Jonker et al., 2010), *B. subtilis* (Reddy, 2010), *B. pumilus* (Daskalakis et al., 2015), *B. lentus* (Sarda et al., 2009), and some unidentified species (Achal et al., 2009; Stabnikov et al., 2011; Stabnikov et al., 2013; Lisdiyanti et al., 2011) as well. Among the bacteria, *S. pasteurii* is the most frequently used in MICP for its ability to act as nucleation sites. Four factors are necessary to benefit *S. pasteurii* to be nucleation sites during MICP (Ma et al., 2020):

i. A better correlation between biomass growth and urease production of *S. pasteurii* provides sufficient biomass and urease simultaneously for improved biomineralization.
ii. The highly negative cell surface charge of *S. pasteurii* is suitable for binding calcium ions.
iii. The robust cell structure.
iv. The weak mobility.

Recently, the interest in applying the MICP technique in soil improvement has increased. The microbial activities include ureolysis, photosynthesis, denitrification, ammonification, sulfate reduction, and methane oxidation (Dejong et al., 2010; Anbu et al., 2016; Zhu et al., 2016). In addition, the different types of biocement (other than MICP) usually require specific microorganisms to activate the process. These include nitrate-reducing bacteria, iron-reducing bacteria, sulfate-reducing bacteria, acetate or formate-oxidizing bacteria, acid-tolerant UPB, iron-oxidizing bacteria, and phototropic cyanobacteria (Ivanov et al., 2017).

The urease enzymes can hydrolyze urea into ammonia and carbonate ions. The presence of ammonia raises the pH of the pore fluid favoring bacterial growth and survival. The carbonate ions react with the freely available Ca^{2+} in soil and are responsible for $CaCO_3$ precipitation. The calcium ion is freely soluble in water (i.e., the solubility is 82.8 g/100 mL of water at 20℃). In addition, the rise in pH results in the formation of calcium carbonate due to proton consumption.

2.5.2 Compatibility between pore throats and organism

Compatibility between soil pore throats and bacterial size to allow free movement is essential for an effective MICP process, as depicted in Figure 2.1. The processes by which biology can modify the engineering properties of soil depend on the size of organisms, both in dimension and relative to the particle size. Unicellular microbial organisms in soil consist primarily of bacteria and archaea (Woese et al., 1990). They typically range in diameter from 0.5 to 3 μm and have morphologies usually spherical (coccus) or cylindrical. The cylindrical shapes may be straight (rods), curved (vibrio), or corkscrew-shaped (spirilla). They possess an extensive spectrum of biogeochemical reactions, the ability to grow inside the soil, and fast metabolic

rates to produce various mineral carbonates such as $CaCO_3$. The chemotrophic prokaryotes (Ivanov, 2008) were used for MICP treatment owing to their smallest size, typically from 0.5 to 2 µm. The cyanobacteria, a kind of phototrophic prokaryotes, grow on the soil surface only because light penetrates only a few millimeters into the soil. These bacteria can produce a rigid crust on the surface of soil or sediment, which diminishes soil infiltration rate and improves slope stability. Cyanobacteria can also create millimeter-scale laminated carbonate build-ups called stromatolites. Their formation in the shallow marine environment is due to sedimentation sequence, biofilm growth, production of a layer of exopolymers, and lithification of sediments by the precipitation of microcrystalline carbonate (Reid et al., 2000)

The physiological classification of chemotrophic prokaryotes is helpful in the ecological design of the bioclogging and biocementation process. This classification has two features: (1) relation to oxygen connected with energy generation and (2) type of cell wall. Based on the relation of oxygen and type of energy generation, prokaryotes can be classified as follows: (i) fermenting anaerobes; (ii) anaerobic respiring prokaryotes that produce energy by anaerobic oxidation of chemical substances using such electron acceptors as nitrate (NO_3), nitrite (NO_2^-), ferric (Fe_3^+), sulphate (SO_4^{2-}), sulphur (S), or carbon dioxide (CO_2); (iii) microaerophilic and facultative anaerobic prokaryotes; (iv) aerobes, for example, the *S. pasteurii* strain is classified under aerobic respiration prokaryotes.

The urease-producing bacteria are present in soil either through entrapment during deposition of fine-grained offshore sediments (Rebata-Landa and Santamarina, 2007) or migration through pore space during hydraulic flow transport or self-motility. The increase in enzymatic activity within a given cell or an increased

number of cells increases the bulk reaction rate. Geometric compatibility between bacteria and archaea and the pore throats dictates mobility (Mitchell and Santamarina, 2005; DeJong et al., 2010), survivability (Rebata-Landa and Santamarina, 2012) to regulate geochemical reaction by urease producing bacteria. The location of the enzyme, usually within the cell membrane or within the membrane-bound cytoplasm, regulates (through diffusion or active transport) the rate at which the reaction can occur. Mobility of the microbes from the surface to the sub-surface through a network of the pores fixed the soil depth for MICP treatment.

Further, the cells of *S. pasteurii* do not aggregate, resulting in a high cell surface-to-volume ratio, which is necessary to initiate effective cementation. As bacteria possess low Reynold's number, a thin watery layer surrounding the cells forms an interface between the bacteria and the microenvironment. Hence, variable concentrations of pH, dissolved inorganic carbonates (DIC), and Ca^{+2} would coexist in the microenvironment.

2.6 MICP PATHWAYS, KINETICS, AND MICROORGANISMS

In the MICP process, calcium carbonate crystals form through the reaction of metabolites generated by microorganisms (CO_3^{2-}) and their surrounding environment enriched in Ca^{2+}. The four key factors, including the concentration of Ca^{2+} and dissolved inorganic carbon (DIC), medium pH, and the availability of nucleation sites, have been reported by Hammes and Verstraete (2002) as the main influencing parameters on calcium carbonate precipitation. The precipitation of calcium carbonate is under autotrophic and heterotrophic pathways. Seifan et al., (2016) extensively reported the mechanisms involved in the autotrophic biosynthesis of calcium carbonate through methanogenesis, oxygenic photosynthesis, and

anoxygenic photosynthesis pathways. The sulfur and nitrogen cycle are responsible for the different metabolic pathways for heterotrophic precipitation of calcium carbonate. Jain and Arnepalli (2019) studied the biochemically induced carbonate precipitation in aerobic and anaerobic environments using *S. pasteurii*. Besides, the microbial growth, ureolytic activity, and precipitation of minerals were compared over aerated, anoxic, and anaerobic conditions. They have shown an insignificant result under an anaerobic environment than the remaining exposure conditions. In addition, there was a significant inhibition of the MICP process without oxygen or frequent injection of the bacterial cells.

It is necessary to use an appropriate growth medium to enhance the urease activity and specific urease activity of *S. pasteurii* and its application on MICP for sand treatment. Urease activity is the amount of ammonium produced from a 1 M solution of urea per minute. Stabnikov et al., (2016) reported that the amount of ammonium produced from urea shows a linear correlation ($R^2 = 0.9997$) between the molar concentrations of NH_4^+ and the changes in electric conductivity solutions. Therefore, urease activity has increased calcium carbonate production in the biotreated specimens.

The biosynthesis of calcium carbonate in the nitrogen cycle is through different pathways (Seifan et al., 2016), namely (i) ammonification of amino acids, (ii) dissimilatory reduction of nitrate (denitrification), and (iii) ureolysis (urea degradation). Some gram-negative aerobic microbial strains can use amino acids as their sole source of energy to initiate the biomineralization of calcium carbonate. The biochemical reaction to produce ammonia and carbon dioxide using oxidative deamination of amino acids is shown in Eq. (2.1). The production of ammonia creates an alkaline microenvironment around the cell that can increase the pH and

CO_2, a favorable condition for calcium carbonate precipitation. In the presence of water, the ammonia converted to ammonium (NH_4^+) and elevated the pH, as shown in Eq. (2.2). CO_2 is a readily available molecule in the atmosphere which can interact with aqueous systems. It can dissolve as aqueous CO_2, followed by further speciation as bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions based on the microenvironment conditions as in Eq. (2.3). Therefore, the various dissolved CO_2 species are dissolved inorganic carbon (DIC). The CO_2 reacts with hydroxyl ions (OH^-) as Eq. (2.3) and transforms into HCO_3^- or CO_3^{2-}. Finally, the carbonates reacted with the free calcium ions present in the soil and converted them into $CaCO_3$, as shown in Eq. (2.4). The reaction formulas for ammonification of amino acids are (Rodriguez-Navarro et al., 2003):

$$\text{Aminoacids} + O_2 \rightarrow NH_3 + CO_2 + H_2O \quad (2.1)$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^- \quad (2.2)$$

$$CO_2 + OH^- \rightarrow HCO_3^- \overset{\text{Alkaline condition}}{\Longleftrightarrow} CO_3^{2-} + H^+ \quad (2.3)$$

$$Ca^{2+} + HCO_3^- + OH^- \rightarrow CaCO_3 \downarrow + H_2O \quad (2.4)$$

The denitrification pathway is another subclass of the biosynthesis of calcium carbonate in the nitrogen cycle, and it mainly occurs where nitrate and organic carbon are available. During this metabolic pathway, nitrate is used as an electron acceptor by denitrifier bacteria, such as *Bacillus, Alcaligenes, Denitro bacillus, Thiobacillus, Spirillum, Micrococcus, Pseudomonas denitrificans, Castellaniella denitrificans,* and *Achromobacter*, for oxidizing organic compounds to provide energy and support microbial growth (van Paassen et al., 2010; Zhu and Dittrich, 2016).

Another microbial metabolism to denitrification is ureolysis, wherein the urease enzyme from ureolytic microorganisms initiates the biomineralization process. Dhami et al., 2013 suggested two different opinions on the role of bacteria in the precipitation of calcium carbonate via the ureolysis pathway: the precipitation is

i. an unwanted and accidental byproduct of metabolism and
ii. a specific process with ecological benefits for precipitating organisms.

The biochemical reaction in soil undergoes two processes, namely bioaugmentation and biostimulation, to precipitate $CaCO_3$. First, the exogenous microbes (non-native) play an essential role in the bioaugmentation process. But in biostimulation, the native microbes are applied with nutrients to create a suitable microenvironment for growth and reproduction in the subsurface environment. While the former has been used as the primary strategy in exploring geotechnical applications, even though there is an increase in biostimulation of microbes for use in the geoenvironmental field. Bioaugmentation is less favorable than biostimulation. Due to the introduction of non-native microbes, the potential for die-off or dormancy if the environment is not favorable for their growth and reproduction. In addition, there will be an extra cost of application of the microbes and the difficulty of uniform application in the subsurface. Therefore, biostimulation is generally preferable, owing to the stimulation of native microbes to adapt to the subsurface environment. However, the challenges exist in biostimulation associated with stimulation and growth of microbes and applying uniformly over a treatment area to improve its soil properties. Therefore, it is better to adopt bioaugmentation at a low concentration, followed by stimulation in situ or micro-dosing (Martinez et al., 2013). Biogeochemical interactions regulated through biostimulation or bioaugmentation often result in multiple products. The primary outcome is the precipitation of $CaCO_3$, which

enhances the bonding and filling of interparticle and pores present in loose sandy soils. However, the generation and transport of ammonium ions as an intermediate product during the biochemical reaction for $CaCO_3$ precipitation is unavoidable, but it is reusable as a composition for fertilizer production.

2.6.1 MICP pathway by urea hydrolysis

The mechanism of $CaCO_3$ precipitation by urea hydrolysis is into two stages: (i) urea hydrolysis using urease enzymes, and (ii) the end product of the biochemical reaction is $CaCO_3$ precipitation (Hammes et al., 2003; Hillgartner et al., 2001). Carbonates, silicates, and ion-oxides are usually natural cementing agents. Therefore, the microbial activity in soil forms naturally cemented soils. The addition of urea into the culture medium provided an alkaline environment suitable for the cultivation of *S. pasteurii*. Bacteria need carbon to form the cells and energy to sustain life for growth and reproduction. The biogeochemical reaction in the pore fluid usually consists of bacteria, urea, and $CaCl_2$ under saturated conditions to produce biomineralized products as inorganic precipitation, organic precipitation, and gas generation. The factors for bacterial growth are the availability of nutrients as an energy source, water, pH, temperature, presence of organic contaminants and heavy metals, space of solids, the concentration of dissolved organic carbon and calcium ions, and the presence of nucleolus sites (Mitchell et al., 2005; DeJong et al., 2006, 2010).

Under the proper cultivation process, S. pasteurii produces enzyme urease through its metabolic activity. The enzyme urease triggers the MICP biochemical reaction by hydrolyzing urea (NH_2-CO-NH_2) into ammonia and carbon dioxide. During the process, bacteria consume urea for energy sources and produce ammonia

(NH_3), which increases pH level and carbon dioxide (CO_2), as in Eq. (2.5). CO_2 is a readily available molecule in the atmosphere which can interact with aqueous systems by dissolving as aqueous CO_2, followed by further speciation as bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions based on the microenvironment conditions Eq. (2.6). The various dissolved CO_2 species are referred to as dissolved inorganic carbon. In the presence of water, the ammonia produced by bacteria will convert to ammonium (NH_4^+) in Eq. (2.6), and pH will increase. In addition, the bacteria exhibited a negative cell surface charge that can bind the positive calcium ions, weak mobility, and intact cell structure as nucleation sites during the MICP process.

CO_2 reacts with hydroxyl ions (OH^-) in Eq. (2.7), resulting in the formation of carbonate required for the precipitation of $CaCO_3$ in Eq. (2.8). The reaction formulas are as follows (Achal and Mukherjee, 2015; Jiang et al., 2016)

$$NH_2 - CO - NH_2 + H_2O \rightarrow 2NH_3 + CO_2 \quad (2.5)$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^- \quad (2.6)$$

$$CO_2 + OH^- \rightarrow HCO_3^- \quad (2.7)$$

$$Ca^{2+} + HCO_3^- + OH^- \rightarrow H_2O + CaCO_3 \downarrow \quad (2.8)$$

The overall process of urea hydrolysis and formation of calcium carbonate is in Eq. (2.9)

$$2NH_2 - CO - NH_2 + 2H_2O + Ca^{2+} \rightarrow 2NH_4^+ + CaCO_3 \downarrow \quad (2.9)$$

Urea hydrolysis using urea and $CaCl_2$ may also occur in the following ways:

$$NH_2 - CO - NH_2 + 2H_2O \xrightarrow{urease} 2NH_4OH + CO_2 \uparrow \quad (2.10)$$

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \xrightarrow{Carbonic-anhydrase} H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-} \quad (2.11)$$

$$CaCl_2 + H_2CO_3 \rightarrow 2HCl + CaCO_3 \downarrow \quad (2.12)$$

$$2HCl + 2NH_4OH \rightarrow 2NH_4Cl + 2H_2O \quad (2.13)$$

From the above reactions, it can conclude that: (i) if the pH increases, the concentration of carbonates increases; (ii) if the concentration of DIC increases in a buffered system, the carbonates also increase.

The overall reaction formula of Eqs. (2.10) to (2.13) for urea hydrolysis to precipitate $CaCO_3$ is as follows:

$$NH_2\text{-}CO\text{-}NH_2 + 2H_2O + CaCl_2 \xrightarrow{\text{Urease-and-carbonic-anhydrase}} 2NH_4Cl + CaCO_3 \downarrow \quad (2.14)$$

Carbonic anhydrase is another enzyme with an essential role in MICP, catalyzing the reversible hydration of CO_2 (Dhami et al., 2014). In a study of factors controlling urease enzyme and microbial deposition of $CaCO_3$ using *S. pasteurii*, Okwadha and Li, 2010 found that the CO_2 sequestration rate caused by urea hydrolysis was directly proportional to microbial $CaCO_3$ formation. In addition, the CO_2 released during urea hydrolysis is trapped in $CaCO_3$, preventing CO_2 into the atmosphere and facilitating CO_2 sequestration.

Under suitable conditions, most bacteria can induce carbonate precipitation on the external surface of bacterial cells by successive stratification, and bacteria can be embedded in growing carbonate crystals (Castanier et al., 1999). Possible ion-exchanged reactions in urea-$CaCl_2$ medium to precipitate $CaCO_3$ through the cell membrane can be summarized as follows:

$$Ca^{2+} + Cell \rightarrow Cell - Ca^{2+} \quad (2.15)$$

$$Cl^- + HCO_3^- + NH_3 \rightarrow 2NH_4Cl + CO_3^{2-} \quad (2.16)$$

$$Cell - Ca^{2+} + CO_3^{2-} \rightarrow Cell - CaCO_3 \downarrow \quad (2.17)$$

Since the cell wall of bacteria is charged negative and carbonaceous, it has the affinity to attract cations from the environment, including Ca^{2+}, to deposit on their cell surface as in Eq. (2.15) and released CO_3^{2-} as in Eq. (2.16). During the process,

the Ca^{2+} ions react CO_3^{2-}, which causes the precipitation of $CaCO_3$, which will serve as a nucleation site (Kroll, 1990; DeJong et al., 2010) as shown in Eq. (2.17).

2.7 FACTORS AFFECTING THE EFFICIENCY OF THE MICP METHOD

The factors affecting the efficiency of urease activity and $CaCO_3$ precipitation are concentrations of the bacterial cell, urea, and calcium reagents, temperature, and pH for the bacterial activity to release urease enzymes during the MICP process.

2.7.1 Bacterial cell concentration

The bacterial strain *S. pasteurii* (MTCC 1761) is a urease-producing bacteria used during the MICP process in soil (Shougrakpam and Trivedi, 2020). Under sterile aerobic conditions, the culture medium used to cultivate the bacteria consisted of nutrient broth (NB), urea, NH_4Cl, and $NaHCO_3$. The addition of urea into the culture medium provides an alkaline environment suitable for *S. pasteurii*. Compared to *S. pasteurii* cultivated without urea, *S pasteurii* grown with urea observed faster growth and urease production, better shape, more negative surface charge, and higher biomineralization ability (Ma et al., 2020). Furthermore, to survive the unfavorable growth environment due to the absence of urea, *S pasteurii* up-regulated the expression of genes involved in urease production, ATPase synthesis, and flagella, possibly occupying resources that can utilize for MICP. In addition, as compared to non-mineralizing bacteria, *S. pasteurii* exhibited more negative cell surface charges for binding calcium ions and a more robust cell structure as nucleation sites.

The culture medium was sterilized in an autoclave and urea by filtration to avoid thermal decay. The inoculated growth medium with the bacterial cells was

harvested as the bacterial solution. Okwadha and Li, 2010 used several concentrations of bacteria (10^6–10^8 cells/mL) and found that the 10^8 cells/mL concentration was optimal, with the attainment of 30% $CaCO_3$. Al Qabany et al., 2012 used a bacteria solution with OD_{600} (0.8 to 1.2) and achieved a high urease activity (5–20 mM urea/h). OD_{600} is the optical density of the biomass measured at 600 nm wavelength using an ultraviolet-visible spectrophotometer. The OD_{600} of the bacteria solution is usually used to characterize the input biomass in MICP studies. One unit of urease activity corresponds to the enzyme that hydrolyzes 1 µM of urea per minute. The value of OD_{600} was converted into cells/mL by the following Eq. (2.18) for *S. pasteurii* (Okwadha and Li, 2010):

$$C_{(cells/mL)} = 8.59 \times 10^7 \times (OD_{600})^{1.3627} \quad (2.18)$$

Many researchers use other microbiological methods to quantify bacteria concentration, such as the plate count method, using CFU/mL (colony-forming units per mL) to represent bacteria concentration (Soon et al., 2014). For example, Okwadha and Li, 2010 found that injecting more bacteria to increase the rate of ureolysis is more efficient than providing more urea to the system during MICP. Adding $CaCl_2$ to the bacterial solution increases the bacterial cell aggregation attached to the sand particles. In addition, the pH of the solution is to be maintained below 7 to avoid precipitation of $CaCO_3$ in amorphous states before the injection of BS.

2.7.2 Urea and calcium reagent concentrations

Cementation reagent plays a significant role in soil calcification and solidification by using urea as a carbon and nitrogen source and $CaCl_2$ as a calcium source. The molar concentration of the CRS used in soil treatment influences the calcite formation and

the subsequent gain in its soil strength. Soon et al., 2014 reported that calcite formation at lower CRS concentrations (0.05–0.25 M Ca) was more efficient than at higher concentrations (0.5–1.0 M Ca). Zhao et al., 2014 also found that the reagents in the cementation media used for specimen treatment were not fully utilized when their concentration was higher than 1 M Ca. However, if it is as low as 0.125 M Ca, the MICP-treated specimens failed due to insufficient particle bonding into a solid soil matrix. Therefore, lower concentrations of the cementation media below 1 M over many treatments will give uniform calcite precipitation of smaller crystal sizes and result in more homogeneous cementation (Al Qabany et al., 2012; Shougrakpam and Trivedi, 2020, 2021). Wen et al., 2019 repeatedly applied up to 4 times with 0.25 M cementation media concentration. However, it was reduced to two when media concentration was increased to 0.5 M Ca or 0.75 M Ca with an efficient improvement of mechanical strength due to the reduction of interparticle voids. Therefore, it is required to find an optimum molar concentration of CRS that can precipitate a significant amount of $CaCO_3$ and strength in soil.

Zhao et al., 2014 found that higher cementation media concentration from 0.25 M Ca to 0.5 M Ca improves strength, and the median UCS increased from 0.13 MPa to 1.36 MPa. However, when cementation media concentration increased from 0.5 M Ca to 1.5 M Ca, the UCS increased from 1.36 MPa to 2.13 MPa. Moreover, the *S. pasteurii* urease can tolerate CRS of up to 3 M urea and 2 M Ca. The $CaCO_3$ precipitation occurred in CRS concentrations of up to 1.5 M Ca (Whiffin, 2004). However, a higher concentration of $CaCl_2$ in the CRS inhibits the urease activity of the bacterial enzyme (Whiffin, 2004; van Paassen, 2009; Soon et al., 2014).

Calcium ions (Ca^{2+}) are present in low concentrations (0–50 ppm) in surface water, wastewater, groundwater, and soil. It is at higher concentrations (100–2000

ppm) in oceans, saline lakes, and alkaline soils (Grobe and Machel, 2002). Anthropogenic water sources rich in calcium (500–1500 ppm) include effluents from reverse osmosis, bone processing, paper recycling, and drinking water (Habets and Knelissen, 1997). So, the various water sources containing calcium ions may be cost-effective alternative sources for MICP.

2.7.3 Temperature

The catalysis of urea hydrolysis by urease enzymes is temperature-dependent. The optimum temperature for most bacteria is 37℃ (human body temperature). The optimum range of the enzymatic reaction depends on the temperature range between 20℃ to 37℃, bacterial concentration, and chemical reactants in the system (Okwadha and Li, 2010). Such a condition may ensure a sufficient microbial urease activity for catalyzing the biochemical interactions between bacteria and the cementing reagents for calcite precipitation. The optimum incubation temperature was 25℃ for bacterial precipitation of $CaCO_3$ (Baskar et al., 2006). For *S. pasteurii*, the urease activity was increased proportionally with a temperature that varies from 25 to 60℃ (Whiffin, 2004). However, if the temperature is below 5℃, the urease activity is negligible (van Paassen, 2009). Further, any variation in the temperature may influence the crystal formation of different calcium carbonate polymorphs.

2.7.4 pH

During treatment, the pH of the pore fluid in the initial, intermediate, and final plays a significant role in soil calcification using MICP treatment. The formation of $CaCO_3$ was the primary cause of changes in the properties of the sand. Specifically, the $CaCO_3$ filled the pores or bridges between the sand particles, and hence the pore

volume decreased. The $CaCO_3$ precipitated in the soil results from the hydrolysis of urea catalyzed by the urease enzyme released by bacteria. The microbial processes are possible at a specific pH range; urease enzymes are only active at certain pH like all other enzymes. The pH for active urease enzyme ranges between 7 and 8.5 (Soon et al., 2013; Shougrakpam and Trivedi, 2020). Except for a small group of acid ureases, microbial ureases generally possess an optimum pH of near neutrality (Mobley et al., 1995), and *S. pasteurii*, an alkalotolerant bacteria, has an optimum pH value of 8 (Stocks-Fischer et al.,1999; Ciurli et al., 1996). If the pH drops below 5, the microbial ureases could be irreversibly denatured (Mobley et al., 1995). The production of ammonia from urea hydrolysis will raise the pH of the pore fluid during the reaction. On the other hand, the bicarbonates released during urea hydrolysis and microbial respiration act as a buffer to the pH rise.

2.8 MECHANISMS OF MICP PROCESS

Cement is widely used to strengthen the soil, but cement production has environmental impacts during all stages of the manufacturing process. Additionally, global cement production accounts for about 5% of the total industrial energy consumption and 5% of anthropogenic CO_2 emissions (Worrel et al., 2009). In many studies related to ground improvement techniques, chemical grouts using admixtures, deep mixing, soil-cement micro-piles, etc., have been used for soil stabilization. Soil improvement using chemical grout employs sodium silicate, calcium chloride, calcium hydroxide, acrylates, and acrylamides to bind soil particles (Karol, 2003; Ivanov and Chu, 2008). However, this method is expensive and toxic to humans and the environment (Karol, 2003; DeJong et al., 2006; Ivanov and Chu, 2008).

Therefore, investigate economic and sustainable alternatives to chemical grouting, cement stabilization, and other conventional methods.

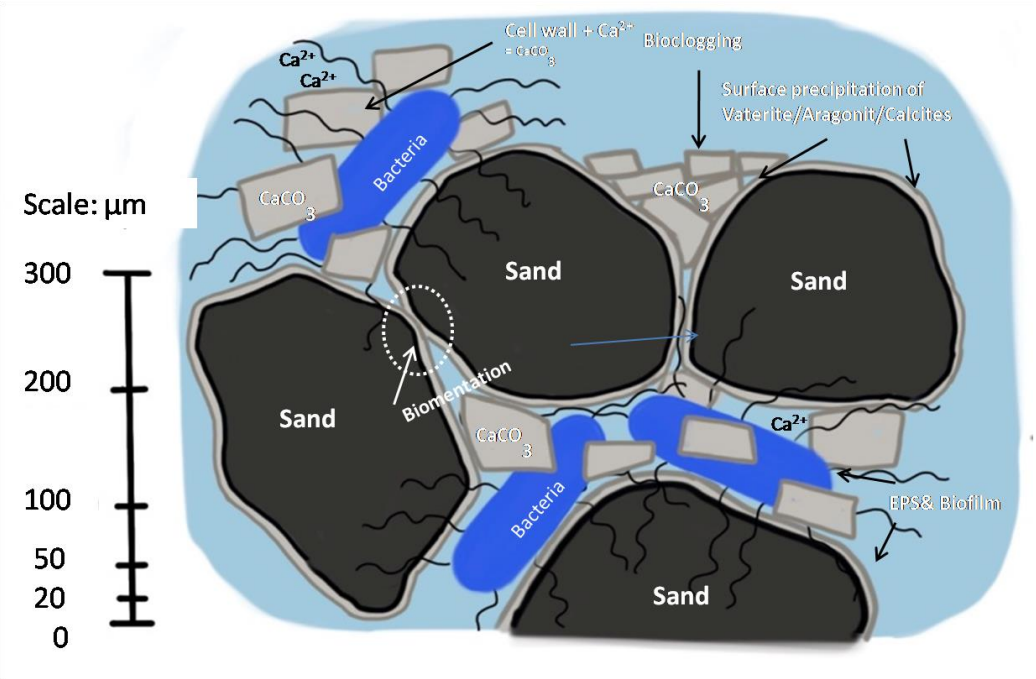


Figure 2.2 The MICP pathway for biomineral secretion with precipitation and growing of calcium carbonate crystals on the sand particles and cell surfaces as a soil strengthening process during MICP treatment

The MICP pathway consists of biomineral secretion with precipitation and growing of calcium carbonate crystals on sand and cell surfaces, at particle-particle contacts (biocementation), and infilling the pore spaces (bioclogging) in the soil matrix as a soil strengthening process during MICP treatment as in Figure 2.2.

2.8.1 Biocementation

The soil stabilization method using the biocementation process via the MICP method has been quest by many geotechnical engineers, biologists, chemical engineers, microbiologists (Dejong et al., 2006; Canakci et al., 2015, Montoya et al., 2013, van Paassen et al., 2010, Burbank et al., 2013, Martinez et al., 2013). Biocement is an alternative to cement and chemical grouts (De Muynck et al., 2010; Stabnikov et al.,

2011) that can produce binder materials via MICP treatment to improve the strength and durability of cementing materials with soil particles (Phillips et al., 2013; Dhami et al., 2014). Soil cementation materials include carbonates, hydroxides, phosphates, silicates, and sulfides (Ivanov and Chu, 2008). Calcium carbonate is primarily used in biocementation because it is readily available in nature. Soil biocementation can improve soil and increase shear stiffness. The biochemical reaction of a urease-producing bacteria and the cementation reagent solution (CRS) triggered $CaCO_3$ in soil (Soon et al., 2013). The binding of soil at particle contact with calcite precipitates is the biocementation process that increases the strength and stiffness of soil. The calcite generated is responsible for particle bonding and coating on the particle surface and densifies the soil into sandstone-like materials. Moreover, the calcite crystals embedded bacteria as nucleation sites, provoking cellular death due to a lack of nutrients during the bio-mineralization process (Stocks-Fischer et al., 1999; Soon et al., 2013). The bonded soil particles prevent the movement of particles and hence enhance the strength and stiffness properties of soil (Harkes et al., 2010; Mitchell and Santamarina, 2005; Whiffin et al., 2007; Ivanov and Chu, 2008; De Muynck et al., 2008, 2010; Sarda et al., 2009; Achal et al., 2010; Chu et al., 2012; 2014; DeJong et al., 2010, 2013; van Paassen et al., 2010; Dhami et al., 2012). Hence, the dynamic soil behaviour would consolidate soil from a loose to a dense state. In addition, the formation of a low-permeable biocemented crust layer (~ 2mm thick) may mitigate the wind erosion potential of sand and control leakage from leachate ponds and landfill sites (Shougrakpam and Trivedi, 2020).

2.8.2 Bioclogging

During the biomineralization reaction of the MICP process, the carbonate ions are released, which react with excess dissolved or free calcium ions present in the soil and forms $CaCO_3$ precipitates as a cementing material. Cementation mechanisms that coat or bridge separate soil particles could be naturally occurring cementation or microbially induced cementation; these progressively reduce the pore space in the soil fabric and decrease the permeability of the soil (DeJong et al., 2006). The bioclogging process can decrease the ability of soil and permeable rocks to allow the passage of fluid passing through them. In addition, the pore throat sizes are reduced due to microbial production of water-insoluble polysaccharides in situ (Ivanov and Chu, 2008). Therefore, the sand pores are filled with the calcites, thus, reducing the void spaces, likewise, calcite precipitates can close leaky construction pits, aquaculture ponds, landfills, or dikes.

The rearrangement of sand particles, breakage of $CaCO_3$ cementation due to interparticle friction, and possible abrasion or attrition at the interface between $CaCO_3$ and sand grains decrease the pore spaces. Consequently, there will be a decrease in the void ratio when applying vertical stresses to the soil. As the vertical load increases, there will be grain breakage in fracture mode with a reduction in void ratio, reducing soil compressibility. In addition, the detached $CaCO_3$ from the grain surfaces, premature biochemical reactions, breakage of $CaCO_3$ cementation into smaller sizes, and abrasion of sand grains will exist in soil fabric. Such loose materials may further clog the pores and soil or porous matrix channels. The deposition of biominerals on sand grains has implications for soil strengthening and solidifying desert aeolian sand (Li et al., 2018), sandstone, and bio-cemented construction materials (Bu et al., 2018).

The clogging in porous soil may be physical, chemical, and microbiological. The microbial clogging process in-situ may also occur due to the accumulation of cells as biomass in the pore space by the extracellular polymeric substances (EPS), biogas, and loose calcites as water barriers, thus reducing the pore volume (Soon et al., 2013; DeJong et al., 2010). Hence, the plugging of the soil restricts the water flow through the soil to reduce its permeability. Subsequently, the clogging process may reduce drain channel erosion, control soil erosion, prevent slope failure and piping of earth dams and dikes, and reduce water infiltration into slopes. In addition, it forms grout curtains to reduce the migration of heavy metals and organic pollutants from soil and water contaminants.

2.9 MICP METHODS OF TREATMENT

The soil properties such as soil type, soil texture, bulk density, climate, and fauna can influence soil macroporosity and generally lead to heterogeneity of treatment solution to flow within the soil matrix. They may be responsible for the structural voids present in a particular soil. In addition, the size of macropores and their connectivity may affect the infiltration behaviour of the bacteria and cementation solution to react in pore fluids to produce $CaCO_3$. The infiltration behaviour may vary even within a few centimeters of depth from the surface, affecting the efficiency and distribution of calcites in soil.

Initially, the introduction and retention of ureolytic bacteria or urease enzymes inside the soil matrix are essential for the MICP process. Later, the cementation solution can apply and react with the bacteria to induce $CaCO_3$ precipitates. However, improper methods of bacteria retention could lead to the bacteria being flushed away or detached by a subsequent injection of the cementation solution,

leading to uneven distribution of bacteria. Such distribution may lead to non-uniform $CaCO_3$ precipitation and strength within the biocemented soil. Therefore, to improve the uniform distribution of bacterial solutions, many researchers have adopted two methods for bacterial application in soil: injection and premixing. The injection method warranted the flushing of bacteria solution top-down on the soil matrix and retained. The bacteria can retain to attach to the sand grains for a few hours before applying the cementation solution. The retention period will allow bacteria to be transported along with the sand specimen and firmly attached before applying the cementation solution (Al Qabany et al., 2012). In the premixing method, the bacteria and the soil are mixed mechanically before applying the treatment solution. So, the application of both bacterial solution and CRS may adopt one of the methods to get uniform $CaCO_3$ precipitation and strength, as discussed below.

2.9.1 Injection method

After the initial injection of the bacterial solution, $CaCl_2$ (50 mM) was added as the fixation solution to attach the bacteria to the sand grains (Harkes et al., 2010). The Microbes are attached to the sand grains via an increased ionic strength of Ca^{2+} ions, which encourages bacterial adsorption onto the surface of the sand. Whiffin et al., 2007 injected both the bacteria culture and cementation solution from top to bottom alternately into the entire length of a 5 m sand column for treatment. However, the $CaCO_3$ distribution was significantly lower in the bottom section than at the top of the column due to more reactants in the upper portions. This finding is consistent with the depthwise $CaCO_3$ distribution in treated sand columns, decreasing from top-down, i.e., 14% to 3.4% below the top surface (Shougrakpam and Trivedi, 2020, van Paassen et al., 2010) have observed a high variation in the peak strength of the UCS

values suggesting non-uniformity along a biotreated sand volume. However there may be an uneven distribution of bacteria and in-homogeneity of $CaCO_3$ distribution, which results in strength variation throughout the length of tested columns (Whiffin et al., 2007; Shougrakpam and Trivedi, 2020; van Paassen et al., 2010). When bacteria are injected through the pore space of the sand matrix, they are likely to be filtered through the sand with a long-linear reduction of microbe concentration along the injection path. In addition, the reaction of cementation solution with bacteria leading to more $CaCO_3$ deposits near upper portions during the penetration eventually leads to pore plugging at the region near the injection source, leading to uneven distribution of $CaCO_3$ precipitation (Cheng and Cord-Ruwisch, 2014). Therefore, Harkes et al., 2010 allowed a slow injection rate of bacterial suspension to avoid pore plugging along the penetration to allow for a sufficient bacteria delivery to more distant locations along the treated soil column to counter th non-uniform distribution of $CaCO_3$. However, Whiffin et al., 2007 recommended an increased flow rate of cementation solution to allow more reagents to reach deeper locations and to achieve a uniform $CaCO_3$ distribution into the treated soil column.

2.9.2 Premixing method

In this method, the bacteria are premixed mechanically with soil to achieve the desired homogeneity. By applying this method, Yasuhara et al., 2012 obtained biocemented sand specimens of UCS values ranging from 400 kPa to 1.6 MPa and 83% of the $CaCO_3$ precipitates (Zhao et al., 2014) a homogeneous distribution. Even though the premixing method solved the homogeneity problem, it remains the least favorable MICP method because it causes disturbance to the local soil. The soil disturbance may lead to a pseudo stress development in the soil sample due to the

vigorous mixing between the soil and the cementing agent. Unmeasured stresses applied during the mixing of soil samples will complicate the soil stress history and make it difficult to discern during mechanical testing. In addition, the mechanical mixing of bacteria with soil requires a higher energy source and cost than the trickling method, though its effectiveness is higher, specifically in deeper soil zones.

2.9.3 Submersion method

An improved immersing treatment method was developed to enhance the MICP process to trigger calcite precipitates (Wen et al., 2019). Zhao et al., 2014 fully submerged the premixed bacteria-sand matrix into geotextile wrapped 100 mm length soil columns in a mechanically operated tank reactor containing cementation solution. Then, the columns were freely diffused under the concentration gradient by the action of a magnetic stirrer. As a result, the amount of $CaCO_3$ obtained was 6.6–8 % and achieved in UCS strength between 1.76 MPa and 2.04 MPa in the various sand columns. However, it is impractical to apply the diffusion method in the field. It requires an installment of a geotextile wrapping as a protective membrane that can accelerate the rate of diffusion between the chemical substances into the treated samples. In addition, the preinstalled geotextile wrapping may disturb the integrity of treated soil and induce a pseudo-stress history that may contribute to an inaccurate strength value.

2.9.4 Surface percolation method

The surface percolation method employs free-draining of water movement by spraying or ponding the bacterial solution followed by cementation solution for sand column treatments. Spraying or trickling bacterial suspension and cementation

solution alternately onto the soil surface followed by the solution penetration into the soil driven by gravity, the soil was cemented up to 2 m deep (Cheng and Cord-Ruwisch, 2014). The injection method does not require heavy machinery during solution injection due to the free-draining of water movement for in situ large-scale applications. However, this method may be applicable for sandy soils because of the low infiltration rate of fine-grained soils (e.g., silt or clay). The treatment was achieved along a 2 m long coarse sand column with UCS between 0.850 MPa and 2.067 MPa. In addition, the surface percolation was also tested in a large container and showed a reasonably homogeneous distribution of $CaCO_3$ and strength on a 3D scale due to the self-adjustable preferential flow path during the treatment (Cheng and Cord-Ruwisch, 2014). In reality, fine-grained soils are usually encountered deep into soil deposits. Therefore, possible applications of the surface percolation method may include dust suppression, track basement stabilization, and embankment construction.

2.10 ENGINEERING PROPERTIES OF BIOTREATED SOILS

The engineering properties of soil that can be improved using the MICP process are porosity, permeability, shear strength, stiffness, unconfined compressive strength, shear wave velocity, etc., as discussed in the following sections:

2.10.1 Porosity

Porosity is the number of voids in a soil matrix. MICP can reduce soil voids by reducing pore sizes and pore volumes. Qian et al., 2010 characterized the effectiveness of cementation in terms of the porosity of cemented sand specimens, which was reduced to 25% after MICP treatment. Tagliaferri et al., 2011 used X-ray

imaging and quantitative 3D digital image analysis to analyze the crushed biocemented bonds and found an overall porosity of biocemented soil reduced to 30%. The reduction in porosity was due to the $CaCO_3$ that clogged the sand pores. Porosity governs the effectiveness of MICP treated samples by replacement of the pore content of the sand matrix with $CaCO_3$. As the amount of precipitated $CaCO_3$ increases, the degree of cementation increases. In addition, a higher amount of $CaCO_3$ crystals replaces the pore content in the soil structure, which will increase the strength and stiffness of the soil. In addition, reducing the pore ratio and pore spaces will cause a reduction in soil permeability.

2.10.2 Permeability

Permeability measures the ability of porous materials to allow the passage of fluid through the pores. In MICP, permeability is of utmost importance because the technique is preferable for pervious or semi-permeable soils, such as sand and gravel. Porous materials with high permeability can prevent the development of excess pore water pressure during loading. In general, MICP can increase soil strength while maintaining sufficient permeability (in soil biocementation) or completely blocking the soil pores (in soil bioclogging). Moreover, soil drainage condition is related to its packing density. The macro-scale behaviour of soil mass results from the interaction between the soil at interparticle levels. According to Chu et al., 2013, a good drainage path with a hydraulic conductivity value of at least $1x10^{-4}$ m/s must maintain to penetrate the desired sand depth for the bacteria and cementation solution to ensure homogeneous $CaCO_3$ precipitation. In soil biocementation, MICP facilitates permeability retention for biocemented soil samples better than the other cementitious materials such as Ordinary Portland cement (OPC).

The reduction in permeability in biocemented soils is because of $CaCO_3$ crystallization in the soil pore spaces. The $CaCO_3$ crystals cause a slight volume change in the pore spaces instead of the hydrates, ensuring good drainage that allows a liquid passage through the biocemented soil matrix. Van Paassen (2009) reported a 60% reduction in the initial permeability of biotreated soils at approximately 100 kg/m^3 of $CaCO_3$ precipitation. In contrast, Ivanov et al., 2010 recorded a 50–99% reduction in permeability using a 1 M cementation solution. Al Qabany and Soga, 2013 used a 0.5 M cementation solution and found a reduction of 20% in the initial permeability value at 2% $CaCO_3$ precipitation. Larger $CaCO_3$ crystals clogged the pores with larger crystals with a high concentration solution. Therefore, for samples treated with a solution of high concentrations (0.5–1 M), the reduction in permeability is usually more significant than those treated with a solution of low concentrations (0.1–0.5 M). However, in-homogeneity along the sand column samples can still be attributed to the localized clogging. Therefore, it is recommended that a low concentration solution be used if less permeability reduction is desirable to ensure a uniform consistency of $CaCO_3$ precipitation. A solution with a low concentration may produce a more uniform precipitation pattern and higher strength samples for a given amount of $CaCO_3$ precipitation. Although the MICP technique could retain sufficient soil permeability after treatment, it can be used for soil clogging, significantly reducing the hydraulic conductivity or permeability of porous soil media. After treatment with bacteria, Ivanov and Chu, 2008 showed a significant permeability reduction ($5x10^{-5}$ m/s to $1.4x10^{-7}$ m/s) in loose clean sand samples signaling the potential use as a sealant for wastewater or agriculture treatment ponds and landfill sites.

Similarly, Chu et al., 2013 used *Bacillus* sp. bacteria strain to promote bioclogging in the sand. It was observed that the permeability of biotreated sand varied with the content of precipitated calcium. It was also suggested that for bioclogging of sand to occur, precipitated $CaCO_3$ of 9.3% w/w or higher is required. Wen et al., 2019 reported that under cementation media, concentrations of 0.25 M Ca, 0.5 M Ca, and 0.75 M Ca can improve the utilization of cementation media and reduce the hydraulic conductivity of MICP-treated sand from untreated $1.4x10^{-3}$ m/s to $1x10^{-5}$ m/s after four MICP treatments.

2.10.3 Shear strength

Shear strength is the magnitude of shear stress that a soil can sustain and depends strictly on the shear strength parameters of soil, including the cohesion (*c*) and friction angle (*Ø*). Duraisamy and Airey, 2012 correlated the shear strength of liquefiable sandy soil treated with MICP to the degree of cementation using the shear wave velocity technique. The shear strength of biocemented soil was strictly affected by the increase in soil cohesion resulting from the increase in the cement content, while the friction angle was not significantly affected by the cementation process. In contrast, Chou et al., 2011 reported a significant increase in soil friction angle. Still, a slight increase in soil cohesion in all treated samples using MICP catalyzed using three conditions of *S. pasteurii* (growing, resting, and dead cells). Further, the peak shear strength of biocemented soil was higher than untreated specimens and generally higher in the growing cell treatment than in other treatment methods.

Soon et al., 2014 applied MICP using *Bacillus megaterium* to treat a residual soil and found that the shear strength ratio of treated to untreated soils was increased at values ranging from 1.40 to 2.64. Montoya and DeJong (2015) observed that the

shear strength of MICP-treated sand was significantly improved with the increase in MICP cementation. In addition, the peak shear strength increased with increasing cementation, leading to a transition in the stress-strain behaviour from strain hardening to strain softening. Cheng et al., 2013 also discussed the cohesion and friction angle of biocemented soil samples treated under different degrees of saturation and showed that at lower saturation degrees, the precipitated $CaCO_3$ crystals contributed more to improving the soil cohesion than the friction angle. On the other hand, regardless of the saturation degree, both the cohesion and friction angle increased at higher $CaCO_3$ content due to the filling effect of the calcite crystals in the soil pore spaces. Thus, MICP also increases the undrained cohesion of soil.

2.10.4 Stiffness

Soil stiffness, commonly known as soil elastic modulus (E), is the ratio of stress over strain. Soil stiffness is closely related to the bonding strength between loose soil grains. Cheng et al., 2013 compared the elastic modulus of biocemented sand with other geomaterials such as concrete, gravel, and soft rock. The biotreated sand is the most flexible among the materials tested.

In earthquake-prone areas, less stiff soil can provide extra time for evacuation due to its ability to maintain significant residual strength even after failure. Lee et al., 2013 performed MICP on residual soil and found that the stiffness behaviour of biocemented residual soil is similar to that of biocemented natural sand. Previously, researchers studied the effects of cementation on the strength and stiffness of granular soils using a variety of different cementing agents, namely the Portland cement, gypsum, and sodium silicate (Amini and Hamidi, 2014; Fernandez and

Santamarina, 2001). The strength and stiffness of cemented materials increase with the amount of cementing material in the soil matrix. However, the amount of cementing material required to produce a particular cementing effect may vary.

Based on this fact, Montoya and DeJong, 2015 studied the effect of biocementation on the stress-strain behaviour of biotreated sand. They found that stiffness was significantly improved with increased biocementation using $CaCO_3$. It is worth noting that the effective stress path and the drainage condition influence the MICP- treated soils to reduce the rate of stiffness due to the degradation of cementation before failure. The stress paths of a given soil depend on the initial, in situ, and final state of the soil sample. A study carried out by Ruistuen et al., 1999 suggested that the stress path-dependent behaviour of weakly cemented soil is due to the shear-enhanced compaction, and the increase in cementation was shown experimentally to reduce the stress sensitivity.

2.10.5 Unconfined compressive strength testing

The unconfined compressive strength (UCS) testing is the most commonly used test to describe the strength of biocemented soils, as reported by many researchers (Cheng et al., 2013; Harkes et al., 2010; Ivanov et al., 2015; Whiffin et al., 2007; Zhao et al., 2014). However, the biotreated soil always fails at a low axial strain in unconfined compression tests. The axial stress drops quickly after peak stress (Mortensen et al., 2011; Shougrakpam and Trivedi, 2020).

Many researchers (Zhao et al., 2014; Yasuhara et al., 2011, and Shougrakpam and Trivedi, 2020) observed improvement in the UCS values in the range of 0.4–2.18 MPa based on 4.0–8.0% calcite content at different treatment conditions and reaction times. Therefore, the strength of the microbially induced calcite formations is

comparable to soft rocks. However, the distribution of $CaCO_3$ is reduced from the top surface to lower depths. Therefore, the mechanical response of MICP-treated soil can vary significantly depending on the effective $CaCO_3$ precipitation mechanism. The UCS testing is used to characterize the strength property of cemented soils because it allows many samples to be tested within a short time (Al Qabany and Soga, 2013). Triaxial testing is also recommended to investigate further the response of biocemented soils toward the monotonic and cyclic loadings as it simulates the natural behaviour of soil in the field.

2.10.6 Shear wave velocity

The shear wave velocity is a property of soil that can indirectly identify the cementation level and, more directly, the stiffness. Geophysical methods, such as imaging the shear wave velocity profile of the subsurface, are widely used in liquefaction assessment and to identify the general characteristics of soil during in-situ testing. The stiffness increase of soil specimens due to biocementation treatment can be effectively captured by bender elements which allow non-destructive measurement of shear wave velocity and its variation during curing and shearing. The non-destructive technique using the bender elements has been employed to determine the progressive strength development of soil (Dejong et al., 2006). Martinez et al., 2013 performed real-time monitoring of one-dimensional flow for a half-meter-scale column improved with MICP using S-wave velocity measurements. It was concluded that the S-wave velocity is a proxy to increase the small-strain shear stiffness as cementation occurs at the particle to particle contacts. The study also pointed out that the S-wave velocity measurements are effective monitoring indicators of MICP soil improvement for temporally and spatially based conditions.

DeJong et al., 2006 injected *S. pasteurii* solution into a column of fine-grain sand and found that shear wave velocity had increased 200% after MICP. Montoya and DeJong, 2015 captured the change in the small strain stiffness of biocemented soil during shearing using the S-wave velocity method. It was concluded that an indication of cementation degradation as a function of the strain level could be deduced from the change in the small-strain stiffness. The advantages of this technique include the non-destructive examination of biocemented soil samples and the capability to measure the soil strength as a function of time in a real-time domain. Hence, it can be applied to determine the changes in ground improvement in the field over a long period. A reasonable empirical correlation between shear wave velocity and the amount of calcite precipitated is shown in Eq. 2.19 (Al Qabany et al., 2011). This empirical equation from the bender elements test also shows that shear wave velocity can be a good indicator of calcite produced and can thus serve as a cementation index.

$$V_s = 9.7(CaCO_3)+147 \tag{2.19}$$

where V_s = Shear wave velocity (m/s) and

$CaCO_3$ = Calcium carbonate concentration (kg/m^3)

Depending on the stress level, typical loose sands may have shear wave velocity between 100-150 m/s. For example, Table 2.1 shows the soil profile classification system used by the National Earthquake Hazard Reduction Program (NEHRP) guidelines (refer to Table 2.1). Liquefiable soil is any soil falling under a shear velocity of 500 m/s. Therefore, to improve the soil, shear wave velocity must be above 500 m/s and maintain a velocity of 500–1000 m/s throughout any earthquake event.

Table 2.1 Site classification based on shear wave velocity (NEHRP, 2003)

Site class	Soil Profile	Shear wave velocity, V_s (m/s)
A	Hard rock	$V_s > 1524$
B	Rock	$762 < V_s \leq 1524$
C	Very dense soil and soft rock	$366 < V_s \leq 762$
D	Stiff soil	$183 < V_s < 366$
E	Soft soil	$V_s < 183$

2.11 SUMMARY

This review confirmed urease activity is the main factor governing biocementation using ureolytic bacteria. Urease enzymes directly control the rate of urea hydrolysis and, as such, precipitation of different polymorphs of $CaCO_3$, as discussed in section 2.3. It also indirectly controls the pH of the environment depending on urea concentration, thus influencing the type of crystals precipitated. The different environmental factors affecting the efficiency of the MICP process are also discussed in section 2.7. *S. pasteurii*, used in most MICP studies, is one such bacterium with high urease activity and is not pathogenic to the environment. Section 2.5 discussed the soil microorganisms, and section 2.6 discussed the different MICP pathways, kinetics, and potential microorganisms used for MICP treatment. However, it is not the only option available, and there is a need for further research to explore other microbes. Furthermore, the data on the chemical reagents used to optimize biocementation has only been limited. Still, it has been shown that the concentration of urea has no direct effect on urease activity and that the optimum pH for urea hydrolysis by the urease enzyme is 8.5. The primary waste product in soil strengthening using biocementation is ammonium salt. Therefore, MICP using the

urea hydrolysis pathway depends on the effective approach to reducing ammonium salt and other toxic materials in the environment.

Widely investigated treatment methods have their limitations as, even in the latest research conducted, the strength improvement is not uniform throughout the treated soil columns. Section 2.10 explains the different engineering properties of soil improved by the MICP process, which is influenced by the supply of cementation reactants versus the bacterial activity in the soil column. Therefore, there is a need to explore alternative methods of introducing biocement into the soil before any approach can be effectively utilized for ground improvement. Proper investigation of the method before its field application will make the soil treatment more efficient and cost-effective.

CHAPTER 3

Engineering Properties of Bacterially Induced Calcite Formations

This chapter is based on the published paper, Engineering properties of bacterially induced calcite formations. Shougrakpam, S. and Trivedi, A. (2020), *Current Science*, 118, 1060–1067.

Abstract

This article presents the engineering properties of bacterially induced calcite formations, often called microbially induced calcite precipitation (MICP), via ureolysis on granular formations consisting of loose and collapsible river sand. Two sets of experiments consisting of five sand columns each were treated using urease-producing bacteria, urea ($CO(NH_2)_2$), and calcium chloride ($CaCl_2$). The reaction produced biomineralized calcium carbonate crystals that bind and stiffen the sand grains. The reaction was checked by measuring the pH. The pH of the effluent solution taken from the initial stage to day 14 of treatment ranged from 7 to 8. There

was significant calcite formation in the alkaline range when the pH was 7 and above. The strength gained in the treated specimens has been estimated from the unconfined compression tests and was obtained in the range of 1.1–2.18 MPa based on 4.0–8.0% of calcite content at different reaction times. The calcite in the biocemented sand was confirmed by the scanning electron microscopic images. The grain-size distribution curves of the untreated sand were compared to the biotreated sands to observe the increase in grain sizes after biotreatment. There was an increase in grain sizes of the biotreated sands indicating that grain size is a function of MICP. The collapse potential of sand was reduced after MICP treatment. The strength of the bacterially induced calcite formations was comparable to soft rocks.

3.1 INTRODUCTION

Calcite is a metabolic by-product of microorganisms that occurs during the biogeochemical reaction that initiates when bacteria release the urease enzyme, which reacts with cementation reagents resulting in precipitation formation, referred to as microbially induced calcite precipitation (MICP). MICP is possible due to the interaction of urease-producing bacteria (UPB), urea, and $CaCl_2$. The cementation reagents consist of urea and $CaCl_2$. The urease enzymes released by bacteria catalyze the hydrolysis of urea. Compared to the traditional ground improvement techniques, soil improvement through biocementation and bioclogging using microorganisms is an innovative, environmental-friendly, and cost-effective technique.

During the MICP process, the urease enzymes released by UPB can hydrolyze urea to ammonium and carbonate ions in the presence of water. The carbonate ion reacts with the calcium ions from $CaCl_2$ to produce insoluble calcite grains ($CaCO_3$)

that bind the sand particles together, which improves the soil strength in loose sandy soil formations (Al Qabany et al., 2012; Burbank et al., 2011; Chu et al., 2013; Soon et al., 2013; DeJong et al., 2006; Feng and Montoya, 2015; Ivanov et al., 2015; Li et al., 2016; Mitchell and Santamarina, 2005; van Paassen et al., 2010). In addition, indigenous or exogenous microbes create nucleation in the ureolysis process via the urease enzyme to produce calcite precipitation. This process is also known as MICP and can be called biomineralization or biocalcification (DeJong et al., 2006; Whiffin, 2004). This binding process is referred to as biocementation, which in turn increases the density of the soil. This soil densification helps reduce the pore volume, reduce the pore water pressure to increase resisting liquefaction, and reduce post-shaking settlements during an earthquake (Montoya et al., 2013).

The present study aims to improve the engineering properties of the loose sandy soil by increasing calcite content in the soil through the MICP process. In addition, it is an effort made to achieve an economical and environmentally friendly method of soil treatment using fewer reagent concentrations. Therefore, the following parameters are considered for the treatment of specimens in two sets of experimental studies:

i. The concentration of the bacterial solution (BS) and the cementation reagent solution (CRS)
ii. Mode of application of the two solutions in the specimens
iii. Treatment durations
iv. Effect of pH to promote calcite precipitation during MICP treatment at different reaction times.

The MICP treated specimens were used to assess the gain in compressive strength at different levels of cementation. In addition, the scanning electron microscopic (SEM)

images of the treated specimens will assess the distribution pattern and morphology of calcite precipitates in the soil matrix.

3.2 MATERIALS

3.2.1 Sand

The sand collected from the Yamuna river basin in Delhi, India, was used to prepare the sand columns for MICP treatment. First, the sand was washed through a 75 μm IS-sieve under tap water till cleared water, then dried in an oven at 105° C till its volume remained the same. Later, the dried sand was sieved through various sieve sizes stacked according to IS 2720-Part IV:1985 and was grouped in three gradations as 0.425–1.18 mm (~3%), 0.150–0.425 mm (~77%), and ≤0.15 mm (~20%), respectively (Figure 3.1). Table 3.1 presents a detailed description of soil gradation used in the present study compared to standard material. According to IS 383:1970, the sand used in the present study conforms to grading zone IV. In addition, almost 97% of the selected sand was within the ideal size range of 0.050–0.400 mm (Rebata-Landa, 2007), and such sand types are preferred for free bacterial movement, as reported by Maier et al., 2009.

As shown in Table 3.1, the observed values of coefficient of uniformity (Cu) = 1.33 and coefficient of curvature (C_c) = 1.05 for the selected sand, and therefore such sands are classified as poorly graded sand (SP) according to Unified Soil Classification System (ASTM D2487, 2006). Therefore, such poorly graded sand (SP) was considered a suitable target for MICP treatment.

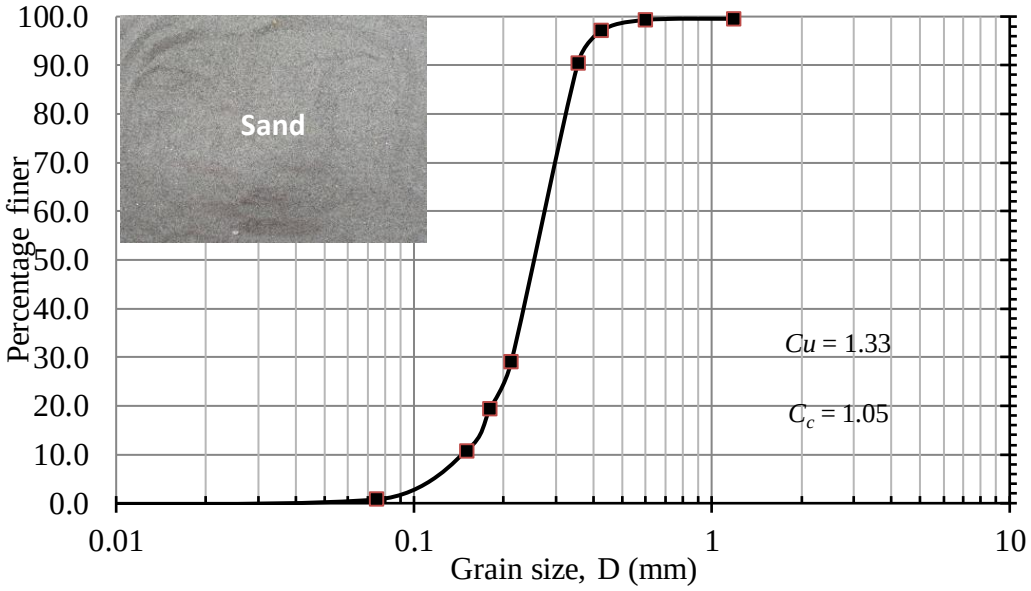


Figure 3.1 The grain-size distribution curve of the untreated Yamuna sand

Table 3.1 Physical indices of various sands used for MICP treatment

River sand	D_{60} (mm)	D_{50} (mm)	D_{30} (mm)	D_{10} (mm)	C_u	C_c	G_s	Reference
Ottawa river (Canada)	–	0.12	–	–	1.6	0.8	2.65	DeJong et al., 2013
Snake river (United States)	0.26	–	0.18	0.11	2.4	–	–	Montoya et al., 2013
Atlas river (Canada)	0.37	–	0.28	0.20	1.9	–	–	Montoya et al., 2013
Ottawa river (Canada)	–	0.22	–	–	1.4	0.9	2.65	Feng et al., 2016
Ottawa river (Canada)	–	0.46	–	0.30	–	–	–	Zhao et al., 2014
Mississippi river (United States)	–	0.33	–	0.20	–	–	–	Zhao et al., 2014
Yamuna river (India)	0.28	0.26	0.21	0.15	1.3	1.05	2.65	Present work

3.2.2 Sand-column preparation

Two experimental set-ups consisting of five sand columns were packed with 400 g of sterilized sand autoclaved at 121℃ for 20 min in polyvinyl chloride (PVC) molds of 55 mm in diameter x 150 mm height be used for MICP treatment is, as shown in Figure 3.2. Figure 3.2 (a) shows PVC molds and sand specimens in polythene bags; (b) Prepared sand-column specimens for set I and set II for MICP treatment. The

sands filled the PVC columns under a free flow from a height of 50 mm above the top level of the mold. The resulting column height and the average density of the sand columns were 120 mm and 14.04 kN/m^3, respectively. Then, seating weights were successively applied on top of each specimen to give incremental pressures of 7, 14, 28, and 56 kPa. After applying the incremental pressures, the column height was reduced to 110 mm, while the density increased to 15.30 kN/m^3. However, after three days of treatment, the final height of the specimens was reduced to ~100 mm, and the density was increased to ~16.84 kN/m^3 with the added mass of the calcium

Figure 3.2 (a) PVC molds and sand specimens in polythene bags

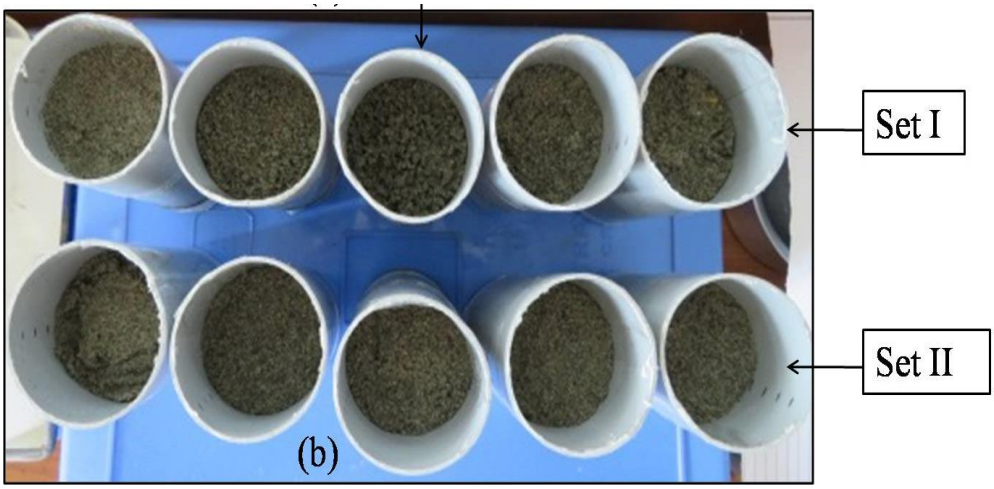


Figure 3.2 (b) Prepared sand column specimens in PVC molds as experimental Set I and Set II for MICP treatment

carbonate. After three days, there was no significant change in height. Moreover, the depth of the PVC columns above the top level of the sand columns was the ponding depth for storing the cementation solution before the percolation of the treatment solution.

3.2.3 Bacteria

The present study used the microbial strain *Sporosarcina (S.) pasteurii* (MTCC 1761) in MICP treatment. A discussion on the important characteristics of the aerobic soil bacterium *S. pasteurii* is in section 2.5.1. It can produce the enzyme urease, which can hydrolyze urea during the MICP treatment to produce calcite precipitates, as reported by Ferris et al., 1996. In addition, due to the formation of extracellular calcite precipitation, the size of the microbe increases. If the pore throat is less than the size of calcite material will hinder its mobilization if a detachment of the calcites from the particle-matrix (DeJong et al., 2006). A microenvironment may prevail with different pH concentrations, dissolved inorganic carbon (DIC), and Ca^{+2}. All bacterial cells are electronegative and can induce carbonate precipitation by adsorbing the free Ca^{2+} ions present in the soil to produce $CaCO_3$ (Boquet et al., 1973). Thus, the bacterial cell itself acts as a crystal nucleation site and encapsulates the carbonate minerals surrounding the cell wall on a microscale (Castanier et al., 1999). The production of calcium carbonate crystals by soil bacteria is a natural phenomenon, as in the formation of caves (Ferris et al., 1996). The encapsulation of bacteria results in their death and the rapid reduction of active bacteria having 'active' precipitation linked to ion transport (specifically Ca^{2+}) across cellular membranes (Castanier et al., 1999, 2000; McConnaughey et al., 1997).

3.2.4 Preparation of bacterial solution

The *S. pasteurii* (MTCC 1761) cells obtained in dried form were grown in nutrient broth (NB), an enrichment food for bacterial growth. Table 3.2 shows the bacterial solution (BS) composition used for MICP treatment.

Table 3.2 Bacterial solution composition used in various MICP treatment

Chemical doses in gL^{-1}					Reference
Urea	Calcium Chloride	Nutrient Broth	NH_4Cl	$NaHCO_3$	
11.2	27.6	4.5	3.8	1.60	Li et al., 2016
20	3.7	3.0	10	2.12	Gunjo et al., 2016
20	3.3	3.0	10	2.12	Soon et al., 2014
20	1.4, 2.8, 5.6	3.0	10	2.12	Stocks-Fischer et al., 1999
20	11.25	3.0	10	2.12	Present work

The bacterial strain was grown in 3 g of NB, 10 g of NH_4Cl, and 2.12 g of $NaHCO_3$ per liter of deionized water and was harvested at desirable concentrations. Sodium bicarbonate and ammonium chloride are used to act as a buffer. The test liquid was autoclaved to sterilize at 121°C for 20 min after adjusting the pH to 6.5 using 1N HCl. After being autoclaved, the test liquid was cooled down to 35°C. The liquid medium was mixed with bacterial cells for growth, and then 20 g/L of urea was

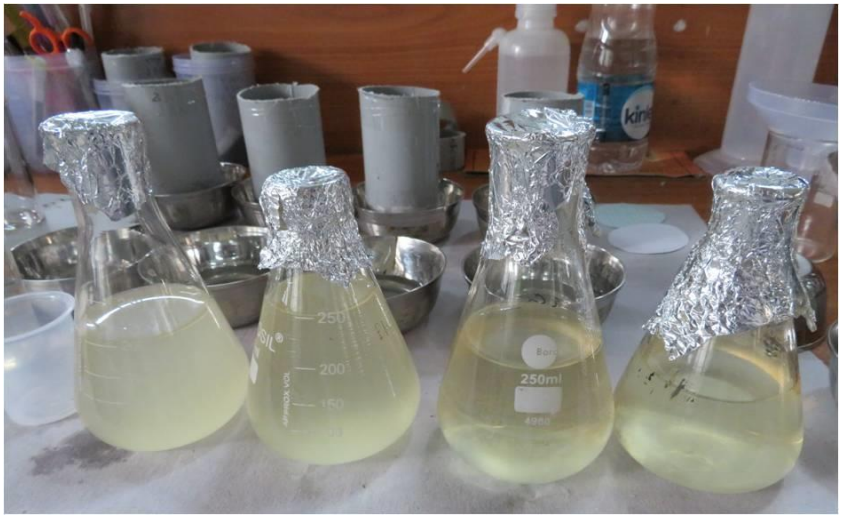


Figure 3.3 The prepared bacterial solution in Erlenmeyer flasks for incubation

added to initiate urea hydrolysis by the urease enzyme released by the bacteria. The urea was not autoclaved as it decomposes at higher temperatures because of the changes in chemical structures. The resulting test liquid was transferred to 250 mL Erlenmeyer flasks for incubation at 35°C on horizontal shakers at 120 rpm for 48 h, as shown in Figure 3.3.

The test liquid without cells was stored in a test tube and incubated parallel to avoid contamination. The test liquid medium containing incubated *S. pasteurii* cells is the bacterial solution (BS). The BS was harvested at a cell concentration of 0.7 to 1.0 at a wavelength of 600 nm, referred to as OD_{600} (approximately estimated as$1x10^7$ cells/mL). The BS was stored at 4°C till its use. The urease activity of the culture was in the range of 10–15 μM urea/min. Immediately before injection, 11.25 g/L of $CaCl_2$ was mixed thoroughly with the BS to enhance the aggregation of bacterial cells and allow percolation through the soil matrix.

3.2.5 Preparation of cementation reagent solution

The urea-Ca^{2+} molar ratio of the cementation reagent solution (CRS) was 1:1. Then, the reagents were added by 0.22 filter sterilization. Table 3.3 summarizes the MICP treatment process using CRS from a higher concentration (0.5 M in the set I and 0.75 M in set II specimens during 8–24 h) to a lower concentration (0.25 M in all specimens after 24 h onwards) based on the efficacy of biochemical reaction.

Table 3.3 Composition of urea and $CaCl_2$ in the CRS for MICP treatment process

Urea (g/L)	$CaCl_2$ (g/L)	Biochemical reaction of CRS with bacteria
30	73.50	At 0.5 M CRS during initial 8–24 h (stage II) in the set I
45	110.20	At 0.75 M CRS during initial 8–24 h (stage II) in set II
15	37	At 0.25 M CRS from 24 h (stage III), overreaction times of 3, 7, 14, 21, and 28days specimens in each set

3.3 MICROBIALLY INDUCED CALCITE PRECIPITATION

During the biochemical reaction, urease enzymes released by the bacteria hydrolyze urea into ammonium and carbonate ions in the presence of water. Then, the carbonate ions react with Ca^{2+} from $CaCl_2$ in the solution to form $CaCO_3$ (Kroll, 1990; Kantzas et al., 1992), and the bacterial cells serve as nucleation sites in growing carbonate crystals (Castainer et al., 1999; DeJong et al., 2006) at the cell surface. The reaction process between bacterial cells and Ca^{2+} and subsequent production of $CaCO_3$ are in Eqs. 2.15–2.17 under section 2.6.1.

3.4 SOIL TREATMENT PROCEDURES

The sand column treatment using the MICP process was in three stages at room temperature 25°C. During stage I, 50 mL of the BS (~$1x10^7$ cells/mL) was added and retained for 8 h to adsorb the cells in the sand particles. During stage II (8–24 h) and stage III (after 24 h), 150 ml of CRS was added every 4 h to allow the bacterial cells to promote calcium carbonate precipitation. During 24 h of stage II, the treatment solution of 0.50 M CRS in set I and 0.75 M in set II was applied from the top of the columns to keep the specimens undersaturation. And then, after 24 h, the stage III treatment was started but changed the solution to a lower concentration of 0.25 M CRS in all specimens equally in both sets. In each set, one specimen was assigned for a different treatment duration of 3, 7, 14, 21, and 28 days, respectively. Initially, the specimens were kept submerged in the treatment solution. Then, it was allowed to percolate through the sand columns from top-down by gravity flow up to the completion of their respective treatments. Figure 3.4 shows the flow chart of the MICP treatment process.

Now, the sand pores were filled-in with the CRS solution that allows the reaction of Ca^{2+} ions with the carbonates to induce the $CaCO_3$ precipitation. The pH

of the effluent solutions was measured at an interval of 4 h. The active hydrolysis was ensured if the pH was ≥ 7, and it remained above 7 for 14 days.

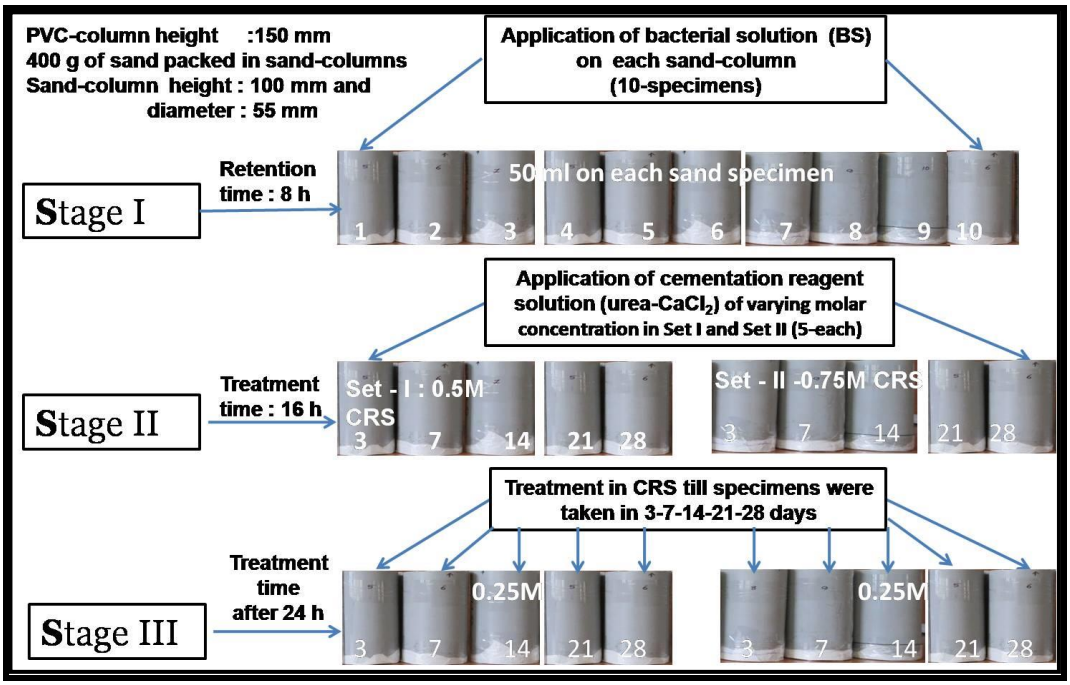


Figure 3.4 The MICP treatment process using BS and CRS in specimens kept for 3, 7, 14, 21, and 28 days

However, after 14 days of treatment in the 21 and 28 days specimens, pH began to drop below 7. The environment has become unsuitable for bacterial survival, slowing the biochemical reaction. Therefore, for a continuous and efficient MICP process, fresh doses of BS of 50 mL each were applied intermittently when the pH of the effluent solution dropped below 7. Furthermore, the addition of fresh bacteria may increase the bacterial activity in releasing urease enzymes for ureolysis. Meanwhile, the treatment solution was continued to apply for 21and 28 days. However, the pH dropped below 7 after 14 days of treatment in the 21 and 28 days specimens, which shows the discontinuity of the reaction despite supplying BS and CRS. Finally, the MICP-treated specimens were dried for the unconfined compressive strength (UCS) test.

3.5 METHODS

3.5.1 Determination of unconfined compressive strength

The unconfined compressive strength (UCS) of the biotreated specimens 14, 21, and 28 days was determined using the UCS test according to IS 4332 (Part-V): 1970 under strain-controlled conditions at a uniform loading rate of 1.25 mm/min.

3.5.2 Determination of change in grain size due to biocementation

The grain size distribution of the MICP treated sand was determined using the wet sieving method after pulverization according to IS 2720 (Part-IV):1985. In addition, the grain-size distribution curves of the sand specimens before and after the treatment were used to examine the increase in grain sizes after treatment.

3.5.3 Determination of calcium carbonate content

The calcium carbonate content was determined by washing 100 g of treated sand in HCl solution using 0.1 N from each column. The calcium carbonate dissolved in the acid wash solution was rinsed and drained from the soil through a #200 sieve (75 µm sieve). Then the retained sand on the sieve was oven-dried. The difference in mass of the oven-dried specimen before and after the acid wash was determined to estimate the mass of the precipitated calcites (Zhao et al., 2014; Burbank et al., 2011 and Rebata-Landa, 2007), and the increase in the calcite content was calculated in percentage.

3.5.4 Scanning electron microscopy

Scanning Electron Microscope (SEM) is a technique that uses electrons instead of light to form an output image. SEM is an indispensable tool for the characterization of materials from nanometer to micrometer scale. It is one of the most versatile instruments available for the examination and analysis of the microstructure morphology of different particle shapes, surface morphology, sizes, and size distributions in biocemented soil structures consisting of soil particles, bacteria, and calcite crystals can be visualized of the present study.

SEM analysis evidenced the direct involvement of bacteria in $CaCO_3$ precipitation. Such images visualize the calcite bonds and their distribution in the soil qualitatively. This study suggests that calcite production through biomineralization processes is highly effective and may provide a useful strategy as a sealing agent for filling the gaps or cracks and fissures in any construction structure.

(a)

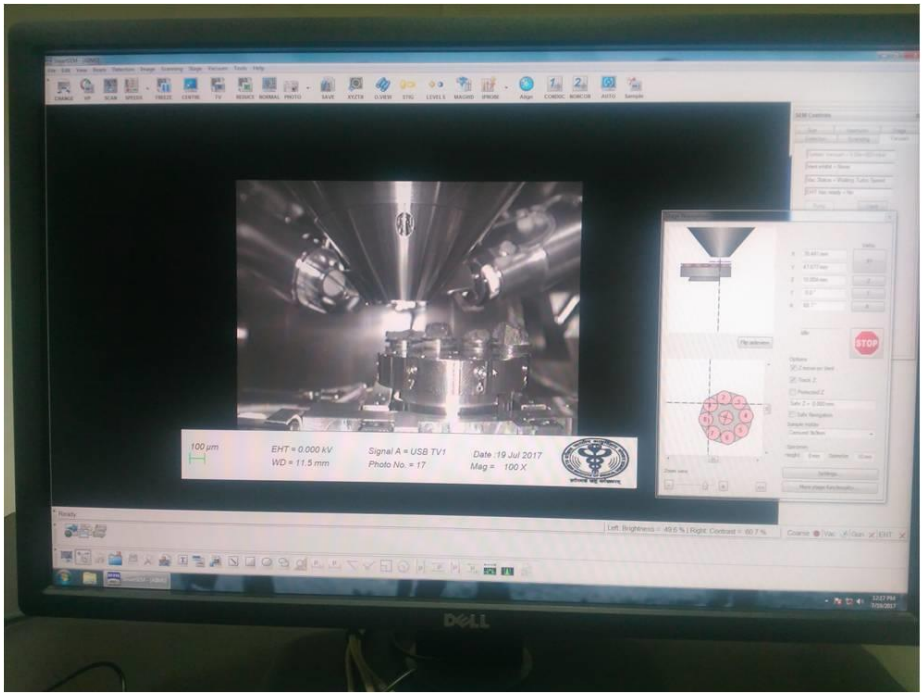
Figure 3.5 (a) Treated specimens oven-dried at 60°C for 1 h

(b)

Figure 3.5 (b) Mounting the oven-dried specimens to use in agar sputter coating

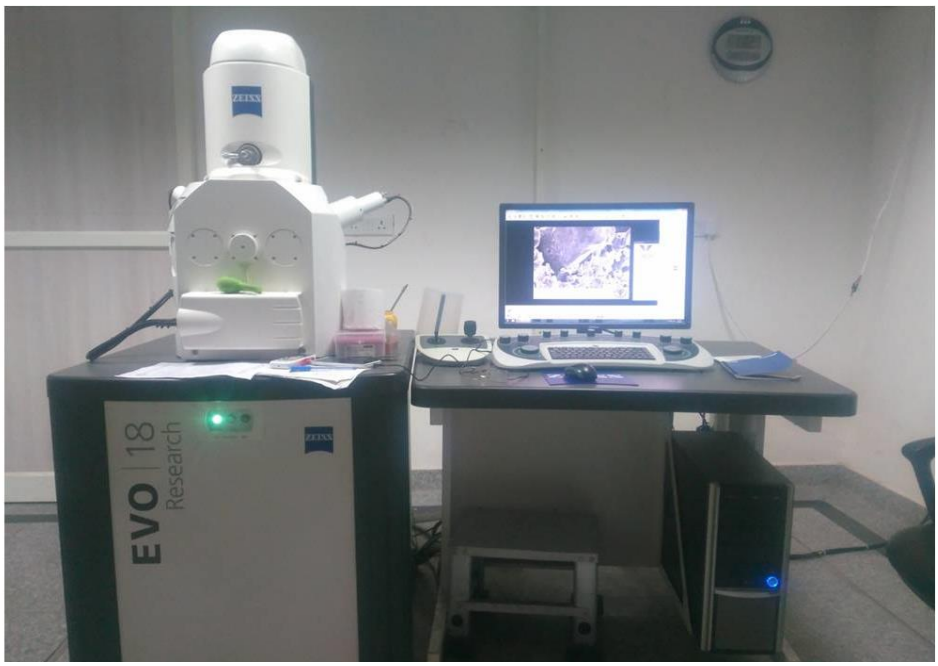
(c)

Figure 3.5 (c) Sputter-coating with silver using Agar Sputter Coater



(d)

Figure 3.5 (d) Image capturing using the software EVO 18 Research



(e)

Figure 3.5 (e) The captured image is displayed on the computer screen

The fragments of sand from a few treated specimens were prepared for processing in scanning electron microscopes (SEM) for microimaging. Such images show the morphology and distribution of calcites in the sand matrix. Initially, the specimens were oven-dried at 60°C for 1 h to remove the moisture content till the weight of the sample remained constant, as shown in Figure 3.5 (a). It was followed by sputter-coating with silver using Agar Sputter Coater, as shown in Figures 3.5 (b) and (c).

Next, image processing by using SEM Zeiss (EVO 18 Research), as shown in Figure 3.5 (d). Finally, the captured image was displayed for visualization on the computer screen, as shown in Figure 3.5 (e), to analyze the morphology and distribution pattern of the calcite precipitates in the biocemented soil specimens.

3.6 RESULTS AND DISCUSSIONS

3.6.1 Effect of pH on MICP

During the MICP process, *S. pasteurii* produces a urease enzyme that hydrolyzes urea into ammonium and hydroxide ions. The formation of hydroxide ions increases the pH and can shift the bicarbonate equilibrium and form a carbonate ion. Moreover, an increase in pH is due to the breakdown of complex proteins in the stationary growth phase (Jain and Arnepalli, 2019). The carbonate ions, in turn, react with free calcium ions present in the soil from $CaCl_2$ and precipitate as calcium carbonate crystals.

During 0–8 hours of stage I in both sets, treatment was done only with the BS, so there was no pH monitoring. However, during 8–24 h of stage II, the pH fluctuated between 7.6 to 8.0 in both sets of specimen treatment due to higher bacterial activity in releasing urease enzymes. The pH remained above 7 till 14 days

of treatment in all specimens. The curve of pH versus duration of treatment (in days) in Figure 3.6 (a) shows that the maximum pH is 8 in specimens of the set I when treated by using 0.5 M CRS, and in Figure 3.6 (b), it is 7.8 for set II specimens when treated with 0.75 M CRS. The pH was higher in the set I specimens than that of set II specimens till 14 days of treatment.

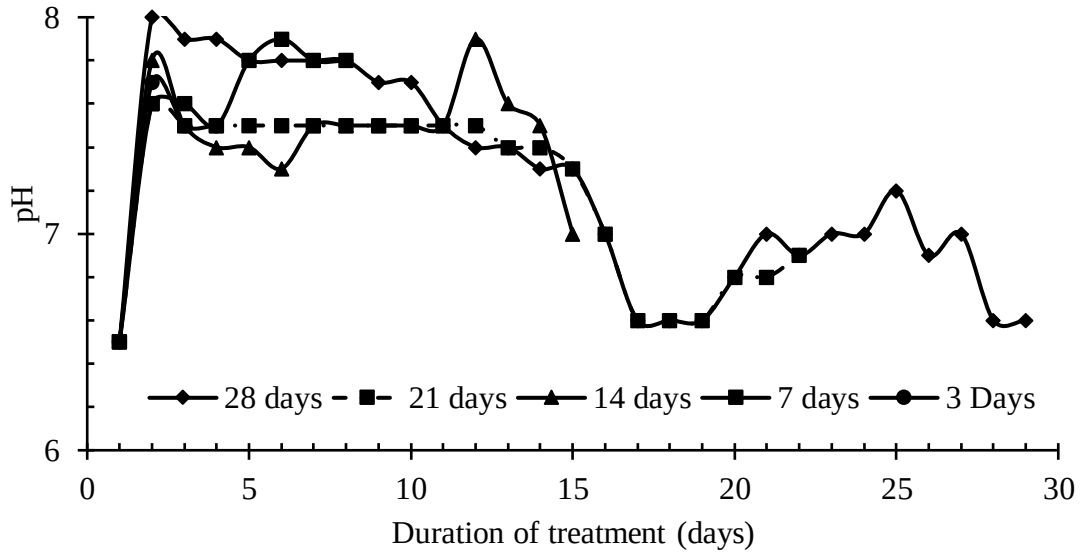


Figure 3.6 (a) pH vs. duration of treatment with initial treatment of specimens at 0.5 M, which was followed by 0.25 M CRS in set I specimens

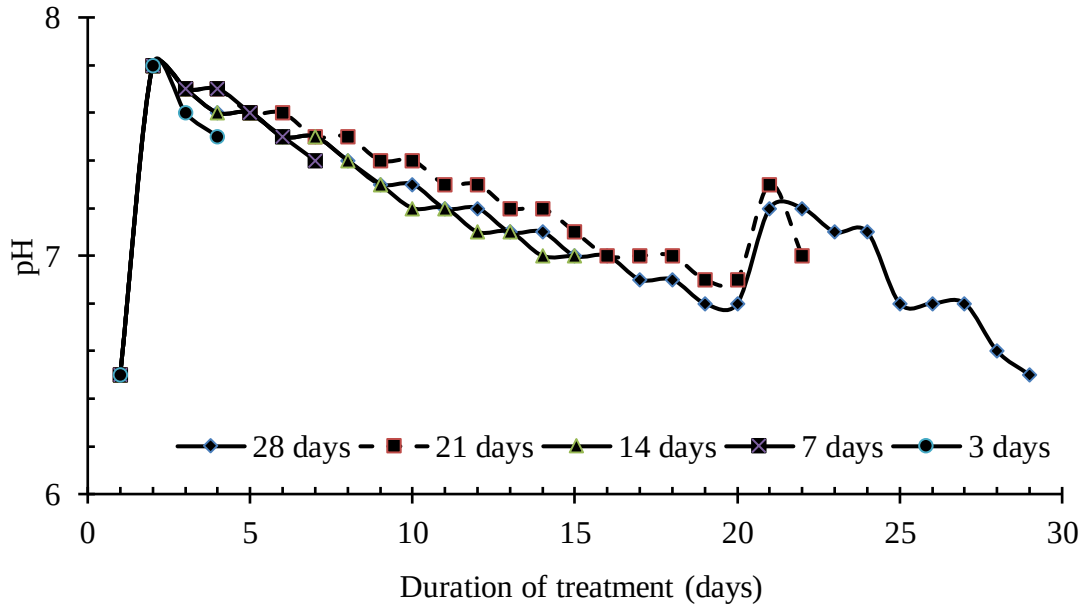


Figure 3.6 (b) pH vs. duration of treatment with initial treatment of specimens at 0.75 M, which was followed by 0.25 M CRS in set II specimens

The higher pH in the set I specimens indicates that treatment combinations at lower concentrations have higher urease activity than at higher concentrations. From 14 to 21 days, pH was below 7 even after adding new doses of BS and CRS. The decrease in pH may be due to bicarbonates from urea hydrolysis and microbial respiration (release of CO_2) and lack of oxygen inhibiting the rise in pH. Thereby, the ureolytic activity of the bacteria decreases in the MICP process (Jain and Arnepalli, 2019; Mobley et al., 1995). Another reason may be the release of protease enzymes by bacteria after 5 days and inhibit the activity of urease enzymes. However, after 14 days of treatment till 21 days in the 21 and 28 days specimens, the pH was increased again above 7. Then, it reduces below 7 from 26 days till the completion of treatment in the 28 days specimens even after adding new doses of BS. Overall, the pH after 14 days is insignificant despite applying new BS and CRS doses in both sets.

Therefore, the treatment beyond 14 days will waste chemicals, time, and cost. In addition, higher concentrations do not favor the reaction when the bacterial activity decreases, so adding more chemicals would be a waste. The pH measured within 14 days of treatment in the present study is comparable to the optimum pH range of 7.5–8 (Jain and Arnepalli, 2019). Urease activity reached its peak at pH 8.0; however, it began to decline at higher pH and as the treatment duration increased. During the treatment, the measured pH was in the optimum range of 6.0 to 8.0, comparable to that found by Mobley et al., 1995 and Evans et al., 1991 required for microbial growth (Maier et al., 2009). Rebata-Landa, 2007 also obtained a similar drop in bacterial activity during a treatment duration of 16 to 32 days. The drop in the bacterial activity is due to the inhibition of the urease activity by the bacterial protease enzymes, which are released after 120 h of treatment time (Achal et al., 2009). Table 4 shows the pH observed during MICP treatment in various studies.

Table 3.4 Observed pH during MICP treatment in various studies

Microorganism	pH	References
S. pasteurii	8.7–9.5	Dupraz et al., 2009
	9.3	Ferris et al., 2004
	9.1	Fujita et al., 2004
	7–8	Present work

3.6.2 Effect of cementation solutions on MICP

The sand column specimens were treated by applying the BS in the first 0 to 8 h, followed by CRS in subsequent treatments consisting of three-stage treatment. The three-stage of the soil treatment procedure is as in Figure 3.4 under section 3.4. During stage I (0–8 h), 50 mL of BS was allowed to percolate through the sand columns and retained for 8 h to attach the bacterial cells to the sand grains. During stage II (8–24 h), the application of CRS was by using two concentrations, i.e., 0.5 M in set I and 0.75 M in set II after every 4 h. Then, after 24 h, during stage III treatment, the concentration was changed to a lower concentration of 0.25 M till the completion of their respective treatment durations for 3, 7, 14, 21, and 28 days. Higher reagent concentrations during 8 to 24 h may be effective for calcite precipitation when the bacterial activity was high to release the urease enzymes. Soon et al., 2014 reported a similar result, stating that treatment within 5 days is more effective. Therefore, the objective of the present study is to improve the soil strength by applying higher reagent concentrations of 0.5 M and 0.75 M CRS during the initial 8 to 24 hours and changing to a lower concentration of 0.25 M CRS in subsequent treatments. Thus, an effort was made to reduce the cost of urea and $CaCl_2$ as reagents by minimizing the wastage of chemicals by replacing higher concentrations with lower concentrations. Such a practice may reduce the harmful effects of chlorine in concrete or soil by minimizing the use of $CaCl_2$. In addition, the

ammonium ions released as a by-product during the biochemical reaction will be lesser due to a decrease in the use of urea. Although a higher reagent concentration between 0.5 to1.0 M of CRS can precipitate higher calcite content, the efficiency for particle-particle contacts may be low (Nemati et al., 2005; Okwadha and Li, 2010). De Muynck et al., 2010 observed that the efficiency of calcite formation in 0.5 M cementation solution was almost half of that in 0.25 M solution. Hence, the present study introduces a higher concentration of CRS of 0.5 M in the set I and 0.75 M in set II specimens during stage II, then at a lower concentration of 0.25 M during stage III treatment to obtain a considerable strength improvement. In addition, the treatment solution was in the alkaline state (pH $\geq$ 7) till 14 days of treatment; however, pH was at higher values in the set I than in the set II specimens. Therefore, the biochemical reactions at lower concentrations provide higher urease activity during calcite precipitation.

3.6.3 Unconfined compressive strength

The biotreated sand columns were used as specimens for the UCS testing. The peak strength before failure when axial loads were applied to the sand columns would indicate the compressive strength in the biotreated columns. In cement grouting, the soil requires 28 days for curing to achieve sufficient strength. But, in the MICP method, the treated soil may take less time to gain significant strength and stiffness by bridging the soil grains with the calcite precipitates.

The stress-strain plots for 14, 21, and 28 days treated specimens are as in Figure 3.6 (a) for the set I and Figure 3.6 (b) for set II specimens. All the curves show a gradual rise in axial stress and strain before reaching sharp peaks at relatively low axial strains ranging between 1.5% to 2.5%, and then sharp peaks were followed

by sharp declines (Dejong et al., 2006). The gained in compressive strength in the set I specimens ranged from 1.1 to 1.44 MPa and 1.1 to 2.18 MPa in set II specimens. Such ranges of peak strength may be classified as soft rocks. The axial stresses were dropped quickly after the peak stress, which indicates that MICP-treated soils are brittle. In addition, the failure of the specimen was due to the incremental load applied to the sand columns, and failure occurs due to the decementation of the soil grains at particle contacts (Wang et al., 2019).

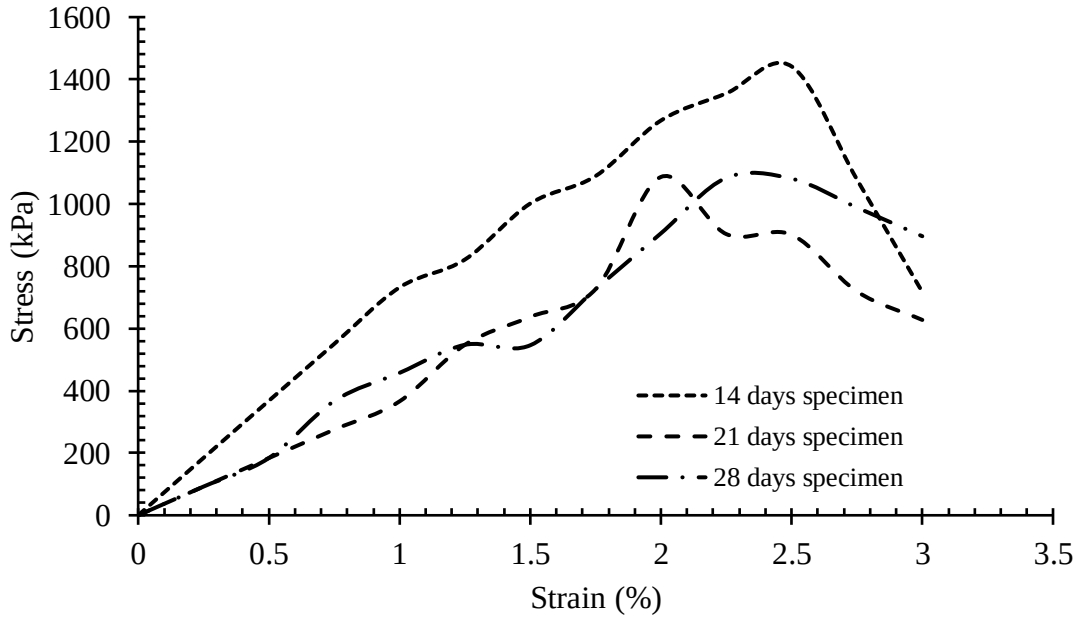


Figure 3.7 (a) Stress-strain curves of 14, 21, and 28 days treated specimens of set I

The mechanism of cementation and damage amid the grain formations may be captured by a numerical hardening–softening process active among the rock masses, as proposed (Trivedi, 2010; 2012, and 2015).

The $CaCO_3$ content in the biotreated specimens was calculated by using the acid wash method and is tabulated in Table 3.5 for each of the different specimens. The same table also tabulated the corresponding UCS values and the $CaCO_3$ content gained in each treated specimen at different durations. The table shows that the UCS of the specimens treated at different reaction times varies from 1.1 to 2.18 MPa for a

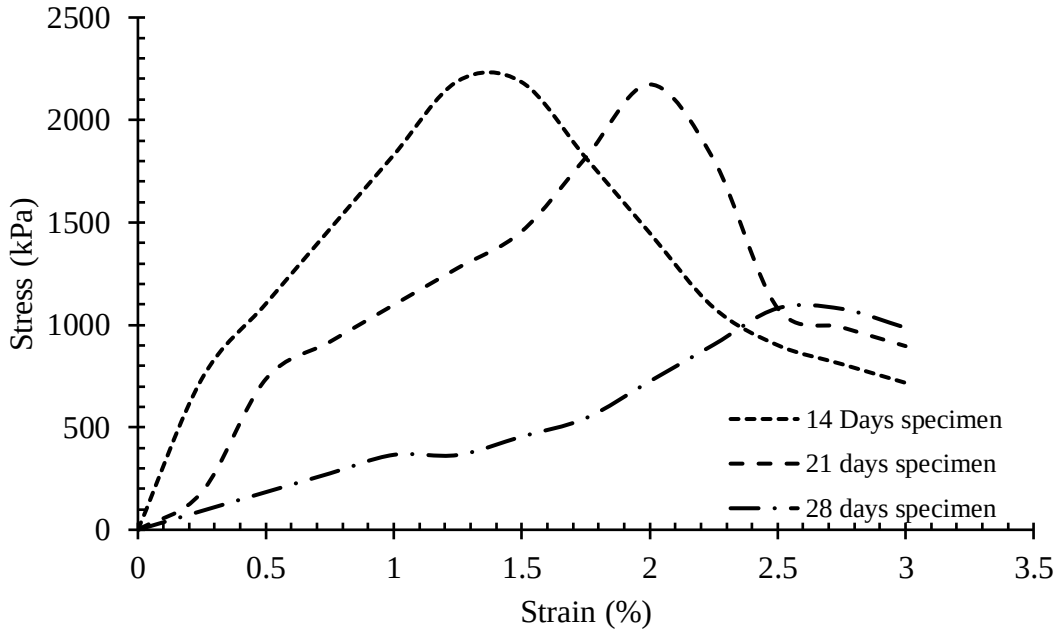


Figure 3.7 (b) Stress-strain curves of 14, 21, and 28 days treated specimens of set II

Table 3.5 Compressive strength and the $CaCO_3$ (%) content of the specimens

Molarity of CRS	Reaction (days)	Axial strain (%)	UCS (MPa)	$CaCO_3$ (%)
(0.5–0.25) M	3	–	–	2.0
	7	–	–	3.2
	14	2.5	1.44	6.0
	21	2.0	1.10	4.0
	28	2.3	1.10	4.5
(0.75–0.25) M	3	–	–	2.3
	7	–	–	3.3
	14	1.5	2.18	8.0
	21	2.0	2.17	7.5
	28	2.5	1.10	4.3

range of calcite content between 4.0%–8.0%; however, the ranges are below ~3 MPa, for normal applications (Mitchell and Santamarina, 2005). Besides, the compressive strength and calcite content do not necessarily increase beyond the median curing of 14 days in the present finding. Similar results were reported by Whiffin et al., 2007, and Mahawish et al., 2017. The strength at higher concentrations (0.75 M in stage II – 0.25 M in stage III) with a calcite content of 8%

is almost two times that in lower concentrations (0.5 M in stage II – 0.25 M in stage III) at 6% calcite content. Here, both strength and calcite content is higher in specimens treated with higher concentration which is contradictory to De Muynck et al., 2010 that calcite formation in higher cementation solution was almost half of that in a lower concentration of 0.25 M. The strength and the calcite content for 14 days were 1.44 MPa at 6% for the set I and 2.18 MPa at 8% for set II specimens. As treatment continued till 21 and 28 days, there was no increment in the strength and the calcite content, as shown in Table 3.5. The $CaCO_3$ may be triggered due to the bacterial activity to react with the cementation solution until 14 days of treatment. However, there may be an insignificant amount of $CaCO_3$ production due to discontinued biochemical reactions between bacterial cells and cementation solution, as the bacterial activity ceases due to the nucleation of bacteria by $CaCO_3$ depositions that results in the death of microorganisms. So, it is better to discontinue the treatment after 14 days to avoid the wastage of chemicals. In addition, the detached calcite particles from the sand surfaces may wash away with the effluent solution reducing the calcite content and hence the strength if treated for a longer duration. Hence, the treatment beyond 14 days does not provide further additive strength or calcite content.

Figure 3.8 (*a*) shows the UCS specimens biotreated for 14 days, and Figure 3.8 (*b*) shows the propagation of shear failure in the 14 days biotreated specimen.

Figure 3.8 (a) The 14 days biotreated sand column for UCS testing

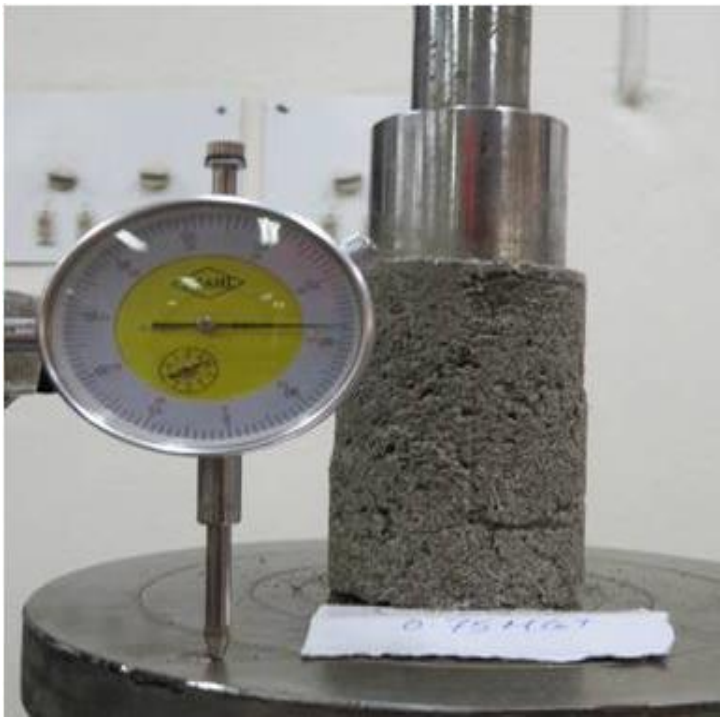


Figure 3.8 (**b)** Propagation of shear failure in the 14 days biotreated specimen during UCS testing

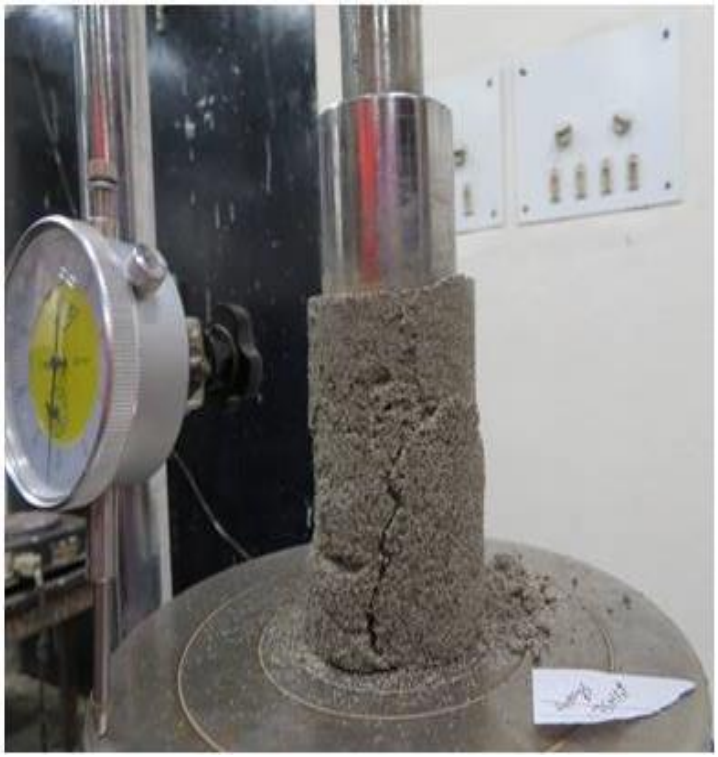


Figure 3.8 (c) Shear failure propagation in the 28 days biotreated specimen (set I)

Figure 3.9 Shear failure in the 28 days specimen (set I) during the UCS testing

Figure 3.8 (c) shows shear failure propagation in the 28 days of the biotreated specimen (set I) during the UCS testing. Figure 3.9 shows the shear failure in the 28 days of the biotreated specimen of set I during the UCS testing. It can be observed that the heavily cemented portions are in the upper and lower portions of the specimens but are lesser in the middle portions. Between 30 and 70 mm depth was the brittle and de-cemented portion of the specimens. In addition, decementation proceeded radially inwards and concentrated the weak zone around the middle portion of the specimens. As a result, there is more cementation near the injection of the treatment solution and outlet portion of the specimen, which may be due to the accumulation of more calcite precipitates in these zones.

The strength gained is 1.1 MPa at 4% calcite content in 21 days and 2.3 MPa at 4.5% in 28 days treated specimens for the set I, while 2.17 MPa at 7.5% calcite content in 21 days, and 1.10 MPa at 4.3% in 28 days treated specimens of set II. In both sets, the compressive strength and calcite content do not increase after 14 days, instead found to be in the decreasing trend as treatment duration increases. The reason is the washing away of more calcites and decementation.

3.6.4 Percentage change in grain sizes of the biotreated soils

After UCS tests, the treated sand of 200 gm of each specimen was pulverized and kept immersed in water for 24 h and then washed under tap water using the wet sieving method and then oven-dried. The oven-dried sands at 105°C were used for grain-size analysis according to IS: 2720-Part IV: 1985 for MICP treated sands. The percentage fractions retained on each sieve were grouped in three gradations following the same process as for untreated sands.

From Table 3.6, it was observed that in the first gradation (0.425–1.180 mm), the percentage increased in grain size from 3 to 9.7, 13.2, 12.8, 10.8, 9.4, respectively for 3, 7, 14, 21, and 28 days specimens of set 1 and to 10.7, 12.2, 13.0, 9.5, and 9.9 respectively for 3, 7, 14, 21 and 28 days specimens of set II as shown in Table 3.7. In the case of the second gradation (0.150–0.425 mm), the increase in percentage size is from 77% to 89, 85.4, 78.9, 78.6, and 79.2 for 3, 7, 14, 21, and 28 days respectively for the set I specimens and to 88, 84.5, 79.8, 80.3 and 79.3 for 3, 7, 14, 21 and 28 days respectively for set II specimens in Table 3.7.

Table 3.6 Percentage change in sizes of biotreated sands of set I specimens

Gradation range (mm)	Untreated sand (%)	Percentage change in sizes of biotreated sands				
		3-days	7-days	14-days	21-days	28-days
0.425–1.180	3	9.7	13.2	12.8	10.8	9.4
0.150–0.425	77	89	85.4	78.9	78.6	79.2
≤0.15	20	1.3	1.4	8.3	10.6	11.4

Table 3.7 Percentage change in sizes of biotreated sands of set II specimens

Gradation range (mm)	Untreated sand (%)	Percentage change in sizes of biotreated sands				
		3-days	7-days	14-days	21-days	28-days
0.425–1.180	3	10.7	12.2	13.0	9.5	9.9
0.150–0.425	77	88	84.5	79.8	80.3	79.3
≤0.15	20	1.3	3.3	7.2	10.2	10.8

From the above observations, it can be observed that there is an increase in the percentage of grain sizes retained in the first (0.425-1.180) and the second (0.150–0.425 mm) gradation ranges. However, in the third gradation range (grain sizes ≤

0.150 mm), there is a decrease in the percentage of finer fractions from 20 to 1.3, 1.4, 8.3,10.6, and 11.4, respectively for 3, 7, 14, 21, and 28 days specimens of the set I as shown in Table 3.6 and to 1.3, 3.3, 7.2, 10.2, 10.8 respectively for 3, 7, 14, 21, and 28 days specimens of set II as shown in Table 3.7. The increase in percentage growth in grain sizes from fine aggregates to coarser aggregates is due to the binding of the sand grains across several sand grains through biocementation. The grain surfaces are also coated with the calcium carbonate precipitates, thus forming coarser sand aggregates. The increase in grain sizes due to coating the sand grain with the calcites and binding of the grains at contact points will contribute to the increase in the compressive strength.

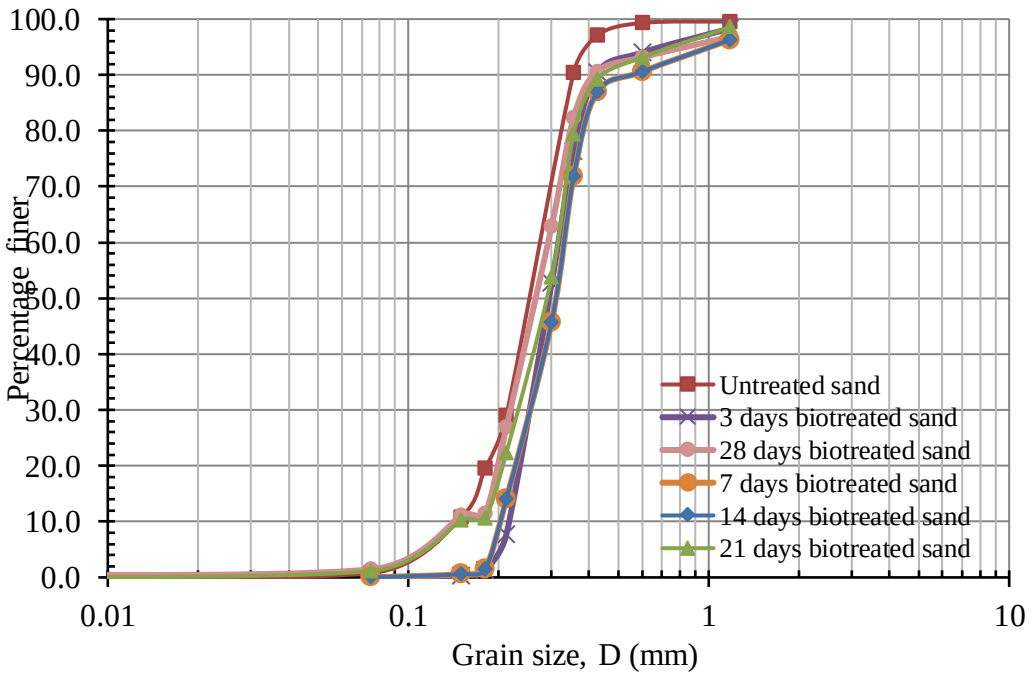


Figure 3.10 (a) Percentage change in grain sizes from the untreated to biotreated specimens over 3, 7, 14, 21, and 28 days in set I (0.5–0.25) M

The increase in grain size can be observed in Figures 3.10 (a) and (b), showing the grain-size distribution curves of untreated and biotreated sand specimens of set I and set II, respectively. All the grain size distribution curves of the treated sands of each

set of experiments lie towards the right of the untreated one, indicating the growth in the grain of different sizes from fine to coarser aggregates, which may give additional roughness and strength.

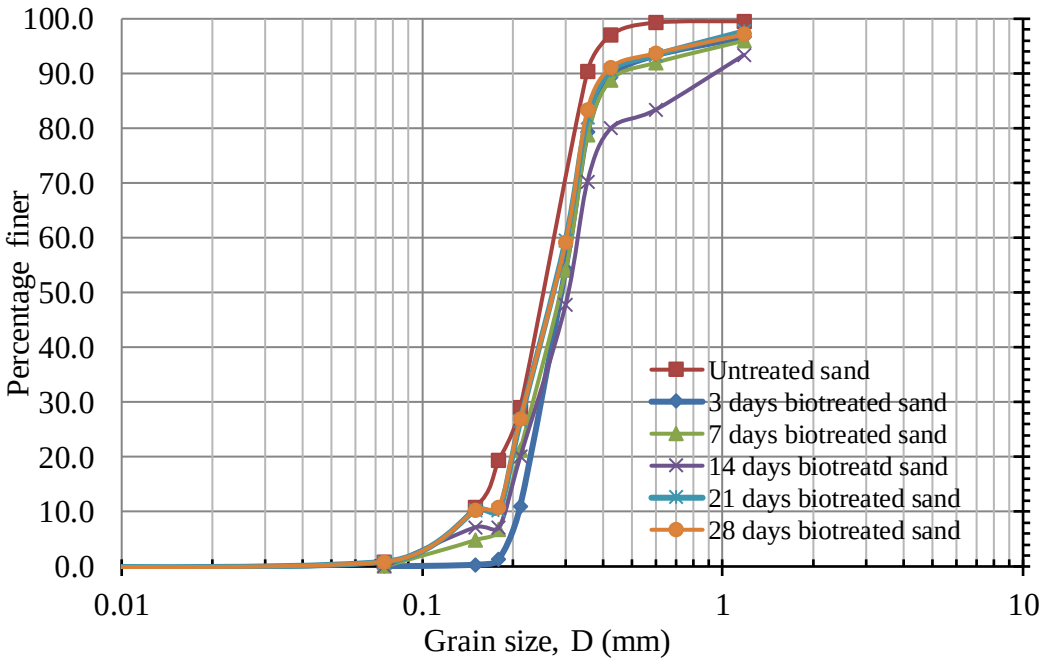
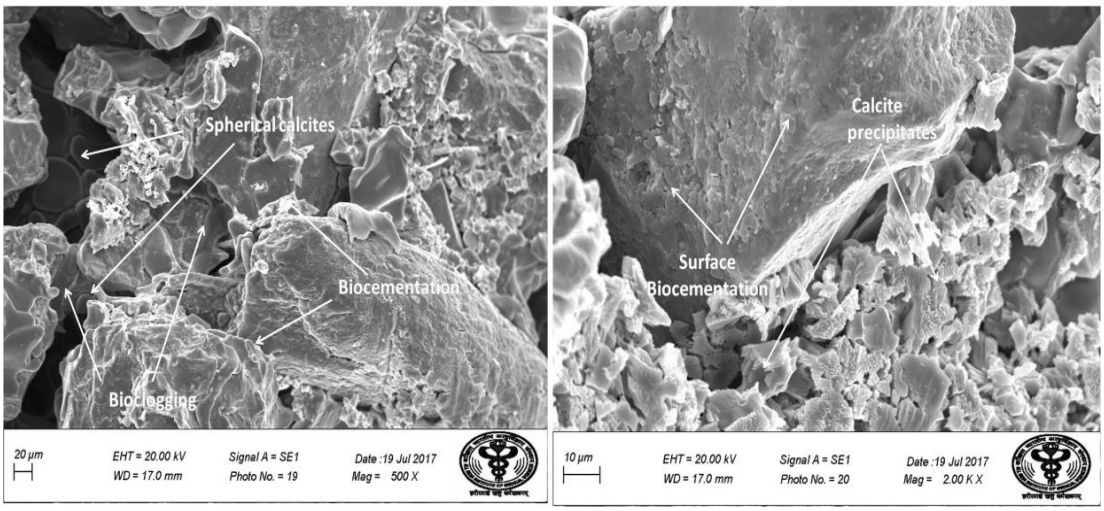


Figure 3.10 (b) Percentage change in grain sizes from the untreated to treated specimens over 3, 7, 14, 21, and 28 days in set II (0.75–0.25) M

3.6.5 Analysis of scanning electron microscope images

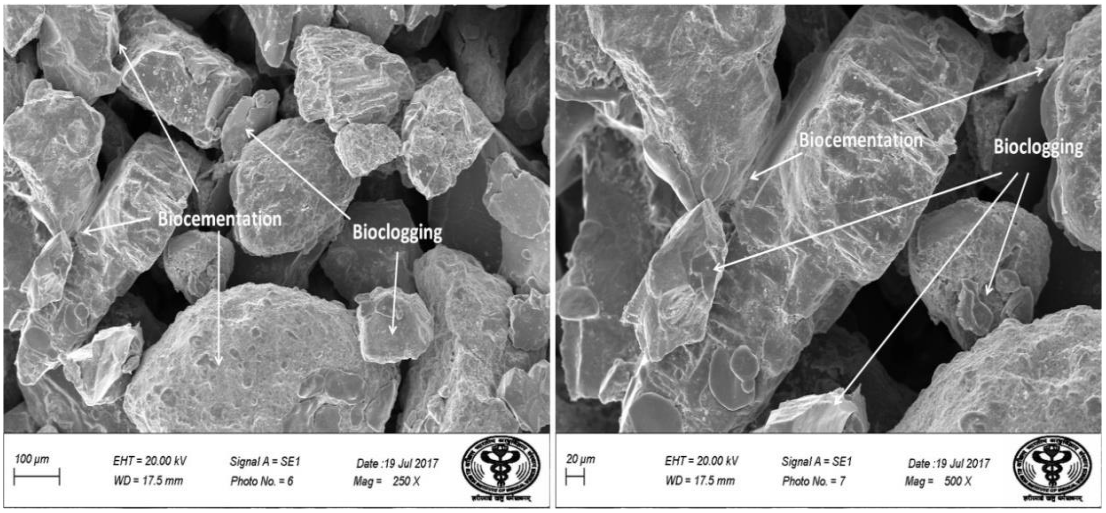
SEM images of three sand specimens were taken to observe the morphology and the distribution of calcite in the sand matrix to validate the presence of calcite precipitates. The SEM images showed the morphology and the distribution pattern of calcite precipitates attached to the surface and bridged the sand grains.

Figure 3.11 (a) shows the SEM images of 28 days specimens of set I. Figures 3.11 (b) and (c) show the 21 days and 28 days specimens of set II. The calcite formation can be seen in lighter grey shades with no stable structure in all the images. In addition, the calcite formation is in flaky structures scattered in layers and clustered together with tiny rough grain structures when viewed at different



(a)

Figure 3.11 (a) SEM images of 28 days treated specimen of set I



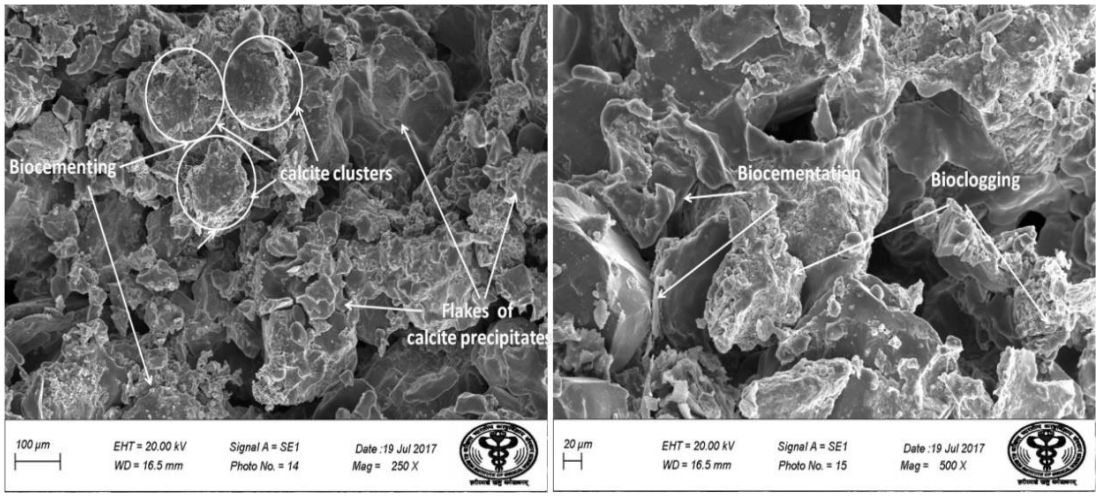
(b)

Figure 3.11 (b) SEM images of 28 days treated specimens of set II

magnifications. In Figure 3.11 (a), the 28 days specimen of set 1 has some spherical shape $CaCO_3$ precipitates lying in the pore portions. Cementation exists on the sand surfaces and in-between the sand particles causing biocementation and bioclogging.

Figure 3.11 (b), the 28 days specimen of set II, shows surface precipitation with biocementation and bioclogging processes. Calcite precipitates are of effective and non-effective types. Effective calcites bridge the sand particles and strengthen

the soil. The non-effective ones that exist on the particle surfaces accumulate into clusters or flakes of calcite precipitates, as shown in Figure 3.11 (c), and are non-effective that do not contribute any strength to the soil. It can be seen that the calcite precipitate bridged the sand grains together and stiffened the joints to increase the strength. Also, the calcites filled the sand pores to reduce the pore volume and hence reduce the permeability.



(c)

Figure 3.11 (c) SEM images of 21 days treated specimen of set II

3.7 CONCLUSIONS

The following conclusions can be made from the present study:

- The improvement in the UCS is by biocementation of the sand grains by the calcite precipitates in loose and collapsible sandy soils. The calcite precipitates on the bacterial cell surface and in-between the sand grains to bind and fill the soil pores.
- The higher compressive strength and calcite content were in 14 days treated specimens. Initially, a higher concentration of reagents (0.5 M in the set I and 0.75 M in set II) was applied during the first 8–24 h. After 24 h,

treatments were followed by a lower concentration of 0.25 M until completion of treatment in set I and set II specimens. Using a lesser reagent concentration of treatment solution after 24 h may be cost-effective when treating in large areas. In addition, the release of toxic chemicals will be reduced as a by-product to cause less harm to the soil environment.

- The calcite content obtained in various specimens of the set I varied from 2%–6% and 2.3%–8.0% for set II specimens. In all specimens, the cementation level was above 1.0% (15 kg/m^3), which can provide measurable improvements in soil shear strength and hydraulic conductivity as reported (Soon et al., 2014).
- The calcite precipitates on the grain surfaces, and the sand pores have reduced the pore volume and pore throat sizes, thereby facilitating the reduction in pore pressure and soil permeability. The reduction in pore pressure decreases the liquefaction and settlement problems of structures constructed in loose sandy soils.

Many challenges arise in the field application of MICP, as it is difficult to control the in-situ microbial activity and biochemical reactions for calcite precipitation at desirable levels. In addition, the soil structure depends on the interaction between minerals and organic/inorganic matter prevalent in the soil environment. Furthermore the soil provides a spatially heterogeneous habitat for various soil microorganisms with varying substrate needs, enrichment nutrients, oxygen concentrations at different depths and pH, thus affecting bacterial diversity and structure (Gunjo and Heejung et al., 2016). Thus, the achievable cementation level, uniformity in distribution of calcite precipitates, durability, and degree of improvement in the engineering properties of sandy soil formations are challenging but not

unpredictable. In the future, this technique would help solve various geotechnical problems such as liquefaction, the swelling potential of clayey silt, mitigating wind erosion potential of loose sand deposits, and pretreatment of the subsurface to reduce settlement of highway structures and slope stabilization.

Despite the challenges faced in field applications, this method has tremendous potential in the future. The MICP soil treatment method may solve many geotechnical problems with further studies. In addition, this method would serve as a cost-effective and environmental-friendly approach to improve the engineering properties of sandy soil formations.

CHAPTER 4

Harnessing Microbially Induced Calcite Precipitates to Use in Improving the Engineering Properties of Loose Sandy Soils

This chapter is based on the published paper, "Harnessing microbially induced calcite precipitates to use in improving the engineering properties of loose sandy soils." Shougrakpam, S. and Trivedi, A. (2021), *Sādhanā*, 46 (41), 1–14.

Abstract

This study investigates the harnessing of microbially induced calcite precipitation (MICP) in soil treatment to improve the engineering properties of loose sandy soils. Experiments were conducted in sand specimens using a channel, a pond, and four sand columns. Initial treatment with a bacterial solution followed by a cementation reagent solution (CRS) was carried out to trigger calcite ($CaCO_3$) precipitates. The submerged and surface percolation treatment methods were carried out in sand using CRS as the treatment solution. The treatment solution was maintained in an alkaline

range (pH ≥ 7). The alkaline condition ensures active microbially induced calcite precipitation in the sand. The calcite precipitates bind the sand particles to increase the strength and stiffness of the soil matrix. The calcite bridged the sand particles and formed a biocemented water-impermeable crust layer (~ 2 mm thick). The calcite act as pore-filling material through the bioclogging process to reduce porosity and permeability. Permeability tests evaluate the effect of seepage control. The permeability was reduced to three order-of-magnitude (~99%) on the 7th day with slight variation (~ 100%) until the 14th day. The compressive strength of the biotreated columns was between 585 and 875 kPa. The calcite content in the upper 10 mm thick in different biotreated columns was between 11% and 14% and was gradually reduced from 9.8% to 3.4% below 10 mm. Hence, the observed 5–15% calcite content in natural biocemented products is comparable to MICP-treated specimens. The scanning electron microscopic images show the calcite distribution patterns in the sand matrix.

4.1 INTRODUCTION

The microbially induced calcite precipitates (MICP) method of soil treatment improves the engineering properties of sandy soils, which may also be suitable for the construction of channels and aquaculture ponds built-in loose sandy soils. The ureolytic bacteria react with urea and Ca^{+2} (from a calcium source such as $CaCl_2$) to induce calcite precipitates that can undergo the biocementation and biologging process in soil (Whiffin et al., 2007; Dejong et al., 2010; Burbank et al., 2011; Al Qabany et al., 2012; Soon et al., 2013; Tang et al., 2017; Liu et al., 2019). The microorganisms found in the soil produce mineral deposits as a metabolic by-product, usually consisting of calcium carbonates ($CaCO_3$) in soils, caves, stalactites

(Danielli and Edington, 1983; Baskar et al., 2006), and rocks in the presence of water through a prolonged process of biocementation. However, the MICP process can make mineral deposition faster by bioaugmentation or biostimulation that simulates microorganisms' natural processes to precipitate $CaCO_3$ as calcites on the surface and within the bulk of sand below it. The precipitated calcites will function as a cementing and a bioclogging material during the soil treatment. The calcite precipitates bind the sand at particle contacts through biocementation and thus, increase the strength and stiffness of the soil. In addition, the precipitated calcite in the pore fluid filled the sand pores. Therefore, it reduced pore volume and pore throat sizes, reducing the pore pressure and facilitating in mitigation of liquefaction likelihood (Burbank et al., 2013).

Bioclogging is the production of pore-filling using calcite precipitates to reduce the pore volume of the soil. As the pores are filled up, the soil is densified, and hence the permeability of the soil is decreased. Furthermore, the bacterial cells also release the extracellular polymeric substances (EPS), which increase the biomass filling the sand pores, reducing the pore volume, and improving the sand solidification. Further, Bachmeier et al., 2002 observed that almost 1% of the dry cell biomass is the urease content in ureolytic bacteria, *S. pasteurii*. In addition, the microorganism releases gaseous by-products in tiny bubbles, which are insoluble that can replace the pores and reduce the pore volume and pore throat sizes. Therefore, the pore pressure may reduce the liquefaction potential in liquefiable sandy soils. Besides, bacteria also produce polysaccharides that could provide a low-cost seepage control technique and reduce hydraulic conductivity. Therefore, biocementation and bioclogging are two MICP processes that strengthen, plug and improve soil properties (Whiffin et al., 2007; Dejong et al., 2010; Ferris et al., 1996; Nemati and Voordouw, 2003). Hence,

MICP may be applicable as a sealing material to reduce leakage and surface erosion in channels and aquaculture ponds.

During the MICP process, the precipitation of calcite is a result of a biogeochemical reaction between urease-producing bacteria (UPB), urea ($CO(NH_2)_2$), and calcium chloride ($CaCl_2$) in the presence of water. Two treatment solutions for soil treatment using the MICP method are the bacterial solution (BS) and the cementation reagent solution (CRS). The BS consists of bacterial cells, while CRS consists of urea and $CaCl_2$ as the two reagents. The bacterial cell used in BS in this study is *S. pasteurii* (MTCC 1761), a urease-producing bacteria that can release a very high capacity of urease enzymes. The urease enzymes hydrolyze urea into ammonia and carbonate ions depending on its urease activity. The presence of ammonia raises the local pH, causing the carbonate ions (CO_3^{2-}) and the free calcium ions (Ca^{2+}) to react in the pore fluid to deposit calcite precipitates. Although, the Ca^{2+} ions are not used in metabolic processes but accumulate near cell surfaces where ureolysis produces an alkaline environment. Thus, the microbial biofilm matrix provides nucleation sites for calcite precipitation. The precipitated calcite binds the sand grains in pore fluid and fills pore spaces during the MICP process.

During microbial urease activity (Mitchell and Santamarina, 2005), bacteria consumed 1 mole of urea ($CO(NH_2)_2$) for energy source and is hydrolyzed intracellularly, and produced 1 mole of ammonia (NH_3) and 1 mole of carbamic acid (NH_2COOH). The carbamate spontaneously hydrolyzes to form an additional 1 mole of ammonia and carbonic acid (H_2CO_3), and further hydrolysis of ammonia produces ammonium (NH_4^+), hydroxide (OH^-), and bicarbonate ions (HCO_3^-), which increases the pH level as a result of the enzymatic reaction that enhances the formation of $CaCO_3$ precipitates. The above reaction equations are shown under section 2.6.1,

which explains the MICP pathway by urea hydrolysis. The ion-exchanged can also produce carbonate particles through the cell membrane. Moreover, the bacterial cells are negatively charged and carbonaceous, attracting Ca^{+2} ions to deposit on their cell surface. The combination of Ca^{+2} ions and carbonate ions finally causes $CaCO_3$ precipitates, which again serve as a nucleation site, and the chemical reactions involved in $CaCO_3$ precipitation are given in section 2.6.1.

4.1.1 Soil microorganisms

Geotechnical engineers explore the biogeochemical interactions to produce microbially induced calcite precipitates (MICP) as a metabolic by-product of microbial activity used for soil treatment to improve soil engineering properties. The microbes exist above 10^{12} microbes per kilogram of soil near the subsurface (DeJong et al., 2006). Many strains of bacteria found naturally in soil and groundwater are ureolytic, meaning they can hydrolyze urea for energy and a source of nitrogen. Catalyzed by the microbially induced urease enzyme, water cleaves urea to form ammonia and carbon dioxide, shifting the carbonate equilibrium toward bicarbonate and carbonate due to a pH increase. Then, calcium carbonate (CaCO3) precipitates with sufficient calcium and carbonate activity, as explained in the introduction section 4.1.

The *Bacilllus* bacteria (Gram+ and Gram-) size ranges from 0.5 to 3.0 µm in length (Al Qabany et al., 2012, Madigan et al., 2003). Compatibility between soil pore throats and bacterial size to allow free movement is essential for an effective MICP process. Moreover, the cells of *S. pasteurii* do not aggregate, resulting in a high cell surface-to-volume ratio, which is necessary to initiate effective cementation. As bacteria possess low Reynold's number, the cells are surrounded by a thin watery

layer to form an interface between the bacterial cell and the microenvironment. Hence, variable concentrations of pH, dissolved inorganic carbonates (DIC), and Ca^{+2} would coexist in the soil microenvironment.

Some of the essential factors to study for an efficient enzymatic reaction for calcite precipitation during the MICP process are:

- The concentration of *S. pasteurii* cells in the bacterial solution (BS)
- The molar concentration of urea and $CaCl_2$ in CRS
- The pH of the microenvironment
- The treatment duration
- The compatibility between grain and pore throat sizes for free movement of the bacteria.

The present work investigates harnessing microbially induced calcite precipitates (MICP) to improve the engineering properties of loose sandy soil. Calcites would act as a biocement that can function in the biocementation and bioclogging mechanisms in the soil matrix. Furthermore, as cementing material, it may potentially seal the interparticle gaps between the sand grains lying on the surfaces/walls and bottom portions of the channels and aquaculture ponds built-in loose and collapsible sand formations to prevent leakage and seepage. Moreover, these processes may facilitate the formation of biocemented water-impermeable crust layers in the cross-plane and in-plane as hydraulic barriers by sealing exposed surfaces and sub-surfaces in channels and aquaculture ponds.

4.2 MATERIALS

4.2.1 Analysis of the sand

In this study, the soil specimen used is from the Yamuna river basin flowing through

Delhi, a northern part of India. The coarser clean sand retained on 75 µm IS sieve after wet sieving was oven-dried at 105℃ for 24 h to be used for sieve analysis. The sand was grouped into three gradations, i.e., 0.425–1.18 mm (~3%), 0.150–0.425 mm (~77%), and 0.075–0.150 mm (~20%). During specimen preparation, the fine fractions passing through a 75 µm IS sieve were oven-dried for use as fine-grain content. From the grain size distribution curve (Figure 4.1), the observed value of D_{10} is 0.15 mm, D_{30} is 0.21 mm, and D_{60} is 0.28 mm, and; hence, the coefficient of uniformity (C_u) and the coefficient of curvature (C_c) are 1.33 and 1.05, respectively.

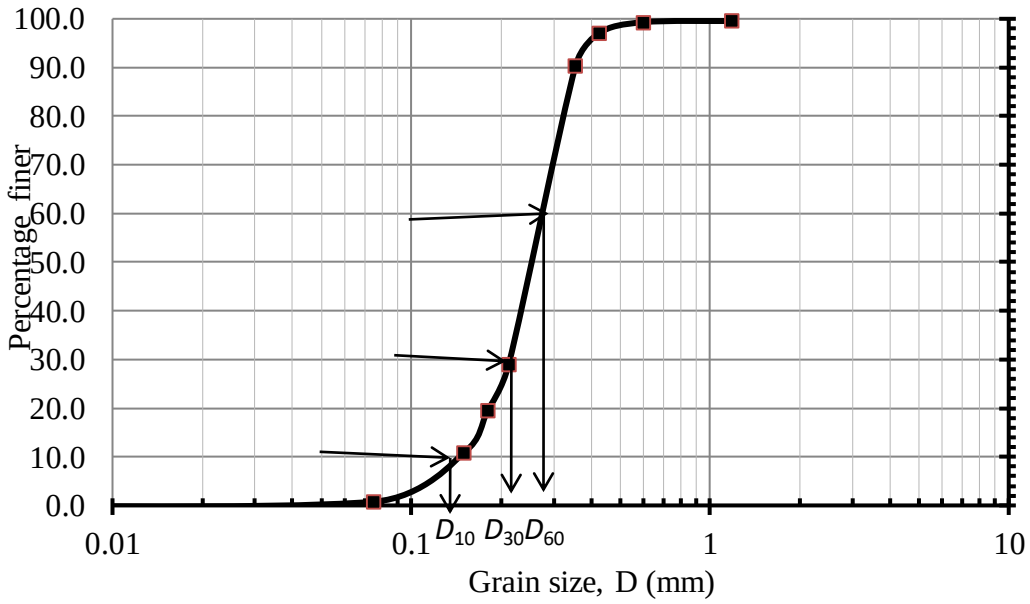


Figure 4.1 The grain size distribution curve of untreated Yamuna river sand

Based on the physical indices, namely, C_u and C_c, the sand is classified as poorly graded based on the Unified Soil Classification System (ASTM D2487, 2006). The poorly graded sand possesses unfavorable engineering properties in geotechnical engineering applications, and such sands are considered suitable for MICP treatment in the present study.

4.2.2 Preparation of specimens

The sand specimen was prepared in two ways. The first type was only with the clean sand specimens retained on the 75 µm IS sieve and designated as Type-A sand. The second type was 90% clean sand retained on 75 µm and 10% finer than 75 µm and designated as Type-B sand. Two types of sand specimen, i.e., with and without fine grains, will assess the variation in the cementation level and strength obtained after MICP treatment. Three sand specimens using Type-B sand, namely a channel, a pond, and a sand column, were prepared in a rectangular metallic tank. The tank was painted with an anti-corrosive agent to avoid any unwanted reaction.

The cylindrical PVC molds were used to prepare four sand columns packed with the two types of sands, namely S_1, S_2, S_3, and S_4, at a density of 1.35 g/cm^3. The mold dimensions were 110 mm in diameter x 100 mm in depth. The resulting sand column sizes were (105 mm diameter x 60 mm depth). The PVC molds consisted of two vertically cut parts glued with temperature-resistant tape to ease the removal of the treated samples. The bottom of the molds was closed with perforated plastic sheets to prevent flushing the bacterium with the effluent solution during treatment. Figure 4.2 shows the channel and the pond prepared with Type-B sand at two separate chambers in a tank and their dimensions.

The channel is in the first chamber, and the pond is in the second chamber, placed inside a tank. The specimen sizes have less significance and were randomly chosen to get a desirable cementation level and compressive strength during MICP treatment. A channel of size (170 mm length x 30 mm depth x 5 mm width) and a base thickness of 20 mm thick were prepared in the first chamber. The channel width of 5 mm was in-between two opposite embankment sections of size (170 mm length x 20 mm width x 50 mm depth). The size of the second chamber was (180 mm in

length x 170 mm width x 50 mm depth), and within that constructed, a cylindrical pond centrally with a size of (110 mm diameter x 30 mm depth) and 20 mm base thickness was constructed. Above the pond base, a PVC mold packed with

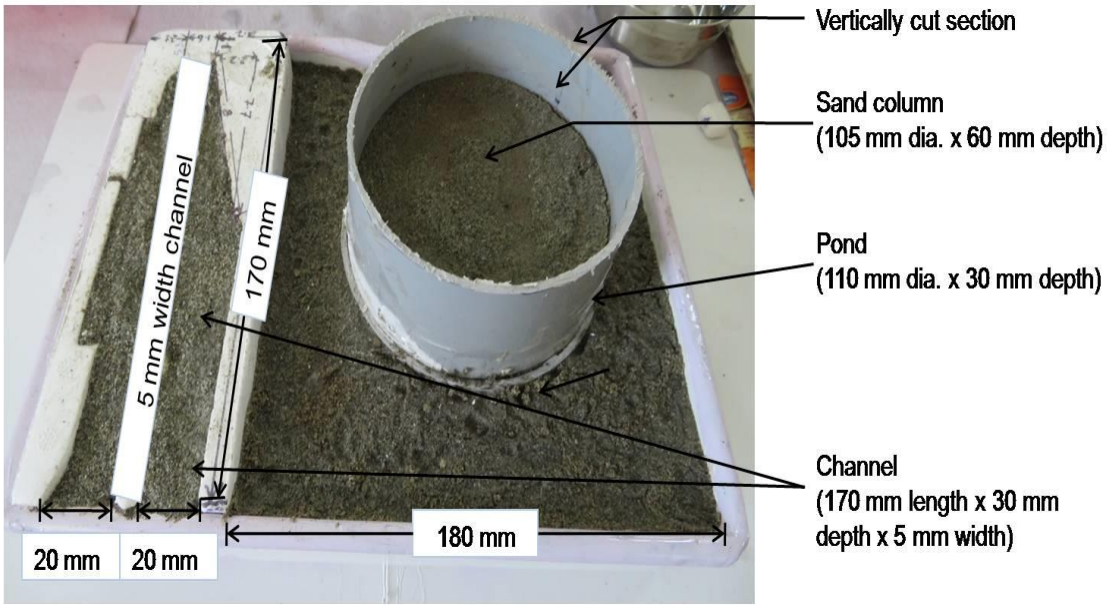


Figure 4.2 Details of the test specimens, namely a channel, a pond, and a sand column, S_1

Type-B sand, same as the channel, and the pond were placed and designated as sand-column S_1. The pond surrounding was packed with Type-B sand to 50 mm in depth. Finally, the tank containing the three specimens was placed inside a rectangular reservoir of size (280 mm length x 230 mm width x 155 mm depth). The reservoir was filled with 10 liters of treatment solution with 0.66 M CRS to treat the specimens under submerged conditions. After treatment, the PVC mold containing the S_1 column will remove, and the portion left will form a cylindrical pond. The portion lying outside of the pond will act as its sidewalls. A second sand column, designated as S_2, was packed with Type-A sand in a PVC mold and treated with 0.66 M CRS under a submerged condition in a second treatment reservoir of size (150 mm diameter x 170 mm depth) filled with 2.5 liters of treatment solution.

Two more PVC molds were packed with Type-B and Type-A sands to form S_3 and S_4 sand columns. Both the columns will be treated under the surface percolation method using a treatment solution of 0.75 M CRS. Table 4.1 summarizes the sand types used in the preparation of the specimens.

Table 4.1 The sand types used in the preparation of sand specimens

Specimens	Sand Types
Sand-columns: S_1 and S_3	Type-B
Sand-columns: S_2 and S_4	Type-A
Pond and its chamber	Type-B
Channel and its embankment	Type-B

Note: Type-A sand denotes the clean sand specimen
Type-B sand denotes the clean sand mixed with 10% fine-grain content

4.2.3 Preparation of bacterial solution

The growth media for the bacteria *S. pasteurii* (MTCC 1761) consist of 20 g/L urea, 10 g/L NH_4Cl, 2.12 g/L $NaHCO_3$, and 3 g/L nutrient broth (NB) under sterile aerobic conditions. Before the cultivation, the medium containing NB, NH_4Cl, and $NaHCO_3$ was sterilized at 121°C for 20 min in an autoclave and urea by filtration through a 0.2 µm Millipore filter to avoid thermal decay. The resulting growth medium was inoculated with the bacterial cells. The resulting growth medium was incubated in a shaker at 200 rpm for 48 hours at 30°C before harvesting. The bacterial solution was harvested at a cell concentration of approximately 10^9–10^{10} cells/mL and stored at 4°C until its use to avoid contamination. The optical density (OD_{600}) of the harvested culture was between 1.0 and 1.4. The urease activity was measured by a conductivity meter and adjusted to approximately 12–20 µM of urea/min. One unit of urease activity corresponds to the amount of enzyme that hydrolyzes 1 µM of urea per minute. Before its use, 5.25 g/L of $CaCl_2$ was mixed thoroughly with the bacterial

solution to enhance bacterial cell aggregation and their attachment to sand particles after its application. The initial pH was adjusted to 6.5 by 1 N HCl. At this pH, there was no precipitation of $CaCO_3$ in amorphous states.

4.2.4 Preparation of cementation reagent solution

The cementation reagent solution was prepared by adding urea and calcium chloride as reagents in distilled water at a 1:1 molar ratio. Two molar concentrations of the cementation solution were adopted: 0.66 M (39.6 g/L urea and 97.02 g/L $CaCl_2$) and 0.75 M (45.0 g/L urea and 110.20 g/L $CaCl_2$). Al Qabany et al., 2012 reported the optimum concentration of cementation solution to be 0.66 M CRS for the bacterial strain *S. pasteurii*. Therefore, the same CRS concentration was adopted in this study to treat S_1 along with the channel and pond enclosed in the same chamber and S_2 columns under a submerged treatment method. A second treatment procedure will be by using the surface percolation method for S_3 and S_4 columns using a higher molar concentration of 0.75 M CRS. The two concentrations would assess cementation level and strength enhancement after MICP treatment. The pH of the cementing solutions was maintained at 6.5 by 1 N HCl.

4.2.5 Soil sample from natural biocemented products

12 samples were collected from the natural biocemented stones, rocks, and caves by scraping them to a depth of 20-50 mm at three sites, as shown in Figure 4.3 (a, b, and c). The chemical analysis using the acid-wash method of the samples was carried out in the laboratory to check the presence of calcium carbonate.

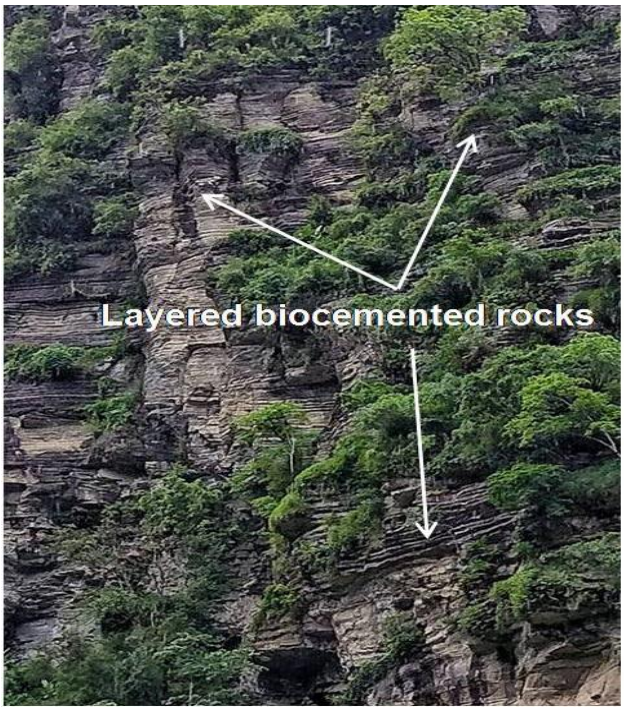


Figure 4.3 (a) Natural biocemented layered rocks at Nung Dolan (stone-staircase) in Manipur, India, where samples were collected to check the presence of $CaCO_3$

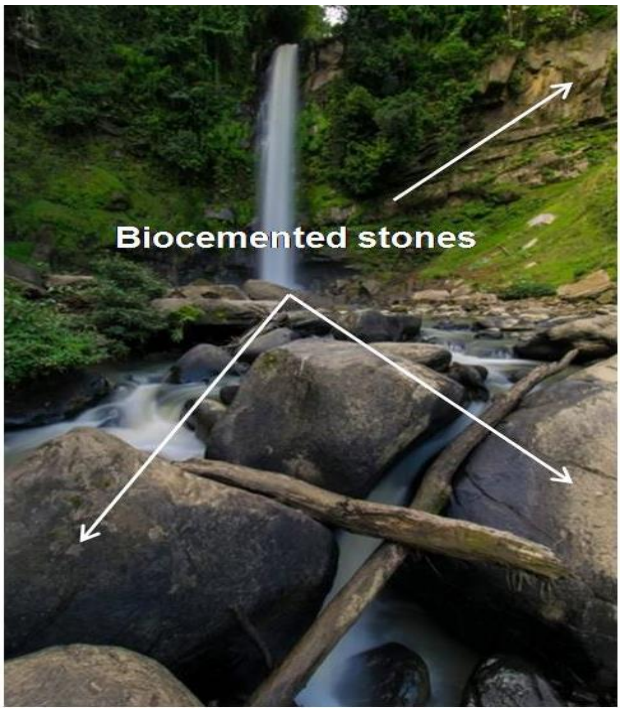


Figure 4.3 (b) Rocks and stones at Khoupam waterfall in Manipur, India, where samples were collected to check the presence of $CaCO_3$

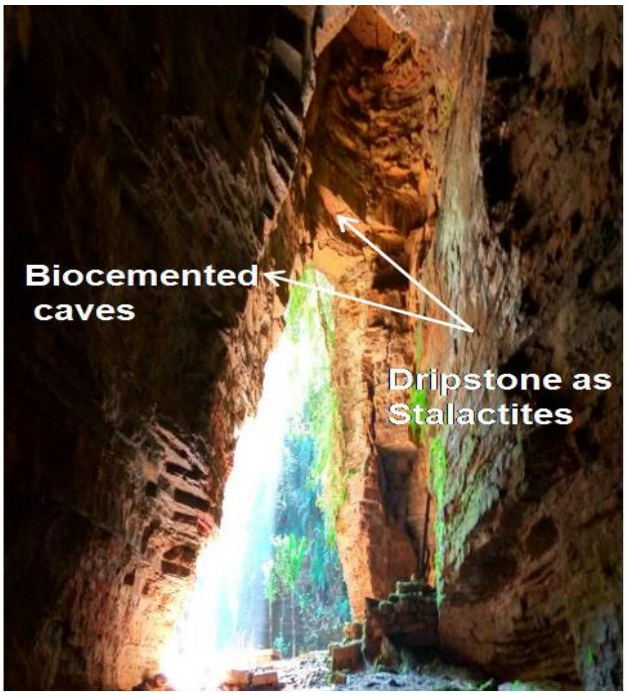


Figure 4.3 (c) Stalactites at Tharon cave in Manipur, India, where the samples were collected to check the presence of $CaCO_3$ in the cave soil

4.3 METHODS

4.3.1 MICP treatment process

The MICP treatment process requires the initial soil treatment with the bacterial solution (BS) and then a cementation reagent solution (CRS) in subsequent treatment until completion. Therefore, during the MICP process, the biological activity in the soil was initiated by applying the BS. The bacterial concentration was within 10^9–10^{10} cells/mL with a urease activity of approximately 12–20 µM of urea/min. The BS of 100 ml each was used and sprayed uniformly over the specimens at the outset. Then, the BS was allowed to retain for 8 h to attach the cells to the sand particles. After 8 h of bacterial retention, the subsequent treatment was followed by using CRS of 0.66 M and 0.75 M CRS for different specimens. The specimens consisting of the channel, the pond, the S_1, and the S_2 columns were treated under the submerged condition using the 0.66 M CRS. At the same time, the S_3 and S_4 columns were treated using a higher CRS concentration of 0.75 M by the surface percolation

method. Al Qabany and Soga, 2013 studied the effect of chemical treatment used in MICP on the engineering properties of cemented soils by using lower concentrations below 0.5 M. Lower concentrations below 0.5 M of the CRS facilitate obtaining a uniform calcite distribution and yield higher strength than a higher molar concentration of 1 M. MICP treatment for microbial calcification of soil with 0.2 M, and 1.0 M CRS improve sandy-slope surface erosion resistance. However, it was observed that the treatment with a higher CRS of 2 M did not increase the surface erosion resistance; instead, the soil was washed away in cemented aggregates under rainfall impact (Jiang et al., 2019). Hence, selecting the two treatment solutions of 0.66 M and 0.75 M CRS is suitable for enhancing the uniform calcite precipitation in embankment slopes and the base layer of the channel and aquaculture pond built in sandy formations to prevent surface erosion and seepage.

MICP treatment began with the bacterial treatment, followed by the CRS treatment in submerged and surface percolation methods. The specimens were kept under submerged conditions with 0.66 M CRS, which was initially filled up to 5 mm below the surfaces and kept for 24 h. Therefore, the biochemical reaction between the bacterial cells and the reagents in the CRS would occur in the bulk of sand below 5 mm from the top surface to trigger calcite precipitates. Then, the treatment solution was filled 60 mm above the surface of S_1 and S_2 columns, which were in separate reservoirs, kept submerged for 14 days, as in Figure. 4.4. The calcite precipitation would occur both on the surface and in the bulk of sand below it.

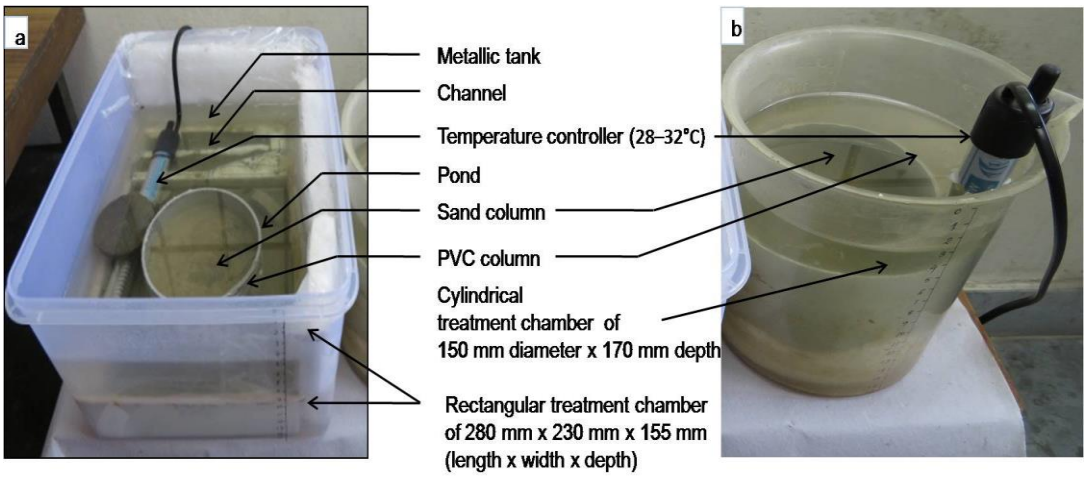


Figure 4.4 Sand specimens submerged in 0.66 M CRS at a controlled temperature of 32°C

The temperature of the treatment solution was controlled at 32°C. The surface of the treatment solution was exposed so the evaporation losses would occur, and to compensate for the loss, more solution was added. The solution was stirred and aerated continuously to increase its interaction with the bacteria for uniform calcite precipitation.

After the bacterial treatment of S_3 and S_4 columns for 8 h, subsequent treatment was followed by applying a cementing solution of 0.75 M CRS using the surface percolation method. The PVC molds consist of an additional depth of 40 mm to reserve the treatment solution above the column surfaces to percolate through the depth. Therefore, a 300 ml of the CRS stored in the reserve portion was allowed to percolate through the specimens every 4 h. The treatment was done at a controlled temperature of 32°C, within the optimum temperature range of 24°C and 37°C for significant enzymatic reaction to hydrolyze urea for microbial carbonate precipitation (Okwadha and Li, 2010). The microbial calcite precipitates bind the sand-sand particles lying on specimen surfaces for biocementation, leading to the formation of a water-impermeable crust surface and soft rock in the bulk of sand.

A manual stirring enables the microbes to adsorb and react with the cementing solution. When the pH of the treatment solution was ≥ 7, then an alkaline condition ensures the enzymatic reaction is in active condition. However, when the pH was < 7, 100 ml of the fresh BS was added and stirred continuously with the treatment solution to regain the microbial activity by raising the pH ≥ 7, continuously inducing the calcite precipitates in the soil matrix.

During 7 days of treatment, the pH was measured between 7 and 8.5. However, after 7 days of treatment, the pH was measured continuously, but it has remained below 7. pH was not able to increase even after adding fresh doses of BS and CRS for up to 14 days. The lowered pH after 7 days may be due to the low urease activity of microbes to react with CRS or the inhibition of urease enzymes by the protease enzymes released by the bacteria after 120 h.

After 14 days of the treatment, the solution was emptied from the reservoirs and refilled with water to cure the specimens in the respective reservoirs for another 14 days. The curing was done to check whether the calcite materials ($CaCO_3$) break down after the biocementation and bioclogging process. Finally, the biotreated sand columns S_1, S_2, S_3, and S_4 were dried in an oven at 60°C for 48 h to use as specimens for UCS tests. The strength and cementation level obtained in the S_1 column are valid for the channel and the pond that were treated together under the same condition.

4.3.2 Temperature

The temperature significantly influences urease activity affecting the biochemical reaction for calcite precipitation during the MICP process. During the MICP treatment, the temperature of the treatment solution for the specimens in reservoir I (channel, pond, and S_1 column) and the S_2 column in reservoir II were monitored at

32°C by using a temperature controller as in Fig 4.4. However, the sand columns S_3 and S_4 were treated at room temperature at 32°C. For the bacterial activity to release urease enzymes, the temperature of the treatment solution was maintained at 32°C (Shougrakpam and Trivedi, 2020). Baskar et al., 2006 have reported that the incubation temperature of 25°C is optimum for bacteria to form $CaCO_3$ precipitation. However, the urease activity is negligible if the temperature is below 5°C (van Paassen, 2010). The urease activity in *S. pasteurii* increased proportionally with a temperature range from 25°C to 60°C, according to Whiffin, 2004. The study on the effect of water content on the strength of bio-cemented sand by Zeng et al., 2018 at two drying temperatures (i.e., 30°C and 60°C) found that the strength enhancement was more for drying at 30°C than that at 60°C under the same water content. In the present study, the temperature of the treatment solution was controlled at 32°C, within the optimum temperature range of 24°C and 37°C for microbial carbonate precipitation (Okwadha and Li, 2010). Therefore, the temperature variation in soil may influence the formation of different polymorphs of calcium carbonate crystals of different shapes, sizes, and thermal stability.

4.3.3 Unconfined compressive strength tests

The unconfined compressive strength (UCS) testing was conducted on biotreated samples as per IS:2720-Part IV:1970 at Soil Mechanics Laboratory of Delhi Technological University, New Delhi. This test was conducted to estimate the increase in sand strength after 14 days of treatment. The oven-dried bio-cemented sand columns obtained after the MICP treatment were used for UCS tests. The tests were conducted under strain-controlled conditions at a uniform loading rate of 1.25

mm/min in oven-dried specimens for 24 h. The specimens were dried to remove moisture and to stop the microbial activity.

4.3.4 Determination of calcium carbonate content

After UCS tests, the fragments of the biocemented sands from each column were determined by using the acid-wash test. Subsamples were collected from the top surface after every 10 mm depth (i.e., 10 mm, 20 mm, 30 mm, 40 mm, 50 mm, and 60 mm) of the oven-dried UCS specimen to determine the calcium carbonate content and its uniformity. The upper depth of 10 mm was observed to have more cementation than the bulk of sand below it. The fragments of each subsample were oven-dried at 60°C till their weights became constant. Take 50 gm of each dried sample from different depths at every 10 mm depth from the location of the injection point to determine the $CaCO_3$ content. The specimens were acid-washed in 0.1 M HCL solution to dissolve the precipitated $CaCO_3$. Then the samples were rinsed and oven-dried. The final weight without $CaCO_3$ was measured. The difference between the initial and the final weight was considered the $CaCO_3$ precipitated in the specimens and was expressed as a percentage increase in $CaCO_3$ content. Similarly, the 12 samples of the natural biocemented rocks collected from natural sources will be determined to ascertain the calcite content at every 10 mm from top to 60 mm depth. The samples were pulverized and oven-dried at 105°C before being acid-washed for chemical analysis to determine the calcite content.

4.3.5 Preparation of biotreated sands for checking changes in particle sizes

The changes in particle sizes of the biotreated sand were examined using the sieve analysis method. After UCS tests, the specimens from each column were grounded

manually with a steel rod and then submerged in water for 24 h. Therefore, the $CaCO_3$ in amorphous states would be removed from the specimens. Then, the samples were oven-dried for 24 h at 105℃ before sieve analysis. Analysis was carried out to check for any changes in the particle sizes of the untreated soil after biotreatment. Then, the change in grain sizes would be checked from the grain size distribution curves of the different biotreated sand specimens to that of the untreated soil. Such curves may give useful information to observe the change in grain sizes in biotreated sands by biocementation.

4.3.6 Permeability Test

Permeability testing was conducted to evaluate the effect of MICP on seepage control. The permeability tests were conducted at two stages for S_3 and S_4 columns within the PVC molds using surface percolation methods. In the first stage, a bacterial solution of 100 mL was applied to the sand columns and was allowed to retain for 8 h to attach the bacterial cells to sand grains. In the second stage, 300 ml of 0.75 M CRS of the treatment solution was percolated through the sand columns every 4 h till 14 days of treatment.

4.3.7 Microscopy of biocemented specimens

The biocemented sand fragments remained after the UCS test was used to examine micro characterization and the distribution pattern of the calcite precipitates in the sand specimens using the scanning electron microscopy (SEM) images. The specimens were oven-dried at 60℃ for 1 h to remove moisture for SEM analysis. Zeiss EVO 18 Research SEM available at All India Institute of Medical Sciences (AIIMS), New Delhi, was used for imaging the specimens. Coupled with the ease of

use provided by SmartSem software, EVO 18 Research was suitable for this study. Fractions of specimens were sputter-coated with silver using an Agar Sputter Coater for SEM analysis to determine the micro characteristics of sand specimens and the distribution pattern of calcite precipitates in between the network of sand grains.

4.4 RESULTS AND DISCUSSIONS

4.4.1 UCS and calcite content in the biotreated sand columns

The microbially treated specimens in PVC tubes were extruded and tested for unconfined compressive strength and calcite content determination at different depth ranges (0–10 mm, 10–20 mm, 20–30 mm, 30–40 mm, 40–50 mm, and 50–60 mm) from the injection surface. On the top surface of the treated columns, a thin and hard impermeable bio-cemented crust layer of about 2 mm thick and a hardened solid block in the bulk of sand below it was formed, which resembles the characteristics of soft rocks. The calcite content in the upper 10 mm varied between 11% to 15%, decreasing as depth increased from the top surface, as shown in Table 4.2. Figures 4.5–4.7 show the failure patterns of the specimens during UCS testing conducted in the laboratory. The specimens S_1 and S_2, S_3, and S_4 columns have maximum stresses at low axial strains within 3–4% during UCS testing, as shown in Figure 4.8.

A brittle failure was observed in all bio-cemented columns as there was a rapid drop in the applied load with a further increase in the strain. Brittleness and decementation of the tested specimens were initiated from the periphery before moving inwards.

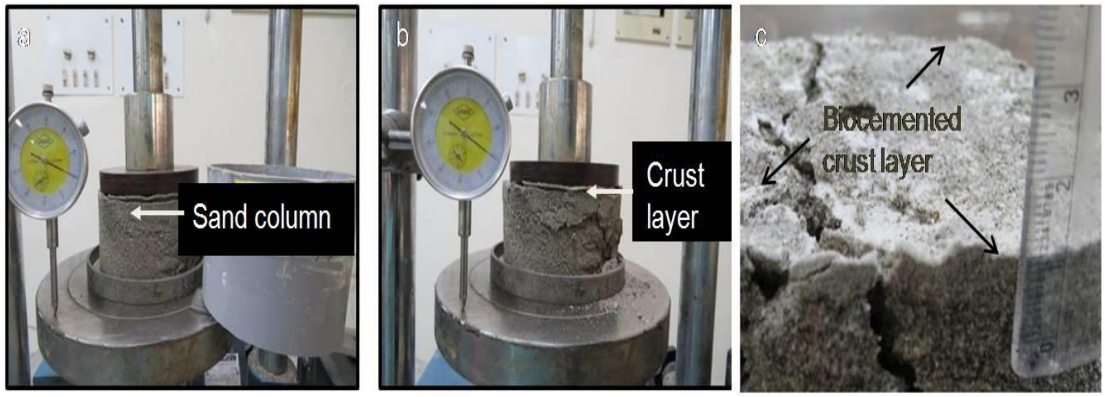


Figure 4.5 (a) Mounting of specimen column, S_1; **(b)** Failure paths of the specimen under an applied load **(c)** Bio-cemented crust layer on the sand surfaces

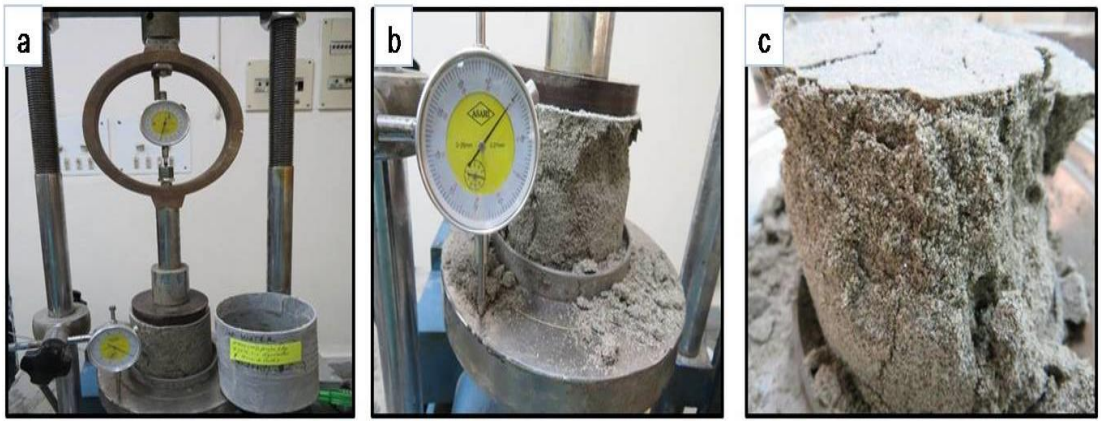


Figure 4.6. (a) Mounting of specimen column, S_2; **(b)** Failure of the specimen along the weak paths; **(c)** Failure of the biotreated sample after UCS testing

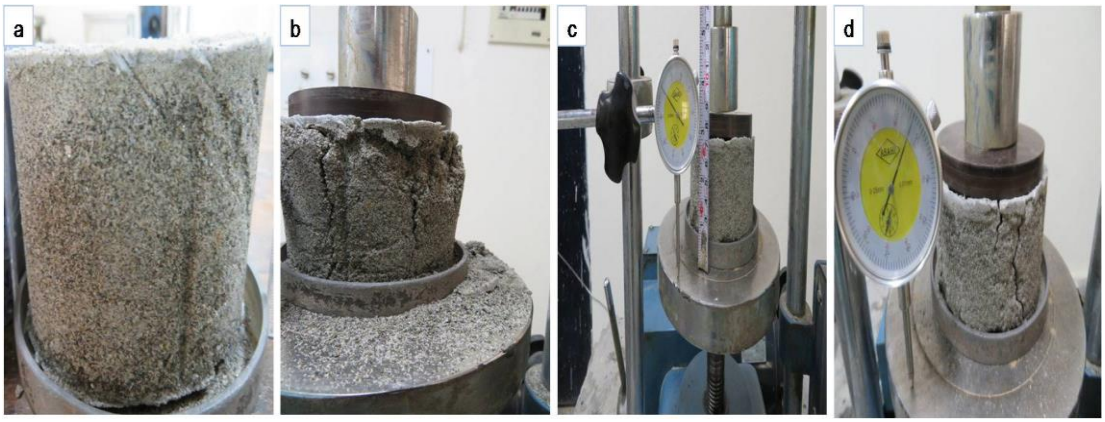


Figure 4.7 (**a** and **c**) Specimen columns S_3 and S_4. (**b** and **d**) Failure of specimens S_3 and S_4 under applied stresses along the weak paths.

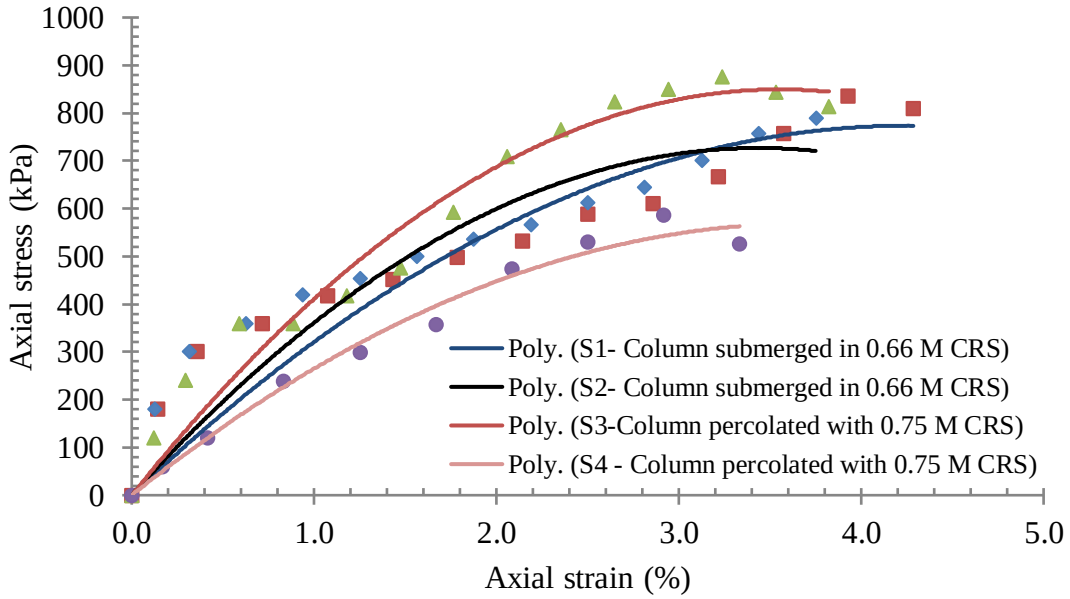


Figure 4.8 Stress-strain behaviour of biocemented sand columns S_1, S_2, S_3, and S_4

However, the soil structure of the inner portions remains intact even after achieving peak strength. The reason may be a more effective and uniform distribution of calcite precipitates at particle to particle contacts in the interior portions to enhance strength and stiffness. The correlation between $CaCO_3$ content and UCS at low axial strains obtained in the bio-treated specimens is reported (Shougrakpam and Trividi, 2020), each treated at different reaction times (3–7–14–21–28 days). These specimens were treated using different concentrations at two stages from higher (stage I) to lower (stage II) concentrations as (0.5–0.25) M and (0.75–0.25) M for 5 columns each in two sets. It was found that the UCS and $CaCO_3$ (%) were maximum for the specimens treated for up to 14 days duration (Shougrakpam and Trividi, 2020). Moreover, the treatment beyond 14 days for 21 and 28 days specimens has demonstrated no significant enhancement in strength or calcite content. Hence, 14 days of treatment is sufficient for microbial precipitation of calcites.

The calcite content and the improvement in UCS obtained in various biotreated specimens that were treated for 14 days are shown in Table 4.2. The average calcite

content in S_1, S_2, S_3, and S_4 columns are 7.9%, 7.4%, 8.6%, and 6.2%, respectively. The results have revealed that the UCS increased with an increase in calcite content. The solidified sand bearing UCS obtained after 14 days of treatment in S_1 is ~ 20% more than S_2 columns treated under submerged conditions. Further, S_3 has ~ 50% more compressive strength than S_4 columns treated under surface percolation methods. The specimens, S_1 (835 kPa) and S_3 (875 kPa) columns with 10% fine-grained content, have achieved more calcite content and compressive strength than S_2 (701 kPa) and S4 (585 kPa), as shown in Table 4.2. The presence of fine particles provided more surface area to precipitate calcite and hence induced more calcite for biocementation for strength enhancement. Therefore, the results demonstrate that the fine particles act as a binder and a filler material between the particles in the sand mass. Hence, calcite plays a vital role in enhancing strength and stiffness in the soil, as observed by Trivedi, 2013 and 2015.

Table 4.2 The effect of cementation level on the UCS of the biocemented columns

Molarity of CRS	Sand-columns	Clean Sand with	$CaCO_3$ (%) at 0-10 mm depth	Average $CaCO_3$ (%)	Axial strain (%)	UCS (kPa)
0.66 M	S_1	10% fine content	14	7.9	3.9	835
	S_2	Clean sand only	12	7.4	3.1	701
0.75 M	S_3	10% fine content	15	8.6	3.2	875
	S_4	Clean sand only	11	6.2	2.9	585

Table 4.2 shows the average calcite content obtained in the upper 10 mm of the specimens was 14%, 12%,15%, and 11% for columns S_1, S_2, S_3, and S_4, respectively. The reduction in the calcite content was from the upper towards downward for every

10 mm depth in various treated columns was in the range of 14% to 5.1% for S_1, 12% to 5.3% for S_2, 15% to 5.3% for S_3, and 11% to 3.4% for S_4.

Table 4.3 The depthwise variation in the $CaCO_3$ (%) content in the biocemented sand columns

Molarity of CRS	Sand columns	Depthwise range (mm) of $CaCO_3$ (%) content						Average $CaCO_3$ (%)
		0-10	10-20	20-30	30-40	40-50	50-60	
0.66 M	S_1	14	9.8	7.0	6.2	5.4	5.1	7.9
	S_2	12	8.6	7.2	6.3	5.1	5.3	7.4
0.75 M	S_3	15	9.3	8.1	7.7	6.1	5.3	8.6
	S_4	11	7.8	5.5	4.8	4.5	3.4	6.2

It was observed that specimen S_3, with the highest average calcite content of 15% at the upper 10 mm and an average of 8.6%, has the highest strength of 875 kPa. In addition, the percentage of $CaCO_3$ content in the various specimens at the upper 10 mm thick was ~ 2 times the average calcite content in the bulk of sand below it, i.e., within 10 to 60 mm, the average calcite content is 6.7%. Therefore, it can be concluded that there is more calcification in the upper zones than in the lower ones.

The stress-strain curves of the four biocemented sand columns (S_1, S_2, S_3, and S_4) obtained during UCS testing are shown in Figure 4.8. In addition, Table 4.3 highlighted the depthwise variation in the $CaCO_3$ (%) content in the biotreated columns at every 10 mm depth from the top surface.

Further, the results of the chemical analysis using the acid wash method of the 12 soil samples collected from the naturally biocemented products of rocks, stones, and caves were tested positive for the natural calcite content with 5–15%.

4.4.2 Permeability test results

The permeability tests were carried out on untreated and treated samples (1st, 7th, and 14th day). The initial permeability of untreated sand samples was 2.74×10^{-6} m/s. A 300 ml of 0.75 M CRS of the treatment solution was allowed to percolate through the sand columns S_3 and S_4 every 4 h. Initially, the solution was infiltrated rapidly through the sand columns S_3 and S_4 at 10^{-3}–10^{-4} m/s during the first 8 h. It was reduced to $0.6x10^{-4}$ m/s after five treatments during 8–24 h. Then, on the 7th day, the permeability was reduced to three order-of-magnitude (from 0.6 x 10^{-4} m/s to 1.1 x 10^{-7} m/s), which was approximately a 99% reduction. On the 14th day, the permeability was reduced to $0.7x10^{-7}$ m/s (100% reduction) compared to the initial day. This 100% reduction in soil permeability was due to forming a thin and water-impermeable biocemented crust layer with the calcite precipitates on the surface and a biocemented solid block in the bulk of sand below it. In addition, the calcites filled the pores and facilitated the soil consolidation process. Thus, the time of consolidation decreases with a reduction in soil permeability as treatment time increases. Chu et al., 2012 also reported a decrease in permeability to 14 mm/day (or $1.6x10^{-7}$ m/s) in both surface and the bulk of sand below it. This reduction was due to cementation mechanisms that coat or bridge individual soil particles that gradually reduce the pore throat, thereby lowering the hydraulic conductivity of soil (Al Qabany et al., 2012; Martinez et al., 2013). Whiffin et al., 2004 also used *S. pasteurii* cells that could reduce the permeability from $2x10^{-5}$ m/s to $9x10^{-6}$ m/s, and the improvement in UCS was 570 kPa. The results are in agreement with previous studies. Besides, the biocemented specimens can achieve significant strength while maintaining permeability. The retention of such soil permeability is advantageous for strength enhancement, as additional treatment injection can be possible for further

strength improvement. Hence, the amount of strengthening can be controlled and achieved. Kucharski et al., 2006 also demonstrated that MICP could be applied to the soil without disturbance to nearby structures, unlike other conventional methods such as deep soil mixing, soil replacement, and compaction methods. The enhanced strength of soil is achieved while maintaining permeability.

The bacterium *S. pasteurii* can also grow under subsurface conditions in sealing wellbore by using the enzymatic precipitation of $CaCO_3$ (Verba et al., 2016). The distribution of bacteria in slope soil is drastically decreased with the sloping depth since the number of cells per unit gram of soil at 10 mm below the slope surface is $1.4x10^6$, and the bottom slope is $5x10^3$. Therefore, it demonstrates that more bacterial cells were retained in the surface zone during the percolation process than in the lower depths and facilitates calcite precipitation for binding the sand particles. Therefore, the upper surfaces of the sloping surface consist of a thin and water-impermeable biocemented crust layer that may resist surface erosion and slope failure.

4.4.3 Sieve analysis for biotreated sand samples

Figure 4.9 shows the grain size distribution curves of the untreated soil and the different biotreated sand-column specimens. The observed values of sand particle sizes (D_{10}, D_{30}, and D_{60}) for different biotreated columns after sieve analysis for the grain size distribution curves are shown in Figure 4.9. In addition, the percentage increased in different grain sizes of each sand column calculated based on untreated soil having particle sizes (D_{10} = 0.15 mm, D_{30} = 0.21 mm, and D_{60} = 0.28 mm) are also presented in Table 4.4.

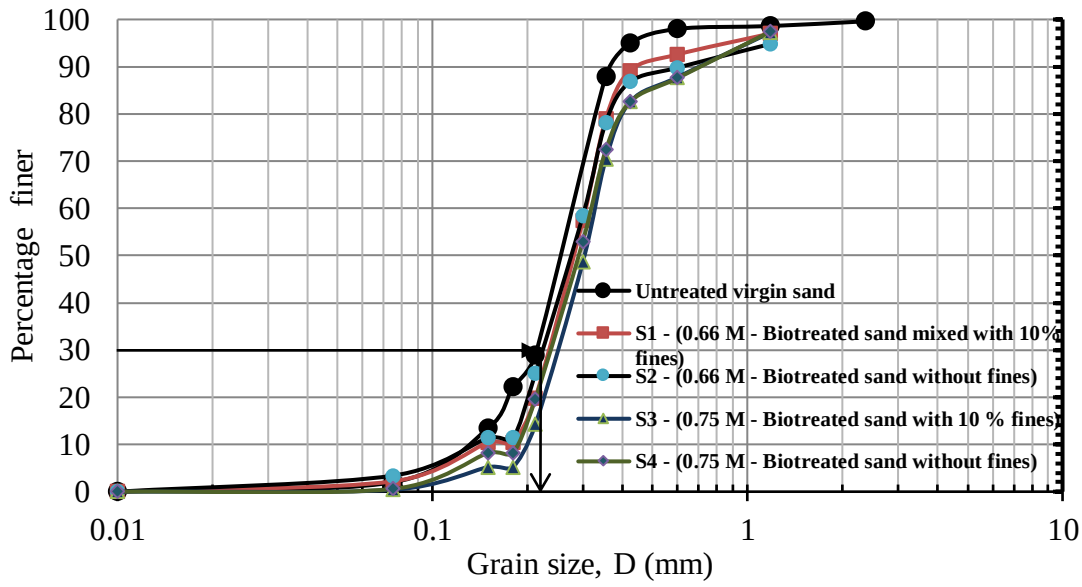


Figure 4. 9 Grain size distribution curves of the untreated and biotreated soils

Table 4.4 Percentage increase in grain sizes of the biotreated sand obtained in biocemented sand

Specimen types	Molarity of CRS	D_{10} (%)	D_{30} (%)	D_{60} (%)	C_u	C_c	Avg. calcite content (%) of 0-10 mm depth	UCS (kPa)
S_1 - (Type-B sand)	0.66 M	26	10	14	1.68	0.87	14	835
S_2 - (Type-A sand)	0.66 M	20	4.7	07	1.67	0.83	12	701
S_3 - (Type-B sand)	0.75 M	33	24	18	1.65	1.02	15	875
S_4 - (Type-A sand)	0.75 M	20	4.7	14	1.77	0.84	11	586

Table 4.4 shows a percentage increase in particle sizes (D_{10}, D_{30}, and D_{60}) in all sand columns. Grain sizes finer than 30% lie below 0.21 mm, while the effective size (D_{10}) is below 0.15 mm with 10% fine-grain content. The uniformity coefficient ranges from 1.852 to 3.307 for Yamuna sand with a silt percentage of 10%. In addition, a significant amount of biocementation is by the soil particles passing through D_{30} (sizes ≤ 0.21 mm), as depicted by the grain size distribution curves in Figure 4.9. The presence of higher fine particles provided more surface area to bind with the precipitated calcites. A significant percentage increase in particle sizes was observed for S_1 and S_3 columns, and both have gained higher UCS of 835 kPa and

875 kPa and average percentage calcite content (w/w) of 14 and 15% in the upper 10 mm depths. The S_2 and S_4 columns have almost similar calcite content even though S_2 has gained (~ 20%) more compressive strength than S_4 due to the formation of more uniform and effective calcite precipitates. Hence, the S_1 and S_3 sand columns with 10% fine particles would provide a higher surface area for binding.

Further, the higher calcite content and UCS value for the S_3 column treated using the percolation method have revealed its effectiveness over the submerged method. The average calcite content of the upper 10 mm portion of the treated specimens was between 11% and 15% and comparable to the range of calcite content between 5% and 15% in the naturally biocemented rocks, stones, and caves. Therefore, the shear strength parameters may be obtained for slightly cemented sand as recommended for silty sand using relative compaction (Ojha and Trivedi, 2013).

4.4.4 Scanning electron microscopy

Figures 4.10 and 4.11 show the scanning electron microscopy (SEM) images of the biocemented subsamples after UCS testing of the sand columns S_1 and S_2. The calcite distribution in the biocemented channel and the pond may be similar to the S_1 column treated under the same condition in a tank. At the same time, the S_2 column was treated in another reservoir by maintaining the same treatment condition as S_1. The SEM images of the S_1 and S_2 columns show the dense formation of calcite precipitates (i.e., in paste form of cementing materials, aggregates, and flakes of calcites). The calcite crystals are deposited at particle contacts (i.e., binding crystals) and on their (i.e., surface crystals) surfaces. The crystals at particle contacts are more effective for biocementation than those formed on their surfaces, as shown in Figure 4. 11(b). Effective calcite crystals play an important role in improving the strength

and stiffness of the soil. The detached crystals from the particle surfaces, in turn, clog in or fill the sand pores. The bioclogging process within the sand pores facilitates the reduction of soil porosity and thereby decreases the permeability of the soil. SEM images show the position of the crystals and have demonstrated that both biocementation and bioclogging processes occurred during MICP treatment. In Figure 4.10 (b), when the image was viewed on a 10 µm scale, biocementation was observed in the form of a cemented paste layer with numerous imprints of the rod-shaped bacteria. The bacterial cells might have burned out during the oven drying of the specimens before UCS tests and SEM imaging. Distinct calcite crystals are not visible but rather in the form of flakes of calcites aggregated together on the grain surfaces, as shown in Figures 4.10 (a) and 4.11(a).

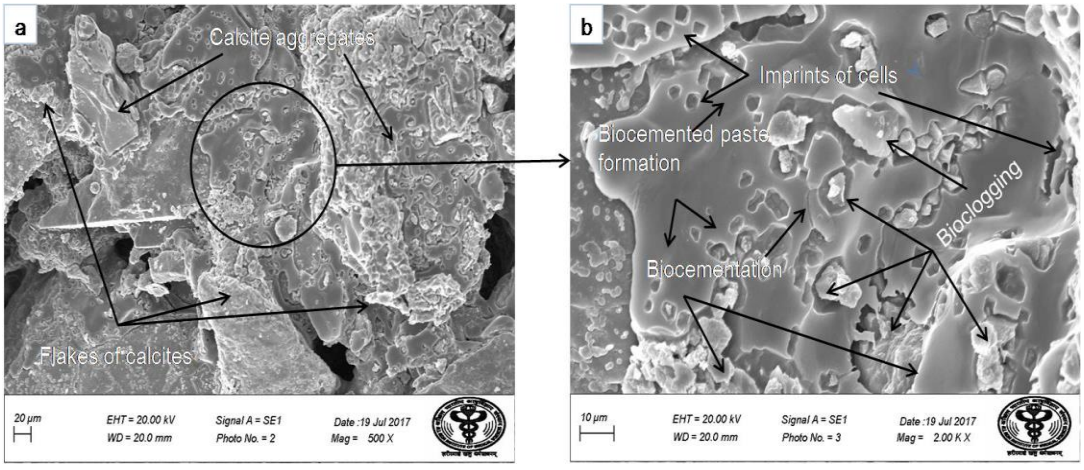


Figure 4.10 SEM images of the treated sand column S_1: **(a)** 500 x and **(b)** 2 K x.

Figure 4.11(b) shows non-effective calcite crystals attached to the grain surfaces and effective crystals that bind the particles. Thereby, the calcite precipitates facilitate both biocementation and bioclogging.

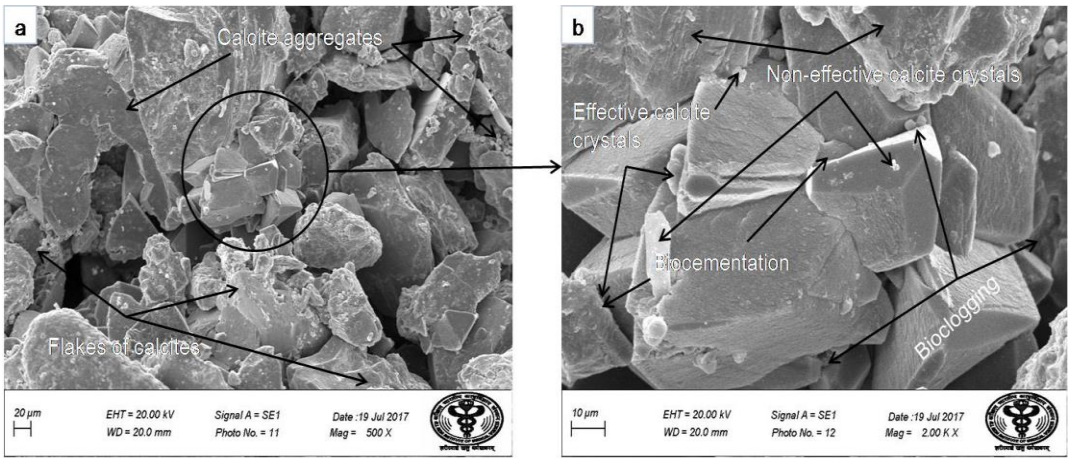


Figure 4.11 SEM images of the treated sand column S_2: **(a)** 500 x and **(b)** 2 K x.

4.5 CONCLUSIONS

This study investigated the performance and mechanisms of MICP in harnessing natural biogeochemical systems to provide sustainable solutions in increasing the strength and reducing the soil permeability to built-in structures on sandy soil formations. The specimens were treated using the submerged and surface percolation methods at two concentrations (0.5 M and 0.75 M CRS) using the strain *S. pasteurii* has improved the UCS by biocementation and reduced permeability by bioclogging with calcites. The conclusions of the present study are as follows:

- The specimens with 10% fine-grain content (Type-B sand) exhibit higher strength and stiffness (~19% for 835 kPa and 49% for 875 kPa in S_3 for submerged and surface percolation methods, respectively) than the specimens without fine content (Type-A sand), i.e., 701 kPa for S_2 and 585 kPa for S_4 columns. S_1 is treated similar to S_2 and S_3 to S_4 under similar treatment conditions. Filling the voids in the coarser sand with the 10% fine particle content may increase the density of the soil and provide more surface area for coating and binding the sand particles. It was found that a considerable increase in cohesion of treated soil can be achieved for soil samples with

maximum 10% fine-grain content (Hataf and Jamali, 2018). The binding of sand particles will further increase the strength of the soil. Similarly, the average calcite content increased by ~ 11.89% from S_1 to S_2 for submerged conditions and ~ 37.65% from S_3 to S_4 columns for percolation methods in specimens with Type-B sand than Type-A. The increase in calcite content and enhancement in soil strength may be attributed to the higher surface area of the sand grains due to fine particles coated with calcite precipitates and binding at particle contacts.

- The compressive strengths in S_1, S_2, S_3, and S_4 columns are 835 kPa, 701 kPa, 875 kPa, and 585 kPa, and such range of peak strengths may be classified as soft rocks. The mechanism of cementation and damage amid the grain binding and solidification may be assessed by a hardening-softening process active among the rock masses (Trivedi, 2013, and 2015; Trivedi et al., 2015). The average calcite content in the S_3 (8.6%) treated by the percolation method is higher than the S_1 (7.9%) column treated by the submerged method. There was a gradual top-down reduction in calcite content in all specimens. The average calcite content obtained in biocemented specimens was between 6.2% and 8.6%, which is in the range of 5% to15% calcite content of the natural biocemented products. Higher-strength and calcite content in S_1 may be due to a more uniform distribution of bacterial cells and higher urease activity within the pore fluids to react with CRS for calcite precipitation. In the case of the submerged method, the bacterial cells are more concentrated on the surface of the sand specimens and prevent further entry of the bacteria into the bulk of soil below the surfaces. The previous study showed less variation in compressive strength, calcite content, and grain sizes among the bio-treated

sand specimens at 0.66 M and 0.75 M CRS. Hence, using 0.66 M CRS may be economical in large-scale MICP treatment.

- After 24 h treatment, permeability reduction in sand columns S_3 and S_4 was $0.6x10^{-4}$ m/s treated under percolation methods, and their further reduction to three order-of-magnitude ($1.1x10^{-7}$ m/s) after 7 days of treatment. However, the permeability remained the same magnitude up to 14 days of treatment at $0.7x10^{-7}$ m/s. Hence, there was no significant reduction in permeability after 7 days. Therefore, the subsequent treatments beyond 7 days were considered a wastage. On the other hand, soils with permeability rates obtained after 7 days are comparable to acceptable leakage values reported for mariculture ponds (Weisburd and Laws, 1990; Bear, 1972) for very low permeability. Hence, the developed method would potentially reduce seepage in aquaculture ponds and channels constructed in sandy soil.
- The biochemical reaction of the bacterial cells with the reagents of the cementing solution was more in the surface zone, resulting in a high cementation zone forming a water-impermeable bio-cemented crust layer. Thus, the crust layer may ensure the long-term stability of the water bodies such as the channel and the pond against surface erosion, seepage loss, and reduction in permeability. In addition, the calcite precipitates bind the sand grains into coarser particle sizes, which can provide good mechanical properties to resist wind-erosion, rainfall-erosion, moisture, and ecological compatibility in loose sandy soil regions.
- The urease activity to hydrolyze urea depends on the pH of the treatment solution (CRS). The biochemical reaction to precipitate calcite is ensured if the pH was ≥ 7 during the MICP process, and it was varied in the range of 7 to 8.5

during 7 days of treatment. However, the pH remained below 7 even after the daily addition of new doses of BS and CRS after 7 days. Thus, lowering pH may be due to a slowdown of urease activity as treatment time increases beyond 7 days. A similar observation was observed for a slowdown of the urease activity after 5 days due to encapsulation of bacterial cells by calcite precipitates as treatment time increases, resulting in the death of bacteria (Zhao et al., 2014).

The experimental results demonstrate that the MICP treatment process effectively reduces the permeability to prevent seepage from aquaculture ponds and channels. It also stabilizes the sand surfaces by forming biocemented crust layers to resist surface erosion and prevent seepage. The biocemented soils may prevent levee failure due to overtopping and surface-erosion; and sub-grade water infiltration during road construction. The bio-stabilized crust layer forms water-resistant barriers by impeding the penetration of gravity water and capillary moisture. The low viscosity of biocemented solution can penetrate the ground without mixing with the soil to simplify the construction process. The potential application of the methods presented here could include constructing runoff collection ponds in deserts, watershed areas, aquaculture ponds, or algae biofuel production ponds and channels in sandy soil formations.

CHAPTER 5

Engineering Behaviour of MICP Treated Sand Columns by Sterile and Non-Sterile Treatment Solution

Abstract

Microbially induced calcite precipitation (MICP) is one of the innovative and sustainable soil treatment methods for improving the engineering properties of soil. Laboratory experiments were conducted in soil columns to analyze the influence of sterile and non-sterile treatment solutions as cementation reagent solution (CRS) to form calcite precipitate within the soil matrix for biocementation and bioclogging process within the soil particles. The specimens were treated using a bacterial solution (BS) and a CRS containing urea and $CaCl_2$. After biocementation, the unconfined compressive strength and direct shear test were conducted to analyze the engineering properties of the soil. The results demonstrated that the specimens

treated with sterile solution achieved higher UCS, cohesion, and friction angles than the non-sterile specimen. In addition, the formation of $CaCO_3$ was identified by micro characterization using SEM images.

5.1 INTRODUCTION

The biological technique utilizes the metabolic activities of microorganisms to produce microbially induced calcium carbonate precipitates as calcites in large soil deposits for improving the engineering properties of loose and collapsible sandy soils. Calcite is a biomineralization reaction product of bacterial enzymes, nutrients, and cementing materials present in subsurface soils. Many researchers have carried out studies on applications of MICP for soil improvement in loose sandy soil formations to transform into calcium carbonate sediments, deposits, and rocks. Therefore, the calcite present in the soil plays a vital role in improving the strength and stiffness of incompetent soil by binding the sand at particle contacts.

When a construction activity has been accomplished on the soft soil, cement or chemicals are used as a binder to increase its bearing capacity. Grouting is another ground improvement technique used to increase soil bearing capacity by injecting a mixture of cement, bentonite, silicate, or other chemicals with water. Grouting can either displace soil for compaction, mix with soil under high pressure, or penetrate the soil, filling the void between soil grains (Karol, 2003). However, cement or chemicals for soil improvement is expensive and time-consuming. The MICP is an environmentally friendly biogrout that injects the soil, similar to the grouting fluid mixture. Biogrouting hardens and strengthens the sand formations and allows water to penetrate them, which is impossible in silicate cement. In addition, biogrouting is

advantageous to strengthen soil where space is limited to avoid disturbance and vibration to nearby structures.

During the MICP process, CO_2 sequestration and storage through solubility and mineral trapping of carbon dioxide induced by bacterial ureolysis and carbonate formation; thereby, the rate of production of $CaCO_3$ is directly proportionate to CO_2 released (Milleet et al., 2012). In addition, Okwadha and Li, 2010 also found that the CO_2 sequestration rate caused by ureolytic hydrolysis was directly proportional to microbial $CaCO_3$ formation.

The process of diagenesis and biocementation has undergone in biocemented sand increases the contact area between particles and bondage between neighboring particles. Therefore, a light biocementation is often sufficient to significantly increase the small-strain stiffness of soils, dilative tendency, and liquefaction resistance. Moreover, a higher small-strain stiffness of cemented soils may hinder the early build-up of pore-water pressure and, consequently, reduce the liquefaction potential of sandy soil formations.

Cementation can profoundly affect analysis and design, but the misinterpretation of cementation effects can lead to unsafe design. Loose, lightly cemented soils often exhibit collapse, and a progressive failure may accompany shear. Therefore, it is necessary to significantly achieve a targeted cementation level in loose sands to stiffen soils using the MICP treatment method. In addition, it may benefit from reducing the cost of improving the engineering properties of initially loose and collapsible sand formations into a cemented soil matrix of higher strength.

This chapter presents the study on the effect of MICP treatment in soil using sterile and non-sterile treatment solution as cementation reagent solution (CRS) for calcite precipitation and the strength improvement in the biotreated specimens. In

addition, considerable importance is taken for bacterial cell concentration, nutrients, the molar concentration of urea and calcium chloride, and other environmental factors such as temperature and pH to generate calcite precipitates.

5.2 MICROBIALLY INDUCED CALCITE PRECIPITATION (MICP)

The MICP is a biomineralization reaction process to precipitate calcites in-between and around sand particles as a metabolic by-product of urease-producing bacteria. Urease-producing bacteria are also known as ureolytic bacteria. Calcite is an insoluble and thermally stable cementing material. The biomineralization process is possible using the urease enzymes released by the highly active urease-producing bacteria to induce the calcite precipitates. In the present study, the urease-producing bacteria used for the MICP process is *S. pasteurii* (MTCC 1761), a soil-based bacteria and non-pathogenic that can simulate indigenous field conditions. These bacteria can move through pore thresholds larger than their sizes. In addition, cells of *S. pasteurii* do not aggregate, which results in a high cell surface-to-volume ratio, which is essential for effective cementation initiation of calcium carbonate. *S. pasteurii* at the time of injection is about 1–3 μm enabling free passage through a selected sand pore structure. When extracellular calcite precipitation forms, the microbe size enables expansion to approximately 20 times its original, hindering the mobilization of calcite solutes if detached from the soil particles. The non-pathogenic and soil-based bacteria *S. pasteurii* were chosen to simulate indigenous field conditions.

As outlined in previous chapters, a MICP reaction occurs between bacterial cells and a cementation reagent solution (CRS) containing urea and $CaCl_2$ as reagents in the pore fluid. The specimen consists of sand columns. The specimens

were inoculated with cell concentrations representing both sufficient urease proteins and crystal nucleation sites. Free urease enzyme in the aqueous environment hydrolyzes urea into ammonium and carbonate ions, thus, increasing the pH of the local environment and shifting the carbonic acid equilibrium to form carbonates. Carbonates react with Ca^{2+} present in CRS to form $CaCO_3$ as calcite within the interparticle spaces and bacterial surfaces. Thus, the precipitated calcites bind the sand particles, thus increasing the strength and stiffness of the treated soil. In addition, the calcite materials fill the pore spaces, thus reducing the pore volume and permeability. The urease enzyme can facilitate a biochemical reaction to precipitate as $CaCO_3$ (Kroll, 1990). In the presence of calcium, or other divalent cations, precipitation of solid carbonate species occurs once a critical saturation state has been reached for the precipitation of $CaCO_3$. Meanwhile, CO_2 produced is trapped in $CaCO_3$, preventing to release of CO_2 into the atmosphere to mitigate carbon sequestration. Rebata-Landa , 2007 showed a relation between grain size and $CaCO_3$ content, and the maximum carbonate deposition observed on grains was approximately 100 μm in size.

Along the Indo-Gangetic planes, the Indian subcontinent has vast deposits of silty sands along the bank of perennial Himalayan Rivers, where the river sands are obtained with varied proportions of non-plastic silts. The sand and silt are coarse and fine-grained granular materials per soil classification systems. They occur with varied surface textures and shapes ranging from angular to spherical with moisture in void space. Yamuna sand is chiefly found alongside the river Yamuna in Delhi. It has a low load carrying capacity and also gets eroded easily. Moreover, Delhi lies in seismic zone 4, which has a high risk of earthquake damage.

Moreover, the Yamuna river basin flowing through the National Capital Region of Delhi contains a remarkable quantity of silt consisting of 0-20% fines. Thus, it is susceptible to liquefaction below the groundwater table during earth tremors. In several places, groundwater is shallow, and structures built over sand under such conditions are not safe. Therefore, understanding the shear behaviour of Yamuna sand and its improvement in shear strength is necessary. Therefore, the MICP technique is one of the alternatives to the conventional methods of improving sandy soils by blending them with biocement, which is eco-friendly and cost-effective.

The study aims to compare the effectiveness of MICP treatment using the sterile and non-sterile treatment solutions in two sand columns. The first sand column was treated with a sterile treatment solution, whereas the second column was with a non-sterile solution prepared using tap water. Soil properties of the treated specimens may depend on the quality of the treatment solutions.

5.3 MATERIALS

5.3.1 Analysis of Sand

The two sand columns, namely C_1 and C_2, were prepared using the Yamuna soil. The C_1 column was treated with a non-sterile CRS solution, while the C_2 column with a sterile CRS solution. Soil samples were first sieved through a 2 mm sieve size and washed under tap water (pH = 7.7) using a 75 µm sieve size. The portion of sand retained on the 75 µm sieve was kept immersed in water for 24 h to remove any unwanted material and then oven-dried at 105°C till its weight remained constant. In addition, the soil particles finer than 75 µm (10%) was also oven-dried. The

specimen soil was a mixture of 90% clean sand and 10% fine-grained and packed in PVC columns to prepare the sand columns.

The sands grains were angular to sub-angular quartz. The particle size analysis followed the IS: 2720 - Part IV: 1985. The grain size distribution (GSD) curve of the Yamuna river basin is in Figure 5.1 (a). The curve lies between the boundaries of potentially and most liquefiable soils, which signifies that the untreated soil used in the present study is susceptible to liquefaction under seismic conditions (Tsuchida, 1970). In addition, the soil from the Yamuna river basin was classified under Type D - a liquefiable soil as per the seismic map where Delhi is in Zone IV of the seismic zone.

The curvature coefficient (*Cc)* and uniformity coefficient (*C*u) of the soil were calculated as 1.05 and 1.33, respectively, and according to the IS classification system (IS:1498-1970 2002), the soil was poorly graded sand (SP). The three parameters, namely $C_{u,}$ $C_{c,}$ and $D_{10,}$ were the characteristic parameters that influence the particle arrangement and the inter-particle gaps, determining the adequate bonding of particles with $CaCO_3$. In addition, D_{10} is a characteristic size for the gap between particles ≤0.15, and the mean grain size (D_{50}) was 0.22 mm. The relative density of sand was determined according to IS: 2720-Part XIV: 1983 and calculated in the range of 30%–80%, indicating the soil collected from the Yamuna river basin to be in a loosest to the densest state. The specific gravity of sand was 2.65, and the PI value was less than 12 or non-plastic.

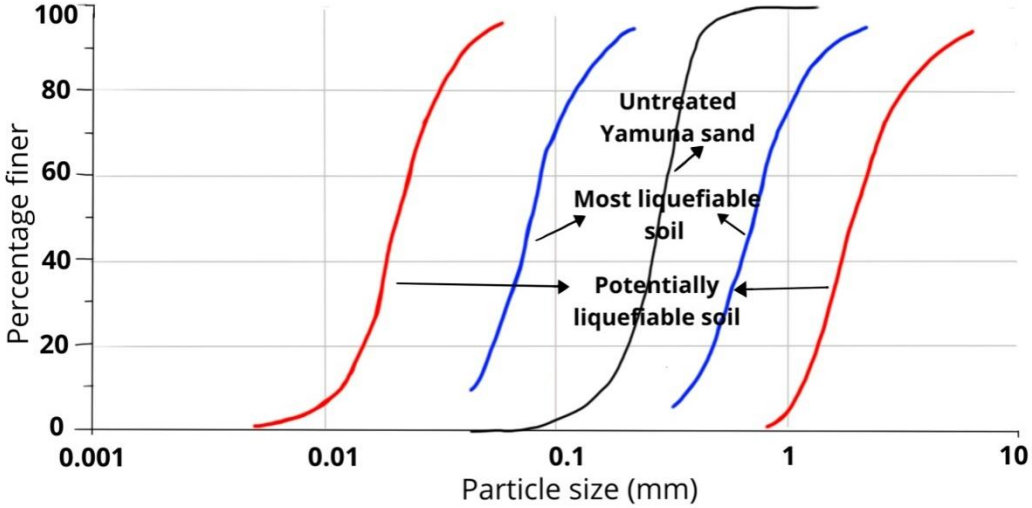


Figure 5.1 Particle size distribution curve of the untreated soil from Yamuna river basin is lying within the liquefiable soil boundaries as depicted by Tsuchida, 1970

Figure 5.2 shows the maximum dry density of the sand to be 15.5 kN/m^3 and the optimum moisture content (OMC) at 10% using the Standard Proctor Compaction Test of soil (IS 2720 - Part-VII: 1980).

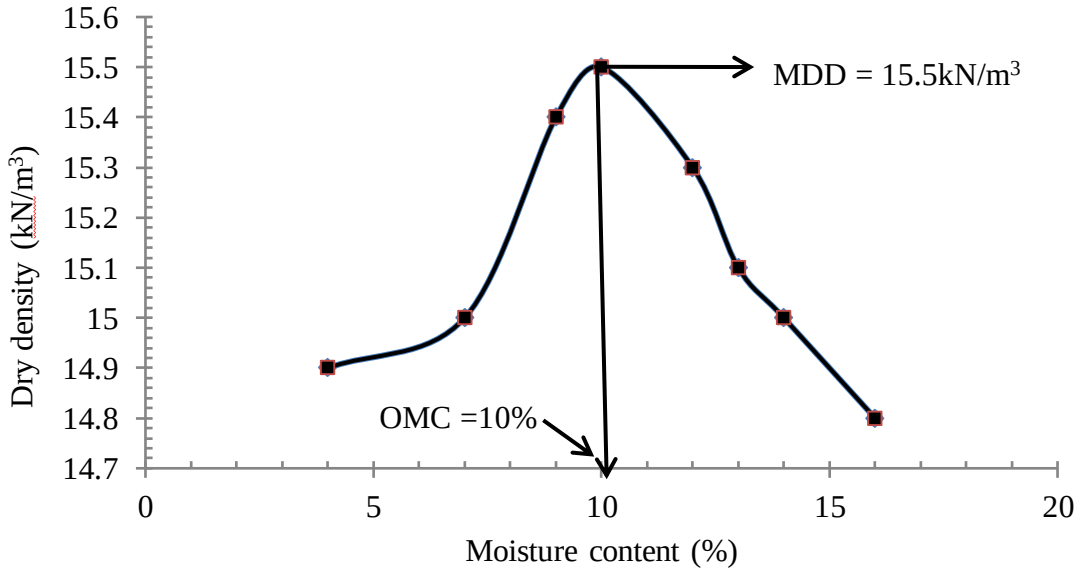


Figure 5.2 Dry density versus moisture content of the untreated soil

5.3.2 Preparation of Bacterial Cell Suspension

S. pasteurii strain bacteria is grown in nutrient broth (NB) medium, a general medium that serves as the nutrient for the bacteria to stimulate cellular metabolism. The preparation of the initial inoculation using growth cells was possible by using the bacterial strain in powdered form procured from MTCC Chandigarh, India.

Table 5.1 Composition of the bacterial solution as was used in MICP treatment

Urea (g/L)	$CaCl_2$ (g/L)	NB (g/L)	NH_4Cl (g/L)	$NaHCO_3$ (g/L)
20	11.25	3	10	2.12

The bacterial strain *S. pasteurii* (MTCC 1761) was grown under sterile conditions to obtain a significant cell concentration according to the protocol provided by the supplier. The test liquid consisted of 3g of nutrient broth, 10g of NH_4Cl, and 2.12g of $NaHCO_3$ per liter of de-ionized water, as shown in Table 5.1, and was sterilized in an autoclave at 121℃ for 20 min. The pH of the medium was adjusted to 6.5 before sterilization by 1N HCl. After autoclaving, the test liquid was cooled down to 35°C. Next, the bacterial cells were inoculated to the liquid media and added 20 g of urea ($NH_2(CO)NH_2$). Resulted test liquid was transferred to 250 ml Erlenmeyer flasks and was incubated at 35℃ on horizontal shakers at 120 rpm for 48 hours. Then, the flasks were removed from the incubator and stored at 4°C. Test liquid without cells was stored in a test tube and was incubated in parallel to control the contamination.

The optical density (OD_{600}) of the bacterial strain was measured through a spectrophotometer at 600 nm. The OD_{600} of *S. pasteurii* was in the range of 1.0–1.2. Bacterial cell concentration was calculated by OD_{600}, which signifies the growth of living bacteria cells after the incubation process. The bacterial cell concentration (Y)

present per ml of solution can also be calculated using the following formula (Ramachandran et al., 2001).

$$Y = 8.59 \times 10^{7} \times OD_{600}^{1.3627} \quad (5.1)$$

The centrifugation of bacterial solution, removing the supernatant, and replacement with a fresh nutrient solution was avoided to save the cost, energy, and time for large-scale application; thus, centrifugation was not used for application in the PVC pipe setup. Instead, the cultured bacteria solution was augmented directly without centrifugation.

Before use, 11.25 g/L of calcium chloride ($CaCl_2$) was mixed thoroughly with the bacterial solution (BS), and the pH was adjusted to 6.5. Mixing 11.25 g/L of $CaCl_2$ enhanced the aggregation of bacterial cells and their attachment to the sand grains after being added to sand (Stabnikov, 2011). The urease activity of the culture was between 13 to 18 µM urea/min. Next, 300 ml of the BS was injected at the top of the sand columns and percolated through its depth. The bacterial cells were allowed to retain for adsorption on the sand surface for 8 hours; then, the subsequent treatments were continued with the cementation solution.

5.3.3 Preparation of cementation reagent solution

Two cementation reagent solutions were prepared using sterile distilled water or non-sterile tap water. The non-sterile conditions would represent typical field conditions to examine its feasibility for treatment on a large scale. Urea (NH_2-CO-NH_2) and calcium chloride ($CaCl_2$) were used as reagents in the preparation of the cementation reagent solution (CRS) solution at a molar concentration of 0.66 M. The urea-Ca^{2+} molar ratio was at 1:1. The pH of the calcium source solution was adjusted to 6.5 by

1 N HCL to avoid the formation of $CaCO_3$ in the amorphous form before starting the actual reaction. The composition of the cementation reagent solution is shown in Table 5.2.

Table 5.2 Composition of cementation reagent solution

Molar Concentration	Urea (g/L)	$CaCl_2$ (g/L)	Solution used	pH	Condition
0.66 M was adjusted to pH=6.5 by 1N HCl	39.6	97.02	Distilled water	7	Sterile
	39.6	97.02	Tap water	7.7	Non-sterile

From the previous study, as outlined in Chapter 4, there was an insignificant variation in calcite content and strength in the specimens treated with 0.66 M and 0.75 M; therefore, to avoid wastage and to be economical for large scale treatment, a 0.66 M CRS was adopted for the cementing solution consisting of urea and $CaCl_2$, which was also the optimum concentration obtained by Al Qabany et al., 2012. Further, the strength improvement was retarded at a higher concentration of 1 M. The reason is the high salinity of the reagents that have an inhibitory effect on the microbial activities (De Muynck et al., 2010). Al Qabany and Soka, 2013 obtained a higher strength in the specimens treated with lower concentrations below 1M due to a more uniform distribution of calcite precipitates that binds at particle contacts.

The urea and $CaCl_2$ at a molar ratio of 1:1 were added as reagents in the treatment solutions at a concentration of 0.66 M. First solution was prepared using the sterile de-ionized water for treatment of column C_2 and the second solution using the non-sterile tap water was for treatment of column C_1. De-ionized water was sterilized in an autoclave at 121°C for 20 minutes before mixing the reagents. In both the solutions, urea was added post autoclaved by 0.22 millipore filter to avoid

thermal decomposition, while $CaCl_2$ was added immediately before their application. The purpose of treating soil columns using sterile and non-sterile treatment solutions was to test which solution gives more calcite content and strength improvement while reducing permeability. In addition, treatments were done to check the feasibility of using non-sterile tap water for large-scale treatments.

5.4 Methods

5.4.1 Sand Column Preparation

The sand specimen was prepared by mixing 10% fine contents (i.e., < 75μm) with 90% clean sand (i.e., sizes > 75 μm) to pack inside the PVC columns to mimic the field conditions. The column to be treated with the non-sterile solution was designated as non-sterile column C_1, whereas the one treated with the sterile solution was designated as sterile column C_2. The PVC columns were chosen as molds to avoid unwanted chemical interference during the biochemical reaction. The C_2 column was packed with sterile sand after being autoclaved at 121℃ for 20 minutes, while the C_1 column was with non-sterile sand. The sands were allowed to flow freely through a funnel to fill the molds to prepare C_1 and C_2 columns with 1000 g and 700 g of soil specimen. The sands were packed into the column in three consecutive layers, ensuring that each layer was compacted evenly to achieve at least 95% of the maximum dry density to maintain the consistency of the experiments. The resulting sand column size for C_1 was 105 mm diameter x 80 mm depth and for C_2 was 105 mm diameter x 60 mm depth, as shown in Figures 5.3 (a) and (b), respectively. The mold height was higher than the sample height to allow ponding of solution during biotreatment.

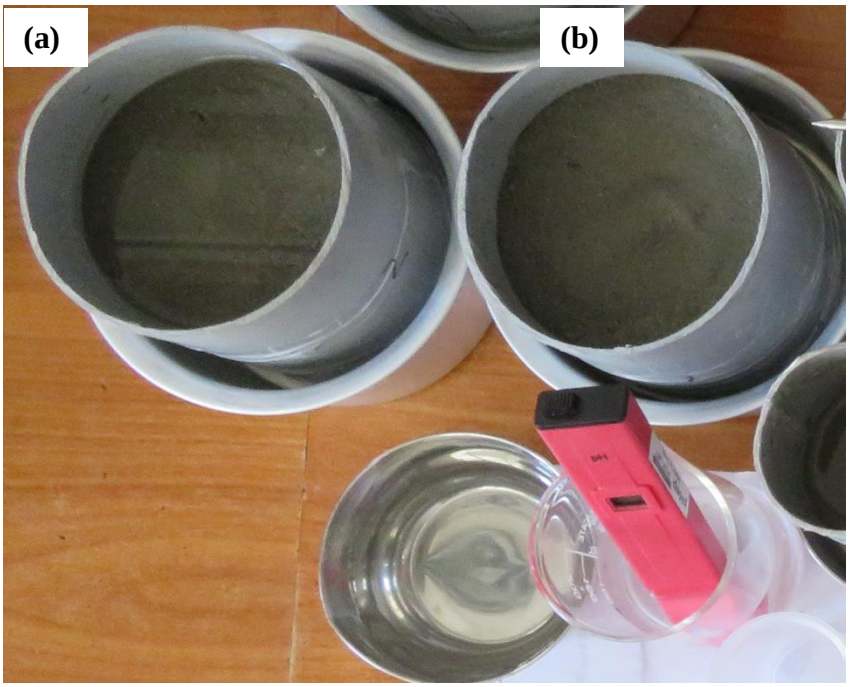


Figure 5.3 (a) C_1 column (105 mm diameter x 80 mm height); **(b)** C_2 column (105 mm diameter x 60 mm height)

The difference in the column sizes has no significant effect on biotreatment and was a random choice. Treatment solutions were applied on the sand columns C_1 and C_2 using the surface percolation method. The PVC molds consisted of two vertically cut parts glued with temperature-resistant tape from the exterior and filled the interior gap using the standard paste to avoid leakage. The treated specimens can be removed by opening the vertical cuts. The bottom of the columns was closed with perforated plastic-woven sheets to prevent fine particles and bacteria from flushing with the effluent solution.

5.4.2 MICP Treatment Method

The initial requirements to conduct the MICP process in sand-packed PVC columns are:

i. The preparation of sand-packed column specimens inside PVC molds

ii. The preparation of bacteria cell solution (BS)

iii. The preparation of cementation reagent solution (CRS) consisting of Urea-$CaCl_2$ as two chemical reagents

iv. The application of BS followed by CRS in subsequent treatments

v. The monitoring of the pH and temperature of the treatment solution

Initially, the sand columns within the PVC molds were inoculated with 150 mL of the bacterial solution (BS). The solution was allowed to retain for 8 hours to give sufficient time to attach the cells to the particle surfaces. Later, in subsequent treatments, the cementation solution was allowed to percolate through the specimens. From the previous study, outlined in Chapter 4, the calcite content and compressive strength of the MICP treated specimens by surface percolation gave better results than by immersion method. Therefore, the surface percolation method was used as a suitable method for large-scale treatment. The treatment duration was kept for 14 days. Moreover, a prolonged treatment beyond 14 days has no additive gain in calcite content and compressive strength, as observed in the previous study (Shougrakpam and Trivedi, 2021).

The non-sterile solution is used as a treatment solution to investigate its efficacy for large-scale in-situ treatment because treatment with a sterile solution is possible only in laboratory scale treatments. Therefore, the experiments were performed at room temperature that ranged between 20°C and 35°C in February-April in Delhi. Such a temperature range is within the optimum temperature range of 24°C and 37°C, suitable for microbial carbonate precipitation, as recommended by Okwada and Li, 2010.

During treatment with the cementation solution, 300 mL of the CRS was introduced in a cycle of 8 hours and reserved on top of each column before its percolation using the non-sterile solution in the C_1 column and with the sterile

solution in the C_2 column. The percolated effluent solution was collected in a jar, and measured the pH and the temperature. The pH was found to vary from 7 to 8.5 in both the samples from the initial treatment till the seventh day. However, in subsequent treatments beyond 7 days, pH was dropped below 7 even after adding a fresh dose of 150 mL of the BS on the 8^{th} day. It was an attempt to increase the bacterial activity to release the urease enzymes for uninterrupted urea hydrolysis for the further precipitation of $CaCO_3$.

Zhao et al., 2014 also found that slowing down the bacterial activity after 5 days may be due to the encapsulation of bacteria and hence the death of enzyme-releasing bacteria. After 14 days of treatment, both the columns were cured for another 14 days by submersing in non-sterile tap water to examine the possibility of decementation in the biocemented sands. After curing, the columns were removed from the water and air-dried for 5 days. Later, the specimens were oven-dried at 105° C for another 5 days before the UCS test, following Choi et al., 2016. The oven-dried specimens were used for UCS tests. After UCS tests, the sand was used to measure the $CaCO_3$ content using the acid wash method.

5.4.3 Unconfined compressive strength (UCS) tests

The UCS testing was carried out on the biotreated specimens as per IS:2720 - Part 10 :1991 to estimate the shear strength of the soil treated for 14 days. First, the specimens were sheared at a strain rate of 1.25 mm/min. Then, the specimens were oven-dried for 24 h to discontinue the microbial activity and remove moisture. The resulting biotreated specimens C_1 and C_2 are as shown in Figures 5.4 (a) and (b).

(a) **(b)**

Figure 5.4 Oven-dried biotreated specimens (a) C_1 column; and (b) C_2 column

5.4.4 Direct shear tests

A standard direct shear box test of 60 mm x 60 mm was carried out on biotreated soils as per IS: 2720 - Part 13:1986 to determine the shear strength of biotreated sand specimens. The biocemented sand was pulverized and oven-dried before filling the direct shear chambers. The soil specimens were compacted in the shear box in three layers of equal thickness by tamping on the top of each layer with a wooden tamper. The number of tamps per layer was adjusted until the dry density equaled 90% of the maximum dry unit weight (γ_{dmax}) determined by Standard Proctor Compaction. The shearing of the soil was at a constant displacement rate of 1.25 mm/min. Shear strength was at peak stress for all direct shear tests.

5.4.5 Determination of calcium carbonate content

The samples of the biocemented soils after UCS tests from each column, C_1, and C_2, were collected from the top, middle, and bottom portions of the two soil columns.

The quantitative measurements of the carbonate content in the soil specimens were carried out by gravimetric analysis using the acid-treatment weight loss technique.

5.4.6 Microscopy

The fractions of the biotreated soil columns, $C_{1,}$ and $C_{2,}$ obtained after the UCS tests, were used for imaging by scanning electron microscope (SEM). The SEM images can observe the morphology and distribution behaviour of the calcites precipitated within the biocemented soil matrix. The specimens were oven-dried at 60°C for 1 h before using sputter-coating with silver. The coating was done using an Agar Sputter Coater before taking the SEM images to view the behaviour of calcite precipitates and their distribution patterns.

5.5 EXPERIMENTAL RESULTS AND DISCUSSION

5.5.1 Analysis of calcium carbonate content

The samples were collected from the top, middle, and bottom layers of the biotreated columns to investigate calcite precipitation's spatial distribution and uniformity throughout the column depths. Table 5.3 shows the calcite content variations in specimens biotreated for 14 days in sterile and non-sterile solution, and the calcite precipitation occurs uniformly throughout the biotreated sand columns. This is an important observation as the calcite content uniformity is the major challenge of the MICP technique. As shown in Table 5.3, the calcite content of the biocemented specimens was almost similar in each layer, with a decreasing trend from the top to the bottom layers. However, a higher accumulation of calcite precipitation was observed in the top layers.

Table 5.3 Variation in the three-layer calcite content of biotreated columns

Columns	Top (%)	Middle (%)	Bottom (%)	Average (%)
C_1	8.5	8.2	7.8	08.17
C_2	11.5	11.3	11.2	11.33

The uniform distribution of calcite precipitation has indicated an efficient biochemical reaction with the cementing solution using the surface percolation technique. The average calcite content was 38% more in column C_2 than in the C_2 column. The higher calcite content in the C_2 column may be for treatment under sterile conditions. The sterile condition gives a more effective biochemical reaction for calcite precipitation than the non-sterile column C_1. Therefore, the results have indicated that treatment, even with non-sterile tap water, could release a significant amount of calcites, making it promising for its use in large-scale applications. Furthermore, using non-sterile tap water will make the treatment more economical by skipping the sterilization steps and reducing costs.

5.5.2 Grain size distribution of biotreated soils

Grading curves can indicate the geometric properties of soils and is very useful for soil description. The biotreated specimens used after direct shear tests were used for sieve analysis to check for increased in particle sizes in biotreated specimens.

Table 5.4 shows a percentage increase in grain sizes of D_{60}, D_{50}, D_{30}, and D_{10}, C_u and Cc values of the biotreated sand grains due to bridging of sand grains by calcites at particle contacts due to biocementation. The values of C_u are 1.75, 1.78, and 1.70 for the untreated soil, C_1, and C_2 columns, respectively. The range of C_u values is very close to each other, indicating that the sandy soil is uniform and has very narrow particle size ranges. The C_c values are 1.08, 0.92, and 1.15 for untreated

soil and biotreated C_1 and C_2 columns, respectively. The range of C_c values is less than 6, indicating to be poorly graded soils.

Table 5.4 Percentage increase in grain sizes of the biotreated soils

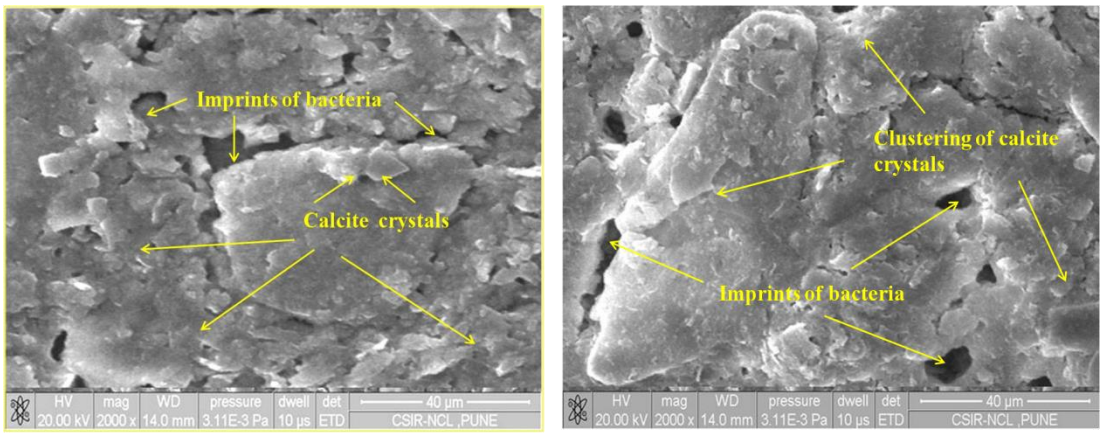
Grading sizes	Untreated soils	Biotreated grain sizes (mm)		The percentage increased in grain sizes	
		C_1 column	C_2 column	C_1 column	C_2 column
D_{60}	0.28	0.32	0.34	14.28	21
D_{50}	0.26	0.28	0.32	07.69	23
D_{30}	0.22	0.23	0.28	04.54	27
D_{10}	0.16	0.18	0.20	12.50	25
C_u	1.75	1.78	1.70	–	–
C_c	1.08	0.92	1.15	–	–

Table 5.4 has observed that the particle sizes of D_{60}, D_{50}, D_{30}, and D_{10} of treated C_1 and C_2 have increased as compared to untreated soils. The percentage increase in grain sizes was ranged between ~ 4–14% and 21– 27% for C_1 and C_2 columns, respectively. Also, a higher percentage increase in grain sizes was observed in different grain sizes of C_2 than in C_1 column as compared to untreated soils. The results have indicated that MICP plays a vital role in increasing particle sizes and roughness. The growth in particle sizes may be due to the encapsulation of sand surfaces or bridging of sand grains by the calcite precipitates. In addition, the precipitated calcites filled in and clogged the pore spaces reducing the pore volume but increasing the density. The decrease in pore volume will reduce the pore pressure and soil permeability.

5.5.3 Scanning electron microscopy

SEM images revealed the formation of $CaCO_3$ crystals present in the biotreated specimens. Despite the apparent exterior resemblance between samples treated with

the same chemical concentration of 0.66 M CRS, it is clear from the SEM images that the calcite distribution is different at the microscale in C_1 and C_2 columns, as shown in Figures 5.5 (a) and (b).



(a) (b)

Figure 5.5 SEM images of the biotreated specimens (a) Column C_1 and (b) Column C_2 (magnification – 2000 X)

Figures 5.5 (a) and (b) show the presence of calcite crystals, and imprints of bacteria can also be seen in the biocemented soil mass. SEM images were taken at a similar magnification of 2000 X to discern the calcite crystals under different biotreatment conditions. In addition, the presence of microbial beds can also be seen with numerous imprints of the bacteria. These microbial beds were found in 1–5 mm size and appeared on the calcite crystal surfaces in hollow cylindrical spaces. In both C_1 and C_2 columns, calcite distribution is observed on the surface and in-between the sand grains, and also the calcite crystals are clustered together in agglomerates. Heavy cementation is observed more in the C_2 column than in the C_1 column. The reason may be due to a more effective biochemical reaction between the bacterial cells and the reagents in the sterile solution for calcite precipitation than in the non-sterile solution. Moreover, the images were taken using the specimens preserved for 36 months and found that the cementation between the grains and among the calcites

has remained intact. Therefore, the soil treatment using the MICP method may have the potential for application in long-term solidification and hardening of soil to increase the soil strength.

The bacterial activity of *S. pasteurii* has resulted in the growth of large size crystals due to high urease production and the generation of calcite precipitation within the soil mass. The variations in the crystal sizes are related to the competition of growth of already formed crystals and the new ones. The generation of new crystals and the growth of old crystals depend on bacteria and the reagents in the cementing solution. Therefore, heavy precipitation is observed in the C_2 column than in the C_1 column, which can directly influence the strength behaviour in the biotreated specimens

5.5.4 Unconfined compressive strength tests

The oven-dried biotreated soil columns C_1 and C_2 were used for UCS testing, as shown in Figures 5.6 (a) and (b), respectively. The mounting of the specimens C_1 and C_2 for axial loading is in Figures 5.7 (a) and (b), respectively. Finally, the failure of the specimens due to the application of incremental loads is as shown in Figures 5.8 (a) and (b), respectively.

The unconfined compressive strength of the soil is the peak stress or the stress that yields 20% of axial strain, whichever is lower. In Figure 5.9, there is a gradual rise in the stress as strain increases in column C_1 and C_2 before reaching their respective peak stresses of 835 kPa at 2.75% axial strain and 1630 kPa at 2.5% axial strain. For normal application, the UCS strength required is less than 3 MPa. The characteristics of the curves are similar to the findings of Mortensen et al., 2011. The MICP treated soil always fails at the low axial strain during UCS testing, and the

axial stress was dropped quickly after collapse during shearing. When the load was axially applied to the soil columns during UCS tests, brittleness and decementation of the biocemented soil particles were initiated in depth between 10 mm–20 mm below the top surface before the collapse. The degradation of cohesion of biocemented soils was observed with increasing inelastic deformation, which may occur before peak strength is reached.

(a) (b)

Figure 5.6 Treated specimens for UCS testing (a) C_1 column; and (b) C_2 column

(a) (b)

Figure 5.7 (a) Mount C_1 column; and (b) Mount C_2 column to apply the axial load

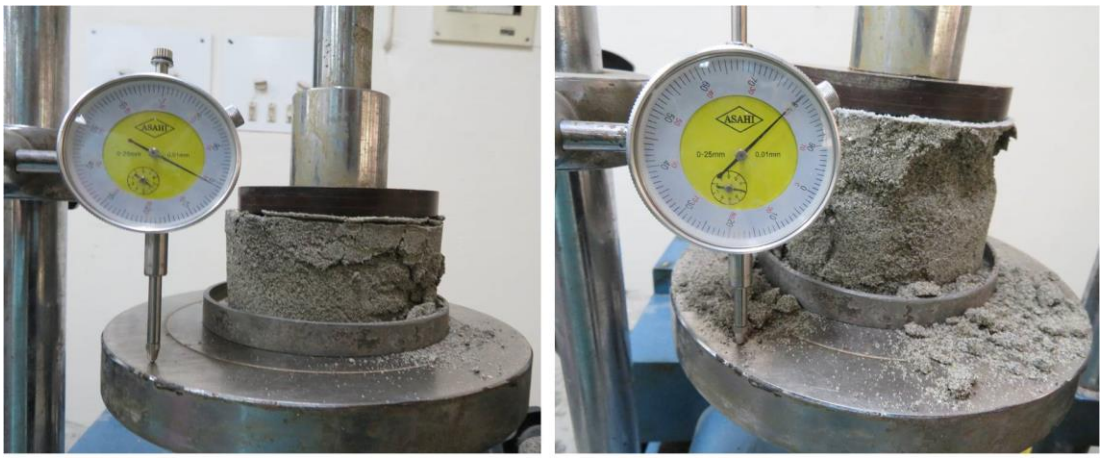


Figure 5.8 Failure mode of the sheared specimens under axial loading during UCS testing **(a)** C_1 column; and **(b)** C_2 column

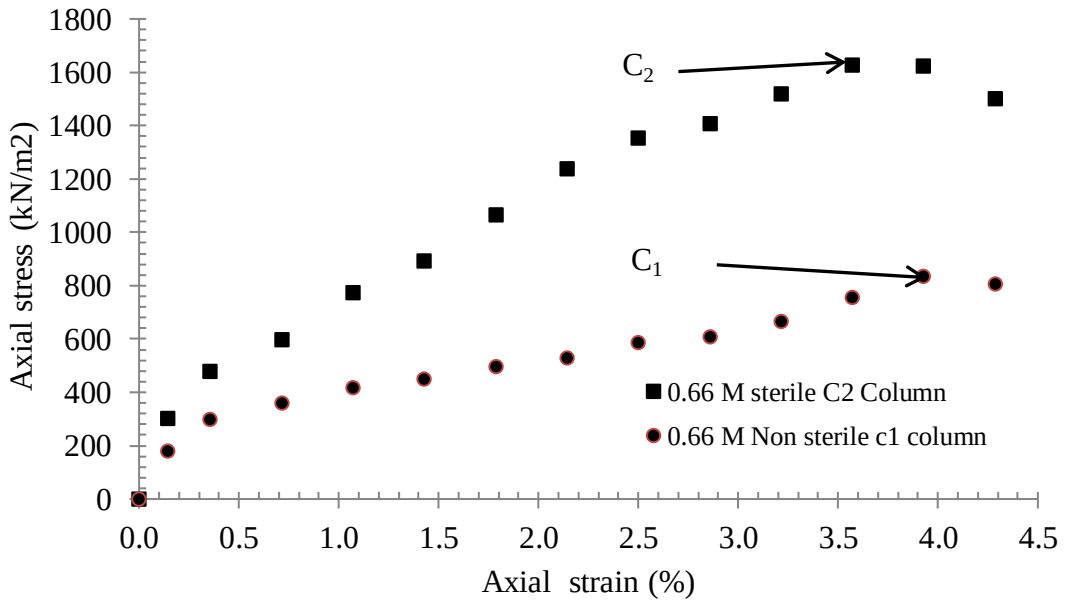


Figure 5.9 Stress-strain curves for biotreated specimens of C_1 and C_2 columns

for the specimens. Zhao et al., 2014 also experienced the brittleness properties of the MICP-treated soils. Soil columns exhibit non-collapse strain properties of the MICP-treated soils. Hence, the soil columns exhibit a non-collapse strain-softening shear behaviour, with a higher initial shear stiffness and ultimate shear capacity, and drop suddenly at lower shear strains comparable to the typically cemented sands as reported by Dejong et al., 2006 as in Figures 5.8 (a) and (b).

5.5.5 Shear strength of MICP treated soils

The MICP treated geomaterial was examined to improve the shear strength for a set of selected parameters in the study. The cohesion and angle of internal friction are the strength parameters governing the engineering behaviour of the soils as per the Mohr-Coulomb strength criteria. Therefore, the shear strength parameters of MICP treated geomaterials were estimated using direct shear tests.

The biotreated soils obtained after MICP treatment is a geomaterial consisting of sand and fines having similar grain size distribution as that of silty sand obtained from the Yamuna river basin. The shear strength parameters of various geomaterials are found using direct shear, triaxial shear, or vane shear tests. However, the strength is usually captured using linear Mohr-Coulomb parameters, namely the cohesion and the angle of internal friction, since the sandy soils containing non-plastic fines behave as a frictional geomaterial. Therefore, the strength of MICP soils may also be interpreted using dilatancy parameters for non-linear strength envelopes.

The peak and critical shear strength of a frictional geomaterial is expressed as,

$$\tau = c + \sigma' \tan\phi_{peak} \tag{5.1}$$

$$\tau = \sigma' \tan\phi_{peak} \tag{5.2a}$$

$$\tau = \sigma' \tan\phi_{c} \tag{5.2b}$$

where c and ϕ are the shear strength parameters, τ and σ' are shear stress and normal effective stresses in direct shear test, respectively, which have units of shear strength. ϕ_{peak} and ϕ_c are the peak and critical friction angles, respectively. The cohesion intercept tends to zero for the post-failure residual strength of MICP soils in Eq. (5.1). The friction angle reaches the critical ϕ_c which appears only after the soil

has fully dilated. At high shear strains, no volume change occurs, and the soil does not dilate anymore.

The rate at which a material dilates in shear primarily depends on the observed peak friction angle ϕ_{peak}. Bolton (1986) defined stress-dilatancy relation in terms of peak (ϕ_{peak}), critical friction angles (ϕ_c), an angle of dilatancy (ϕ_d) at peak strength, and the term I_{rd} referred to as relative dilatancy depending upon relative density (R_d). It may also be expressed in terms of I_{af} as relative dilatancy depending upon relative compaction (R_{cf}) for MICP treated silty soils since relative density is not clearly defined for silty soils. The relationship between peak and critical angle is expressed in terms of dilatancy index, I_{rd} and I_{af} as,

$$\phi_{peak} - \phi_c = AI_{rd} \tag{5.3}$$

$$\phi_{peak} - \phi_c = AI_{af} \tag{5.4}$$

where $I_{rd} = R_{df}\ (Q_{df} - \ln 100\ p'/\ P_A) - R_{df}$ (5.5)

$$I_{af} = R_{cf}\ (Q_{af} - \ln 100\ p'/\ P_A) - R_{af} \tag{5.6}$$

where A is an empirical constant having a value of 3 for triaxial strain conditions and takes care of the scaling effect and shape factor; R_{cf} is the relative compaction defined as the ratio of natural dry unit weight to maximum unit weight, expressed as a number between zero and 1; p' is the mean effective stress at peak strength in kPa; P_A is the reference stress (100 kPa) in the same units as p'; R_d, R_{cf}, Q_f, R_f, Q_{af} and R_{af} are non-dimensional.

The non-linear shear strength parameters for sand with silt were applied on MICP-treated soils for a varied proportion of fines using Eq. (5.5 and 5.6). Ojha (2015) observed that the dilation angle at peak strength was a function of the relative density R_d or relative compaction R_{cf}, and the mean confinement stress p' corresponds to the strain conditions. Therefore, the maximum dilatancy angle of 10° was found

useful to relate the strength of silty sand. In addition, the strength of MICP treated geomaterial is observed for varied packing densities with relative compaction of 0.90 –0.98 and friction angle of 28–34°.

The shear strength of the soil is the principal criterion for selecting a suitable type of substructure for buildings, bridges, roads, and related infrastructure works such as water treatment plants, sewage treatment plants. It is recommended to use strength and dilatancy concepts for the soil treated with MICP. The difference between triaxial and plane strain friction angles is related to the dilatational characteristics of the silty sand, variation of unit weight with the angle of internal friction, and variation of the angle of internal friction with relative compaction (Trivedi and Ojha, 2021). The geomaterial collected from the Yamuna river basin has a relative density (R_d) in the range 30%–80%, e_{min} of 0.5–0.30, e_{max} of 0.8–0.62, and D_m of 0.26–0.208 with the value of ϕ_c = 24.2–30.7 (Ojha and Trivedi 2013a; 2013b; Shougrakpam and Trivedi, 2020).

For evaluating the angle of friction and cohesion, direct shear tests were observed on untreated soil, S_0, and biotreated soil specimens of C_1, C_2, S_3 & S_{14}. All the specimens were treated for 14 days. Here, S_{14} denotes the biotreated specimen using 0.5 M sterilized CRS of set I specimens (as explained in Chapter 3). The details of the soil specimens are listed in Table 5.5.

Table 5.5 Details of the various specimens used for direct shear tests

Specimens	Molarity of CRS	Treatment condition	Reference
S_0	–	Sterile	–
S_{14}	0.5 M	Sterile	Chapter 3
S_3	0.75 M	Sterile	Chapter 4
C_2	0.66 M	Sterile	Present
C_1	0.66 M	Non-Sterile	Present

The fine content of the specimens was in the range of 10–15%. Table 5.6 shows the variation of shear strength parameters with relative density (R_d) of 30–80% (Ojha and Trivedi, 2013), which applies to MICP treated soils for a varied proportion of 0–20% silt content. The Q_f and R_f are fitting parameters to calculate the shear strength and found that a value of $R_f = 0.5$ works well for all gradations and within the limited range of relative density.

Table 5.6 Variation of shear strength parameter (Q_f) of silty sand based on relative density as applicable to MICP treated soils with 0-20% of silt content

Silt (%)	Yamuna	Ottawa	Tread line with $R_{df} = 0.5$ Yamuna Sand	Tread line with $R_{df} = 0.5$ Ottawa Sand
0	11.674	9	7.096	9
5	11.397	9	7.047	11
10	10.934	8.3	7.433	10.6
15	11.041	11.4	6.817	10.3
20	15.435	10.1	5.426	9.5

The relative compaction is used to reduce error in the overall estimation of strength and deformation properties of MICP treated soils which contain plastic and non-plastic silts, by using relative compaction in Eq.5.4. Table 5.7 shows the variation of shear strength parameters based on relative compaction of 90 to 98% of the silty sand for a varied proportion of fines containing 0–20% (Ojha and Trivedi, 2013) as applicable to MICP treated soils.

The peak shear stress was determined at different vertical stresses (25, 50, 100, and 150 kPa) of different specimens to determine the failure envelopes of untreated soil and the MICP treated specimens. The friction angle (ϕ) and cohesion (c) parameters were estimated using Mohr-Coulomb failure envelopes for different soil specimens, as shown in Figure 5.10. Figure 5.11 shows the residual strength of MICP

treated soils when cohesion intercept c = 0, similar to cohesionless soil with increased grain sizes.

Table 5.7 Variation of shear strength parameter (Q_{af}) of silty sand based on relative compaction as applicable to MICP treated soils with 0-20% of silt content

Silt (%)	Yamuna	Ottawa	Tread line with R_{af}= 40 Yamuna	Tread line with $_{Raf}$= 40 Ottawa
0	49.609	51.187	49.833	48.53
5	44.341	52.959	49.797	49.43
10	45.620	45.093	50.163	49.72
15	40.651	40.671	49.800	49.74
20	49.844	45.036	48.742	49.88

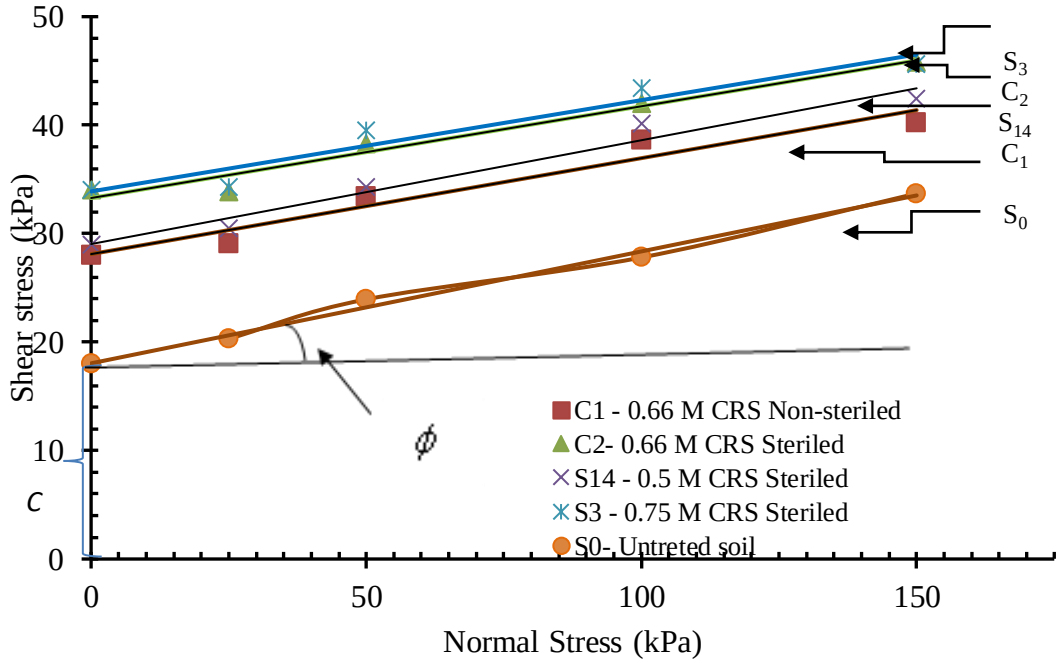


Figure 5.10 Mohr-Coulomb failure envelopes to determine c and ϕ of untreated specimen S_0 and MICP-treated soil specimens C_1, C_2, S_3 & S_{14}.

In Figure 5.10, the failure envelope of untreated soil S_0 observed a cohesion of 18 kPa and a friction angle of 9°. If the cohesion intercept was kept zero, the angle of internal friction was 22°, as shown in Figure 5.11. From the failure envelopes of

MICP treated specimens as shown in Figure 5.10, it can be observed that the cohesion and friction angle of the C_1 column be 28 kPa and 9°, respectively. However, specimens C_2, S_3, & S_{14} has obtained cohesion and friction angles in the range of 29–34 kPa and 9–10°, respectively. Figure 5.11 shows curves fitting shear strength parameters with zero-intercept for the specimens, namely S_0, C_1, C_2, S_3,& S_{14}. The cohesion intercept is considered zero for the sandy soils; the corresponding linear Mohr-Coulomb strength envelope, the angle of internal friction was mapped between 25°–29° of the MICP treated soils which indicates the strength development in the MICP treated soils, as observed in Figure 5.11.

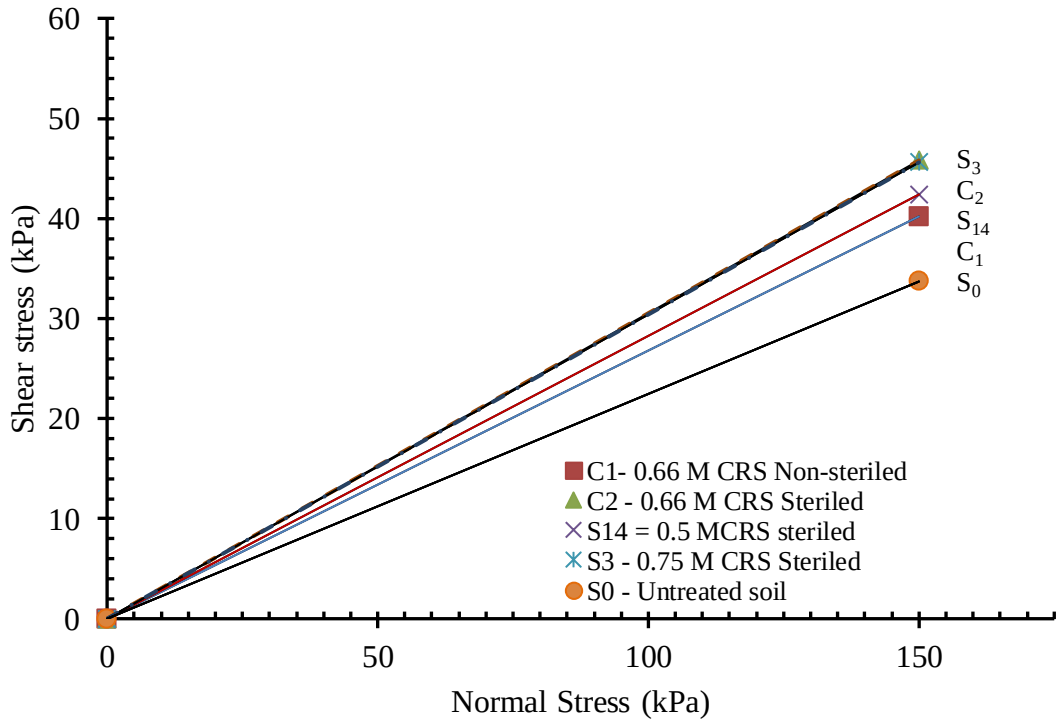


Figure 5.11 Residual strength parameters with zero-intercept for the specimens, namely S_0, C_1, C_2, S_3, & S_{14}

Further, Mingdong et al., (2016) examined a cohesion that ranges between 29–45 kPa, and the angle of internal friction between 7.6–11° of biotreated sand, which is significantly higher than the results obtained in the present study, which may be due to variation in the treatment conditions.

The computed values of Mohr-Coulomb strength parameters of the various specimens in direct shear tests are tabulated in Table 5.8. The residual strength of the MICP-treated soils is as shown in Table 5.9.

Table 5.8 Strength parameters of the various specimens in direct shear tests

Specimens	Molarity of CRS	Treatment condition	Peak friction angle	Cohision intercept (kPa)
S_0	–	Sterile	9°	18
S_{14}	0.50 M	Sterile	10°	29
C_2	0.66 M	Sterile	9°	33
S_3	0.75 M	Sterile	10°	34
C_1	0.66 M	Non-Sterile	9°	28

Table 5.9 Residual strength of the various specimens in direct shear tests

Specimens	Molarity of CRS	Treatment condition	Residual friction angle
S_0	–	Sterile	22°
S_{14}	0.5 M	Sterile	27°
C_2	0.66 M	Sterile	28.7°
S_3	0.75 M	Sterile	29°
C_1	0.66 M	Non-Sterile	25°

5.6 CONCLUSION

Challenges faced in applying MICP for soil improvement include monitoring in-situ microbial and chemical interactions for uniform distribution of the calcite precipitates throughout the soil mass. In addition, a desirable level of cementation is to be achieved to increase the strength and stiffness of the treated soils. However, the uniformity and distribution of the calcite precipitation within the treated soil deposits are unpredictable. In the present study, two soil columns, C_1 and C_2, were treated using the same concentration of BS and CRS. A comparative study on soil properties of treated soils, such as strength and permeability of the soil based on the

cementation level, was made between sand columns C_1 and C_2, treated with sterile and non-sterile treatment solutions, respectively. A 10% fine-grain content was added in the soil specimens and studied its important role in determining the angle of internal friction and cohesiveness in the treated soils. The results of the tests indicate that the angle of internal friction, shear strength, and compressive strength of the soil increases after treatment. Results of the laboratory tests by Gupta and Trivedi (2009) show that maximum and minimum void ratios of clean sand decrease as fine-grain content increases from 0 to 20% and increase if fine-grain content exceeds 20%. Therefore, a 10% fine-grain content was mixed with the soil specimens of the biotreated columns in the present study to ensure a decrease in the void ratio. The results obtained from the various laboratory tests are summarised as follows:

- The UCS obtained in C_1, and C_2 columns are 835 kPa at 2.75% axial strain and 1630 kPa at 2.5% axial strain, respectively, which is less than 3 MPa is applicable for normal (Chu et al., 2016). The strength gained in the treated column C_2 is 2 x the strength gained in the C_1 column, a higher strength in C_2 column is due to the use of sterile treatment solution.
- A three-layered calcite content variation in the biotreated columns was examined. The calcite content was varied in decreasing order from top to lower layers, though the variations were insignificant. The percentage increase in the average calcite content of C_2 is 38% more than that of C_1, the reasoned being of more effective biochemical reaction to induce the calcite precipitates in C_2, which may be due to the use of a sterile treatment solution. Calcium carbonate content was analyzed from different layers of the treated soil columns. It was found that the average calcite content in C_2 was exceeded by 38.67% from C_1. However, there was a slight variation of calcite

content from the upper to the lower layers, indicating a uniform distribution throughout the column depth.

- The grain size of D_{60}, D_{50}, D_{30}, and D_{10} were analyzed and compared between the untreated and treated columns of C_1 and C_2. It has been observed that the grain sizes of the treated specimens are coarser than the untreated soils. However, the percentage increase in the grain sizes of C_2 is more than C_1. which indicates the specimen treated under sterilized condition has higher calcite precipitates for biocementation.
- The microbial imprints appeared on the calcite crystal surfaces in hollow spaces. Heavy precipitation was observed in the C_2 column than in the C_1 column. It was clear from the SEM images that the calcite distribution was different in the microscale of the C_2 and C_1 columns. SEM imaging was taken from the specimens preserved for 36 months and found that the morphology of the calcites and the level of biocementation remained intact. Such findings have indicated that the MICP-treated soils have increased the strength and stiffness for long-term solidification and hardening of soil.
- The stress profiles of the biotreated specimens indicate that the MICP treatment effectively improves the shear strength. The cohesion and the friction angle of the untreated soil were observed as 18 kPa and 9°, respectively. When the cohesion intercept was kept at zero, the angle of internal friction was 22°. The cohesion and friction angle of MICP treated soil specimens were approximated in 28–34 kPa and 9–10°, respectively. If the cohesion intercept is zero for non-cohesive soils, the corresponding values for linear Mohr-Coulomb strength envelope, the angle of internal friction was mapped between 25°–29° of the MICP treated specimens. The cohesion and

angle of internal friction for C_1 were 28 kPa and 9°, respectively, while the friction angle was 25° when the cohesion intercept was kept at zero. The increase in cohesion values and angle of internal friction has indicated strength development in the MICP-treated soil. Therefore, it can be concluded that the MICP treatment solution prepared in non-sterile tap water can be used for large-scale treatments at a lower cost with less time by skipping the sterilization process.

CHAPTER 6

Conclusion

6.1 INTRODUCTION

In recent years, many researchers have started giving importance to the role of microorganisms in improving the engineering properties of sandy soils by transforming them into biocemented soils using microbially induced calcite precipitation (MICP). The current study investigates new approaches to improve the engineering properties of loose sandy soils collected from the Yamuna river basin near Delhi Technological University located in Delhi in India, using the MICP technique. The biotreated sand specimens were tested for shear strength based on unconfined compression strength (UCS) and direct shear tests. The improvement in the unconfined compressive strength (UCS) in all the biotreated sand specimens achieved by biocementation has indicated the effectiveness of MICP treatment in increasing the soil strength. Also, a significant reduction in permeability was achieved by bioclogging in the loose sandy soils has confirmed the potential of

MICP treatment in mitigating seepage and leakage from hydraulic structures and aquaculture ponds built in sandy soils. The increase in soil strength after MICP treatment can contribute to a more significant soil bearing capacity, while a reduction in hydraulic conductivity could minimize settlement, shrink well tendency, seepage, and infiltration of rainfall into embankment slopes and ground for the sandy soil formations of Yamuna river basin which supports most engineering structures in the National Capital Region of India. Permeability retention for MICP-treated soil allows continuous or periodic treatments for strength enhancement and increased liquefaction resistance (Whiffin, 2004). Improving strength while maintaining permeability can be achieved without disturbing the soil and nearby structures (Kucharski et al., 2006). From the grain-size analysis (IS: 2720-Part IV: 1985), the sand used was conformed to grading zone IV (IS-383: 1970). According to the Unified Soil Classification System (ASTM D2487), the soil was poorly graded sand (SP). In addition, ~97% of the sand particle sizes were within the ideal size range of 0.050–0.400 mm for MICP treatment (Rebata-Landa, 2007). Therefore, the selected sand was considered suitable for free bacterial movement in the soil and hence, a desirable target for MICP treatment (Maier et al., 2009).

The significant achievements of the present study are listed below:

i. The physical properties of the soil collected from the Yamuna river basin were investigated, and the soil type was found to be poorly graded (SP) and liquefiable.

ii. There was a significant improvement in UCS by biocementation and a reduction in hydraulic conductivity by the bioclogging process in MICP-treated specimens. The results have indicated the feasibility of using *S. pasteurii* in

the MICP soil treatment method by replacing cement and chemical grouts to design a sustainable and durable soil system.

iii. *S. Pasteurii* being a soil bacteria, can simulate the indigenous in-situ field conditions to precipitate calcites in the soil matrix by harnessing the microbial activity to release urease enzymes for urea hydrolysis, which plays a vital role during MICP treatment.

iv. The microbial activity releases a high amount of urease enzyme during the initial 24 h to facilitate urea in the pore fluid containing urea and $CaCl_2$ reagents. The urease activity remained high until 7 days, confirmed by the pH $\geq$ 7 of the effluent treatment solution. When the pH dropped below 7, it was attempted to increase the pH by adding new doses of BS and CRS until 14 days. However, after 14 days, the pH could not regain above 7 after adding new doses. Besides, bacteria release protease enzymes generally after 5 days of treatment, which hinders the activity of the urease enzymes. From the above discussions, 14 days of MICP treatment can be considered the optimum treatment time.

v. The cementation level and the corresponding UCS achieved in the various MICP-treated specimens were significant to give sufficient strength and stiffness in the soil if treated for 14 days. In addition, the experimental results have shown a decreasing trend in calcite content and UCS for the specimens treated beyond 14 days. Therefore, 14 days of treatment will save time, cost, and wastage of chemicals. In addition, using a lesser quantity of urea will reduce the release of harmful ammonium ions in the soil system.

vi. The calcite content in the naturally biocemented products like rocks and caves was determined to be between 5 and 15%, comparable to the calcite content obtained in the various MICP-treated soil specimens in a few days.

vii. The formation of biocemented water-impermeable crust layers may be feasible for treating aquaculture ponds, channels, and slope stabilization by reducing surface erosion, soil erosion, and seepage from water bodies.

viii. The gain in calcite content and UCS was more in the specimen treated with the sterile solution than in the non-sterile solution. However, treatment with either solution could give significant soil strength. Therefore, the non-sterile treatment solution using tap water can replace the sterile solution for large-scale soil treatment using MICP by skipping the costly and time-consuming sterilization process.

The literature review in chapter 2 paved the way for the present research to move forward with new approaches to soil treatment using MICP to be a sustainable and environmentally friendly method for improving the engineering properties of loose sandy soils.

Chapters 3, 4, and 5 include the results and discussion section of different experimental programs conducted for MICP treatment using different soil specimens. Again, the data are illustrated with discussions to understand the behaviour of MICP-treated biocemented soils.

In chapter 3, the experimental results have shown the role of pH in effective MICP soil treatment. In the present study, pH was varied between 7.6 and 8, comparable to the optimum pH range obtained by Jain and Arnepalli, 2019; Mobley et al., 1995; Evans et al., 1991 required for microbial growth. The improvement in UCS through biocementation and bioclogging relative to calcite crystals obtained in

various treated specimens in two sets of experiments at different durations is shown in Table 3.5. During the initial 24 h, treatments were with either 0.5 M CRS for set I or 0.75 M CRS for set II when the bacterial activity was high in releasing urease enzymes. When the urease activity was slowed down, treatment was continued with a lower molar concentration of 0.25 M CRS till the completion of treatment. The gain in UCS of the biotreated specimens ranged between 1.10 to 2.18 MPa, with calcite content ranging from 4.0% to 8.0%, obtained within the range for typical applications (Mitchell and Santamarina, 2005) by biocementation and bioclogging. Thus, applying treatment solution using a higher concentration to a lower concentration of the cementing solution will reduce the cost and wastage of treatment reagents of urea and $CaCl_2$. In addition, such a practice will reduce the harmful effect of chlorine in concrete or soil by minimizing $CaCl_2$. Besides, the harmful effect of ammonium ions in the soil environment will be reduced due to the lesser use of urea. Although higher concentrations may precipitate larger calcite, there will be a less efficient non-uniform distribution of calcites in the soil matrix, reducing the soil strength. The growth in grain sizes by biocementation and coating of sand surfaces transformed the fine particles into coarser ones, as shown in the SEM images in Figures 3.11 (a), (b), and (c). In addition, the increase in size increases the roughness of the sand grains, reducing the friction between the sand grains and giving more strength to the biotreated specimens. The improvement in shear strength and diminution in hydraulic conductivity of soil increased with increased density of the soil matrix when mixed with the calcite crystals.

In chapter 4, the biotreated sand specimens were improved in UCS by biocementation and by reducing the permeability by bioclogging. Some specimens with 10% fine-grain particles and found they possess higher strength than the

specimens that consisted of cohesionless, collapsible, and clean sands only. The presence of fine particles promotes more surface area to bind with the calcites through biocementation, which improves the strength. The permeability was reduced to three order-of-magnitude from the initial day to the seventh day of treatment. The reduction was due to the potential of biocementation that coats or bridges the individual soil particles with the calcites that gradually reduce the pore throat and volume; thereby, the permeability is reduced to acceptable leakage rates reported for mariculture ponds (Weisburd and Laws, 1990) and Bear (1972) for permeability of soils to be impervious. Hence, MICP treatment can reduce seepage in aquaculture ponds, channels, leaching ponds, and landfill sites. In addition, the average calcite content achieved in biotreated sand columns was comparable to that of calcium carbonate content in natural rocks. Therefore, the strength improvement in the biocemented columns was comparable to soft rocks, as depicted by Trivedi, 2013. The SEM images further verified the formation of biocemented crust layers, as shown in Figures 4.10 and 4.11. The presence of small particles leads to more interparticle $CaCO_3$ bonds whose breakage would again still allow the small particles to fill the interparticle void spaces (Xiao et al., 2020) and thus increase the strength while reducing permeability. The addition of fine-grain resulted in a more effective biocementation and bioclogging process that might reduce the cost associated with the MICP process. Therefore, the strength gained in the biocemented specimens was also enhanced by adding fine particles.

In chapter 5, the effect of sterile and non-sterile treatment solutions as cementing reagents was investigated by treating the sand column specimens. The particle distribution sizes of the sand were in the liquefiable soil boundaries as depicted by Tsuchida, 1970 as was shown in Figure 5.1. The improvement in UCS

while reducing the permeability in the biotreated soils has proved the effective use of MICP in improving the engineering properties of loose sandy soils. The particle size distribution curves of the biotreated sand specimens lie to the right of the curve of the untreated soil, and hence the treated sands possessed higher values of D_{10}, $D_{30,}$ and D_{60}. This finding has indicated that the particle sizes and roughness increase after treatment.

The experimental results in chapter 5 have indicated that MICP effectively improved the shear strength of the untreated soil in terms of cohesion and friction angles treated with sterile and non-sterile treatment solutions. The improvement in UCS in the C2 column was 1630 kPa, almost 2x that of the C_1 column at 835 kPa. The column treated under sterile conditions gives more effective calcite content and strength than the column treated with a non-sterile solution**.** A significant improvement in UCS in the soil column treated with a non-sterile solution has indicated the potential to replace the sterile treatment condition in large-scale treatments. Thus, the treatment process may skip the sterilization process to reduce the cost and duration of MICP treatment. Table 5.3 shows a three-layered calcite content that decreases from the first to the third. The reason may be the accumulation of more calcites near the top surface and the non-uniform distribution of calcites throughout the depth. A significant amount of calcite crystals improved the UCS in the non-sterile column. Improvement in the particle sizes was also observed in the biotreated sands than in the untreated ones though the increment was more in the sterile column. The reason may be more efficient soil bridging at particle contacts or coating grain surfaces with the calcite precipitates. In the SEM images, as shown in Figure 5.5 (a and b), the column treated with the sterile solution was more heavily

cemented than the non-sterile due to the release of more calcites under sterile conditions. So, gain in strength is related to biocalcification in the soil structure.

Further, the UCS improvement in various biotreated columns was observed between 585 kPa and 2180 kPa. Therefore, MICP treatment can mitigate excessive bulging of stone/sand columns and possible slumping during their installation (Mahawish et al., 2017). Further, the shear strength parameters obtained in the biocemented sands are recommended for silty sand using relative density and compaction (Ojha and Trivedi, 2013a) as applicable to MICP-treated soils.

The MICP treated soils have improved the shear strength. Untreated soil cohesion and friction angles were 18 kPa and 8°, respectively. When the cohesion intercept was zero, the angle of internal friction was observed to be 26°. The observed improvement in cohesion and friction angle of MICP treated soil ranged between 28 to 34 kPa and 9-10°, respectively. The cohesion intercept is usually considered zero for the loose sandy soils; corresponding values for linear Mohr-Coulomb strength envelope, the angle of internal friction was mapped between 25°–29° of the MICP treated specimens.

SEM images of treated specimens found no breakage of biocementation or detached the calcite precipitates from the sand surfaces. However, biocementation may prevent the movement of particles and reduce interparticle friction and possible abrasion at the interface between $CaCO_3$ and soil grains. In addition, if breakage of calcites and decementation if occurred, it may increase clogging of the void spaces, thus reducing permeability. Thus, from the above discussions, it can be concluded that the strength increases while permeability reduces during the biocementation and bioclogging process.

Lastly, laboratory and field results obtained by various researchers, including the present study, are promising and viable, suggesting the potential of MICP for various engineering applications such as in improving construction materials, calcification of porous soil media, improving in strength and stiffness of engineered soils, reduction in soil permeability, waste containment, liquefaction, and erosion mitigation. Furthermore, compressibility decreases as calcification increases based on the same void ratio (Xiao et al., 2020). The potential applications of MICP are discussed in the following section.

6.2 POTENTIAL APPLICATIONS OF MICP

Over recent years, researchers have investigated various biomediated techniques for soil improvement, such as biocementation, bioclogging, bioremediation, and phytoremediation. Biocementation via MICP provides a great potential to alter the engineering properties of soil. The exploitation of bacteria to induce biominerals in soil fills the pore space and binds the soil particles together. The implementation of MICP by biocementation and bioclogging also has the potential for enhancing the stability of retaining walls, embankments, and dams; treating pavement surface; strengthening tailings dams to prevent erosion and slope failure; increasing the bearing capacity of piled or nonpiled foundations; reinforcing or stabilizing soil to facilitate the stability of tunnels or underground constructions; reducing the liquefaction potential of soil, and controlling erosion in coastal areas and rivers. In addition, the consolidated sand by MICP has the potential for cement mortar crack remediation. Microbially enhanced crack remediation (MECR) utilized $CaCO_3$ as a biological byproduct, showing a wide range of application potential as a sealant (Shougrakpam and Trivedi, 2020).

6.2.1 Soil strengthening using MICP

The improper engineering properties of soil in many regions and industrial sites can cause serious issues. Under this condition, the dikes, dunes, and slopes can become unstable; the roads and railways undergo settlement, and the rivers are likely to be subjected to erosion (van Paassen et al., 2010). Therefore, the feasibility of solving the above issues is by increasing soil strength through biocementatiooon and reducing permeability by the bioclogging mechanism of MICP treatment.

6.2.2 Liquefaction mitigation and erosion control

Liquefaction and erosion mitigation is an emerging area of research for MICP application. The MICP method of soil treatment can be used to mitigate the liquefaction problem in the Yamuna river basin. The permeability of the biotreated soil was reduced to three-order-of magnitude. In addition, the gain in the UCS was in the range of dense soil and rock type, which can be classified as site class C according to soil classification by NEHRP, 2003 (refer to Table 2.1). It has indicated that the shear wave velocity of the MICP-treated soil may range between 366 m/s to 762 m/s. Therefore, the MICP treated soil may mitigate soil deformation triggered by rapid earthquakes and vibrations and cyclic loading on saturated cohesionless soils in undrained conditions. The soil tends to act like a liquid; hence the shear strength and stiffness are significantly reduced.

6.2.3 Carbon dioxide sequestration

Several researchers studied carbon dioxide sequestration. Due to rapid urbanization, concrete consumption has increased to construct civil engineering infrastructures. Soils improved through urease-induced mineralization or cementation are environmentally friendly, as they can substitute ordinary Portland cement, which

produces large quantities of carbon dioxide through its production processes (Park et al., 2014). In addition, concrete is the largest source of anthropogenic greenhouse gas emissions, disturbing the ecosystem. The CO_2, in turn, can bind with the free Ca^{+2} present in the soil for microbial precipitation of $CaCO_3$. As a result, MICP helps in CO_2 sequestration.

6.2.4 Bioremediation of contaminants from soil and groundwater

The increased rate of environmental degeneration resulting from industrial activities, mainly from the exploration of fossil fuels, has increased the threat to the ecosystem and the health of all creatures, including humans. Bioremediation is a process that uses microbial metabolism in the presence of optimal environmental conditions and adequate nutrients to biodegrade or biotransform contaminants, not only petroleum hydrocarbons but also other metals metalloids. Bioremediation is a technique employed to immobilize contaminants in soil using microbes.

6.2.5 Removal of calcium from industrial waste

Calcium-rich effluents are associated with landfill leachates, reverse osmosis concentrates and industrial processes. The high concentrations of Ca^{2+} are a severe hazard for the environment or, in some cases, may negatively affect the processes. For example, in aerobic or anaerobic reactors, Ca^{2+} tends to clog the pipelines, boilers, and heat exchangers and therefore causes scaling or malfunctioning of instrumentations (Hammes et al., 2003). As a result, Ca^{2+} needs to capture, and MICP serves as a new emerging solution to address this problem. Hammes et al., 2003 reported the positive effect of the ureolytic microbial community on removing excess calcium from industrial effluents.

6.2.6 Waste containment applications and contaminant attenuation

During the last few decades, deterioration and contamination of soil and groundwater have increased due to different sources of pollution, mainly from urbanization, industry, and intensive agriculture. The contamination sources in soil and groundwater are mainly radionuclides and heavy metals. As most contaminants are toxic, non-degradable, and persistent to accumulate, the research on toxic waste degradation approaches using biological techniques is prioritized for immediate conservation of the environment. Therefore, biological techniques including phytoremediation, bioaccumulation, biocoagulation, bioleaching, biosorbents, and bioimmobilization have been developed to supplement chemical approaches.

6.2.7 Potential of biocement to repair foundations

There has been increasing interest in self-healing concrete materials that can repair cracks to significantly increase concrete durability by preventing corrosive agents from accessing the reinforcement and improving their water tightness, consequently reducing the need for inspection and maintenance. Research into engineering self-healing in concrete was first initiated to reduce the amount of cement needed in concrete mixes as part of global efforts to reduce greenhouse gas generation, as cement production is currently responsible for about 5% of global CO_2 emissions (IPCC, 2013). The inspiration for the study of self-healing concrete is the ability to live organisms to detect and repair the damage rapidly.

6.3 SUMMARY

The following is the summary of the main findings from the present study:

- The different bacterial species could precipitate different amounts, shapes, and carbonate crystals even in a similar soil environment. The calcium carbonate precipitation is very much associated with bacterial cells of ureolytic bacteria, which is *S. pasteurii* in the present case. There is a limit in the calcite precipitation (15%) beyond which no remarkable increase in strength due to the weak bond produced by bacteria-induced cementation (Dejong et al., 2006).
- The reaction formulas for ammonification of amino acids under section 2.6 Eq. (2.1–2.4) and for MICP pathway by urea hydrolysis using urease enzymes whose end product of the biochemical reaction was shown as $CaCO_3$ under sub-section 2.6.1. Again, Eqs. 2.5–2.14 shows the reaction of CO_2 with hydroxyl ions that results in the formation of carbonates to react with Ca^{+2} present in the soil environment to form $CaCO_3$. In addition, Dhami et al., 2014 found that the carbonic anhydrase is another enzyme that plays a vital role in MICP, catalyzing the reversible hydration of CO_2 (Eq. 2.14). Okwadha and Li, 2010 found that the CO_2 sequestration rate caused by urea hydrolysis was directly proportional to microbial $CaCO_3$ formation by trapping the CO_2 released during urea hydrolysis in $CaCO_3$. Therefore, the MICP process facilitates preventing the release of CO_2 into the atmosphere and promotes CO_2 sequestration.
- The temperature was 32°C during MICP treatment, sufficient for the microbial release of urease enzymes to hydrolyze urea. In addition, the enzymatic

reaction depends on environmental conditions, the concentration of bacteria, and reactants in the system.

- Soon et al., 2013 reported that the pH for active urease enzyme ranges between 7 and 8.5, which is comparable to the pH range of the present study. However, Mobley et al., 1995 observed that the microbial ureases could be irreversibly denatured if the pH is below 5.
- Lower concentrations of the cementation media below 1M used in the present study, i.e., 0.25 M, 0.5 M, 0.66 M, and 0.75 M over a large number of treatments, would provide more uniform calcite precipitation and more homogeneous cementation with smaller crystal sizes (Al Qabany et al., 2012).
- Bernardi et al., 2014 observed that 75–80% of injected urea was at the upper portion of the sand columns, and 20–25% of injected urea was used in the lower portions of the sand columns. As a result, a significantly lower $CaCO_3$ precipitation was in the bottom portions than in the upper portions. This finding is consistent with the depth-wise $CaCO_3$ distribution in the biotreated sand columns. The $CaCO_3$ content in the upper 10 mm varied between 14% and 11%, which was further reduced towards the bottom portion from 9.8% to 3.4% (Shougrakpam and Trivedi, 2020) as was discussed in section 4.4.1 (refer to Table 4.2). This non-uniformity of calcite distribution in various biotreated specimens caused strength variation in the treated specimens.
- A significant increase in shear strength parameters of the biocemented soil specimens has indicated the development of soil strength. The results have revealed an insignificant variation in cohesion and friction angles in columns treated with sterile and non-sterile solutions. In addition, treatment using 0.75 M and 0.66 M achieved an insignificant variation in shear strength parameters.

Hence, for large-scale field applications, a non-sterile treatment solution of 0.66 M CRS prepared in tap water can be recommended to reduce the wastage of chemicals and skip the costly and time-consuming sterilization process

- Many researchers revealed a linear relationship between the $CaCO_3$ content and UCS values in biotreated soil specimens. For example, Zhao et al., 2014; Yasuhara et al., 2011; Shougrakpam and Trivedi, 2020 achieved UCS improvement between 0.4 MPa and 2.18 MPa based on an average calcite content that ranged between 4.0 to 11.4% obtained under different treatment conditions, which is significant for normal applications.

6.4 RECOMMENDATIONS FOR FUTURE RESEARCH

In the future, new researchers should overcome the various challenges faced in the large-scale field application of the MICP technique for improving the engineering properties of biocemented soils using microorganisms, such as *S. pasteurii*. Therefore, new challenges of soil treatment using the MICP technique should be studied for efficient process optimization and careful monitoring. The challenges are in terms of cementation solution, presence of various microbes, non-sterile conditions, uncontrolled environment (i.e., temperature), and non-uniformity of calcite precipitation. In addition, pH adjustment, ammonium release, and optimum use of calcium and urea sources as chemical reagents to avoid wastage of reagents in the treatment solution.

The following are the recommendations for future research on MICP :

- The systematic evaluation of the soil properties for the feasibility of treatment by selecting suitable soil microorganisms that can release a high capacity of urease enzymes for urea hydrolysis during MICP reactions to produce

effective and uniform calcite precipitates.

- The fundamental research for understanding the behaviour of the soil-bacteria-calcite under control laboratory-scale studies for improvement in the engineering and mechanical property of MICP-treated soil.
- Adoption of new field-scale applications for effective in-situ soil treatment using MICP method with new methods for assessment of in-situ soil improvement in real-time during MICP treatment to achieve desired strength improvement.
- To conduct a feasibility test for slope stabilization using the MICP method to prevent slope failures, landslides, mud-flow hazards, and soil erosion by reinforcing soil with the calcite precipitates in the hilly regions as suggested (Shougrakpam et al., 2021).
- The feasibility of using MICP to seal surface cracks and fissures in concrete and rocks, base and sub-base stabilization of pavements, and soil consolidation at construction sites.
- The feasibility to replace the chemical grouting that uses toxic and harmful chemicals by microbially induced $CaCO_3$ as biocement using constructive, ecological, or combined techniques for soil stabilization.
- To face the challenges for future applications to reduce the mass loss and penetration resistance for transporting nutrients and the metabolic activity of microorganisms for deep-soil treatment.
- The feasibility of CO_2 sequestration by $CaCO_3$
- To address environmental issues, such as environmental remediation, including immobilization of toxic metals, and waste containment to control soil and groundwater pollution.

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Publications based on the work in the thesis

Authors	Title of the paper	Details of the journal	IF/Indexed
Shougrakpam, S. and Trivedi, A. (2018)	Formation of Biomineralized Calcium Carbonate Precipitation and Its Potential to Strengthen Loose Sandy Soils.	In: Wu W., Yu HS. (eds) Proceedings of China-Europe Conference on Geotechnical Engineering. *Springer Series in Geomechanics and Geoengineering*. Springer, Cham. https://doi.org/10.1007/978-3-319-97112-4_186	Indexed by Scopus I F =0.21 H-index=10 Springer Verlag, Germany
Shougrakpam, S., and Trivedi, A. (2020)	Engineering Properties of Bacterially Induced Calcite Formations	*Current Science Journal.* **118**(7): 1060−1068. https://wwwops.currentscience.ac.in/Volumes/118/07/1060.pdf A journal of Indian Academy of Science, Bangalore and Co-published with Springer	IF(2021)= 1.102 and Indexed by Scopus (Springer)
Shougrakpam, S., and Trivedi, A. (2021)	Harnessing microbially induced calcite precipitates to use in improving the engineering properties of loose sandy soils	*Sādhanā* **46 (41):** 1−14. https://doi.org/10.1007/s12046-021-01563-x A journal of Indian Academy of Science, Bangalore and Co-published with Springer	IF(2021)= 1.188 and Indexed by Scopus and SCI (Springer)
Shougrakpam, S., Trivedi, A., and Yendrembam, A. (2021)	Chapter 23: Manipur	"Chapter 23: Manipur" as a book chapter in "Geotechnical Characteristics of Soils and Rocks of India," Edited by Sanjay Kumar Shukla, pp. 451−472. ISBN 978-1-032-01098-4 https://doi.org/10.1201/9781003177159-23	CRC Press, Taylor and Francis Group

Shougrakpam, S., and Trivedi, A.	Sustainable ground improvement characteristics of MICP stabilized soils using *Sporosarcina pasteurii*	To be Communicated
Shougrakpam, S., and Trivedi, A.	Engineering behaviour of MICP-treated sand columns by sterile and non-sterile treatment solution	To be Communicated