

## **Conservation and restoration of cultural heritage: A biotechnological approach**

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### **ABSTRACT**

*Carbonate precipitation is a natural phenomenon with a great importance in many chemical and engineering applications. Precipitation can be induced by bacteria as a byproduct of common microbial processes, such as ureolysis. In this process, bacteria hydrolyze urea through a series of reactions which raise the pH of the system. In the presence of calcium ions, this rise in pH shifts the saturation state of the system, allowing for solid calcium carbonate (CaCO<sub>3</sub>) to form. The use of these bacteria in biotechnical applications is appealing because urea is a fairly inexpensive substrate, and ureolytic bacteria are common in soil and aquatic environments. Bacteriogenic mineral plugging is an innovative use for this process. The tools of biotechnology have a great and largely untapped potential for the preservation and restoration of our cultural heritage. In this article it has been described a unique example of a group of scientists and review some of the new applications in biotechnology for the preservation of cultural heritage. Here, it has been predicted an expansion in this field and the further development of biotechnological techniques, which will open up new opportunities to biologists for conservation and restoration of cultural heritage sites.*

**Keywords:** Calcium, Monuments, Carbonate precipitation, Urolysis, Bio cementation

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### **INTRODUCTION**

The use of miscellaneous stone materials as medium for artistic terms and as building material ranges from small statues over historic monuments to all kind of different buildings from the beginning of mankind to the present. Historical events are immortalized in monuments. Very few examples include Eiffel Tower (Paris); Status of Liberty (USA); Westminster Abbey (London); Great Pyramids (Egypt) [1, 2, 3] etc. In India Some of these ancient heritages include The Taj Mahal, Agra; Qutub Minar, Delhi; Qutb Minar, Sanchi and Mathura; Ajantaa and Ellora Caves, Nasik, Maharashtra; The Jantar Mantar, Delhi, Jaipur; The Red Fort, Delhi; The Charminar, Hyderabad and others. Like any other living and non – living things, these monuments are also subjected to harsh weather and they are therefore liable to deterioration. Damage in a typical heritage building and monuments, which has existed for thousands of years, may occur by Cracks, Loose building blocks, particularly in roof, Seepage and leakage of water, Tilting and Corrosion and discoloring [4].

These structures are often large and located outdoors, for this reason they pose particular conservation challenges that require collaborative efforts between conservators and scientists. The weathering of rocks to soil is a well-known degradation process essential for the evolution of life and erosion formed earth over billions of years till the present state and also in future. On the other hand the decay of culturally meaningful stone artifacts, buildings etc. represents an irreversible loss of our cultural heritage [5]. Weathering processes, including wind, sunlight,

temperature, rain, snow and moisture are grouped into physical and chemical factors that have influence on the materials. These agents affect the stability of the rock matrix as well as oxidation and hydration reactions, dissolution of carbonates and solubilisation of some elements cause chemical corrosion of the stone-forming minerals [6]. Additionally, anthropogenic factors (e.g. air pollution due to cars, electric utilities, heating and many more) leading to a higher atmospheric concentration of inorganic and organic compounds, that deposit on stone surfaces, contribute to the decay of exposed stone materials [7,8,9].

Many conservation treatments have been applied to the protection and consolidation of stone before extensive granular disintegration causes loss of surface material and therefore irreversible damage [10]. Protection refers to treatments that waterproof and/or strengthen stone surfaces in order to keep water or other weathering agents from entering the core of the stone. Consolidation is the impregnation and thus strengthening of a friable decayed porous stone with a cementing and/or hardening product. Both treatments have been performed in the past using organic and inorganic materials [11], such as acrylic or epoxy resins [12] and  $\text{Ba}(\text{OH})_2$  solutions [13]. However, none of the treatments available to date have proven to be satisfactory. Organic treatments commonly result in the formation of incompatible, often harmful surface films. In addition, they generally release noxious solvents. Inorganic consolidation may be preferable since stone minerals and protective or consolidating materials share some physical-chemical affinity [14]. For instance, the so-called limewater treatment [15] composed of  $\text{Ca}(\text{OH})_2$  solutions, has been used to consolidate carbonate stones because calcium hydroxide easily carbonates in the presence of atmospheric  $\text{CO}_2$ , resulting in calcite ( $\text{CaCO}_3$ ) formation. However, the limewater technique often leads to the formation of a superficial, micrometer-thick, friable aggregate of submicron-size calcite crystals and has an insufficient protection and/or consolidation effect [16].

Construction materials such as stone and concrete are subjected to the weathering action of several physical, chemical and biological factors [8, 17]. Because of their composition and textural characteristics, carbonate stones (limestones, dolo stones and marbles) are particularly susceptible to weathering. Progressive dissolution of the mineral matrix as a consequence of weathering leads to an increase of the porosity, and as a result, a decrease of the mechanical features [18]. In order to decrease the susceptibility to decay, many conservation treatments have been applied with the aim of modifying some of the stone characteristics.

Historic stone supports large and diverse communities of microorganisms that colonize both the stone surface and the porous interior [19]. Numerous diverse bacterial species participate in the precipitation of mineral carbonates in various natural environments, including soils, geological formations, freshwater biofilms, oceans and saline lakes [20]. Recently, microbial mineral precipitation resulting from metabolic activities of some specific microorganisms and use of this bio mineralogy concept leads to the potential invention of a new material [21]. Bacterial concrete is an inherent and self-repairing biomaterial that can remediate the cracks and fissures in concrete [22]. Though concrete is quite strong mechanically, it suffers from several drawbacks, such as low tensile strength, permeability to liquid and consequent corrosion of reinforcement, susceptibility to chemical attack and low durability [18].

Carbonaceous minerals are commonly found in oceans, soils and geological formations, and as such, represent a significant fraction of the global carbon pool. More than sixty carbonate minerals are known, most commonly represented by calcium carbonate ( $\text{CaCO}_3$ ) [29]. Calcium carbonate ( $\text{CaCO}_3$ ) is one of the most common minerals widespread on earth, constituting 4% by weight of the earth's crust. It is naturally found in extensive sedimentary rock masses, as limestone, marble and calcareous sandstone in marine, freshwater and terrestrial environment [22, 50, 51].

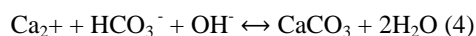
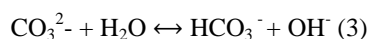
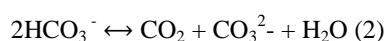
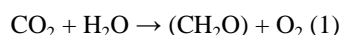
#### **MICROBIOLOGICALLY INDUCED CALCIUM CARBONATE PRECIPITATION (MICP)**

Bacteria is accomplished by two different mechanisms, active and passive [31, 38]. The passive production involvement of two metabolic cycles, nitrogen and sulfur cycle using three pathways, 1) ammonification of amino acids under anaerobic conditions in the presence of organic matter and calcium, 2) dissimilatory reduction of nitrates under anaerobic and microaerophilic conditions in the presence of organic matter, calcium and nitrate, and 3) hydrolysis of urea or uric acid in the presence of enzyme urease and in an environment rich in organic matters and calcium [31, 39-40].

Calcium carbonate ( $\text{CaCO}_3$ ) precipitation is a common phenomenon found in nature such as marine water, freshwater, and soils [1, 7, 10]. This precipitation is governed by four key factors: (i) the calcium ( $\text{Ca}^{2+}$ ) concentration, (ii) the concentration of dissolved inorganic carbon (DIC), (iii) the pH ( $\text{pK}_2(\text{CO}) = 10.3$  at  $250^\circ\text{C}$ )

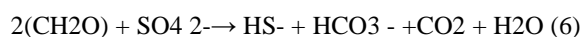
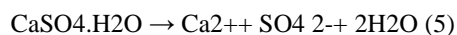
and (iv) the availability of nucleation sites [1, 11]. Numerous species of microorganism have been detected previously and assumed to be associated with natural carbonate precipitates from diverse environments. The primary role of microorganism in carbonate precipitation is mainly due to their ability to create an alkaline environment through their various physiological activities [1, 7].

There are three main groups of microorganism that can induce the carbonate precipitation: (i) photosynthetic microorganism such as cyanobacteria and microalgae; (ii) sulphate reducing bacteria; and (iii) some species of microorganism involved in nitrogen cycle [1, 6, 7]. The most common MICP phenomena appeared in aquatic environments is caused by photosynthetic microorganisms [6, 12]. Photosynthetic microorganisms use  $\text{CO}_2$  in their metabolic process (eq. 1) which is in equilibrium with  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  as described in eq. 2. Carbon dioxide consumed by photosynthetic microorganisms shift the equilibrium and resulting the increment of pH (eq. 3) [6]. When this reaction occurs in the present of calcium ion in the system, calcium carbonate is produced as described at chemical reaction in eq. 4 [7].

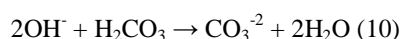
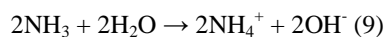
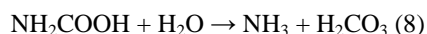
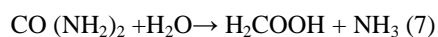


The precipitation of calcite ( $\text{CaCO}_3$ ) can also be induced by heterotrophic organism. This microorganism produces carbonate or bicarbonate and modified the system so that the carbonate precipitation may occur [1]. Abiotic dissolution of gypsum ( $\text{CaSO}_4 \cdot \text{H}_2\text{O}$ ) (eq. 5) causes system rich of sulfate and calcium ion. In the presence of organic matter and the absence of oxygen, sulphate reducing bacteria (SRB) can reduce sulphate to  $\text{H}_2\text{S}$  and  $\text{HCO}_3^-$  as described in eq. 6 [1, 6].

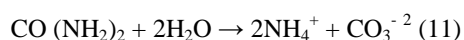
When the  $\text{H}_2\text{S}$  degasses from the environment, pH of system will increase and the precipitation of calcium carbonate will occur [1].



Currently urease enzyme activity in most of microorganism metabolism process has been used as a tool to induce the precipitation of calcium carbonate [9, 12]. The hydrolysis of urea by urease enzyme in heterotrophic microorganism will produce carbonate ion and ammonium. This mechanism will result system with higher pH and rich of carbonate ion [12]. One mole of urea hydrolyzed intra cellularly to one mole ammonia and one mole carbamate (eq. 7), which spontaneously hydrolysed to one mole ammonia and one mole carbonic acid (eq. 8). Ammonia and carbamate subsequently equilibrate in water to form bicarbonate and 2 moles of ammonium and hydroxide ions as described in eq. 9 and 10 [2].



Total reaction:



The presence of calcium ion in the system will lead to the calcium carbonate precipitation once a certain level of super saturation is reached. The calcium carbonate precipitation mechanism induced by urease enzyme activity illustrated in Figure 1.

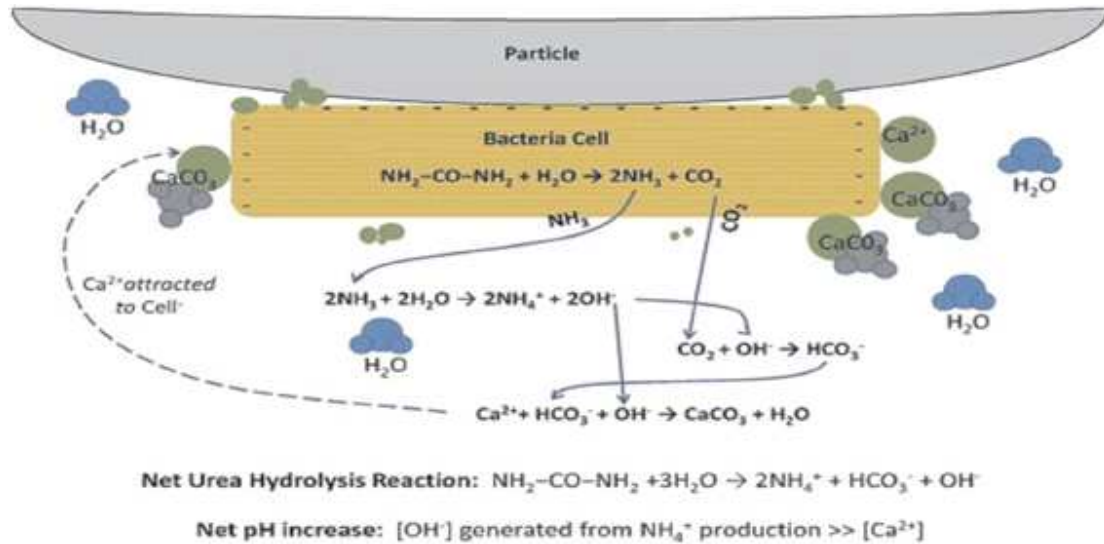


Figure-1 Bacterial serving as nucleation site for CaCO<sub>3</sub> precipitation in the sand particle [40]

Calcium ions in the solution are attracted to microorganism cell wall due to the negative charge of the latter. After the addition of urea to the system, microorganism convert urea to dissolved inorganic carbon (DIC) and ammonium (AMM) and released it to the environment (A). The presence of calcium ion cause the supersaturation condition and precipitation of calcium carbonate in microorganism cell wall (B). After a while, the whole cell becomes encapsulated by calcium carbonate precipitate (C). As whole cell encapsulated, nutrient transfer becomes limited and resulting in cell death. Image (D) shows the imprints of microorganism cell involved in carbonate precipitation [7].

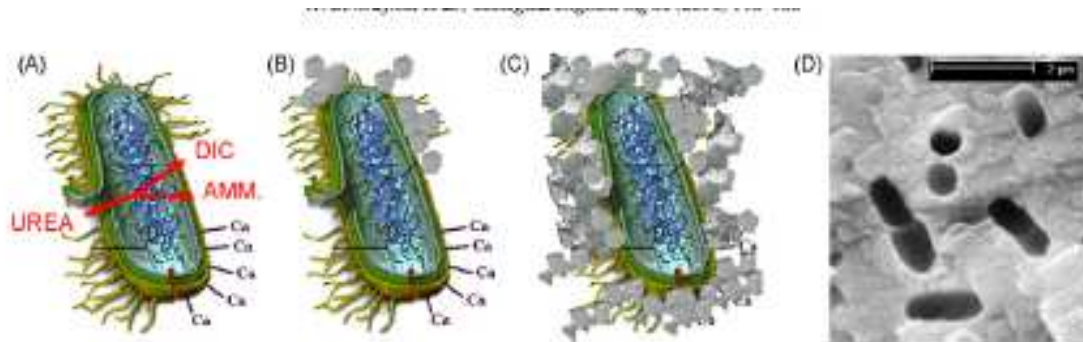


Figure-2 Simplified representation of events occurring during ureolytic induce carbonate precipitation [14]

**BIO CEMENTATION**

In the biosphere, bacteria can act as geo-chemical agents, resulting in the concentration of materials. This induces the formation of special minerals, which constitute an area of research of growing interest known as bio mineralization [18]. Bio mineralization is the science of precipitation of minerals by living organisms. Both eukaryotic and prokaryotic organisms deposit minerals [44]. Some examples: (i) Eukaryotic organisms: like plants that produce cystolith inclusion in leaves and animals that form bones, teeth and shells [52] ; (ii) Prokaryotic organisms like bacteria they have the remarkable ability to precipitate minerals such as calcites, carbonates, phosphates, oxides, sulphides, silicates, silver and gold [16].

Bio cement is a product innovation from developing bioprocess technology called bio cementation. Bio cement refers to a  $\text{CaCO}_3$  deposit that formed due to microorganism activity in the system rich of calcium ion [27]. In MICP, the mineral calcium carbonate is precipitated by the microorganisms [44]. Bio cementation or bio grout is a sand consolidation technology, in which carbonate released from microbial urea hydrolysis precipitates with an excess of calcium ions to form in situ calcite ( $\text{CaCO}_3$ ) precipitation [45]. Another definition of bio cementation is suggested by Ivanov and Chu, [53] and Ariyanti *et al.*, [27] that is the generation of particle-binding materials through microbial processes *in situ* so that the shear strength of soil can be increased and by [54] that is bio cementation is an innovative technology based mainly on application of urease-producing microorganisms together with urea and calcium ions in a permeable soil.

### Bio cementation mechanism

Naturally, bio mineralization process occurs at a very slow rate over geological times like the formation of limestone, sandstone, etc. Natural carbonation occurs by the reaction between atmospheric  $\text{CO}_2$  and alkaline materials, which is called “weathering” [55]. The difference of Bio cement respect to nature principles is that the microbial carbonate precipitation reaction takes a relatively short period of time instead of millions of years.

## PRINCIPAL PARAMETERS GOVERNING $\text{CaCO}_3$ PRECIPITATION FOR BIO CEMENTATION

### i. The calcium and carbonate concentration

The calcium and carbonate concentrations can have a positive or negative effect on the precipitation rate and / or inhibitory effect on the  $\text{CaCO}_3$  production by the bacteria. Urea hydrolysis generates carbonate ions at a 1:1 molar ratio. Hence with increased urea, carbonate concentrations can be increased to facilitate  $\text{CaCO}_3$  saturation [16].

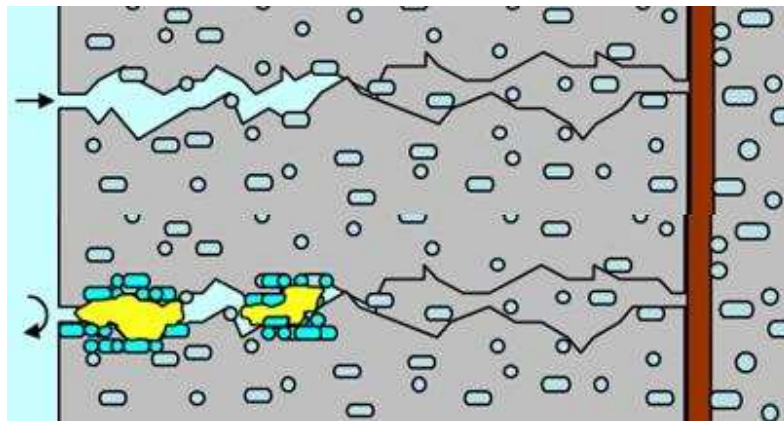


Figure-3 Top: A crack is formed on the concrete surface. Water infiltrates the crack and activates the bacteria. Bottom: Calcite produced by the bacteria fills the crack and repairs the damaged structure[47]

### ii. The pH of the environment

This controls carbonate speciation, and calcium carbonate solubility. The pH of the environment has a positive or negative effect on the rate of carbonation by the bacteria, precipitation of  $\text{CaCO}_3$  in the medium and type of crystals formed.

### iii. The presence of nucleation sites

The presence of nucleation sites is important as it governs the homogeneity of the carbonation and also the strength of the carbonate being produced (by the production of  $\text{CaCO}_3$  at specific points). Depending on the type of microorganism being used the above outcomes will vary, and so also the material properties of the  $\text{CaCO}_3$  produced. In microbial  $\text{CaCO}_3$  precipitation, the first two factors are the key for  $\text{CaCO}_3$  precipitation, while the third factor is not a key factor because the bacteria themselves behave as nucleation sites [14, 27, 56, 57, 58, 59, 60].

Recently, the role of microorganisms in the bio conservation and restoration of monuments and stone works has been acknowledged and evaluated. These microorganisms, though not limited to any specific taxonomic group share a common property: that of extracellular precipitation of calcium carbonate. The majority of microorganisms which



are known to precipitate calcium carbonate thereby confer protection to monuments and stone works against bio deterioration.

**Table 2. Potent bio protectors of the heritage monuments and art work**

| Sr.No. | Organisms                           | Phylum         | Applications   | References |
|--------|-------------------------------------|----------------|--|------------|
| 1      | <i>Desulfovibrio Desulfuricans</i>  | Proteobacteria | Removal of black crust from marble surface   | [61]       |
| 2      | <i>Pseudomonas Fluorescens</i>      | Proteobacteria | Biocleaning and restoration of fresco Calcite precipitation  | [62]       |
| 2      | <i>Bacillus Cereus</i>              | Firmicutes     | Biocement (Biocalcin) formation and limestone consolidation  | [63]       |
| 3      | <i>Pseudomonas and Bacillus sp.</i> | Proteobacteria | Removal of phenantrene deposit from weathered stones   | [64]       |
| 4      | <i>Pseudomonas sp.</i>              | Proteobacteria | Removal of nitrates from the weathered stones  | [65]       |
| 5      | <i>Micrococcus</i>                  | Actinobacteria | Reduction in water absorption of "Pietra di Lecce", a calcareous stone thereby rendering consolidation | [13]       |
| 6      | <i>Bacillus Subtilis</i>            | Firmicutes     | Reduction in water absorption of "Pietra di Lecce", a calcareous stone thereby rendering consolidation | [14]       |
| 7      | <i>Bacillus Amiloliquifaciens</i>   | Firmicutes     | Conservation of ornamental stones  | [86]       |
| 8      | <i>Bacillus Megaterium</i>          | Firmicutes     | Calcite Precipitation  | [66]       |
| 9      | <i>Bacillus Sphaericus</i>          | Firmicutes     | Concrete consolidation Biocement (Biocalcin) formation   | [29,32,67] |
| 10     | <i>Myxococcus Xanthus</i>           | Proteobacteria | Consolidation of ornamental stones Bioconservation of cultural heritage Structures                     | [68,69]    |
| 11     | <i>Halobacillus Trueperi</i>        | Proteobacteria | Biomineralization  | [70]       |
| 12     | <i>Pseudomonas Cepacia</i>          | Proteobacteria | Biocleaning, restoration and removal of animal glue from fresco  | [71]       |
| 13     | <i>Pseudomonas Flavescens</i>       | Proteobacteria | Biocleaning, restoration and removal of animal glue from fresco  | [71]       |
| 14     | <i>Pseudomonas Stutzeri</i>         | Proteobacteria | Biocleaning, restoration and removal of animal glue from fresco  | [71]       |
| 15     | <i>Thiobacillus sp.</i>             | Proteobacteria | Removal of fouled layer of lichen from weathered concrete specimens                                    | [72]       |
| 16     | <i>Bacillus Lentus</i>              | Firmicutes     | Biocement (Biocalcin) formation and limestone consolidation  | [32]       |
| 17     | <i>Bacillus Pumilus</i>             | Firmicutes     | Calcite precipitation  | [74]       |
| 18     | <i>Bacillus Thuringiensis</i>       | Firmicutes     | Calcite precipitation  | [74]       |
| 19     | <i>Pseudomonas Halophile</i>        | Proteobacteria | Calcite precipitation  | [75]       |
| 20     | <i>Desulfovibrio Vulgaris</i>       | Proteobacteria | Removal of black crust from marble Surface   | [76]       |
| 21     | <i>Rhodococcus Erythropolis</i>     | Actinobacteria | Consolidation of "Pietra di Lecce", a calcareous stone   | [77]       |
| 22     | <i>Bacillus Pasteurii</i>           | Firmicutes     | Biocement formation and consolidation of sand column and repair of concrete cracks                     | [78]       |
| 23     | <i>Morcella sp.</i>                 | Ascomycetes    | Formation of carbonate concretions   | [79]       |
| 24     | <i>Pseudomonas sp.</i>              | Proteobacteria | Limestone consolidation  | [80]       |
| 25     | <i>Acinetobacter</i>                | Proteobacteria | Limestone consolidation  | [80]       |
| 26     | <i>Myxococcus xanthus</i>           |                | Limestone restoration by ureolysis   | [81]       |
| 27     | <i>Sporosarcina pasteurii</i>       |                | Limestone restoration by ureolysis   | [82]       |
| 28     | <i>Synechococcus</i>                |                | Concrete restoration by photosynthesis   | [83]       |

These bacteria are widely distributed and have been ubiquitous since the Precambrian. They are found in soil [33-34], in fresh water [35], and in sea water [36]. These micro-organisms are currently the subject of extensive studies, which are yielding a fresh understanding about their role in carbonatogenesis, and aiding the development of new technologies for the bio conservation and consolidation of monuments and stone works of art [13-14, 37]. This group of micro-organisms is known as carbonatogenic micro-organisms or calcifying microbes because of their inherent capability of producing calcium carbonate.

#### APPLICATION OF BIO CEMENTATION [53, 54, 84]:

- Stabilizing pollutants from soil by the binding;
- Controlling erosion in coastal area and rivers;
- Creating water filters and bore hole filters;
- Immobilizing bacterial cells into a cemented active biofilter;
- Reducing the liquefaction potential of soil;
- Treating pavement surface;
- Binding of the dust particles on exposed surfaces to reduce dust levels;
- Increasing the resistance to petroleum borehole degradation during drilling and extraction;
- Increasing the resistance of offshore structures to erosion of sediment within or beneath gravity foundations and pipelines;
- Strengthening tailings dams to prevent erosion and slope failure;
- Constructing permeable reactive barriers in mining and environmental engineering.

### ADVANTAGES OF BIO CEMENTATION

The biocementation process (microbial consolidation) is advantageous over the ordinary cementation processes by:

- i. Upgrading of load bearing capacity of soil deprived of making the soil impermeable to fluids by MCP is a unique property compared to other treatment methods [60]. Retention of the permeability was evident by the absorbance of water recorded in the biocemented surfaces [13]. For consolidation of loose material, it is vital to conserve the permeability so that the water moves through the voids in the stone hindering the deterioration due to water logging [85].
- ii. It is possible to reuse cells in-situ, which is a cost saving process as cost of culturing the cells is not considered [85].
- iii. Reactants are aqueous in nature, hence less energy required as low injection pressure is required as they easily infiltrate into pores [60].
- iv. Its economical effective process as compared to Calcite *In-situ* Precipitation System (CIPS) technology [85].
- v. Bio cementation process dependent on bacteria which are more tolerant to the cementation condition than the plant source [56].

### LIMITATION OF SOIL BIO CEMENTATION

There is several limitation or disadvantage of disadvantage of soil bio clogging and bio cementation in comparison with chemical grouting which are summarized as follows;

- i. That the microbial process is usually slower;
- ii. Microbial process is more complex than the chemical one because the microbial activity depends on many environmental factors such as temperature, pH etc. [53].

### CONCLUSION

MICP is a multifaceted bio-chemical route that employs the urea hydrolysis that takes place between the sand particles for enhancement of soil engineering properties. There is an cumulative need for a ground development method, and one of the methods is to improve the strength of soil particles by utilizing the cementation technique. Even though there are various chemical methods available that are currently in practice, many of them have adverse environmental effects. This review paper describe better thoughtful of the effects of numerous parameters on the biological cementation and on accomplishing microbial cementation using several soil microorganisms. Further, MICP is one of the best alternative ground improvement techniques due to its high efficiency and low cost within the geotechnical applications.

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