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A REVIEW OF UREASE-PRODUCING BACTERIA'S POTENTIAL EFFECTS ON SOIL STRENGTH

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ABSTRACT

Biocementation via microbial carbonate precipitation is a new branch of microbial geotechnology that deals with the use of microbiological methods to produce cemented materials for engineering applications. The primary goal of these applications is to improve soil geophysical properties so that it can be used for construction and environmental purposes. Biocementation applications would necessitate interdisciplinary research at the intersection of microbiology, ecology, geochemistry, civil and environmental engineering. This emerging field has the potential to meet society's growing demand for novel treatment processes that improve soil engineering properties. This paper provides an overview of biocementation, specifically microbial calcium carbonate (CaCO3) precipitation, as well as nondestructive geophysical techniques for real-time monitoring of soil engineering properties. The focus is then narrowed to a laboratory-scale test of biocementation of sandy soil and shear wave velocity measurement of strength development (Vs). Other analytical results discussed included microscopic imaging with a scanning electron microscope (SEM) and identification of CaCO3 precipitation in bio-cemented sand with an X-ray diffractometer (XRD). The benefits and potential applications of bio-cemented soil improvement are identified.

***Keywords**: Bio-cementation, Calcium Carbonate, Diffractometer, MICP, SEM*

1. INTRODUCTION

Dr. Cord Ruwisch of Murdoch University created the name "biocement" to describe the calcium carbonate generated by soil-based bacteria; biocement is a self-healing substance that improves the durability of architectural structures and preserves cultural heritage. Mineral carbonates are precipitated by a variety of organisms in a variety of natural habitats, including soils, geological formations, freshwater biofilms, oceans, and saline lakes. Microbial mineral precipitation arising from metabolic activities of some specific bacteria in concrete has recently been a popular study topic for improving the overall behavior of concrete. The use of this biomineralogy concept could lead to the creation of a new material. Bacterial cement is a natural, self-healing biomaterial that can fix concrete fractures and fissures. Thus, concrete is a strong material [Ghosh et al., 2006].

Concrete is perhaps the main restricting specialist utilized around the world for development and framework purposes. More noteworthy mindfulness on the natural and human effects of regular concrete creation has motivated the need to examine choices. Bio-cement (insoluble precious stone) has as of late arisen as another limiting material which is created through microbially induced carbonate precipitation (MICP) for soil changes. It can be permeable materials together and improve their mechanical properties [Gowthaman et al., 2021].

Bio-cement materials can connect soil particles together when the bio-cement materials are appropriately encouraged in the dirt lattice, at last improving the mechanical properties. Not at all like most settled soil adjustment techniques, bio-cementation can happen at encompassing circumstances with insignificant discharge of carbon dioxide Conventional soil adjustment rehearses which utilize mechanical (for example soil compaction) or synthetic (for example fluid silica) techniques require extensive work, include high thickness materials with low soil vulnerability and are hazardous to the climate and people. Then again, bio-cementation is sturdy, less dangerous and reasonable for profound infiltration treatment with fine pores. Throughout the course of recent years, bio-cementation by means of urea hydrolysis has been broadly read up for quite a long time with applications which incorporate concrete and break fix, soil combination and adjustment, and disintegration control [Gowthaman et al., 2021] [Chu et al., 2016].

The basic structure and synthetic creation of encourages delivered by the MICP and MISP processes are investigated by energy dispersive X-beam spectroscopy (EDS) and powder X-beam diffraction examination (XRD). The morphology of encourages can be seen by examining electron magnifying lenses [He et al., 2022].

2. BIO-CEMENT

Making Bio-cement with microorganisms is simple since it takes less time and effort. Because it is created from non-pathogenic and environmentally friendly microorganisms, bio-cement is also cost effective [Ariyanti *et al*., 2012].

Bio-cement are made up of two forms of calcium precipitates:

1. Calcium carbonate precipitate [CCP]
2. Calcium Phosphate Cement [CPC]

Calcium carbonate precipitate:

Calcium carbonate precipitation is caused by urea degradation by the microbe's urease enzyme. Urea is commonly employed as a fertilizer, as a waste product from animals, and as a byproduct of the decomposition of uric acid secreted by birds. Increased urea levels pollute the land and water, resulting in toxic algal blooms [Qiu et al., 2014]. Urease is produced by microorganisms and occurs both within the cell and as an external enzyme when the cell dies. One mole of urea is converted to one mole of ammonia and one mole of carbonate by microbial urease activity, which then hydrolyze spontaneously to another mole of ammonia and carbonic acid as follows:

$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3$

$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3$ [Qiu *et al.*, 2014]

In water, this product equilibrates to generate bicarbonate, one mole of ammonia, and hydroxide, resulting in a pH rise.

$H_2CO_3 \rightarrow 2H^+ + 2CO_3^{2-}$

$NH_3 + H_2O \rightarrow OH^- + NH_4^+$

$CA^{2+} + CO_3^{2-} \rightarrow CaCo_3$[Fisher *et al.*, 2017]

Carbonic acid causes an increase in CO2 conversion and carbonate generation. The buildup of bicarbonate and ammonium ions in the cell as a result of urea hydrolysis favors urea and bicarbonate metabolism. Carbonate production from bicarbonate is pH-dependent, and alkaline conditions result in an increase in carbonate concentration [Qiu et al., 2014]. Naturally, the deposition of CaCO3 in vacant areas causes porous material to clog. Calcium carbonate precipitation is affected by the following factors: (1) calcium content, (2) dissolved inorganic carbon (DIC) concentration, (3) pH, and (4) bacteria as a nucleation site [Fisher et al., 2017].

Calcium Phosphate Cement [CPC]:

CPC is extensively made by combining calcium orthophosphates with an aqueous phase, usually water, to make a paste that can be employed. Depending on the liquid-to-powder ratio, the porosity of CPC might range from 30% to 50%. Commercial CPC comes in a variety of forms, the most common of which is hydroxyapatite [Horiuchi et al., 2014].

3. SOURCES OF BIO-CEMENT

1. Microorganism
2. Plant
3. Sewage sludge

1) Bacteria:

Microbial Induced Calcium Carbonate Precipitates can be produced by three different types of bacteria: [Ariyanti *et al.*, 2012].

- Photosynthetic microorganisms
- Sulfate reducing bacteria
- Microorganisms involved in Nitrogen cycle

Bacteria are unicellular prokaryotic creatures that can be found practically anywhere. Many rocks in nature are cemented by the formation 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 are able to produce Bio-calcite defined by Bharathi, they are *Bacillus pasteurii*, *B. subtilis*, *Bacillus sphaericus* and *B.lentu* [Bharathi *et al.*, 2012]. Bacteria that perform metabolic processes such as urea hydrolysis, denitrification, sulfate reduction, iron reduction, and the production of carbonic anhydrase enzymes can create calcium carbonate precipitates [Qiu *et al.*, 2014]. Non Ureolytic bacteria, such as *B. subtilis*, are helpful in MICP synthesis in mixed culture because they provide a nucleation site for calcium carbonate precipitation. Calcium carbonate precipitates are also produced by halotolerant alkaliphilic bacteria [Carmona *et al.*, 2016].

2) Microalgae:

Microalgae can be employed as a nucleation site for Microbial Induced Calcium Carbonate Precipitation because photosynthetic organisms that produce urease and consume urea can be used to make Bio-calcite. The reaction takes place as follows:

$$CO_2 + H_2O \rightarrow (CH_2O) + O_2$$

$$2HCO_3^- \leftrightarrow CO_2 + CO_3^{2-}$$

$$CO_3^{2-} + H_2O \leftrightarrow HCO^{3-} + OH^-$$

$$Ca^{2+} + HCO^{3-} + OH^- \rightarrow CaCO_3 + 2H_2O$$

Because the amount of pH increase is directly proportional to the amount of calcium precipitation, microorganisms that can survive in high calcium and ammonia concentrations should be chosen, as urea hydrolysis produces ammonia. Microorganisms should also be nonpathogenic. Photosynthetic organisms such as green algae found in both salt and freshwater, such as *Cyanobacteria synechococcus, Scyntonema*, *Chlorella*, *Nannochloris Atomus*, *Synechocystis*, and *Synechococcus*, produce Bio-calcite [Chen *et al*., 2011].

3) Plant:

Plant-derived urease can also be used for Biocement production. Calcium carbonate as well as calcium phosphate cement can be produced. This type of plant is burnt then the biomass having silica mixed with Portland cement to produce biocement. It has the benefits that its size is small and can penetrate in very small cracks. The urease enzyme is widely used in industries such as medicinal, construction, agricultural, food, etc. It can precipitate carbonate (mainly calcite) through urea hydrolysis in several environmental and geotechnical engineering applications. The aim of this section is to summarize the existing and potential applications in CaCO3 precipitation using plant- derived ureases [Dilrukshi *et al*., 2016].

4) Sewage Sludge:

Sewage sludge can be utilized as a raw material to make Biocement, which will minimize carbon dioxide emissions and act as an environmentally benign alternative to Portland cement. Caulobacter, Blastomonas, and Roseobacter are microorganisms found in waste water utilized in biocement manufacturing [Torres et al., 2013]. Municipalities collect a tremendous amount of rubbish, which is then scattered across the land, buried in landfills, or burned. Treatment of sewage waste consumes both energy and money, but it can be used to make biocement, lowering the cost of sewage sludge treatment. Because sewage sludge includes silica, it can be used to make Biosilica and blended with Portland cement. Sewage sludge can be utilized to make Biocement in two different methods:

(a) It incinerates ash with Portland cement.

(b) Adding Portland cement, limestone is co-combusted with sewage [Torres *et al*., 2013].

4. MICP MECHENISM

Cheng analyzed different sands and determined firstly the size of particles. By Cheng the current tests utilized Bacillus urease active strain, which was recovered from a previous study. The results of the UCS experiments on coarse sand treated with various doses of MICP at varied saturation levels: 20%, 40%, 80%, and 100% [Cheng *et al*., 2013]. This section attempts to uncover the rationale for the MICP-treated sand's increased strength and stiffness at lower saturation levels. Microbially induced calcite precipitation (MICP) has been the subject of exploration for quite a long time. The method can modify soil qualities to expand shear, strength and solidness, while keeping up with satisfactory porousness. Cheng and whiffin suggested the method include presenting vigorously developed microbes with exceptionally dynamic urease chemical into soil, tackling the urease catalyst to catalyze the hydrolysis of urea to create ammonium and carbonate particles [whiffin *et al*., 2004].

Varalakshmi is worked on urease utilizing bacteria the principal gatherings of microorganisms that can instigate the carbonate precipitation are photosynthetic microorganisms such as Cyanobacteria and Microalgae; Sulfate-decreasing microbes and a few types of microorganisms included in nitrogen cycle [varalakshmi *et al*., 2014]. Using EDTA titration, determine the water harness. A few components have been recognized by which microorganisms can incite the calcium carbonate precipitation, including urea hydrolysis, denitrification, sulfate creation, and iron decrease is defined by Chu and J. Two unique pathways, or Autotrophic and Heterotrophic pathways, through which calcium carbonate is created, have been recognized [varalakshmi *et al*., 2014].

The created microbially prompted $CaCO_3$ has spanned nearby soil particles by establishing the dirt grains together to frame solidified sand illustrative of calcareous stone is approved by Zaghloul. Controlling the MICP cycle and anticipating the subsequent material properties are fundamental in further developing the designing properties of permeable strong materials [Zaghloul *et al*., 2021]. Chuo is recorded Numerous scientists have researched the exact relationships between how much hastened $CaCO_3$ precious stones and soil designing boundaries, like the dirt porosity, strength, solidness, and porousness Properties of soils and the hastened $CaCO_3$ precious stones can fluctuate in mineral kind, thickness, shape, size appropriation, and surface, which could give a clarification for the noticed contrasts in the subsequent designing properties of MICP-treated soils [Chuo *et al.*,2020].

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^{+} + CO_3^{\ 2+}$$

$$Ca_2^{+} + CO_3^{2-} \rightarrow CaCO_3(s)$$

[Chuo *et al.,* 2020]

Calcium carbonate precipitation is predominantly administered by the accompanying variables was highlighted by Anitha is following:

1) calcium focus,
2) grouping of broken-down inorganic carbon (DIC),
3) pH, and
4) Availability of nucleation site.

Moreover, factors like Microbes type, bacterial focus, temperature, and urea fixation are found to influence the proficiency of MICP [Anitha *et al.,* 2018].

5. CALCITE IN-SITU PRECIPITATION SYSTEM (CIPS)

In this process direct precipitation system followed by CIPS solution of calcium carbonate. CIPS works by the immediate precipitation of calcium carbonate from the CIPS arrangement. Whiffin describes microorganisms which are not repressed in presence of ammonium as follows: sporosarcina pasteurii and helicobacter pylori. There is no response with the constituent particles. The calcium carbonate will shape mineral concrete around the constituent particles, carbonate or different sorts of minerals. These particles and their surfaces are fantastic nucleation locales for the developing calcite gems. There is a high potential for the gems to nucleate and develop on these surfaces rather than amidst the CIPS arrangement in the pore spaces. The calcite gems that have solidified from the CIPS arrangement will become outward from the molecule surfaces and into the pore spaces. They are pressed intently together and structure a covering or skin around every one of the constituent particles in the material; this is the calcite concrete [Whiffin et al., 2004].

The CIPS-technique utilizes two water-based fluids containing every one of the synthetic compounds Calcium Chloride, Urea, Tetra Sodium, Sodium Carbonate, Sodium Sulphone and the catalyst Urease. Palmen makes sense that the established sand gets something similar qualities as normal sandstone, actually keeping up with its porosity bringing about no development of pore pressure. [Palmén et al., 2012]. When silica sand is dealt with calcite in-situ precipitation system (CIPS), Whiffin explained the cross-section with bacterial growth. On the bacterial cell surface nucleation occurs with the three parameters:

1. The calcium concentration
2. The carbonate concentration
3. The PH of the surrounding environment.

PH affects carbonate and calcium carbonate ($CaCO_3$) solubility [Whiffin *et al*., 2004].

6. APPLICATION

Mountassir applied biocement which produced Soil stabilization, Erosion resistance, Permeability reduction in porous media, Rock fracture sealing and Well sealing [Mountassir et al., 2009].

Examination of the utilization of MICP through ureolysis has been broadly read up for soil adjustment, specifically for its capacity to work on compressive strength and shear strength, specifically in granular soils (for example sands and rock). The treatment with MICP by means of ureolysis changes at first free fine sand into an established sand/sandstone. Mountassir performed treatment of sands by means of MICP has brought about expansions in unconfined compressive strength of more prominent than three significant degrees and now and again much north of four significant degrees. Because of the increment in strength and firmness managed by MICP it has additionally been proposed for settlement decrease [Mountassir et al., 2009] [Dhami et al., 2017].

- **Cracks in a Building Remediation:**

As the structure ages, wear and tear in the concrete causes' micro cracks, which lead to the formation of macro-cracks when water enters the micro cracks. If not treated at an early stage, this can lead to a poor appearance of the building and even collapse; thus, microbial culture is injected into the cracks of the building and therefore treated at an early stage. This can also be utilized to preserve India's cultural heritage by repairing cracks in ancient monuments [Ekprasert et al., 2020].

- **Self-healing concrete development:**

Inspired by nature, scientists are working to develop self-healing concrete, which will cure cracks without the need for treatment. To achieve these features, concrete is prepared with MICP bacteria. In normal conditions, microorganisms remain dormant, but when a fracture forms, water intrusion activates the cell, causing Calcium carbonate to be formed and the fissure to mend itself. Buildings with self-healing concrete have a longer lifespan. This method prevents cracks from forming, resulting in the use of environmentally friendly cement and the construction of long-lasting structures [Achal et al., 2016].

- **To create sustainable deserts:**

It is extremely difficult to survive in deserts when there is a storm. Even humans can perish while going through the desert. People living in arid and semiarid environments, who cohabit with shifting sands, face a slew of other issues as they strive to survive in hard conditions. As a result, a fresh architectural concept in desserts can be beneficial. Because dunes move quickly with air, MICP is used in this concept to cement granular material locally. As a result, microorganism-mediated cementation must be a carefully managed process in order to achieve the desired pattern and function as architecture [Larsson et al., 2010].

Table 1 Possible microbial processes that can lead potentially to biocementation

Physiological group of Microorganisms	Mechanism of Biocementation	Essential conditions for Biocementation	Potential Geotechnical Applications	Reference
Sulfate reducing Bacteria	Production of undissolved sulfides of metals	Anaerobic conditions; presence of sulfate and carbon source in soil	Enhance stability for slopes and dams	[Ivanov *et al*., 2008]
Ammonifying Bacteria	Formation of undissolved carbonates of metals in soil due to increase of pH and release of CO2	Presence of urea and dissolved metal salt	Mitigate liquefaction potential of sand. Enhance stability for retaining walls, embankments, and dams; Increase bearing capacity of foundations	[Ivanov *et al*., 2008]
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	[Torres *et al.*, 2013]

7. ADVANTAGES

Designed Bacteria for Ground Improvement and Concrete Repair:

Transmitted microbes' spores along with supplements are added to concrete during blending, to such an extent that when breaks later happen and water is sent into the gap, bacterial movement resumes and biomineralization happens, framing calcium carbonate which fills, seals and mends the breaks in situ. The microbes might become idle as dampness exhausts yet can be reactivated if conditions become great or on the other hand whenever revised with extra microorganisms and supplement arrangements infused into the framework. Salifu defeated this impediment, fostered a designed living structure material (LBM) by immunizing a photosynthetic cyanobacterium (Synechococcus sp.) into an underlying framework made out of sand and gelatin (hydrogel). Gelatin platforms purportedly work on in strength when lack of hydration happens [Salifu et al., 2021].

Engineered Fungal Growth for Ground Improvement:

Suitable fungal spp. also accepts bio-mineralization via MICP to perform bio-cement. An efficient test crusade researching the impact of the designed development of the mycelia organizations of clam mushroom on the hydro-mechanical conduct of sands. Salifu designed contagious development brought about critical change of the water driven conduct of sand, prompting outrageous water repellency, decline in invasion and pressure driven conductivity, expansion in the uniaxial compressive strength of sand [Salifu et al., 2021].

Bio-coating of Surfaces:

Designed Microorganisms biofilms in soil. Biofilms are shaped when microbial cells stick to strong surfaces, repeat, and tie themselves immovably to the surface utilizing the extracellular polymeric substance (EPS) which they discharge, accordingly bringing about a mind boggling 3-layered design. In soils, designed biofilms bring about a decrease in porousness. Designed biofilm arrangement for Staphylococcus epidermidis in a dirt lattice, to explore the impact of the biofilm and EPS on soil mechanical conduct. They found that biofilm-treated soil had less strength contrasted with untreated controls [Salifu et al., 2021].

8. LIMITATION OF BIOCEMENTATION

During the development, the response of sulfuric corrosive (emerging from pyrite oxidation) with limestone contained in a seepage cover, brought about the precipitation of gypsum, iron hydroxide and the arrival of CO2. Environmental factors like temperature, season, PH, water shearing also affect the bio-cement [Mountassir et al., 2009].

Adverse consequences of microbial movement have been connected with the oxidizing or diminishing conduct of microscopic organisms, engaged with for instance, the oxidation of dissolvable Fe^{2+} to Fe^{3+} bringing about precipitation named as 'bio-slime', this is known to add to stopping up of groundwater wells recommended by Mountassir [Mountassir et al., 2009].

9. CONCLUSION

Based on the study of the biocement production investigation, it can be concluded that ureolytic bacteria with a high calcium supply are environmentally advantageous. As a result, we stated that biocement is an environmentally beneficial or energy-saving construction material. Bacterial concrete is self-repairing bio-material that can remediate the cracks automatically filled using exterior environmental materials.

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