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Bio-Mineralization and Bacterial Carbonate Precipitation in Mortar and Concrete

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Abstract

Mortar and concrete are the most widely used building materials all over the world as they are cheap, easily available and convenient to cast. But crack in these materials is a common phenomenon during its service life due to many reasons, if unattended timely, will result in long-term structural deterioration with high level of risk and maintenance cost. Currently, epoxy, resins, epoxy mortar and other synthetic mixtures are used for remediation of cracks. Bacteria from various natural habitats have frequently been reported to precipitate calcium carbonate both in natural and laboratory conditions. Application of bacteria based mineralization concept has lead to the potential invention of a new biomaterial that can fill the cracks and fissures in mortar and concrete materials. The present review include bio-cementation in concrete, bacterial carbonate precipitation, chemical process and their self healing activity on the bacteria incorporated materials by application of microbiologically induced calcite precipitation.

Keywords

Bio-Mineralization, Bio-Cementation, Bacterial Carbonate Precipitation, Self Healing, Cracks

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1. Introduction

Concrete is a strong and cheap construction material and its application is rapidly increasing worldwide. However, they can get destroyed for a variety of reasons including the material limitations, design gaps and construction practices, as well as exposure conditions [1]. *Bacillus*, a common soil bacterium, can continuously precipitate calcium carbonate (calcite) under favorable conditions [2]. This phenomenon is called microbiologically induced calcite precipitation (MICP). It is pollution free and natural. The technique can be used to improve the compressive strength and stiffness of cracked concrete specimens. Due to its inherent ability to precipitate calcite continuously bacterial concrete can be called as a "Smart Bio Material" [3].

Currently, heavy amount of currency is needed to repair cracks and maintenance of the buildings, etc. To decrease the susceptibility of decay, use of water repellents and stone consolidates was carried out. But both of these have often been reported to accelerate stone decay [4-5]. Use of organic solvents in cracks repair materials result in the formation of incompatible and often harmful surface films [6]. Concrete show self healing capacity itself to heal freshly formed micro cracks. This property is mainly due to the presence of non hydrated excess cement particles in material matrix, which undergoes delayed or secondary hydration upon reaction with ingress of water. Many different and innovative strategies like use of hollow fibers, micro encapsulation, expensive agents and mineral admixtures were practiced to check their efficiency for self healing. Problem related to durability such as crack formation are tackled by impregnation of crack with cement or epoxy, resins, epoxy mortars or other synthetic fillers. Among the many protection and consolidation treatments available that mainly

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use inorganic and organic materials, none of them have proven to be satisfactory [7]. Therefore, a sustainable repair methodology is recently being investigated i.e., the use of biological repair technique by the introduction of bacteria in concrete. Use of microorganisms within mortar/concrete leading to the process of bio-mineralization is now a potential field of research in concrete technology [5]. The idea was to incorporate bacteria, which helps in the precipitation of calcium carbonate of dissolved inorganic carbon. The applicability of specifically mineral producing bacteria for sand consolidation and monument repair was practiced [6, 8-9]. The filling of cracks and fissures in concrete has been investigated [10-11]. Ureolytic bacteria are anaerobic and water grown, grows well inside the concrete or mortar matrix without supply of oxygen or food. Also the process will reduces atmospheric carbon dioxide emission considerably as less cement to be consumed for self-healing of cracks in mortar and concrete [9, 12].

2. Self Healing of Mortar and Concrete Materials

The methods for self healing of cracks in damaged concrete include, natural healing, autonomic healing, activated processes, autogeneous healing, etc. Natural healing occurs when a concrete crack is automatically and naturally blocked without use of a chemical matrix or design. The natural ability of hydrates to heal cracks over time is termed as autogenic self healing where as artificial means of crack repair that are man-made inclusions (autonomic). The activated process utilizes a different mechanism, which is previously implemented and placed in the concrete beforehand. The main aim of both, autonomic, autogeneous healing is to increase the durability of concrete. It is seen that many old structures survive for long period of time with limited maintenance, for instance, roman aqueducts and gothic churches, in which healing occurs itself when moisture interacts with un-hydrated cement clinker in the crack. But in recent buildings, the content of cement is reduced as a result of which autogenic healing effect is reduced [13]. There are two main aspects to consider with regard to autonomic healing concept, namely, the encapsulation method and the healing agent that is encapsulated. At present available crack repair agents are not compatible, reduce aesthetic appearance, costly and need constant maintenance. Therefore, the possibility to use bacteria as a sustainable and concrete embedded self healing agent was explored. As the pH value of concrete is very high, only so-called alkaliphilic bacteria are able to survive in the material. Bacterial spores were found viable after mixing with cement paste [13].

3. Bio-mineralization

Bio-mineralization is the science of precipitation of minerals by living organisms. Naturally, bio-mineralization process occurs at a very slow rate over geological times like the formation of limestone, sandstone, etc [14-15]. Bacteria 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. The calcium carbonate deposition occurred due to bacterial activity in the system rich in calcium ion [17]. It is the generation of particle-binding materials through microbial processes in situ [18-19] and an innovative technology based mainly on application of urease-producing microorganisms together with urea and calcium ions in a permeable soil [20].

3.1. Advantages of Bio-Cementation

The bio-cementation process has following advantageous over the ordinary cementation process:

1. It is dependent on bacteria which are more tolerant to the cementation condition [21].
2. Consolidation of porous media can be achieved by the direct use of bacterial culture without the need to concentrate the cells of bacteria. Thus, there is no need for additional processing for the bacterial culture to produce sand-stone [15].
3. Reactants were aqueous in nature, hence less energy required [22].
4. Cost-saving process as the bacterial enzyme was reused in subsequent applications of calcium and urea only [15]. The bacterial cells can be reused 2-3 times [23] and over 20 times [15] in subsequent additions of calcium and urease only. Therefore, reusing the cells in-situ is a cost saving process [15, 24].
5. The deposition of a layer of calcium carbonate on the surface and inside pores of the mortar specimens resulted in a decrease of water absorption and permeability. It was concluded that the presence of a layer of carbonate crystals on the surface by bacterial cells has the potential to improve the resistance of cement and concrete materials towards degradation process [18-19, 24].

3.2. Various Micro-Organisms Used for Bio-Cementation

The groups of chemotrophic prokaryotes are most suitable for bio-cementation because of their smallest cell size (0.5 to 2 μm) and their ability to grow inside of soil and big physiological diversity.

Table 1. Few microbes used in concrete [25-26].

No. Applications	Types of bacteria
1.Microbial concrete as crack healer	*S. pasteurii -Deleya halophila* *Halomonas eurihalina* *Myxococcus xanthus*
2. Microbial concrete as surface treatment	*Bacillus sphaericus, B. subtilis*
3. Microbial concrete as water purifier	*Bacillus subtilis, B. sphaericus* *Thiobacillus sp*

There are three main existing groups of organisms that can induce microbiologically induced calcite precipitation (MICP):

(i) Photosynthetic organisms such as cyanobacteria and algae that remove carbon dioxide, (ii) Sulphate reducing bacteria that are responsible for the dissimilatory reduction of sulphate and (iii) Several micro organisms that were involved in the nitrogen cycle [21, 27-28].

Anaerobic fermenting bacteria are capable in cementation of soil particles under the presence of calcium, magnesium or ferrous ions. This cementation process due to the increase in pH caused by ammonification and carbon-dioxide production in soil added with urea [29]. In the case of sulphate-reducing bacteria sulphide reacts with iron and other metal cations to form insoluble suphides of metals, which clogs the soil pores and binds the soil particles. However, the soil compaction created by the formation of sulphides is unstable because they can be chemically or biologically oxidized to sulphuric acid or sulphates under aerobic conditions [30]. Different types of bacteria as well as abiotic factors (salinity and composition of the medium) seem to contribute in a variety of ways to calcium carbonate precipitation in a wide range of environments [30-31]. The primary role of bacteria in the precipitation process has subsequently been ascribed to their ability to create an alkaline environment through various physiological activities [21, 32-35].

3.3. Principal Parameters Governing Bacterial Carbonate Precipitation for bio-cementation

1. The calcium and carbonate concentration have a significant effect on the precipitation rate and inhibitory effect on the calcium carbonate 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 calcium carbonate saturation.
2. The pH of the environment has a significant effect on the rate of carbonation by the bacteria, precipitation of calcium carbonate in the medium and type of crystals formed.
3. The presence of nucleation sites is important as it governs the homogenity of the carbonation and also the strength of the carbonate being produced. In bacterial calcium carbonate precipitation, the first two factors are the key for calcium carbonate precipitation, while the third factor is not a key factor because the bacteria themselves behave as nucleation sites [36-37].

3.4. Chemical Processes of Bacterial Carbonate Precipitation

3.4.1. MICP in Water

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

$$2HCO_3^- \leftrightarrow CO_2 + CO_3^{2-} + H_2O \qquad (II)$$

$$CO_3^{2-} + H_2O \leftrightarrow HCO_3^- + OH \qquad (III)$$

$$Ca^{2+} + HCO_3^- + OH^- \leftrightarrow CaCO_3 + 2H_2O \qquad (IV)$$

3.4.2. Bacterial Sulphate Reduction

$$CaSO_4.2H_2O \rightarrow Ca^{2+} + SO_4^{2-} + 2H_2O \qquad (V)$$

$$2(CH_2O) + SO_4^{2-} \rightarrow HS^- + HCO_3^- + CO_2 + H_2O \qquad (VI)$$

3.4.3. MICP in Nitrogen Cycle

$$CO(NH_2)_2 + 2H_2O \rightarrow CO_3^{2-} + 2N\,H_4^+ \qquad (VII)$$

3.4.4. Chemistry of MICP by Urea Hydrolysis

B. pasteurii is a gram positive, aerobic, alkaliphilic, common soil bacterium with small size (1.3-4.0/0.5-1.2µm) and spore measuring 0.8-1.3 µm in diameter exhibit urease-producing ability. This characteristic feature of *B. pasteurii* is made use of in crack remediation. Urease is a nickel containing enzyme that catalyses the hydrolysis of urea to produce ammonium and carbon dioxide. MICP is comprised of a series of following complex biochemical reactions:

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3$$

$$H_2CO_3 + H_2O \rightarrow HCO_3^- + H^+$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^-$$

$$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \rightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O$$

Since the cell wall of the bacteria is negatively charged, the bacteria draw cations from the environment, including Ca^{2+}, to deposit on their cell surface [38-39].

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

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

The calcium carbonate precipitation forms a highly impermeable layer. It is highly insoluble in water and has the ability of continuously grow upon itself. The bacteria precipitate calcite in the presence of nutrients. The phosphate buffer or urea and calcium chloride have been found effective as nutrients [39]. During the bio-cementation process, by-product ammonium chloride is produced [40].

$$NH_4Cl\ (s) + H_2O\ (l) \leftrightarrow NH_4^+(aq) + Cl^-\ (aq) + H_2O\ (l)$$

3.5. Catalyst for Bacterial Carbonate Precipitation

The presence of 10μM Nickel ion in the active site of urease aids functional activity as well as the structural integrity of the enzyme thus enhancing specific urease activity. Higher concentration of Nickel ions cause inhibition leading to dramatic drop of urease activity [15]. Nickel ions are supplied in the form of nickel chloride. Solution of urea and calcium chloride in equal molar ratio mixed with water are injected to initiate the bio-cementation process. Nickel as a promoter of urease activity has a profound effect on bacterial growth when incorporated in urea based broth prior to inoculation and the maximum absorbance was found at 1000 μm followed by 5 μm ascertaining that these are the most suitable concentrations for growth of *B.cohnii* and *B.megaterium* [41].

3.6. Immobilization of Bacterial Cells

The optimum pH for growth of *B. pasteurii* is around 9. The alkaline environment of concrete is the major hindering factor, restricting the growth of bacteria. No growth on the outer surface was appeared as it results in death of bacterium when it comes on contact with atmosphere [42]. In order to protect the microbes from the high pH of concrete, it was necessary to immobilize the bacterial cells. Polyurethane (PU) has been used to serve this purpose because of its mechanical strength and chemical inertness. Cylindrical shaped PU foam, containing bacterial cells was then placed in to simulated cracks and used as crack repair technique [10]. Silica was used to immobilize the bacteria, the gel containing the bacteria can be directly sprayed in to the cracks. The silica gel was not only used to immobilize the bacteria but used as filling material to fill cracks before calcite precipitation started [37].

4. XRD and SEM Investigation of mortar and concrete

The X-Ray Diffraction (XRD) analysis was performed on three portland cement mortar cubes: (i) mixed and cured in water (ii) mixed with phosphate buffer and cured in urea-calcium chloride medium and (iii) mixed with *B. pasteurii* suspended and cured in urea-calcium chloride medium [43]. In the presence of bacteria, an insignificant increase of calcite precipitation was detected. In another study, XRD analysis was employed to determine the crystalline form of calcite crystals [44]. XRD analysis is carried out to determine chemical composition of the precipitation that occurred due to bacterial mineralization. Bacterial calcite precipitation was quantified by XRD analysis and visualized by SEM [10].

Impressions of the shape, consistent with the dimensions of the bacteria were found in calcite crystals [45]. It was observed that the specimens with bacteria did not develop any micro cracks, as they did not expand much unlike control specimens when subjected to alkali aggregate reactivity, sulfate attack, drying shrinkage and freeze thaw. In order to prove for bacterial calcite precipitation using biomimetics techniques SEM micrograph of mortar without microorganism and with anaerobic microorganisms of 10^7/ml cell concentration was taken [46]. Treatment of the mortar cubes with bacteria and a calcium source resulted in the presence of a crystalline layer on the surface. The type of bacterial culture and nutritional composition had a profound impact on crystal size and morphology. Presence of nutrient broth resulted in absence of rhombohedral crystals in SEM image although they are clearly present in its absence [11]. FE–SEM was performed to identify different CFB induce stain-specific calcium carbonate formations and found that SEM images also showed the strain-specific differences on the crystal surfaces. The solid agar medium containing the bacterial colonies was purified and crystals were visualized for SEM image. The size and morphology of the crystals showed bacterial strain specific differences. The most researchers used the combination of scanning electron microscopy with one or more instruments of electron disperse X-ray, energy dispersion spectroscopy or energy disperse X ray analyzer to determine the chemical constituents on the selected spectrum on image of SEM [47].

5. Application of Bacterial Carbonate Precipitation in Construction Industry

5.1. As a Construction Material

The use of bacterial carbonate precipitation in Bio Geo Civil engineering has become increasing popular. The use of bio-cementation through MICP with ureolytic bacteria for variety of applications seems to be a compatible alternative to cement. In particular this method used for consolidation of sand (aggregate) and the attainable strengths, make it a practicable alternative to concrete. It is being commercialized on a small scale for molded products, bricks or blocks and instant pavements [48]. In the large scales uniform distribution of strengths is yet to be mastered. Some of the applications of MICP to be help full for crack remediation and self-healing of existing concrete.

5.2. As an Alternative to Concrete

The environmental impact of bio-concrete is proven to be half that of ordinary concrete, even though the production of the inputs in bio-concrete is double that of concrete. This is mainly due to the possibility of bio-concrete being able to be recycled and re-used to produce same hence forming a closed loop by closing its cycle.

6. Discussion

Ingress of weathering substances into the construction material is a major causative factor of degradation of mortar and concrete. The main objective of research on MICP is to identify whether microbes can improve the durability of concrete, by altering permeation properties and to establish whether bacteria incorporated in concrete could act as self healing agent to catalyze the process of autonomous repair of freshly formed cracks. Numerous diverse bacterial species participate in the precipitation of mineral carbonates in various natural environments including soils, geological formations, fresh water biofilms oceans and saline lakes [49-50]. The bacteria are able to influence the precipitation of calcium carbonate by production of a urease enzyme. This enzyme catalyzes the hydrolysis of urea to carbon dioxide and ammonia, resulting in an increase of pH and carbonate concentration in the bacterial environment [1-2]. This additional layer formed by bacteria was highly insoluble and increase the impermeability of the specimen. Increase in compressive strength was also detected, this increase in matrix strength would have resulted in lesser mean expansion and would have eventually increased the overall durability performance of the concrete.

7. Conclusions

Microbial calcite precipitation by urea hydrolysis is enabled by interdisciplinary research at the confluence of microbiology, geochemistry and civil engineering. The bacteria induced microbial carbonate precipitation has been proposed as an environment friendly remediation and self healing of cracks in building materials. Bacterial carbonate precipitation has proved to be better than many conventional technologies because of its eco-friendly nature, self-healing ability and increase in durability of various building material. The traditional crack repair systems have number of disadvantageous aspects such as different thermal expansion coefficient compared to concrete and health and environmental hazards. The applicability of ureolytic, mineral producing bacteria for filling of pores and cracks in concrete can be used as surface treatment or as an integrated healing agent, the advantage of later is that it will save manual inspection, repair, energy, time and moreover increase structure durability. This seems to be a classic strategy to save money and protect environment. Comparative studies exhibited that there are both advantages and limitations about different healing agent and strategy but in order to find out the most reliable and effective self healing more researches have to work in the directions. The bacteria induced calcite precipitation reaction may cause lower amount of capillary pores and clogging of the pores, which reduces chloride ion transport in concrete. The use of bacteria has thus become a viable solution not only to some durability problems but also as an environmentally responsible course of action. The production of self-healing materials will most often exceed the costs of traditional materials.

The use of bacteria induced mineralization to fill the cracks in mortar and concrete is very innovative. It is a new and promising method. The research in this aspect focuses more on durability side rather than on the mechanical properties. The ability of alkaliphilic bacterial strains to tolerate highly alkaline environment may have important implications for remediation of cracks in structures. However, further experiments have to be explore to examine the durability of crack repair technique. Production of bio bricks and crack filling in other building material like granite, brick and marble can also be practiced by adopting MICP as a sole technique.

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