Synthesis and Characterization of Biocement using Bacteria, Cow urine, and Calcium ions refined from Limestone

Daniel Fikremariam Betemariam

A Thesis Submitted to

The Department of Materials Science and Engineering

School of Mechanical, Chemical, and Materials Engineering

Presented in Partial Fulfilment of MSc Degree in Materials Science and Engineering

Office of Graduate Studies

Adama Science and Technology University

May, 2024

Adama, Ethiopia

Synthesis and Characterization of Biocement using Bacteria, Cow urine, and Calcium ions refined from Limestone

Daniel Fikremariam Betemariam

ID: PGR/27953/15

Advisor: Shimelis Lemma Beshahwured (PhD)

Co-Advisor: Dinsefa Mensur Andoshe (PhD)

A Thesis Submitted to

The Department of Materials Science and Engineering

School of Mechanical, Chemical, and Materials Engineering

Office of Graduate Studies

Adama Science and Technology University

May, 2024

Adama, Ethiopia

Declaration

I declare that this Mater Thesis entitled "**Synthesis and Characterization of Biocement using Bacteria, Cow urine, and Calcium ions refined from Limestone**" is my work and has not been submitted to any university for a similar purpose. The references used in this Thesis are duly recognized by proper citations.

______________________	______________________	______________
Name of student	Signature	Date

Recommendation of Advisor

I/we, the major advisor/supervisor of this research Thesis, hereby certify that I have closely advised/supervised the student while developing this Thesis and read the draft thesis entitled "**Synthesis and Characterization of Biocement using Bacteria, Cow urine, and Calcium ions refined from Limestone**" prepared under my guidance by Daniel Fikremariam. Therefore, I recommend the submission of the Thesis to the department for further review and evaluation.

________________________ _____________________ ______________

Major Advisor/Supervisor Signature Date

________________________ _____________________ ______________

Co-advisor/Co-supervisor Signature Date

Approval Page

I/we hereby certify that the recommendation and suggestion given by the Thesis Review Committee are appropriately incorporated into the final Thesis entitled "**Synthesis and Characterization of Biocement using Bacteria, Cow urine, and Calcium ions refined from Limestone**" by Daniel Fikremariam.

____________________	____________________	______________
Major Advisor/Supervisor	Signature	Date
____________________	____________________	______________
Co-advisor/ Co-supervisor	Signature	Date

Approval of Board of Reviewers

We, the undersigned, members of the Board of Reviewers of the Thesis open defence by Daniel Fikremariam have read and evaluated the Thesis entitled "**Synthesis and Characterization of Biocement using Bacteria, Cow urine, and Calcium ions refined from Limestone**" and assessed the understanding of the candidate about the research. This is, therefore, to certify that the Thesis is accepted.

______________________	______________________	______________________
Chairperson	Signature	Date
______________________	______________________	______________________
Reviewer 1	Signature	Date
______________________	______________________	______________________
Reviewer 2	Signature	Date
______________________	______________________	______________________
Department Head	Signature	Date
______________________	______________________	______________________
School Dean	Signature	Date
______________________	______________________	______________________
Office of Postgraduate Studies, Dean	Signature	Date

ACKNOWLEDGMENTS

First and foremost, I would like to thank the Almighty God for guiding me in steps that I have passed through to accomplish this work. After that, my sincerest gratitude and respect go to my advisor and co-advisor, Shimelis Lemma (Ph.D.) and Dinsefa Mensur (Ph.D.); respectively, for their unconditional support, patience, inspiration, deep knowledge, and advice that aided me throughout the research and writing of this thesis.

I would like to give special thanks to Materials Science and Engineering staff members, who gave a compassion and help for us to surpass this challenge. We had completed all of the laboratory research which were related with the microstructural analysis. Besides, I have also a great gratitude for Civil Engineering department laboratories technicians for their impeccable support for me in the geotechnical analysis segment of the research. Furthermore, I would also like to thank Mr. Muktar Mohammed for his undeniable support in the culturing and monitoring of the bacteria. Besides, I would like to extend my sincere thanks to our Department Head, Gabissa Bekele (Ph.D.), for his genuine advices, consistent follow-ups and all rounded encouragements.

At the end, I would like to forward my gratitude to all my parents who have been with me in all my ups and downs thereby providing encouragement and support in all the year. Without them, today I might not be here.

Thank you

Daniel Fikremariam

Table of Contents

ABSTRACT........i

List of Tables........ii

List of Figures........iii

List of Acronyms........iv

CHAPTER ONE........1

1. Introduction........1

1.1 Background........1

1.2 Statement of the problems........3

1.3 General objective........4

1.4 Specific objective........4

1.5 Significance of the study........4

1.6 Scope of the study........4

CHAPTER TWO........5

2. Literature review........5

2.1 Biocementation........5

2.2 Sources of Biocement........5

2.2.1 Microorganisms........5

2.2.2 Plant........6

2.2.3 Sewage sludge........7

2.3 Types of biocement....8
2.3.1 Calcium precipitation....9
2.3.2 Biosilica....10
2.4 Advantages of Biocement....10
2.5 Microbially induced calcium carbonate precipitation (MICP)....11
2.5.1 Urease enzyme....11
2.5.2 Microbial Urease....11
2.5.3 Metabolism of urease positive bacteria....12
2.5.4 Cementation Reaction via Ureolytic Bacteria....13
2.6 Source of urea and its properties....14
2.7 Urine pre-treatment....14
2.8 The importance of MICP in greenhouse gas reduction....14
2.9 Key influencing factors of MICP....15
2.9.1 Bacterial species....15
2.9.2 Bacterial Solution Concentration and Cementation Solution Concentration.....16
2.9.3 Ambient Temperature....17
2.9.4 PH Value....17
2.10 MICP Treatment Program....18
2.11 Research gap....20
CHAPTER THREE....21
3. Experimental Methods and Characterization Techniques....21

3.1 Chemicals and Materials........21

3.2 Preparation of soluble calcium ions........21

3.2.1 Preparation of CaO........21

3.2.2 Preparation of $Ca(OH)_2$........21

3.2.3 Soluble calcium ion formation........22

3.3 Culturing of urease producing bacteria........22

3.3.1 Preparation of activated sludge........22

3.3.2 Preparation of pretreated urine........22

3.3.3 Culturing of urease producing bacteria........22

3.4 Monitoring of urease producing bacteria........23

3.4.1. Qualitative method........23

3.4.2. Quantitative method........23

3.5. Preparation of cementation solution........24

3.6. Biocementation treatment of the sand column........24

3.6.1. Preparation of sand column........24

3.6.2. Alternative addition of bacterial solution and cementation solution........24

3.7. Geotechnical test of sand columns........25

3.7.1. $CaCO_3$ content test........25

3.7.2 Unconfined Compressive Strength test........25

3.7.3. Permeability test........26

3.7.4 Percentage Pore space........26

3.8. Microstructural analyses 26

3.8.1. Powdered X-ray diffractometric and Thermogravimetric/Differential thermogravimetric analysis 26

3.8.2. Fourier transform infrared spectroscopy 27

3.8.3. Scanning electron Microscopy Analysis 27

CHAPTER FOUR 28

4. Results and Discussions 28

4.1. Formation steps of soluble calcium ions 28

4.1.1 Thermal Analysis of the precursors 28

4.1.2 XRD patterns of the limestone, calcium oxide, calcium hydroxide, and calcium chloride and the soluble calcium ion 29

4.2 Bacterial growth and Urease Activity Analysis 30

4.2.1 Qualitative determination of the bacterial growth 30

4.2.2 Quantitative determination of the bacterial growth 31

4.3 Microstructural characterization of the formed biocement 32

4.3.1 XRD patterns of the untreated sand and biocemented sand 32

4.3.2 FTIR analysis 33

4.3.3 SEM analysis 34

4.4. Geotechnical analysis discussion 35

4.4.1. The distributions of calcium carbonate precipitates as a function of the sand column layers 35

4.4.2. Unconfined compressive strength versus percentage of calcium carbonate precipitates 36

4.4.3. Permeability versus percentage of calcium carbonate precipitates........................38

4.4.4. Percentage pore space versus percentage of calcium carbonate precipitates 39

CHAPTER FIVE 40

5. Conclusion 40

CHAPTER SIX..41

6. Recommendation 41

CHAPTER SEVEN 42

7. Reference 42

ABSTRACT

ABSTRACT

Biocement, a promising alternative cement made from micororgansisms is considered simpler and more eco-friendly in production process than conventional cement. In this study, a biocemented sand was produced by using locally available materials: activated sludge as a seed of bacteria, cow urine as a urea source and limestone as a soluble calcium ions source through microbially induced calcium carbonate precipitation method in a non- sterlized media. The presence and amount of bacterial biomass were determined qualitatively as the color of the solution chages from yellow to red and quantitativly through optical density and urease activty measurments. The improved geotechnical proerties as a function of calcium carbonate precipitates had been anylzed with their corresponding microstructural changes for the untreated sand (BCS-0), and that of treated sands (BCS-1), (BCS-2), (BCS-3) after two, four, and six times treatments respectivley. The thermal history of the limestone and the calium oxide had been analyzed by Thermogravimetric analysis in which decompostion had been observed at 700 ^{0}C and 420 ^{0}C respectively. The biocemented sand (BCS-3) had the higher geotecnical properties than the rest samples, which is 0.95MPa in unconfied compressive strength, 6.59×10^{-6} m/s in permeablity, and 25 percent reduction in percentage pore space, this might be for this sample there was plenty time for the precipitates to form a stable and well crystaline structures between the sand grains.

Key word: Biocement, calcium carbonate precipitate, urease activity, microbial-induced carbonate precipitation, unconfined compressive strenght, permeability, percentage pore space.

List of Tables

Table 1 Possible microbial process that can leads to potential biocementation (Sweety Parmar 2017) 8

Table 2 Stages of MICP treatments (Dejong, Fritzges, & Nüsslein, 2006) 19

List of Figures

Figure 1: Illustration of calcium carbonate precipitation mechanism induced by urease enzyme activity in microorganisms. (Ariyanti, NoerAbyor, et al., 2012). 14

Figure 2: One-dimensional flow sand column experiments: (left) specimen preparation; (right) specimens during retention time. 20

Figure 3: Schematic for biocemented sand columns preparation 23

Figure 4: DTG, TGA a) limestone powder b) CaO (quick lime)... 28

Figure 5: XRD patterns (a) limestone (b) CaO (c) $Ca(OH)_2$ (d) $CaCl_2.CaOH.2H_2O$...... 30

Figure 6: Qualitative determination of bacterial growth... 31

Figure 7: Optical density, Urease activity, as a function of cultivation hours......................... 32

Figure 8: XRD patterns of BCS-0, BCS-1, BCS-2, BCS-3 33

Figure 9: FTIR results of the untreated and biocemented sand 34

Figure 10: SEM results of the untreated (BCS-0), and the BCS-1, BCS-2, BCS-3 35

Figure 11: Calcium carbonate precipitates distribution at different sand column layers 36

Figure 12: Unconfined compressive strength as a function of calcium carbonate precipitates 37

Figure 13: Permeability as a function of calcium carbonate precipitates 38

Figure 14: Percentage pore space as a function of calcium carbonate precipitates 39

List of Acronyms

MICP	Microbially induced carbonate precipitation
dbA	A-weighted decibel
ASTU	Adama Science and Technology University
AS	Activated Sludge
UV	Ultra violet
PCC	Precipitated Calcium Carbonate
UCS	Unconfined compressive strength
CPP	Calcium Phosphate Precipitate
EPS	Extracellular polymeric substance
ATCC	American Type Culture Collection
UPB	Urease Producing Bacteria
DSM	German Collection of Microorganism
UAB	Urease Active Bacteria
HDPE	High Definition Poly Ethylene
ASTM	American Society for testing Materials
XRD	X-ray Diffraction
SEM	Scanning Electron Microscope
DTG	Differential Thermal Analysis
TGA	Thermographic Analysis
JCPDS	Joint Committee on Powder Diffraction Standard
CW	Cow urine

BCS-0	Biocement sand before treatment
BCS-1	Biocemented sand after two times treatments
BCS-2	Biocemented sand after four times treatments
BCS-3	Biocemented sand after four times treatments
FTIR	Fourier Transform infrared spectroscopy

CHAPTER ONE

1. Introduction

1.1 Background

The building industry has a significant positive impact on social and economic growth in the world. However, it has a significant carbon footprint because it uses a lot of energy, both during the manufacture of raw materials and during the building of structures. This quickens the process of global warming, ice cap melting, and the subsequent rise in sea levels. A similar environmental issue affects the average person's living conditions worldwide. Concrete, cement mortar, and burned clay brick are the building materials that are found to produce the most carbon dioxide (CO_2) during production. Cement is typically used as a mortar and concrete binding materials. On the other side, burned clay bricks are widely used to form masonry walls for buildings. Concrete is the material that mostly used on the globe, second to water (Krishnapriya, Venkatesh Babu, & G, 2015).

Global cement production is rapidly increasing, and it is anticipated that four billion tons of cement is produced each year (Paradiso et al., 2018). The construction and operation cost of buildings contributed 50 percent and 6 percent, respectively, to the global anthropogenic carbon emissions. Producing one burnt clay brick (17 cm x 14 cm x 28 cm in size) in a factory required to burn 0.2 kg of coal, which emits 1197.7 g of carbon during the process (Zhang et al., 2023). In addition, the demolition of historic structures has led to an insufficient use of land for increasing construction waste. These issues have prompted the demand for green alternatives to traditional building materials (Iqbal, Wong, & Kong, 2021).

Currently, a lot of materials have been undertaking as a substitute of convectional cement materials to overcome the aforementioned problem. A few of them are: (i). Using fly ash as a binding materials. In coal-burning power plants, fly ash is created in vast quantities and frequently ends up in landfills. However, when recovered fly ash is utilized to build concrete, it is a replacement for conventional concrete. Fly ash takes less water and improves the behavior and workability of paste. It can cut the need for cement by 20 percent and water by 10 percent (Lazik & Garrecht, 2021). (ii). Substituting cement with ceramic waste powder. A significant amount of ceramic waste is gathered during the final polishing procedure. The poisonous powder that makes up ceramic waste is bad for the air, water, and soil. However, it has shown promise as a cement substitute in concrete. By lowering the need for raw materials, it also benefits the environment. The properties of concrete's workability and durability have been enhanced by the ceramic waste powder (Aswin, 2018).

These are among the works that are undertaken to a green substitute for the conventional cement. But nowadays using biocement to substitute convectional cement is the most effective and simple method. The term "biocement" describes a $CaCO_3$ deposit that developed as a result of microbial activity in a calcium-rich environment (Ariyanti et al., 2012). This biocement is also referred to as bacterial concrete (T. Alhour, 2013). In-situ calcite ($CaCO_3$) is formed when carbonate, which is produced by microbial urea hydrolysis, precipitates with excess calcium ions to form biocement (Al-Thawadi, 2008). Under the appropriate circumstances, this might cause soil to solidify and bond the sand particles (1Khushboo J. Patel, 2019).

Utilizing microorganisms to manufacture biocement is simple because it takes little effort and resources. Additionally, affordable biocement is made using non-pathogenic, environmentally benign microorganisms (Sweety Parmar 2017).

To increase the durability of building materials and structures, biocement, a unique metabolic by-product of microbially induced carbonate precipitation by way of urease (urea hydrolyzing enzyme), is a potential biotechnology. The presence of urease-producing bacteria in nature makes cement stronger under compression, reduces permeability, and slows down the corrosion of reinforced structures (Achal, Li, & Zhang, 2014).

Based on the idea of microbially induced carbonate precipitation (MICP), biocementation is a procedure used to create binding material (biocement). Many industries, including construction, petroleum, and erosion control, and environment can use this method. When used, calcium carbonate precipitation is coupled with other structural materials like sand.

The main focus of this study is to produce biocement using bacteria cultured from activated sludge obtained from ASTU water treatment plant, urea was extracted from cow urine, and calcium ions which was refined from limestone through the MICP route. In this study, X-ray diffraction (XRD), Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), Thermogravimetric analysis (TGA), Permeability, Unconfined shear strength, and Percentage pore space test was performed to examine the geotechnical properties of the produced biocement materials.

Furthermore, the formation of the urease positive bacteria determined qualitatively by the formed colour change. Besides, the bacterial quantification was made by measuring the optical density and urease activities of the biomass.

1.2 Statement of the problems

Microbially Induced Calcium Carbonate Precipitation (MICP) is a sustainable method to enhance soil geotechnical properties. By using specific bacteria to precipitate calcium carbonate within the soil, MICP increases its strength and stability. (Muynck, Belie, & Verstraete, 2010) The process involves microbial activity, where bacteria like Bacillus species hydrolyse urea to raise soil pH and promote carbonate formation. Calcium ions are provided by materials like lime or calcium chloride, while nutrient optimization ensures maximum microbial activity and carbonate precipitation, reducing the need for frequent treatments. Through careful optimization of these factors, MICP can be an effective and efficient way to improve soil conditions (Stabnikov, Chu, Ivanov, & Li, 2013).

Conventional cement industries consume four hundred pounds of coal to produce one ton of calcium carbonate (Ciobanu, Tudor, Istrate, & Voicu, 2022; Mohamad, Muthusamy, Embong, Kusbiantoro, & Hashim, 2021; Raina, Soni, Vajre, & Sangode, 2022). This results in tremendous amount of carbon dioxide emission to the environment. If the carbon dioxide in the atmosphere increases, the earth will experience more frequent extreme weather events such as extended droughts and damaging storms, rises in global sea level, destruction of life-supporting ecosystems, and many more severe consequences for all the planet’s inhabitants. Besides, the noise and particulate pollution are also the additional reasons that subtle the need for alternative sources of cement production. Current studies revealed that, particulate emissions are approximately 52 g/Nm^3 in kiln stack (Zainudeen, 2020).

The use of coal as a fuelling agent in kiln reactions and the use of additives like gypsums, calcined clay, volcanized ash, fumed silica, and fly ash are not sustainable resources since all of which in limited supply. So, biocementation, an alternative source of cement production, is best to reduce the above problems.

Due to the above mentioned reasons, the use of MICP with reduced number of treatment for producing environmentally friendly cement materials is become an impeccable processing technique in order to solve currently recurring headache of our world.

In this research, microbially induced biocement was synthesized for the first time by blending the locally available urine as a urea source, and limestone, which was function as a source for soluble carbonate ions. So that the final solution was cementious solution which substantially bound the grains between the sand columns.

1.3 General objective

Synthesis and Characterization of Biocement using Bacteria, Cow urine, and Calcium ions refined from Limestone.

1.4 Specific objective

- To form biocemented sand column from sand, and equimolar of urea and soluble calcium ion.
- To evaluate the microstructures of the biocemented sand by XRD, FTIR and SEM.
- To evaluate the unconfined compressive strength, permeability, porosity as a function of the amount calcium carbonate precipitates of the biocemented sand.

1.5 Significance of the study

This work benefits our country first by eliminating the need for coal for cement production so that the corresponding environmental pollution and drain of foreign currency will be alleviated. Second, because its raw materials are manufactured at lower temperatures than those used to make ordinary cement, which can reach temperatures of up to 1500 °C, it is appropriate for in-situ and large-scale productions. Besides, it benefits as a benchmark for our country's academicians who are interested in further investigation on Biocement production.

1.6 Scope of the study

In this work, biocemented sand columns by a non-sterile, microbially induced calcium carbonate precipitates technique were synthesized. In a way that the number of treating frequencies was varied for four sand column samples: with the untreated sand column the other three columns were treated two, four, and six times separately. Finally, the geotechnical properties (percentage $CaCO_3$ precipitates, unconfined compressive strength, permeability, and percentage pore space) with the corresponding microstructural change of the samples was analysed.

CHAPTER TWO

2. Literature review

2.1 Biocementation

Biocementation is a process of producing binding material based on a microbial-induced carbonate precipitation (MICP) mechanism (Reddy† & 2015). The precipitation of calcium carbonate ($CaCO_3$) may be performed due to microorganism activity and it produces massive limestone or small crystal forms. These deposits of calcium carbonate are known as biocement or microbial-induced carbonate precipitation (MICP) (Ariyanti, 2012). The primary role of microorganisms in carbonate precipitation is mainly due to their ability to create an alkaline environment (high pH) through their various physiological activities (Reddy† & 2015). In application, the precipitation of calcium carbonate (biocement) is combined with other supporting materials such as sand (Ariyanti, 2012).

The precipitation of calcium carbonate process can be applied in many fields such as construction, petroleum, and erosion control. Applications in the construction field include wall and building coating methods, soil strengthening, and sand stabilizing in an earthquake-prone zone (Muynck, Belie, & Verstraete, 2010).

2.2 Sources of Biocement

2.2.1 Microorganisms

2.2.1.1 Bacteria

Bacteria are prokaryotic unicellular organisms present in almost every place. In nature, many rocks are cemented by the production of Calcium carbonate. Various bacteria found near natural calcium sources may be able to produce calcium carbonate precipitate which is called Biomineralization. Many species of Bacillus able to produce Bio calcite are Bacillus pasteurii, Bacillus subtilis, Bacillus sphaericus and Bacillus lentus (N, 2014). Bacteria that carry out the metabolic process such as Urea hydrolysis, Denitrification, Sulphate reduction, and Iron reduction can produce Calcium carbonate precipitates (Qiu, Sheng, & Yang, 2014). The majority of research is focused on Urease-producing microorganisms, to know the molecular mechanism. Pseudomonas, sporosarchina, and bacillus are among the microorganisms that can produce biocalcite in various amounts (Hammes, Boon, de Villiers, Verstraete, & Siciliano, 2003). Non-ureolytic bacteria are helpful in MICP production in mixed culture as they provide the nucleation site for calcium carbonate precipitation. Halotolerant alkaliphilic bacteria also produce calcium carbonate precipitates (Stabnikov, Chu, Ivanov, & Li, 2013).

2.2.1.2 Microalgae

Photosynthetic organisms which produce urease and consume urea can be used for the production of biocalcite. Thus, microalgae can be used as a nucleation site for Microbial-induced Calcium Carbonate Precipitation. Photosynthetic microorganisms utilize CO_2 which is in equilibrium with carbonate bicarbonate system as shown in equation (2). Microalgae utilize CO_2 resulting in a shift in equilibrium and thus increment in pH occurs (3), when this occurs in the presence of Calcium there is a production of calcium carbonate (4). The reaction occurs as follows (Ariyanti, Handayani, & Hadiyanto, 2012):

$$CO_2+H_2O \longrightarrow (CH_2O) + O_2 \text{ (l)}$$

$$2HCO^{3-} \longleftrightarrow CO_2 + CO_3^{2-} \text{ (2)}$$

$$CO^{3-}+H_2O \longleftrightarrow HCO^{3-} + OH^- \text{ (3)}$$

$$Ca^{2+}+HCO^{3-}+OH^- \longrightarrow CaCO_3+2H_2O \text{ (4)}$$

The amount of increase in pH is directly proportional to the calcium precipitation, hence microorganisms should be selected that can survive in high calcium and ammonia concentration as urea hydrolysis produces ammonia. Photosynthetic organisms such as green algae from marine as well as freshwater are Cyanobacteria syncococcus, Scyntonema, Chlorella, Nannochlorisatomus, Synechocystis, Synechococcus produce Bio-calcite (Bharathi & Strains, 2014).

2.2.2 Plant

Several plants contain silicon in abundant amounts as some plants can absorb silica from the soil. This type of plant is burnt then the biomass has silica mixed with Portland cement to produce Biocement (Hosseini, Shao, & Whalen, 2011). Plant-derived urease can also be used for Biocement production same way as microbial calcium precipitation occurs the only difference is the source. Plant-derived urease has benefits in that its size is small and can penetrate very small cracks. Families of the plants that produce urease are varieties of beans in leguminous family, watermelons, squash, pumpkin, and cucurbits family. The crude extract of jack bean is a good source of urease. Through plant-derived urease both calcium carbonate as well as calcium phosphate cement can be produced (Ran & Kawasaki, 2016).

2.2.3 Sewage sludge

Municipalities collect a large amount of waste which is disposed of by land spreading, burial in landfills, and incineration. It consumes energy as well as money to treat sewage waste but it can be used for Biocement production and reduces the cost of treatment of sewage sludge. Sewage sludge contains silica hence can be used for the production of biosilica and can blend with Portland cement. Sewage sludge can be used in two ways to produce Biocement; (i) by blending its incinerating ash with Portland cement and (ii) by co-combustion of limestone with sewage before adding in Portland cement (Madani Hosseini, Shao, & Whalen, 2011).

Trace metals are another problem during sludge treatment but when sludge is used for Biocement production then trace metals are immobilized in cement and reduce the risk of metal contamination in the environment. Thus sewage sludge can be used as raw material to produce Biocement and partially eliminate the use of Portland cement which will reduce CO_2 emission and act as environment-friendly (Rivadeneyra Torres et al., 2012).

Iron-reducing bacteria's have the ability to reduced Iron (III) into Fe (II) so that the iron can be oxidized and hydrolyzed to generate ferric hydrates. In soil, ferrous ions and chelates are oxidized chemically or through the catalysis of acidophilic or neutrophlic iron-oxidizing bacteria(Ivanov & Chu, 2008).

Sulfate-reducing Bacteria have the capability to produce undissolved sulphides of metals and enhance stability for slopes and dams. Thermothrix, Beggiotoa, Thioplaca, Thiobacillus, Achromatium, Thiomicrospira, Acidithiobacillus, Thiosphaera and Thiothrix are some of the exaples of sulfate reducing bacteria.

Urease active bacteria have the ability to facilitate rapid urea hydrolysis, and raise the pH during urea to ammonia hydrolysis, and produce calcite in rocks or soil. The manufactured cement using Ammonifying Bacteria has a 5MPa maximum compressive strength. Conglomerate, breccia, sandstone, siltstone, shale, limestone, gypsum, peat, lignite, sand, soil, clay, sediments, and sawdust are just a few of the materials that can be handled by biocementation. The genera of the microorganisms that produce urease are Desulfotomaculum, Sporosarcina, Sporolactobacillus, Clostridium, and Bacillus also summarized in table1 below (Ivanov & Chu, 2008).

Table 1 Possible microbial process that can leads to potential biocementation (Sweety Parmar 2017)

Physiological group of Microorganisms	Mechanism of Biocementation	Essential conditions for Biocementation	Potential Geotechnical Applications
Sulfate-reducing Bacteria	Production of undissolved sulphides of metals	Anaerobic conditions; presence of sulfate and carbon sources in soil	Enhance stability for slopes and dams
Ammonifying Bacteria	Formation of undissolved carbonates of metals in soil due to increase of pH and release of CO_2	The presence of urea and dissolved metal salt	Mitigate liquefaction the potential of sand. Enhance stability for retaining walls, embankments, and dams; Increase bearing capacity of foundations
Iron-reducing Bacteria	Production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil	Anaerobic conditions changed for aerobic conditions; presence of ferric minerals.	-Density soil on reclaimed sites and prevent soil avalanching -Reduce liquefaction potential of soil

2.3 Types of biocement

Based upon the main constituents’ oxides that are present in biocement, it is classified majorly as calcium precipitates and biosilica biocement.

2.3.1 Calcium precipitation

The calcium precipitation used in biocement is further classified into two types: Calcium Carbonate Precipitates and Calcium Phosphate Precipitates.

2.3.1.1 Calcium Carbonate Precipitates

In nature, many rocks are cemented by the formation of calcium carbonate. A building having good strength, due to humidity there is an expansion in structure that leads to crack. When this type of building is treated with MICP producing microorganisms the cracks can heal by reducing porosity (Qiu et al., 2014). Widely studied calcium carbonate precipitation occurs by urea hydrolysis by urease enzyme from the microbes. Urea is widely used as fertilizer, a waste product obtained from mammals, and the decomposition of uric acid excreted by birds. An increase in urea causes contamination of soil, and water and produces harmful algal blooms. Microorganisms produce urease exists both within the cell and as an extracellular enzyme on cell death. During microbial urease activity, one mole of urea converts to one mole of ammonia and one mole of Carbamic acid (1), which spontaneously hydrolyses to another mole of ammonia and carbonic acid as follows (Qiu et al., 2014).

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{Bacteria}} NH_2COOH + NH_3 \qquad (1)$$

$$NH_2COOH + H_2O \longrightarrow NH_3 + H_2CO_3 \qquad (2)$$

This product equilibrates in water to form bicarbonate, producing one mole of ammonia and hydroxide which results in a pH increase.

$$H_2CO_3 \longrightarrow 2H^+ + 2CO_3^{2-} \ (3)$$

$$NH_3 + H_2O \longrightarrow OH^- + NH_4^+ \ (4)$$

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3 \ (5)$$

Carbonic acid increases CO_2 conversion and increases in production of carbonate. Hydrolysis of urea results in the accumulation of both bicarbonate and ammonium ions in the cell favors urea and bicarbonate metabolism. Production of carbonate from bicarbonate is strongly pH dependent, an increase in carbonate concentration occurs under alkaline conditions. Therefore, in an alkaline environment, $CaCO_3$ precipitation occurs outside the cell as calcium and carbonate are present in an abundant amount. Calcium carbonates are deposited on the cell due to the production of Biofilm and extra polymeric substances by bacteria. Naturally, the deposition of $CaCO_3$ in the void spaces leads to clogging of porous material (Bharathi & Strains, 2014).

2.3.1.2 Calcium Phosphate Precipitates

Calcium Phosphate Precipitates (CPP) are widely used in biomedical applications such as Bone regeneration. CPP is produced widely by mixing Calcium orthophosphates with aqueous phase generally water which forms paste and can be used (Jia et al., 2010). The porosity of CPP can vary from 30% to 50 % depending on the ratio of liquid to powder (Vater et al., 2010) . There are various commercial CPPs available in the market (Horiuchi et al., 2014). Very little research has been done on the application of CPP in construction as the cost increases a lot when CPP is used.

2.3.2 Biosilica

There are two steps to produce Biocement by using biosilica as follows: (i) Organic residues are burnt to obtain ash containing Biosilica (ii)Biosilica mixed with Portland cement to produce Biosilica. Thus Biosilica decreases the use of Portland cement. Various organic substances can be burnt to obtain Biosilica. Biosilica act as a pozzolanic material. The pozzolanic reaction between Biosilica and calcium hydroxide is as follows:
$Ca(OH)_2 + H_4SiO_4 \longrightarrow Ca^{2+} + H_2SiO4^{2-} + 2H_2O \longrightarrow CaH_2SiO_4 .2H_2O$. This reaction is optimized to obtain the desired physical and mechanical strength of Biocement.

Si is the second most abundant element on this earth after oxygen. Various plants obtain silica from soil and deposit it in the older leaf. Non-edible parts of a plant contain silica which can be used for the production of Biocement.

The following sources are burnt to produce ash containing biosilica. These are: Sawdust ash as, Paper mill sludge ash as, Rice husk and rice straw ash as, Sugar cane ash as , Oil palm ash as , Bamboo leaf ash. Biosilica partially reduces the emission of CO_2, as it is mixed with Portland cement to reduce the use of Portland cement.(Hosseini et al., 2011).

2.4 Advantages of Biocement

Biocement has many advantages compared to ordinary cement, such as the production process is slightly different from sandstone production, Biocement need a much shorter time; it is suitable for in-situ process; raw material of Biocement are produced at low temperature, and is more efficient compared to an ordinary cement which used temperature up to 1500˚C in the production process; Biocement can be used as eco-construction material since it consumes less energy and less CO_2 emission in the production process rather than other ordinary cement(Ariyanti, NoerAbyor, ayani, & Hadiyanto, 2012). It is easy to produce Biocement using microorganisms as it requires less time and requirement. Biocement is also cost-effective and non-pathogenic and environment-friendly microorganisms are used to produce Biocement (Ariyanti, NoerAbyor, et al., 2012). It increases the unconfined compressive strength of mortar by up to 38% and can also decrease the permeability of soil and also improve porosity(Reddy† & 2015).

2.5 Microbially induced calcium carbonate precipitation (MICP)

MICP is induced as the urease positive bacteria metabolize the urea in urine and release urease enzyme so that the decomposed product, specifically the carbon dioxide, will react with soluble calcium ion to form cementation reaction.

2.5.1 Urease enzyme

An enzyme called urea amidohydrolase, or urea enzyme (EC 3.5.1.5), catalyses the hydrolysis of urea to produce ammonia and carbon dioxide. The first enzyme to crystallize was urease from jack beans (Summer, 1926) and it has since been discovered in bacteria, yeast, and various higher plants. Jabri et al. (1995) determined the structure of urease for the first time, demonstrating that although ureases can be made up of one, two, or three different types of subunits, the proteins are all closely related. For instance, Klebsiella aerogenesi (Mulrooney and Hausinger, 1990), Proteus mirabilis (Jones and Mobley, 1989), Proteus vulgaris (Mörsdorf and Kaltwasser, 1990), and Ureaplasma urealyticum all have three-subunit urease enzymes.

2.5.2 Microbial Urease

Microbial Urease activity is widespread in the environment, including bacteria, yeasts, filamentous bacteria, and algae (Booth and Vishniac, 1987; Bekheet and Syrett, 1977; Jeffries, 1964; Seneca et al., 1962). Microbial ureases play an important role in certain human and animal pathogenic states, in ruminant metabolism, and environmental transformations of certain nitrogenous compounds.

The synthesis of urease in the microbial system is generally regulated in five ways (Mobley and Hausinger, 1989; Mobley et al., 1995). These are the modes:

1. Induction by urea: in this case urease expression is triggered by the presence of the substrate urea or other environmental factors. It has been noted that the activity of the ureases induced by the substrate urea reached levels 5 to 25 times greater in Proteus and Providencia species than those in non-induced culture.
2. Developmental control: where the expression of urease varies relating to the developmental stages. Falkinham and Hoffman (1984) reported that in Proteus species, the urease level and urease transcript was high during swarming state, while negligible activity was found in non-swarmer cells.
3. Regulation by pH: According to Sissons et al. (1990), urease is controlled by the pH of the surrounding environment via controlling the rate of urease synthesis. In this phase, which is present in some oral bacteria, urease contributes to the defence of the cells against an acidic environment.
4. Nitrogen regulation: it occurs when high-quality nitrogen sources (NH_3, NH_4^+) are present. In contrast, under nitrogen-limiting or nitrogen shortage conditions, the urease production is triggered (re-depressed). Numerous ureolytic species, such as Pseudomonas aeruginosa (Janssen et al., 1982), Bacillus subtilis (Atkinson and Fisher, 1991), and Rhizobium meliloti (Miksch and Eberhardt, 1994), have been found to exhibit nitrogen control of urease production.
5. Constitutive expression – where the enzyme expression is constant regardless of the environmental conditions (e.g. Bacillus pasteurii, Sporosarcina. uresa, Morganella morganii, and the chromosomally encoded ureases of some E. coli isolates).

2.5.3 Metabolism of urease positive bacteria

Several ureolytic bacteria have revealed that urea hydrolysis plays a novel role in the production of ATP. Proton motive force (p), which includes the transmembrane pH gradient (pH) and the charge gradient or membrane potential (Ψ), controls the production of ATP.

$$\Delta p = \Delta pH + \Delta\psi$$

The chemiosmotic proton gradient is necessary for ATP synthesis in neutrophilic bacteria. The gradient in proton concentration across the cell membrane (low inside/high outside) is caused by the expulsion of protons from bacterial cells through the electron transport chain. ATP is produced as a result of protons being driven back into the cells by a concentration gradient through ATP synthase. (Prescott et al., 1993). and lower pH (high proton concentration) inside the cell, the protons will spontaneously diffuse from inside to outside the cell due to the proton gradient.

This flux direction is opposite of the normal direction for ATP generation. To survive the reversed proton gradient, alkaliphiles have developed two mechanisms to drive the protons back into the cells. These two mechanisms include:

Increasing the cytoplasm's alkalinity in the cells, which lowers the reversible pH, and by Increasing the $\Delta\psi$ by the efflux of cations other than proton. Due to the increased proton motive force (Δp), the protons will be driven back into the cells against the concentration gradient. Ureolytic bacteria, most are alkaliphiles, utilize urea hydrolysis to generate ATP.

2.5.4 Cementation Reaction via Ureolytic Bacteria

S. pasteurii is one of many ureolytic bacteria that are found in settings like soils and aquatic environments. This particular species of bacterium, which thrives in aerobic environments, is heterotrophic, meaning that it needs organic carbon to flourish. Because of its negative charge, the bacterial cell wall attracts dissolved calcium ions when they are in solution. The microenvironment's pH increases as a result of the bacteria's catalysing of the breakdown of urea to release ammonium and release dissolved inorganic carbon (DIC).

In the presence of calcium, the formation of alkalinity promotes the precipitation of calcite Figure 1. Bachmeier et al. (2002) looked into the function of ureolytic bacteria in the precipitation of calcite. The response is described below:

$$Ca^{2+} + cell \rightarrow Cell - Ca^{2+}$$

$$CO_3^{2-} + Cell - Ca^{2+} \rightarrow Cell - CaCO_3$$

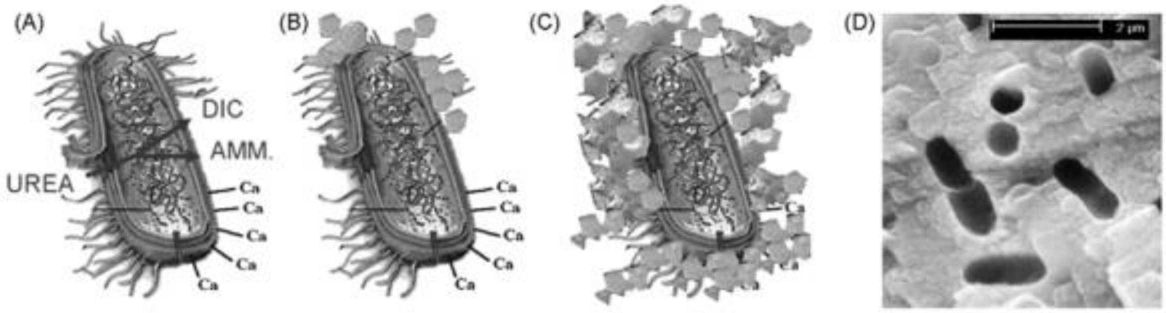


Figure 1: Illustration of calcium carbonate precipitation mechanism induced by urease enzyme activity in microorganisms. (Ariyanti, NoerAbyor, et al., 2012).

2.6 Source of urea and its properties

The composition of CU is 95% water, 2.5% urea, 2.5% enzymes, minerals, 24 different types of salts, and minerals. It also contains lactose, nitrogen, ammonia, manganese, calcium, phosphorus, carbonic acid, potassium, urea, phosphates, potassium, urea, uric acid, amino acids, enzymes, cytokines, and iron. It has a molar mass of 60.06 g/mol. It has a melting point of 134 °C and is a colorless to white crystalline solid with a density of 1.32 g/cm^3. It is readily soluble in both alcohol and water. Urea is a weak base that is insoluble in ethane.

2.7 Urine pre-treatment

Fresh urine was collected, filtered using filter paper with a pore size of 6 m (Grade 3 circle, diameter 23 mm), and then treated to UV radiation for 6 hours before being utilized safely as a growing culture. The UV can kill and render inactive all pathogens, including viruses and bacteria, but it does not affect the chemical composition of urine. It decreased methanogenesis while increasing the efficiency of hydrolysis in terms of microbial community compositions and enzymatic activity.(Yang, Chu, Cheng, & Liu, 2022).

2.8 The importance of MICP in greenhouse gas reduction

Green cement is based on geopolymers, the main advantage in the production of this type of cement is the reduction of CO_2 emissions during its production process since the processing of common cement uses fuels to heat limestone to a temperature of 1500°C to obtain lime, and by decarbonization of limestone, geopolymer-based cement can be manufactured at half the temperature (800°C) and by-products of the manufacturing process they can be incorporated back into the production of this cement, which reduces up to 80% the environmental emissions involved in its development and with a production cost 50% lower than that of common cement (Barberán et al., 2020).

2.9 Key influencing factors of MICP

The conventional precipitation of calcium carbonate is simple, influenced by calcium ion concentration, pH, and nucleation site. However, the micro organismic cement precipitation process (MICP) is complex, influenced by factors like bacterial species, temperature, pH, cementation solution ratio, and cemented materials.

2.9.1 Bacterial species

It significantly influences the crystal form, morphology, and deposition rate of calcium carbonate. The cementation effect of microorganisms on geotechnical materials is determined by the crystal types and calcium carbonate generated by MICP. Studying the mineralization characteristics of different bacteria is crucial for bacterial reinforcement and engineering applications. Scholars worldwide have studied the types and control of calcium carbonate crystals, the adaptability of bacteria to the environment, and MICP expansion.

Bacillus megaterium showed the highest urease activity among bacterial cultures in niobium medium with urea and calcium chloride. It plays a crucial role in building materials repair, soil reinforcement, heavy metal recovery, and surface protection. It also degrades insoluble phosphorus compounds, making them soluble for crops. Cocultures of Bacillus megaterium and Bacillus sphaericus show nitrogen fixation and synergism, making them suitable for microbial fertilizers.

The main constituents of calcium carbonate, according to the experimental investigation, were calcite crystals and a small quantity of aragonite crystals. The quantitative impact of bacterial plugging on the hydraulic conductivity of porous media was confirmed by the study mentioned above. The amount of bacterial cell deposition was inversely correlated with the amount of $CaCO_3$ precipitation. The relative rate of hydraulic conductivity loss was influenced by the number of depositing cells. The density of bacterial cells added to the bacterial solution had an impact on the number of depositing cells(Eryürük et al., 2015). By using ultraviolet light, a positive phenotypic mutant of Bacillus pasteurii (MTCC 1761) was created. This mutant created the calcite by active biomineralization. A more efficient method of fixing the problem could be to screen and cultivate mutant strains.

To locate microorganisms that behave satisfactorily under varied environmental circumstances (not only lab temperature), pertinent studies have been conducted. Bacillus sphaericus was the studied urealytic that was most suited for biodegradation in actual situations. An important component of MICP is urease activity. According to Bachmeier et al. (Bachmeier, Williams, Warmington, & Bang, 2002), using immobilized enzymes as opposed to completely immobilized cells produced a better healing result.

An improved method for filling structural gaps may be found in the screening and development of mutant strains. It also represents a classic example of a biotechnology cross-application with promising future growth. Additionally, it is the main focus of interdisciplinary research between biological science and geotechnical engineering.

2.9.2 Bacterial Solution Concentration and Cementation Solution Concentration.

In the MICP process, bacteria are crucial for the crystallization of calcium carbonate. According to Tourney and Ngwenya, the extracellular polymeric substance (EPS) produced by bacteria had a favorable impact on the shape and crystal type of the $CaCO_3$ precipitation, which was essential for the cementation of geotechnical materials. The MICP procedure benefited from EPS's ability to increase the precipitation of calcite while inhibiting the precipitation of aragonite. The concentration and degree of Ca^{2+}, CO_3^{2-} plasma supersaturation were significantly affected by the number of microorganisms in a mineralized environment (Zhang, Shi, Chen, Huo, & Yu, 2021a). The ionic strength was proportionate to the concentration of urea, calcium, and bacterial cells in the experiment by Okwadha and Jin (Okwadha & Li, 2010) in which Bacillus Pasteurella ATCC strain 11859 was cultured at 25°C. the concentration of bacteria during the urea breakdown rate was higher than the starting urea concentration. The bacterial solution's +e content was discovered to have a significant impact on the shape of calcium carbonate crystals(Zhang, Shi, Chen, Huo, & Yu, 2021b).

Calcium carbonate crystals exhibited spherical or nearly spherical shapes at 100% bacterial solution concentration. Reduced concentrations resulted in irregular shapes, regular cuboids, and aggregates. The composition of culture medium did not directly affect MICP efficiency, but the type and concentration of cement solution affected mortar strength. The concentration of cementation fluid was found to play a crucial role in MICP design and implementation.

Low-concentration cementation fluid produces more uniform and stronger crystals than high-concentration fluid, but requires more steps, time, and costs. The bacterial concentration is key to controlling crystal size, with increasing bacteria concentration in coarse sand being more advantageous. The average size of the sphere increases with cementation solution concentration, with spherical size growth becoming saturated when concentration exceeds 10-25 mM. Therefore, the optimal concentration of bacteria liquid and cementation liquid was considered beneficial for improving the cementation strength and maintaining good air permeability.

2.9.3 Ambient Temperature

The development and metabolic processes of bacteria will be immediately impacted by changes in the ambient temperature. This will alter the rate and yield of calcium carbonate deposition as well as the kind and form of crystals. The effect of microbial mineralization and cementation will be directly impacted by the size of the calcium carbonate crystals and their method of cementation between soil particles as a result of temperature changes. Therefore, it is important to research how temperature affects MICP activity (Dae et al., 2020). According to studies, urease's catalytic activity was at its peak between 20 and 37 degrees Celsius(Okwadha & Li, 2010). The urease activity increased with temperature in this temperature range, which is characteristic of the majority of MICP experiments. A good practical use, however, requires taking into account how bacteria can alter their activity over a wider temperature range. The MICP The impact of temperature on the biodegradation efficiency of various urealytics was examined in research(Khodadadi Tirkolaei, Kavazanjian, van Paassen, & Dejong, 2017), including the reinforcement and protection effect of the treatment, the growth, and the urealytics activity of urealytics at different temperatures (10, 20, 28, and 37°C). Of all the examined microorganisms, the results indicated that Bacillus sphaericus was the most suited for biodegradation under diverse environmental conditions.

2.9.4 PH Value

The metabolism of urease-producing bacteria (UPB), calcium carbonate deposition, and the enhancement of geotechnical qualities are all significantly influenced by pH value. The majority of urease bacteria that are frequently utilized in microbial mineralization can grow in an alkaline environment. For instance, around 9.0 is the ideal pH for Bacillus. For the large-scale culture of Pasteurella, a method for economic growth has been created. The organism had a growth-friendly pH of 9.25 and was somewhat alkalophilic. Through minimum upstream and downstream processing, significant bio coagulation activity may be grown in no sterile settings. The cost of manufacture was cut by 95%. The novel medium produced urease activity at a high level (hydrolysis of 21 mmol urea for 1 minute) for a low cost (0.20 USD per litter) It was essential for MICP's field application. Studying bacterial activity in various pH ranges is important practically since many technical applications must be executed in an acidic environment. Recently, a scientific research team (Lai et al., 2020) examined the mechanical characteristics of iron tailings under low pH circumstances using MICP technology. Under the conditions of 1108 cells/ml bacterial solution, the calcium carbonate content of the Sporosarcina pasteurii (DSM 33) strain increased first and then decreased when the fluid pH changed from 4.5 to 6.5. At pH 6, calcium carbonate content peaked.

In comparison to conventional cementation, the spray approach using a low pH bacteria solution could improve bio-coagulation and make the adhesive more homogeneous. The results provided direction for using MICP technology in acidic settings by demonstrating that this process was particularly suitable for the solidification and dust suppression of iron tailings sand and the reinforcing of tailings pond. Other research has demonstrated that a higher pH raises the growth medium's need for carbon dioxide (Okyay & Rodrigues, 2015). For MICP technology to absorb carbon dioxide and lessen the greenhouse effect, this phenomenon might be useful. Some researchers changed the environment's pH value during the research procedure from 9.5 to 10, and they successfully produced white precipitates by adding calcium ions. XRD analysis indicated that calcite was still the primary constituent of precipitates. Studies on the use of MICP at various pH levels help promote this environmentally beneficial technology into practice (Zhang, Shi, Chen, Huo, Yu, et al., 2021).

2.10 MICP Treatment Program

The MICP treatment can be divided into two stages. In the first (biological phase), 1 group of the bacteria solution can be percolated from the top by gravity and left to set within the Specimen for 24 hours to allow for microbes to attach to the particles (Dejong, Fritzges, & Nüsslein, 2006). In the seconds (cementation) phase, 1 group of the nutrient solution second row of the below table can be injected in the same way and the old solution was allowed to drain out of the specimen.

Table 2 Stages of MICP treatments (Dejong, Fritzges, & Nüsslein, 2006)

Solution	Constituent	Concentration
Initial biological treatment	Yeast extract Ammonium sulphate Tris buffer *S. pasteurii*	20 g/l 10 g/l 0.13 M/l OD600 = 0.8-1.2
Urea-$CaCl_2$ medium	Urea* $CO(NH_2)_2$ Calcium chloride* $CaCl_2$ Sodium bicarbonate $NaHCO_3$ Nutrient broth	0.375 M/L 0.25 M/L 2.12 g/L 3 g/L

To ensure that the sample remained saturated between injections, the plastic tubing was put up and a solution overhead was left at the top, as shown in the below figure. This process of Injection-retention of the nutrient solution was repeated every 24 hours until the desired level of calcite cementation was attained.

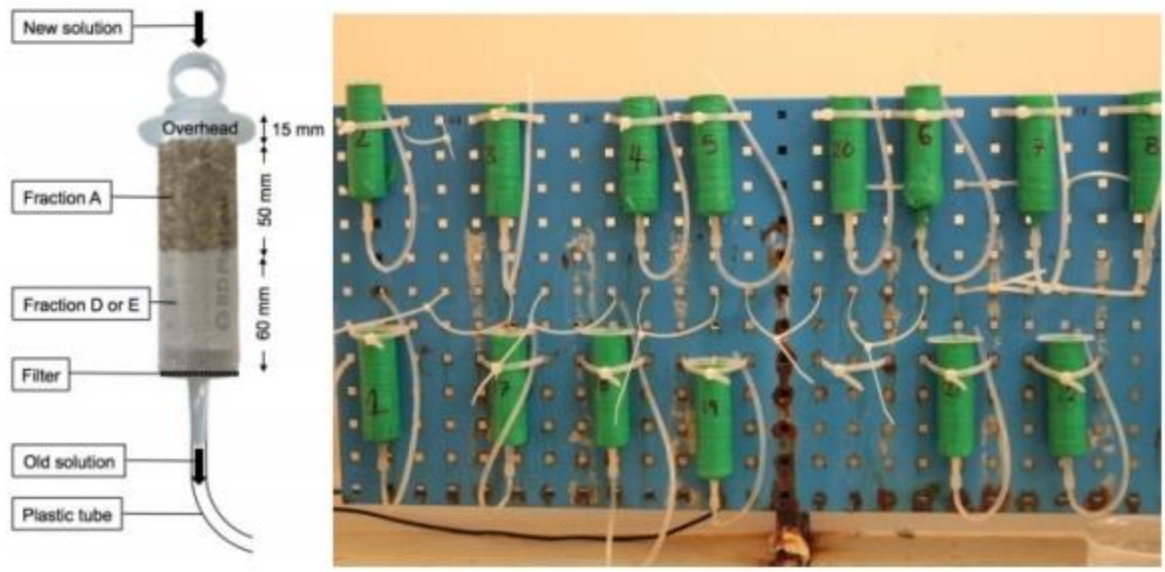


Figure 2: One-dimensional flow sand column experiments: (left) specimen preparation; (right) specimens during retention time.

The termination of each test involved draining all the remaining liquid solution and flushing with DI water to remove excess material. After removing the specimen from the syringe, it was oven-dried to stop the metabolism (Alexandra Clarà Saracho & Dr Stuart Haigh, 2018).

2.11 Research gap

Even though so many studies have been done for biocementation, there is no paper done on the MICP method by locally available substituents: limestone, activated sludge, cow urine, and waste hydrochloric acid all of which are locally available and easily accessible so that by using them it is going to be synthesized a new biocemented sand by formulating the below methods.

CHAPTER THREE

3. Experimental Methods and Characterization Techniques

3.1 Chemicals and Materials

The chemicals that have been used in this research are calcium carbonate, distilled water, waste Hydrochloric acid (68% HCl), cow urine, activated sludge, and 1M NaOH.

The materials that have been used in this research are sample holders, beakers, burette, pipette, micro sieve, filter paper, centrifuge, and centrifuge tube, HDPE columns, flask, and stirrer bar, PH meter.

3.2 Preparation of soluble calcium ions

3.2.1 Preparation of CaO

The limestone rock received from the Mugar area was crushed by using a steel-ball milling machine to achieve the required particle size for processing. Then, the limestone was calcined at 800 ^{o}C for 2 hours and 30 minutes in a Muffle furnace, separated into quicklime (CaO) and carbon dioxide (CO_2).

$$CaCO_3 \xrightarrow{Heat} CaO + CO_2$$

Furthermore; the limestone was grounded in a mortar and pestle, and was sieved using a 100-mesh sieve to obtain uniform particle size distribution for later uses and further characterization.

3.2.2 Preparation of $Ca(OH)_2$

Calcium hydroxide (also known as slaked lime) was prepared after 10g of the quick lime dissolved in 100 mL distilled water and precipitated for 24 hours. The chemical reaction process was as follows:

$$CaO + H_2O \longrightarrow Ca(OH)_2$$

3.2.3 Soluble calcium ion formation

Soluble Calcium ions were prepared by dissolving 74.1g of solid $Ca(OH)_2$ in 25ml of Hydrochloric acid (68% HCl). The mixture was well mixed at 100 ℃ for two days to allow a complete reaction; the residue was filtered using filter paper, leading to the obtained solution containing approximately 0.55M of soluble Calcium ions. This obtained solution after acid-base neutralization reaction formed calcium chloride and calcium hydroxide.$2H_2O$.

3.3 Culturing of urease producing bacteria

3.3.1 Preparation of activated sludge

The activated sludge collected from Adama Science and Technology University Wastewater treatment plant was used as the seeds for the cultivation of urease producing bacteria. The total suspended solids of the sludge was determined by filtering the sludge sample through a pre-weighed glass fiber filter, then by capturing the solids larger than 2 um. Then, the filter was dried in an oven to remove remaining water and weighed again. The weight difference over the sample volume provides the TSS concentration in mg/L. The dissolved organic carbon was determined by removing inorganic carbon with acid, remove the resultant CO, and oxidizing the remaining C and the CO was measured. And finally the PH of the sludge was measured by PH meter.

3.3.2 Preparation of pretreated urine

Before being used safely as a growing culture, fresh cow urine was collected and centrifuged under centrifuge, and then exposed to UV light for 6 hours. All pathogens, including viruses and bacteria, were destroyed and rendered inactive by UV.

3.3.3 Culturing of urease producing bacteria

During the cultivation, 10 mL of activated sludge was mixed with 90 mL of the pretreated urine and 5 g/L of glucose addition with the initial pH of the mixture was adjusted to 10 using 1 M of NaOH, followed by shaking at a speed of 250 rpm at 30 ˚C for 48 hours in shaker incubator. Every six hours, optical density (OD_{600}), pH level and urease activity were continuously measured using the method with slight modification of (Cheng method).

The optimal period for harvesting urease-producing bacteria to create biocement was determined to be when urease activities were peaked.

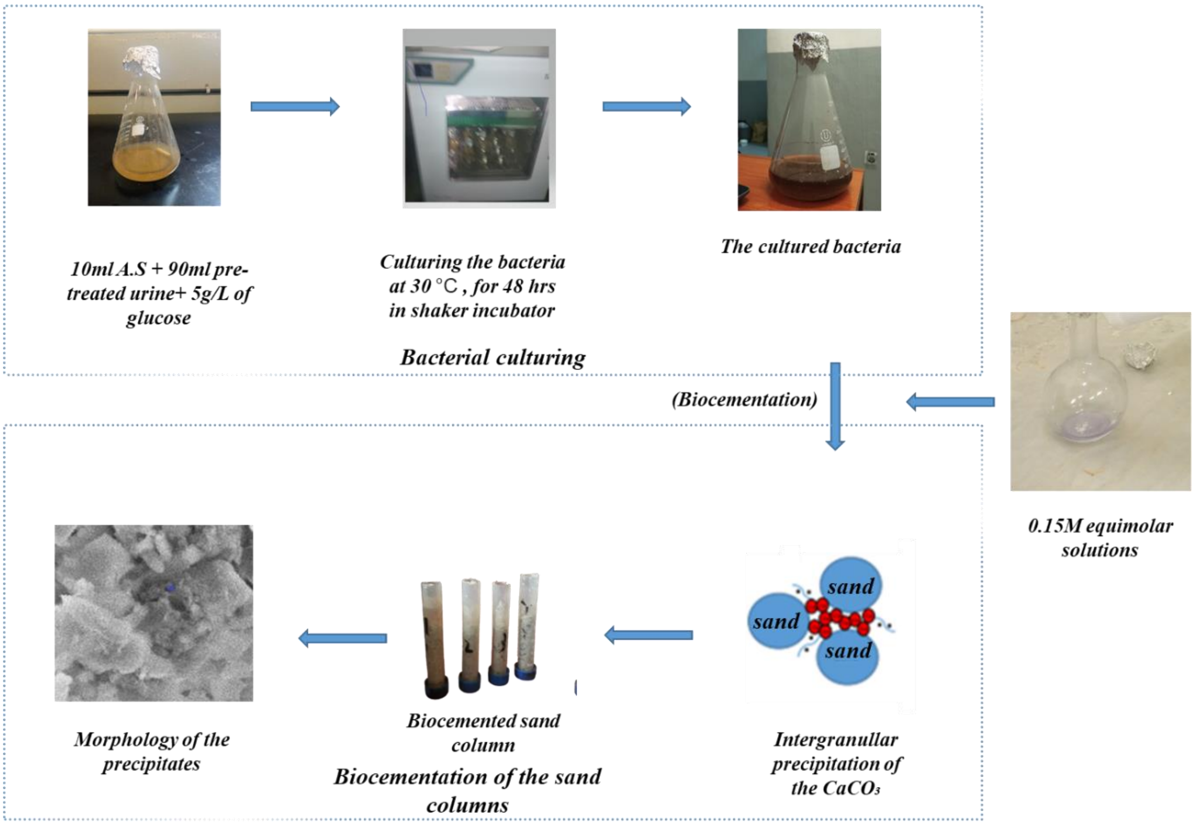


Figure 3: Schematic for biocemented sand columns preparation

3.4 Monitoring of urease producing bacteria

3.4.1. Qualitative method

Urease active bacteria (UAB) media in test tubes was used for the UPB screening. The biomass was putted in a shaker incubator at a temperature of 30 degree centigrade for 48 hours at which possible color change could occur. Bacterial isolates were chosen from ASTU wastewater treatment activated sludge samples.

3.4.2. Quantitative method

3.4.2.1. Urease activity measurement

In the absence of calcium ions, urease activity was determined via solution conductivity.1 mL of bacterial suspension was added to 5 mL of 3 M urea and 4 mL of DI water and the relative conductivity change was recorded over 5 minutes at 25±1^{o}C. The urease activity was then calculated taking the dilution into account. 1U=11.11×E.C mM/(mS/cm) (R_2=0.998)

(Whiffin, 2004).One unit of urease activity is the amount of enzyme that degrades 1 μmol of urea per minute at 25°C.

As the culture was collected at the stationary phase of growth (i.e. all readily available nutrients were consumed from the medium), no further growth of bacterial cells in the sand column during the incubation time could occur.

3.4.2.2. Biomass measurement (OD_{600})

Biomass concentration was determined by measuring optical density of bacterial suspension with a spectrophotometer (Pharmacy department, A.S.T.U, Ethiopia) at a wavelength of 600 nm (Harkes et al., 2010).

3.5. Preparation of cementation solution

The fresh urine was collected and mixed with previously obtained soluble calcium (calcium chloride) to form a mixture in a one litter of urine mixed with 1.11gm of solid calcium hydroxide which forms approximately 0.15 M of equimolar urea and soluble calcium ions, termed a cementation solution.

3.6. Biocementation treatment of the sand column

3.6.1. Preparation of sand column

Sand (Awash melekasa, Eastern Shewa, Ethiopia) was used (>0.3mm: 2.13%, 0.212-0.3 mm: 62.39%, 0.15-0.212 mm: 28.59%, and <0.15 mm: 6.89%) for all experiments. All columns used in this study were made of Poly Vinyl Chloride (PVC) tubing (internal diameter of 15 mm, length of 120 mm). The top and bottom of column were covered with a layer of scouring pads (porous plastic pad) as filters.

3.6.2. Alternative addition of bacterial solution and cementation solution

The top and bottom of the vertically arranged sand columns were completely exposed. From the top of the columns, reagents (cementation solution and bacterial suspension) were added. A combination of capillary forces and gravity caused the liquid to move. The steps that made up the percolation process were as follows:

1) Percolation of the bacterial solution (100 mL for 110 mL sand column)

2) Percolation of cementation solution of 100 mL after half an hour of putting the bacterial solution
3) The reaction process was allowed (urease hydrolysis and calcite precipitation) to occur for 12 hours. Then this cycle was repeated two times for (BCS-1), four times for BCS-2 and six times for (BCS-3).

3.7. Geotechnical test of sand columns

3.7.1. $CaCO_3$ content test

To dissolve the calcium carbonate, 20 mL of 1-M HCl acid was combined with a 5 g sample. After that, distilled water was used to wash the entire solution and the insoluble solid for ten minutes through filter paper with a coarse pore size in a No. 100 sieve.

This washing process was able to remove all soluble calcium from the sand particles. Then all solid particles remaining on the sieve were oven-dried and weighed. The weight difference between the original sand sample (m_0) and post washing sample (m_1) was the mass of calcium carbonate; in percentage it is:

$$\delta_i = \frac{m_0 - m_1}{m_0} \times 100\% \qquad where\ \delta_i = mass\ of\ each\ subparts$$

m_0=initial mass of the subsample

m_0-m_1=reduced mass of the solid particle.

Each subpart is crushed and oven dried at 105 degrees excessive HCl(aq) solution for 24 hrs. The average value of percentage of $CaCO_3$ precipitate is calculated as:

$$\delta = \frac{\sum \delta}{n}$$

3.7.2 Unconfined Compressive Strength test

According to the standards of ASTM D2166/D2166M (ASTM), all of the biocemented sand columns were put through unconfined compressive testing. These tests were conducted utilizing a load frame and a uniaxial loading of 1 mm/min. All column samples were cut to have a length to diameter ratio of 2. The maximum load per unit area is defined as the unconfined compressive strength.

3.7.3. Permeability test

An analysis of the soil sample's hydraulic conductivity (K) is performed using the falling head permeability test. To determine K for each time interval, the following formula has been used:

$$K = \frac{2.3.a.L.\log(\frac{h_1}{h_2})}{A.t}$$

Where: K - Hydraulic conductivity (cm/s); a - Cross-sectional area of the standpipe (cm^2); A- Cross-sectional area of the sample; L - Length of the biocemented sand sample (cm); h_1-initial height; h_2-final height; t - time taken for the water to reach from h_1 to h_2. Then by measuring three times for each samples then the average of each for every single sample was taken.

3.7.4 Percentage Pore space

This is ratio of the weight in air of a unit volume of the impermeable portion of the sand (does not include the permeable pores in sand) to the weight in air of an equal volume of gas-free distilled water at a stated temperature.

$$G_s = \frac{A}{A - C}$$

Where: G_s = Percentage pore space

A = Oven dry weight

C = Weight in water

By measuring this value three times for each samples, then the average for each of them was taken.

3.8. Microstructural analyses

3.8.1. Powdered X-ray diffractometric and Thermogravimetric/Differential thermogravimetric analysis

X-ray diffraction (XRD) examination of samples of biocemented materials and calcium hydroxide was used to verify the chemical composition of the biocemented sand and the generated $Ca(OH)_2$. Bruker Advanced D8 equipment at a scanning rate of 4^0 /min was used to perform the X-ray diffraction (XRD) analysis of the results. Cu K radiation (40 kV, 40 mA) was used. TGA/DTG analysis was performed in order to determine the calcine temperature of the limestone.

3.8.2. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy characterization of the untreated and treated sand samples were performed in order to verify the formation of the functional groups related with the expected precipitates. The sample was positioned within the FTIR spectrometer's light path. The source's infrared radiation travels through the sample, and a broad variety of infrared frequencies are used to assess the transmitted light's intensity. The spectrometer logs the intensity difference between the sample and background spectra. The sample's infrared absorption spectrum is represented by this discrepancy. Plotting the transmitted light's intensity against the infrared frequency yields the obtained FTIR spectrum. It was on the range of 500 to 4000 wavenumber (cm^{-1}) with a scanning speed of 2mm/sec.

3.8.3. Scanning electron Microscopy Analysis

Scanning Electron Microscopy (SEM, Zeiss EV050, UK) observations and analysis were also carried out to image the morphologies of the precipitated calcium carbonate, which is important to study the mechanical properties of biocemented sand in detail. Microstructural specimens were obtained from crushed biocemented samples. All specimens were rinsed with tap water and oven-dried at 105 ◦C for 24 h prior to microstructural tests.

CHAPTER FOUR

4. Results and Discussions

4.1. Formation steps of soluble calcium ions

4.1.1 Thermal Analysis of the precursors

The breakdown of calcium carbonate into CO_2 and CaO is responsible for the weight loss between 700 ^{0}C and 800 ^{0}C, according to the DTA and TGA curves. Additionally, the endothermic peak at approximately 800 ^{0}C verifies that calcium carbonate breaks down into CO_2 and CaO when heat enters the limestone. This result has shown that the limestone exhibits the thermal behaviour of calcite.

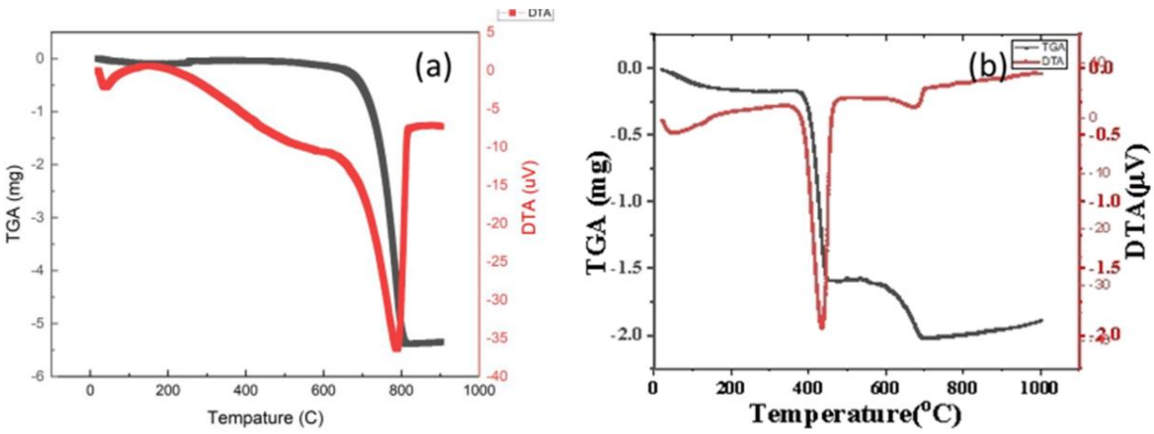


Figure 4: DTG, TGA a) limestone powder b) CaO (quick lime)

Figure 4b shows the thermal history of the decomposed CaO in which the decomposition temperature occurs at around 420 ^{0}C. So that this data can tell as the temperature at which CaO will decomposes. In doing so, it was possible to determine the temperature that we set for $Ca(OH)_2$ compound formation and the acid base neutralization reaction that are next to this calcination step.

4.1.2 XRD patterns of the limestone, calcium oxide, calcium hydroxide, and calcium chloride and the soluble calcium ion

The very intense diffraction maximum in calcite appears at about 2θ = 30°, which helps differentiate it from other polymorphs such as vaterite and aragonite. The X-ray diffraction investigation reveals that the structure has a space group of hexagons. Unit cell parameters are: a = 4.988 Å, c = 17.06 Å and γ = 120^0. The sample spectrum's inter-reticular distance values are provided and contrasted with those found in previous studies. These findings show that there is no evidence of another phase in the sample, indicating that it is entirely composed of calcite. The experimental work demonstrated a successful transformation of CaO from Mugar limestone, as demonstrated by the XRD pattern shown in the above figure 5b. This pattern coincided with JCPDS card 00-037-1497, based on the three highest intensities reflected in the specific pattern of 2θ at 18.0166°, 38.1182°, and 50.8479° on the CaO crystal's XRD pattern. In the figure 5c, the three strongest peaks at 2θ = 17.98°, 34.02°. and 47.04° at specific planes of (001), (101), and (102) respectively coincide with JCPDS card number of #00-049-0082 of that of the calcium hydroxide. So this affirms the formation of our slaked lime. Beside these peaks a minor peak was detected at 2θ = 29.3, which corresponds to the (104) plane of the rhombohedral calcite phase with space group R-3c. This is because calcite is formed as the second phase when portlandite $Ca(OH)_2$ reacts with CO_2 gas from the surrounding air. Where as in figure 5d, the XRD pattern has shown the successful conversion of the reaction between calcium hydroxide and nitric acid into calcium chloride. calcium hydroxide.$2H_2O$. Since the peaks (2θ = 38.2121^0, 38.9078^0 and 39.2825^0), have coincide with JCPDS card number: 74-0566 which is the number that represent the finger print of this compound.

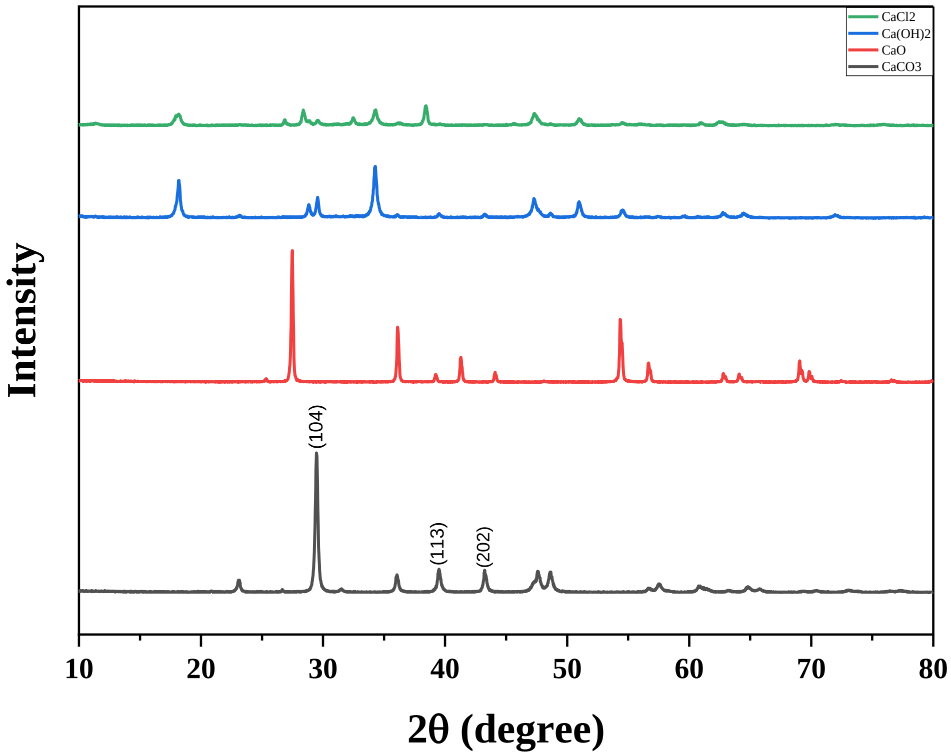


Figure 5: XRD patterns (a) limestone (b) CaO (c) $Ca(OH)_2$ (d) $CaCl_2.CaOH.2H_2O$

4.2 Bacterial growth and Urease Activity Analysis

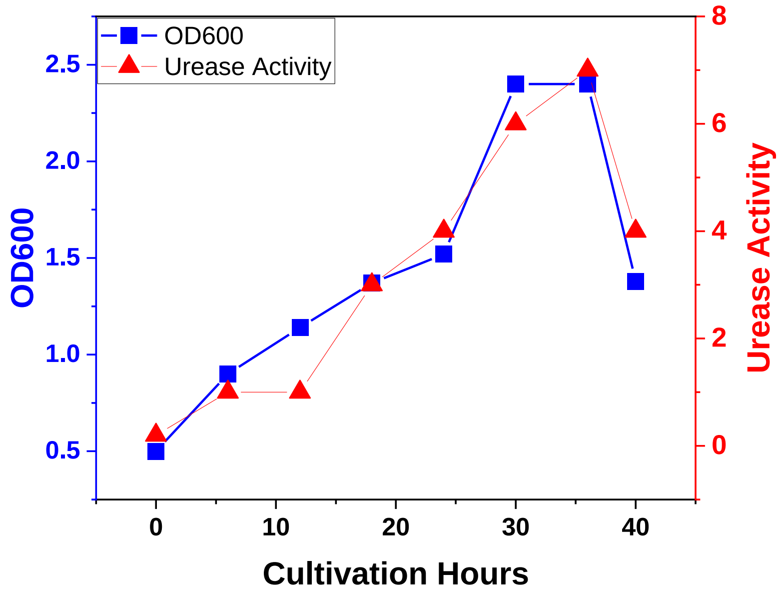
4.2.1 Qualitative determination of the bacterial growth

Qualitatively, the formation of urease active bacteria is confirmed by the color change observed in bacterial solution from yellow to red colour (Yang et al., 2022).

Figure 6: Qualitative determination of bacterial growth

4.2.2 Quantitative determination of the bacterial growth

From the optical density (Y1), which measures the concentration of bacteria in a suspension, from the below graph, the bacteria had a rapid increase in biomass in the cultivation hours ranging from 6 to 24. Then a stationary phase of growth occurs beginning from 24 hours to 36 hours, this steady bacterial biomass concentration is due to the running out of the nutrients. Finally, the bacterial biomass begins to die due to scarcity of the nutrients; this attributes to the decrease in Optical density from 2.3 Optical Density to 1.2 Optical Density after 36 hours on wards. Parallel to this, the amount of urease activity generally increased from 0.6 to 2.4 U/g·min with the increments of bacterial biomass since it was able to generate many amount of decomposed ammonium and carbonate ions per milliter of the biomass. The cultivation at 36 hours was taken as ideal for maximum amount of urease activity to be generated as described from the graph.



1

Figure 7: Optical density, Urease activity, as a function of cultivation hours

4.3 Microstructural characterization of the formed biocement

4.3.1 XRD patterns of the untreated sand and biocemented sand

The XRD plots of the untreated sand (BCS-0), and the biocemented sands BCS-1, BCS-2 and BCS-3 after two, four, and six times treatments are shown in fig 6. In fig 6a, the reflection peaks at 28.07, 31.03, and 49.86 with their respective crystal planes of (101), (100), (001) are the characteristics diffraction peaks of the silica.

As shown in fig 6b, biogenic calcite has appeared in (004) plane with specific diffraction angle of 27.82. Whereas the peaks at the plane of (002) with a diffraction angles of 20.98 confirms the formation of biogenic aragonite. Besides, biogenicon vaterite phase is formed at diffract angle of 23.8 in a respective plane of (002). Here due to less amount of the precipitates after two times treatment, silica phase was detected at the specific diffraction angles of 13.88 and 64.96 with their respective planes of (101) and (121).

When the number of treatments increased, the more stable phase of biognic calcium carbonate precipitates began to occur. Here the silica phase only appears in a fewer amount this is because of the overshadowing effect of the formed precipitates. So, in fig 6c, we have a biogenic calcite peak at two thetas of 27.86 in a specific plane of (004) and biogenic aragonite phase at a diffraction angle of 43.4 in (002) planes and that of vaterite a t 2 theta of 23.8 in (001) plane.

But still we have fewer amount of silica appeared at a diffraction angle of 13.86 and 50.1 with their respective planes of (101), and (111).

Finally, in fig 6d, the most intense and stable phase of the calcite appeared at a diffraction angle of 27.92 in (004) plane. Since the bacteria had got plenty time to completely crystallize so metastable and unstable phase of the precipitates not appeared here. Besides, the silica phases are overshadowed here do to the appearance of strong crystallite here.

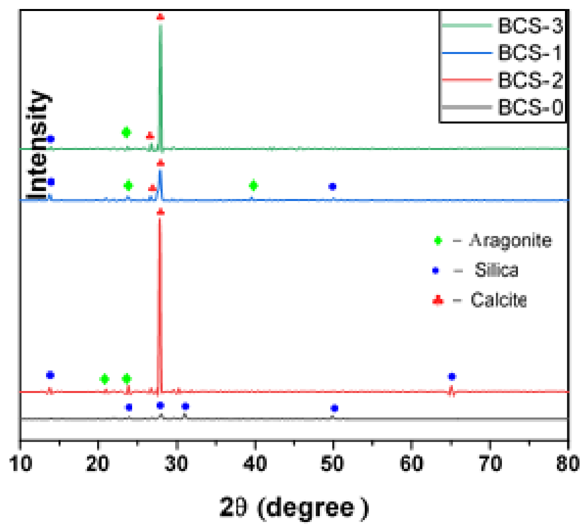


Figure 8: XRD patterns of BCS-0, BCS-1, BCS-2, BCS-3

4.3.2 FTIR analysis

Figure seven depicts the IR spectra of the untreated sand particles and that of the treated sand particles in order to ensure the formation of the functional groups related with the precipitates and for that of the silica. It also infers us the formed precipitates are due to the presence of microorganisms. The asymmetric stretching vibration (v_3) of the carbonate ions, which is due to the formed precipitate, is observed at the IR transmittance peak of 1431.09. The vibration at 694.215 corresponds to the symmetric bending of the Si-O functional group. Whereas, the vibration at 785.28 is due to the in-plane bending vibration (v_2) of the carbonate. The peaks at 3406.47 and at 1628 is attributed to the stretching vibration of O-H and C=O bonds which corresponds to the presence of peptides. Indicating certain organic molecules is involved due to the presence of the bacteria. The peaks at 1431.0277 correspond to non-symmetrical stretching motion of the two oxygen atoms bonded to the central C-atom of the carbonate. The above peaks confirm that the functional groups of carbonates, due to the precipitation, and the functional group of Si-O have also existed in our biocemented sand.

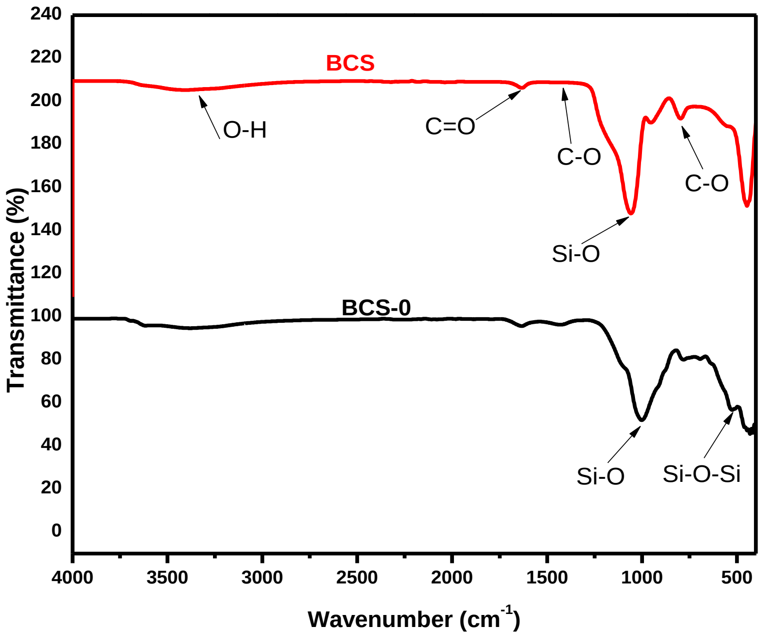


Figure 9: FTIR results of the untreated and biocemented sand

4.3.3 SEM analysis

In the below figure, as we go from a to d, the amount of calcium carbonate precipitate increases in all contact, bridging and rough interlocking forms. As the calcium carbonate precipitates gain plenty time, a blockier shaped precipitate begins to occur this is an indication of the occurrence of a more crystalline and stable phase of the precipitates. (A. Zhang & DIeudonne, 2023).

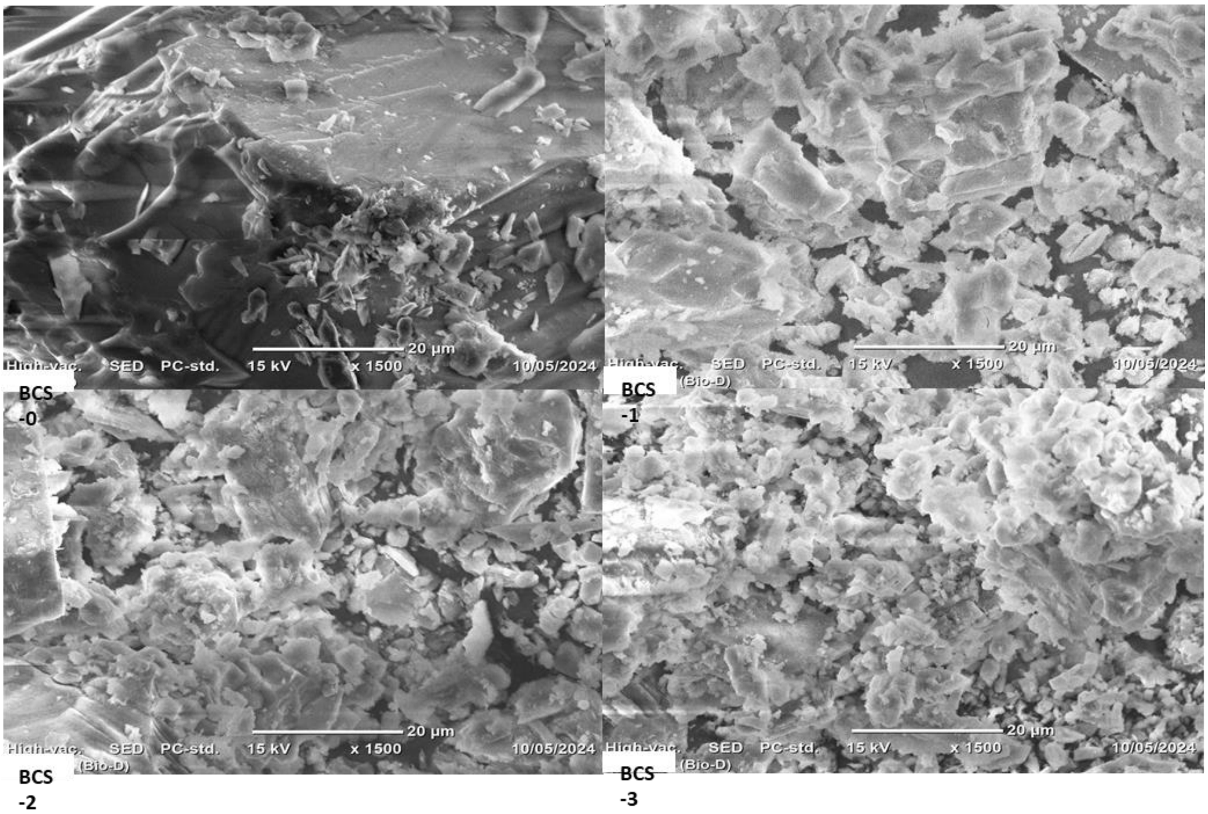


Figure 10: SEM results of the untreated (BCS-0), and the BCS-1, BCS-2, BCS-3

4.4. Geotechnical analysis discussion

4.4.1. The distributions of calcium carbonate precipitates as a function of the sand column layers

The relative increment in precipitation of calcium carbonate at middle layer could be the best accessibility of the nutrients around the middle level for the bacteria. At top it was just begin to dissolve, at lower stage it was declined due to the reduction in urease activity of the bacteria. As we can see from the below figure of 9.8 percent of calcium carbonate precipitate at middle layer of BCS-3 occurred due to repeated number of treatment, which was the maximum percentage precipitate from the given number of samples and treatments.

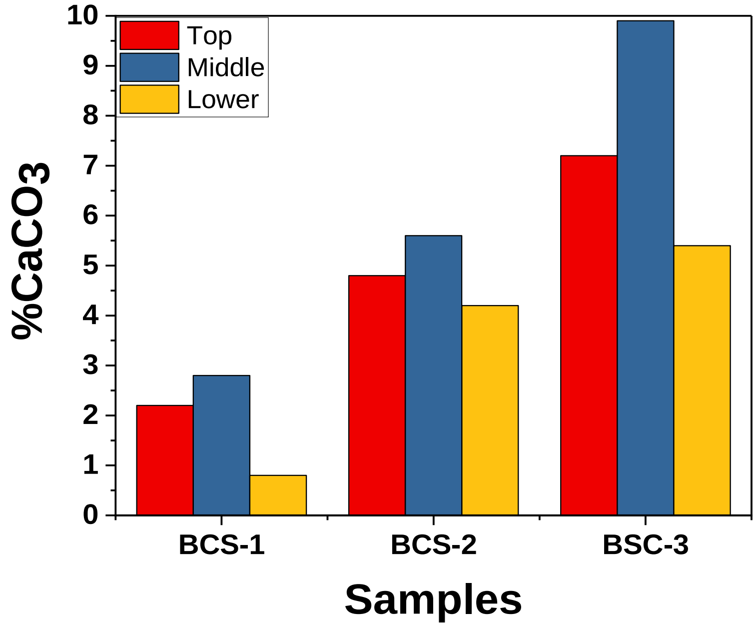


Figure 11: Calcium carbonate precipitates distribution at different sand column layers

4.4.2. Unconfined compressive strength versus percentage of calcium carbonate precipitates

This increment in UCS from 0Mpa to 0.95Mpa, is due to the precipitate of more amount of calcium carbonate between the pores which leads to a stronger particulate interaction between the particles of the calcium and that of the sand and also between the particles of calcium carbonates itself so that the biocemented sand become more resistant to collapsing under external forces. Calcite bonding at particle contacts inhibits slip and particle reorientation, both extending the deformation range of the elastic region and increasing the load-carrying capacity of the assembly at a given global axial strain.

Furthermore, because bond failure at particle contacts is gradual (e.g., Feng et al. 2014; Ning et al. 2017), the bonding serves to continue increase the magnitude of the mobilized strength after yield surface engaged. That a slope can be used to reasonably fit all of the measured strength data for bio-cemented carbonate sands implies that the mechanisms for carbonate precipitates improvement are consistent and complimentary across all of the load paths and cementation levels investigated.

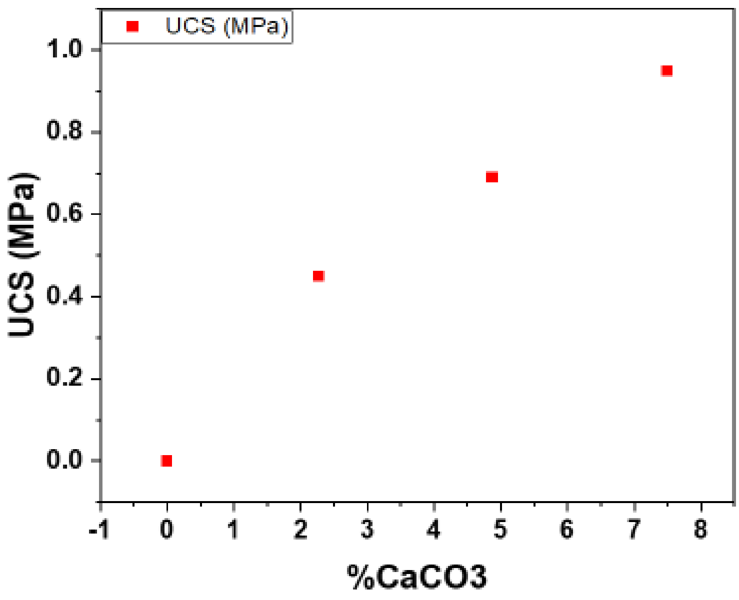


Figure 12: Unconfined compressive strength as a function of calcium carbonate precipitates

4.4.3. Permeability versus percentage of calcium carbonate precipitates

As the calcium carbonate precipitate increases at the contacting point and between the intergranular space of the sand grains, it fills the voids and constrict the pore through, which can be the potential passage for due to this the permeability of our sand column has decreased from 8.82×10^{-4} to 6.59×10^{-6} $m/_{s}$ as the precipitates amount increases (Y. Li & Chen, 2022; Liu, Li, & Jiang, 2021).

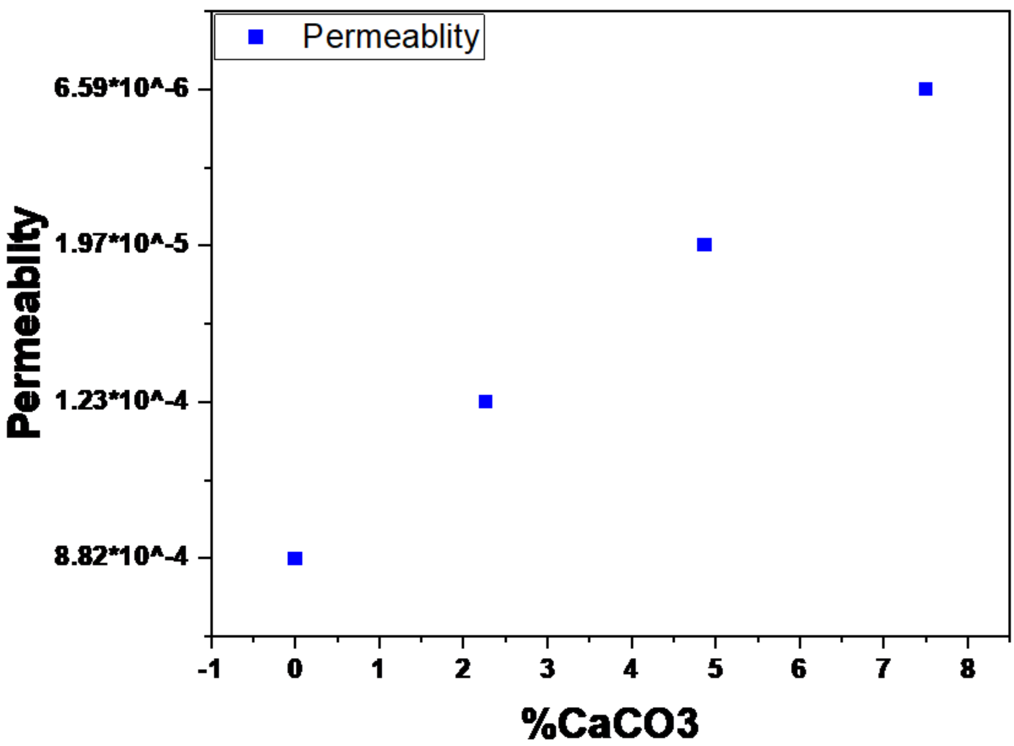


Figure 13: Permeability as a function of calcium carbonate precipitates

4.4.4. Percentage pore space versus percentage of calcium carbonate precipitates

Percentage pore space reduced by twenty five percent as $CaCO_3$ precipitates increase. Since it was able to fill effectively the voids between sand particles. So, this amount of reduction in relative pore space can enable the sand applicable especially in under water construction which requires minimum amount of leakage through the structure.

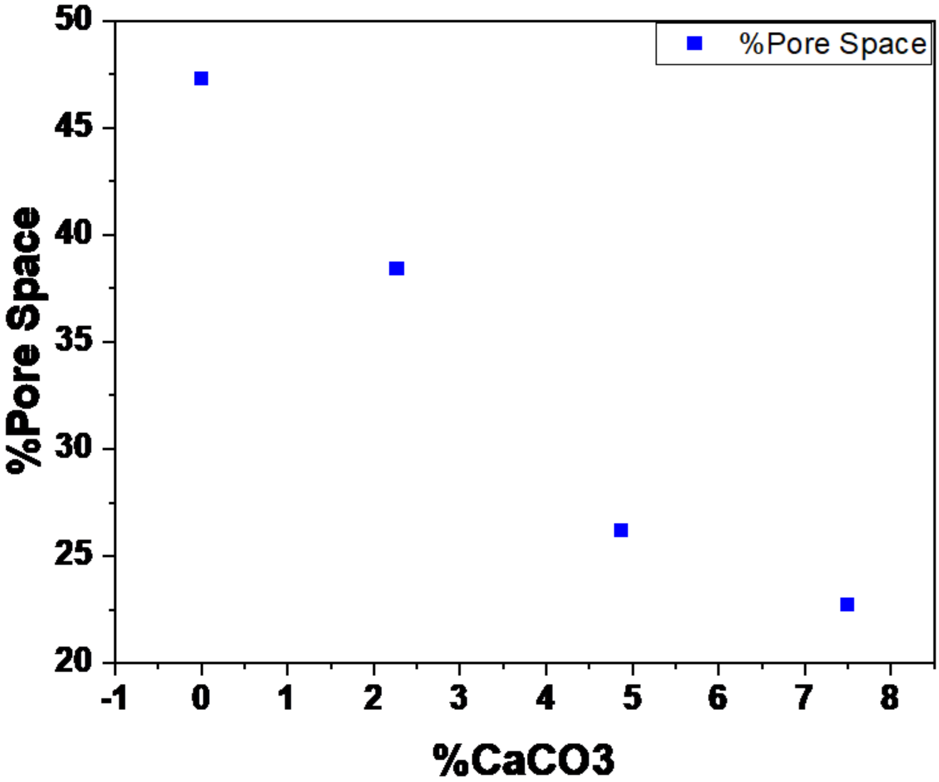


Figure 14: Percentage pore space as a function of calcium carbonate precipitates

CHAPTER FIVE

5. Conclusion

The exploration of biocement as a sustainable alternative to conventional cement production presents a promising avenue for addressing the pressing environmental challenges posed by the construction industry. This study highlights the significant carbon emissions associated with traditional cement manufacturing and the urgent need for innovative solutions. By leveraging microbial-induced carbonate precipitation (MICP), biocement not only reduces reliance on fossil fuels but also utilizes readily available resources, such as cow urine and limestone, to create a binding material that exhibits superior geotechnical properties.

The findings indicate that biocement can effectively bind sand particles, enhancing the stability and durability of construction materials while minimizing environmental impact. The research underscores the potential of biocement in various applications, from soil stabilization to erosion control, thereby broadening its utility across multiple sectors.

Biocemented sand holds significant promise as a sustainable and effective sands geotechnical properties improvement techniques. The combination of 0.15M equimolar of urea and soluble calcium ions after six times treatments was found to be the ideal condition for better geotechnical properties for the sand sample among the formulations examined in this study. This combination produced better results, including a low permeability of 6.59×10^{-6} m/s and a strength of 0.9 MPa and a reduction in percentage pore space of 25 percent, which is about 12.7 percent. And this is ideal for many areas of application which urges for sand stabilization like in drainage and sewage systems, for under water construction in which the reinforcing material get deteriorated unless it is protected by less permeable and good strength materials.

In conclusion, transitioning to biocement represents a critical step towards sustainable construction practices. By promoting the use of environmentally friendly materials, this approach not only mitigates the carbon footprint of the building industry but also paves the way for further innovations in green construction technologies. The continued investigation into biocement production and its applications will be essential in fostering a more sustainable future for construction and environmental preservation.

CHAPTER SIX

6. Recommendation

It will be possible to gain a better understanding of the consistency and effectiveness of biocemented soil by extending the scope of percolating methods research, including the life time analysis and related column tests.

The relevance of this particular study in large-scale soil stabilization requires more research; before being used for large-scale field application, it must be further developed to offer notable cost savings in terms of labours, material, and technology. Therefore, it is imperative to make improvements to MICP approach as a requirement for upcoming pilot tests at field scale level.

Many research on the bio treatment on sands with other grain size and clayey soil has not been investigated yet, further effort should be devoted to it using the MICP technique so as to apply different grain size into producing it.

CHAPTER SEVEN

7. Reference

Cheng, L. (2012). Innovative Ground Enhancement by Improved Microbially Induced CaCO3 Precipitation Technology *2*. Cheng, L., & Cord-Ruwisch, R. (2013). Selective enrichment and production of highly urease active bacteria by non-sterile (open) chemostat culture. *J Ind Microbiol Biotechnol, 40*(10), 1095-1104. doi: 10.1007/s10295-013-1310-6

Choi, S.-G., Park, S.-S., Wu, S., & Chu, J. (2017). Methods for Calcium Carbonate Content Measurement of Biocemented Soils. *Journal of Materials in Civil Engineering, 29*(11). doi: 10.1061/(asce)mt.1943-5533.0002064

Harkes, M. P., van Paassen, L., Booster, J., Whiffin, V., & van Loosdrecht, M. (2010). Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement. *Ecological Engineering, 36*, 112-117. doi: 10.1016/j.ecoleng.2009.01.004

Mustafa2, W. A. M. a. A. M. K. (2015). <PREPARATION OF PRECIPITATED calcium carbonate.pdf>. *iraqi bulletin of geology and mining, vol.11*. omoregie, a. i. (2016). characterization of ureolytic bacteria isolated from limestone caves of sarawak and evaluation of their efficiency in biocementation. randhawa, g. k., & sharma, r. (2015). Chemotherapeutic potential of cow urine: A review. *J Intercult Ethnopharmacol, 4*(2), 180-186. doi: 10.5455/jice.2015022210032

Stabnikov, V., Naeimi, M., Ivanov, V., & Chu, J. (2011). Formation of water-impermeable crust on sand surface using biocement. *Cement and Concrete Research - CEM CONCR RES, 41*, 1143-1149. doi: 10.1016/j.cemconres.2011.06.017

Yang, Y., Chu, J., Cheng, L., & Liu, H. (2022). Utilization of carbide sludge and urine for sustainable biocement production. *Journal of Environmental Chemical Engineering, 10*(3), 107443. doi: 10.1016/j.jece.2022.107443

Chen, C., Habert, G., Bouzidi, Y., & Jullien, A. (2010). Environmental impact of cement production: Detail of the different processes and cement plant variability evaluation. Journal of Cleaner Production, 18, 478-485. doi: 10.1016/j.jclepro.2009.12.014

Ciobanu, C., Tudor, P., Istrate, I., & Voicu, G. (2022). Assessment of Environmental Pollution in Cement Plant Areas in Romania by Co-Processing Waste in Clinker Kilns. Energies, 15, 2656. doi: 10.3390/en15072656

Mohamad, N., Muthusamy, K., Embong, R., Kusbiantoro, A., & Hashim, M. (2021). Environmental impact of cement production and Solutions: A review. Materials Today: Proceedings, 48. doi: 10.1016/j.matpr.2021.02.212

Raina, A. K., Soni, A. K., Vajre, R., & Sangode, A. G. (2022). Impact of topography on attenuation of noise from a cement plant in a hilly terrain. Sci Total Environ, 835, 155532. doi: 10.1016/j.scitotenv.2022.155532

Thai, T., Kučera, P., & Bernatik, A. (2021). Noise Pollution and Its Correlations with Occupational Noise-Induced Hearing Loss in Cement Plants in Vietnam. 18(8). doi: 10.3390/ijerph18084229

Zhu, X., Jinzhong, Y., Huang, Q., & Liu, T. (2022). A Review on Pollution Treatment in Cement Industrial Areas: From Prevention Techniques to Python-Based Monitoring and Controlling Models. Processes, 10, 2682. doi: 10.3390/pr10122682

Achal, V., Li, M., & Zhang, Q. (2014). Biocement, recent research in construction engineering: status of China against rest of world. *Advances in Cement Research, 26*(5), 281-291. doi: 10.1680/adcr.13.00044

Alexandra Clarà Saracho, P. S., & Dr Stuart Haigh, S. L. (2018). Microbially Induced Calcite Precipitation (MICP) to mitigate contact erosion in

earth dams and levees. *U.S. Society on Dams., 3*doi: 10.17863/cam.41847

Ariyanti, D. (2012). Feasibility of Using Microalgae for Biocement Production through Biocementation. *Journal of Bioprocessing & Biotechniques, 02*(01). doi: 10.4172/2155-9821.1000111

Ariyanti, D., Handayani, N., & Hadiyanto, H. (2012). Feasibility of Using Microalgae for Biocement Production through Biocementation. *Bioprocessing & Biotechniques, 2*, 1-4. doi: 10.4172/2155-9821.1000111

Ariyanti, D., NoerAbyor, H., ayani, & Hadiyanto. (2012). Feasibility of Using Microalgae for Biocement Production through Biocementation. *Journal of Bioprocessing & Biotechniques, 2*, 1-4.

Bachmeier, K., Williams, A., Warmington, J., & Bang, S. (2002). Urease Activity in Microbiologically-induced Calcite Precipitation. *Journal of biotechnology, 93*, 171-181. doi: 10.1016/S0168-1656(01)00393-5

Bharathi, N. C. C. P. w. G. P. o., & Strains, I. U. (2014). *Calcium Carbonate Precipitation with Growth Profile of Isolated Ureolytic Strains*.

Dae, K. S., Chang, J. H., Koo, K., Park, J., Kim, J. S., & Yuk, J. M. (2020). Real-Time Observation of CaCO(3) Mineralization in Highly Supersaturated Graphene Liquid Cells. *ACS Omega, 5*(24), 14619-14624. doi: 10.1021/acsomega.0c01300

Dejong, J., Fritzges, M., & Nüsslein, K. (2006). Microbially Induced Cementation to Control Sand Response to Undrained Shear. *Journal of Geotechnical and Geoenvironmental Engineering - J GEOTECH GEOENVIRON ENG, 132*. doi: 10.1061/(ASCE)1090-0241(2006)132:11(1381)

Engineering, D. o. C. (2016). UNCONFINED COMPRESSION TEST. *8*.

Eryürük, K., Yang, S., Suzuki, D., Sakaguchi, I., Akatsuka, T., Tsuchiya, T., & Katayama, A. (2015). Reducing hydraulic conductivity of porous media using $CaCO_3$ precipitation induced by Sporosarcina pasteurii. *J Biosci Bioeng, 119*(3), 331-336. doi: 10.1016/j.jbiosc.2014.08.009

H.P. Ritzema (Editor-in-Chief). (2006). <drainage_principles_and_applications_rd_ed-wageningen_university_and_research_262058.pdf>. *alerra,ILRI, 3*.

Hammes, F., Boon, N., de Villiers, J., Verstraete, W., & Siciliano, S. D. (2003). Strain-specific ureolytic microbial calcium carbonate precipitation. *Appl Environ Microbiol, 69*(8), 4901-4909. doi: 10.1128/aem.69.8.4901-4909.2003

Horiuchi, S., Hiasa, M., Yasue, A., Sekine, K., Hamada, K., Asaoka, K., & Tanaka, E. (2014). Fabrications of zinc-releasing biocement combining zinc calcium phosphate to calcium phosphate cement. *J Mech Behav Biomed Mater, 29*, 151-160. doi: 10.1016/j.jmbbm.2013.09.005

Hosseini, M., Shao, Y., & Whalen, J. (2011). Biocement production from silicon-rich plant residues: Perspectives and future potential in Canada. *Biosystems Engineering, 110*, 351-362. doi: 10.1016/j.biosystemseng.2011.09.010

Jia, J., Zhou, H., Wei, J., Jiang, X., Hua, H., Chen, F., . . . Liu, C. (2010). Development of magnesium calcium phosphate biocement for bone regeneration. *J R Soc Interface, 7*(49), 1171-1180. doi: 10.1098/rsif.2009.0559

Khodadadi Tirkolaei, H., Kavazanjian, E., van Paassen, L., & Dejong, J. (2017). *Bio-Grout Materials: A Review*.

Lai, Y., Yu, J., Liu, S., Liu, J., wang, r., & Dong, B. (2020). Experimental study to improve the mechanical properties of iron tailings sand by using MICP at low pH. *Construction and Building Materials, 273*. doi: 10.1016/j.conbuildmat.2020.121729

Madani Hosseini, M., Shao, Y., & Whalen, J. K. (2011). Biocement production from silicon-rich plant residues: Perspectives and future potential in Canada. *Biosystems Engineering, 110*(4), 351-362. doi: 10.1016/j.biosystemseng.2011.09.010

Mujah, D., Shahin, M. A., & Cheng, L. (2017). State-of-the-art review of biocementation by microbially induced calcite precipitation (MICP) for soil stabilization. *Geomicrobiology Journal, 34*(6), 524-537.

Muynck, W. M. d., Belie, N. D., & Verstraete, W. (2010). Microbial carbonate precipitation in construction materials: A review. *Ecological Engineering, 36*, 118-136.

N, B. (2014). *Calcium Carbonate Precipitation with Growth Profile of Isolated Ureolytic Strains*.

Okwadha, G., & Li, J. (2010). Optimum conditions for microbial carbonate precipitation. *Chemosphere, 81*, 1143-1148. doi: 10.1016/j.chemosphere.2010.09.066

Okyay, T. O., & Rodrigues, D. F. (2015). Biotic and abiotic effects on CO2 sequestration during microbially-induced calcium carbonate precipitation. *FEMS Microbiol Ecol, 91*(3). doi: 10.1093/femsec/fiv017

Qiu, J., Sheng, Q., & Yang, E.-H. (2014). Surface treatment of recycled concrete aggregates through microbial carbonate precipitation. *Construction and Building Materials, 57*, 144–150. doi: 10.1016/j.conbuildmat.2014.01.085

Ran, D., & Kawasaki, S. (2016). Effective Use of Plant-Derived Urease in the Field of Geoenvironmental/ Geotechnical Engineering. *Journal of Civil & Environmental Engineering, 06*(01). doi: 10.4172/2165-784x.1000207

Reddy†, S. R. L., & , A. M. a. M. A. k. (2015). <Bio Cement – An Eco Friendly Construction Material.pdf>. *International Journal of Current Engineering and Technology, Vol.5,*.

Rivadeneyra Torres, A., Martinez-Toledo, M. V., Gonzalez-Martinez, A., Gonzalez-Lopez, J., Martín-Ramos, D., & Rivadeneyra, M. A. (2012). Precipitation of carbonates by bacteria isolated from wastewater samples collected in a conventional wastewater treatment plant. *International Journal of Environmental Science and Technology, 10*(1), 141-150. doi: 10.1007/s13762-012-0084-0

Stabnikov, V., Chu, J., Ivanov, V., & Li, Y. (2013). Halotolerant, alkaliphilic urease-producing bacteria from different climate zones and their application for biocementation of sand. *World J Microbiol Biotechnol, 29*(8), 1453-1460. doi: 10.1007/s11274-013-1309-1

Sweety Parmar , D. M. (2017). <A Novel Technique and.pdf>. *World Journal of Research and Review (WJRR), Volume-4*.

Vater, C., Lode, A., Bernhardt, A., Reinstorf, A., Heinemann, C., & Gelinsky, M. (2010). Influence of different modifications of a calcium phosphate bone cement on adhesion, proliferation, and osteogenic differentiation of human bone marrow stromal cells. *J Biomed Mater Res A, 92*(4), 1452-1460. doi: 10.1002/jbm.a.32469

Yang, Y., Chu, J., Cheng, L., & Liu, H. (2022). Utilization of carbide sludge and urine for sustainable biocement production. *Journal of Environmental Chemical Engineering, 10*(3), 107443. doi: 10.1016/j.jece.2022.107443

Zhang, J., Shi, X., Chen, X., Huo, X., & Yu, Z. (2021a). Microbial-Induced Carbonate Precipitation: A Review on Influencing Factors and Applications. *Advances in Civil Engineering, 2021*, 1-16. doi: 10.1155/2021/9974027

Zhang, J., Shi, X., Chen, X., Huo, X., & Yu, Z. (2021b). Microbial-Induced Carbonate Precipitation: A Review on Influencing Factors and Applications. *Advances in Civil Engineering, 2021*, 9974027. doi: 10.1155/2021/9974027

1Khushboo J. Patel, D. H. K. P. (2019). <Assessment of Biocement Production Capability.pdf>. *JETIR, Volume 6*.

Achal, V., Li, M., & Zhang, Q. (2014). Biocement, recent research in construction engineering: status of China against rest of world. *Advances in Cement Research, 26*(5), 281-291. doi: 10.1680/adcr.13.00044

Anita Khanfari, F. N. K., and Abass Akhavan Sepahye. (2011). an investigation of Biocement production from hard water. *Middle-east Journal of Scientific research, 6*.

Ariyanti, D., Handayani, N., & Hadiyanto, H. (2012). Feasibility of Using Microalgae for Biocement Production through Biocementation. *Bioprocessing & Biotechniques, 2*, 1-4. doi: 10.4172/2155-9821.1000111

Chaklasiyaa, A., & , M. R. A. (2008). A review of urease-producing bacteria's potential effects on soil strength. *International Journal of Research Publication and Reviews, volume3*.

Gerilla, G. P., Teknomo, K., & Hokao, K. (2007). An environmental assessment of wood and steel reinforced concrete housing construction. *Building and Environment, 42*(7), 2778-2784. doi: https://doi.org/10.1016/j.buildenv.2006.07.021

Iqbal, D. M., Wong, L. S., & Kong, S. Y. (2021). Bio-Cementation in Construction Materials: A Review. *Materials (Basel), 14*(9). doi: 10.3390/ma14092175

Krishnapriya, S., Venkatesh Babu, D. L., & G, P. A. (2015). Isolation and identification of bacteria to improve the strength of concrete. *Microbiol Res, 174*, 48-55. doi: 10.1016/j.micres.2015.03.009

Paradiso, P., Santos, R., Horta, R. B., Lopes, J. N. C., Ferreira, P. J., & Colaço, R. (2018). Formation of nanocrystalline tobermorite in calcium silicate binders with low C/S ratio. *Acta Materialia, 152*. doi: 10.1016/j.actamat.2018.04.006

Sweety Parmar , D. M. (2017). <A Novel Technique and.pdf>. *World Journal of Research and Review (WJRR), Volume-4*.

Zhang, K., Tang, C. S., Jiang, N. J., Pan, X. H., Liu, B., Wang, Y. J., & Shi, B. (2023). Microbial-induced carbonate precipitation (MICP) technology: a review on the fundamentals and engineering applications. *Environ Earth Sci, 82*(9), 229. doi: 10.1007/s12665-023-10899-y

Zainudeen, N. (2020). Cement and its effect to the environment: A case study in SriLanka *3*.

Reddy†, S. R. L., & , A. M. a. M. A. k. (2015). <Bio Cement – An Eco Friendly Construction Material.pdf>. *International Journal of Current Engineering and Technology, Vol.5,*.

Aswin, S. (2018). Effects of Ceramic Tile Powder on Properties ofConcrete and Paver Block. *GRD Journal for Engineering, |Volume 3*.

Lazik, P.-R., & Garrecht, H. (2021). Wood ashes from electrostatic filter as a replacement for the fly ashes in concrete. *Journal of Construction Materials, 2*(2). doi: 10.36756/JCM.v2.2.6

Siddique, R. (2008). *Waste Materials and By-Products in Concrete*.

Ivanov, V., & Chu, J. (2008). Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. *Reviews in Environmental Science and Bio/Technology, 7*(2), 139-153. doi: 10.1007/s11157-007-9126-3