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Microbial Calcification: An Insight Into Carbonate Precipitation And Its Emerging Influence In Diverse Applications

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ABSTRACT

Microbially induced carbonate precipitation (MICP) is a biologically induced mineralization process and has found its application in various engineering realms. The process of MICP is often mediated by the enzymes urease and carbonic anhydrase. The bacterial cell wall acts as the nucleation site for the formation of CaCO_3 . This review summarizes the mechanism associated with the microbial CaCO_3 precipitation process and the function of urease. The role of extracellular polymeric substances (EPS) and their ability to influence MICP are also discussed. The various polymorphs of CaCO_3 that are formed in the due course of MICP and the conditions that determine the polymorph selection are discussed as well. Biocalcification by different classes of bacteria isolated from various sources are summarized. This review describes in detail the various applications of MICP including its role in biocementation, removal of heavy metals, CO_2 sequestration, fracture sealing in underground geology, remediation of limestone structures and as a counter measure for erosion. Over the last few years, various process improvements have emerged for improving the quality of MICP and thereby enhancing their potential, viz. optimization of bacterial growth medium, using industrial by-products as growth media components, etc. Some of these process improvements are also discussed in this review. The shortfalls of MICP, particularly those while implementing it at the field level, issues associated with commercialization of the process are also described along with its future perspectives.

Keywords: MICP, urease, CaCO_3 , polymorphs, engineering applications

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INTRODUCTION

Over the last two decades, biomineralization has evolved as an intriguing avenue to investigate for the research fraternity, primarily attributed to the wide range of minerals that are being formed. Biomineralization is defined as the process through which living organisms produce minerals viz. phosphates, sulphates, carbonates, etc. [1-3]. This phenomenon is widespread in nature and syntheses of extracellular minerals have been reported from all classes of organisms. The mechanism of biomineralization can be classified under two major categories: Biologically controlled mineralization (BCM) and biologically induced mineralization (BIM) [4]. In BCM, minerals are synthesized under certain conditions at a specific location within or on the cell while in BIM, there is an extracellular precipitation of minerals due to supersaturation [5].

Biocalcification or Microbially induced carbonate precipitation (MICP) is the process through which microbes form carbonates due to supersaturation associated with certain biochemical activities [6]. MICP is a complex process and their underlying mechanisms are being studied widely. There are reports suggesting that MICP occurs on account of: (1) photosynthesis [7] (2) ureolysis [8] (3) ammonification [9] (4) sulphate reduction [10], etc. Among them, MICP through urea hydrolysis is the commonly found phenomena. Secretion of extracellular polymeric substances (EPS) by the bacteria also acts as the binders of metal ions which facilitate carbonate precipitation [11]. The genomic pathway of MICP is yet to be understood completely, since there are multiple factors which play crucial roles. However, Barbesi *et al.* [12] has reported the role of genes *lcfA*, *ysiA*, *ysiB*, *etfB*, and *etfA* which form a part of *lcfA* operon system in calcite precipitation by *Bacillus subtilis*. The study suggested that the gene *etfA* was crucial for precipitation and there was possible association with fatty acid metabolism.

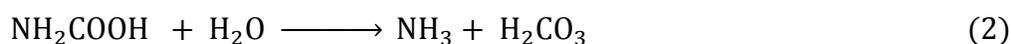
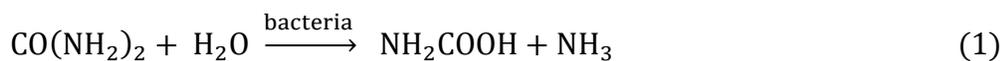
There are four major factors that impact the precipitation of carbonates: (1) pH of the environment, (2) calcium concentration, (3) dissolved inorganic carbon (DIC) concentration and (4) availability of nucleation sites [13]. In MICP, the bacterial cell wall acts as the nucleation site in which the carbonate ion formed due to enzyme activity binds with Ca^{2+} ions to precipitate carbonate crystals [14]. The process of calcium carbonate (CaCO_3) precipitation in microbes is often mediated by the enzyme urease and in certain instances through carbonic anhydrase [15]. However, mediation of biocalcification by urease enzyme has been exhaustively studied in various microorganisms [16, 17]. Urease driven carbonate precipitation process is recognized as environmental friendly and hence used for a multitude of biotechnological applications. Notably, MICP is used for strengthening of concrete materials [18], restoration of limestone buildings [19], self healing of

concrete structures [20], removal of heavy metals and radionucleotides [21], CO₂ sequestration [22], etc. Implementing MICP for sealing fractures in wellbores [23], as a countermeasure for soil erosion [24], an agent to reduce permeability of soil [25] are on the rise, thereby finding its application in subsurface engineering as well.

In spite of its widespread application and demonstration at the lab scale level, several challenges are confronted upon implementing MICP at the field scale applications. The role of environmental factors on the microbial growth, impacts of by-products formed during the process are to be considered for the sustainable application. This review provides an insight into the mechanism of MICP, its applications, recent process improvements, limitations and future prospects.

Urease Driven Carbonate Precipitation

Urease is a common enzyme found to be present in various microbes. Urease mediates the hydrolysis of urea to ammonia and carbamic acid which reacts with water to form carbonic acid.



Carbonic acid and ammonia further equilibrates in water to form bicarbonate and ammonium ions. The formation of hydroxide ion causes an increase in environmental pH, a condition suitable for precipitation to occur [26].



In the presence of calcium ions, carbonate ions combine to form CaCO₃ crystals.



In MICP, the formation of CaCO₃ occurs on the bacterial cell wall which serves as nucleation site. The bacterial cell wall possesses negatively charged ions which bind to available Ca²⁺ ions. The carbonate ions that are formed as a result of urease activity binds to the calcium ions at the bacterial cell wall to form CaCO₃. The process of bacterial calcium metabolism and subsequent precipitation of CaCO₃ is represented in Figure 1. The process of carbonate precipitation through ureolysis by bacteria has been found to be a rapid process to accumulate high amount of CaCO₃ [27].

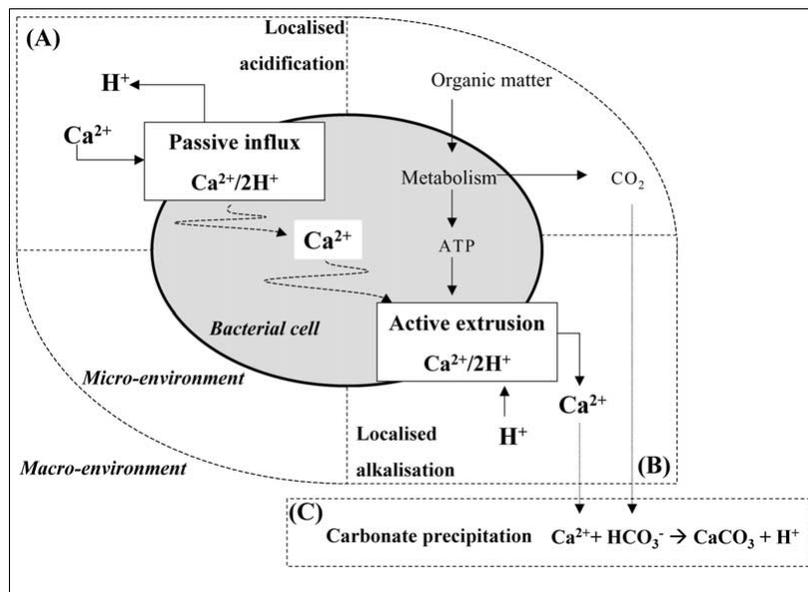


Figure 1: Representation of bacterial metabolism of calcium and CaCO₃ precipitation under high pH and high Ca²⁺ conditions. Image source: Hammes and Verstraete (2002) [13].

The efficiency of bacterial mediated carbonate precipitates and the precipitation caused by free urease enzyme from plant (jack bean) were compared and reported [28]. The use of free enzyme is a straight forward process facilitating to avoid the issues surrounding scaling up of bacterial cultures and their maintenance. In addition, the size of the free urease from plant (12 nm) [29] was significantly smaller than the bacterial urease (0.5- 5 μm) [30] and hence it was found to penetrate the pores in a better manner. Accumulation of CaCO₃ was higher upon using free urease which may be ascribed to the inhibition of bacterial activity due to high urea or calcium ion concentration [30]. The durability can be increased by immobilizing the free enzymes on a suitable substrate [31].

ROLE OF EXTRACELLULAR POLYMERIC SUBSTANCES (EPS) IN CARBONATE PRECIPITATION

EPS have been found to play a crucial role in MICP through enhancing the carbonate precipitation process [11]. EPS, which contains charges residues and sugars possessing carboxyl, amine, hydroxyl groups which helps in trapping of metal ions such as Ca²⁺, Mg²⁺, etc [32]. This trapping of metal ions provide a suitable condition for MICP upon the formation of carbonate ions in the environment. Several classes of bacteria including cyanobacteria, bacilli, etc. are found to produce EPS [11, 33]. Achal et al. [34] has reported the role of EPS in the increased strength of sand column by *Sporosarcina pasteurii*. (Some more references). Kawaguchi et al. (2002) [11] reported that EPS was significant in increasing the precipitation and composition of CaCO₃. The type of EPS produced by the microbes influences the CaCO₃ polymorphs as well. The growth of aragonite

crystals were inhibited by acid polysaccharides [35], while the polysaccharides associated with glycoproteins facilitated calcite formation [36]. EPS is the basic structural component of biofilm formation. Formation of biofilms by bacteria helps them bind the substrate for a significant period and also provides the nucleation site for a longer period of time [37, 38].

CALCIUM CARBONATE POLYMORPHS

Different polymorphs of CaCO_3 have been reported to be produced by the microbes. Predominantly formed polymorphs are calcite, vaterite and aragonite, while monohydrocalcite and hexahydrocalcite [39] are also produced by certain microbes. Calcite is the most commonly found and the most thermodynamically stable polymorph of CaCO_3 [40, 41]. On the other hand, vaterite is metastable form of CaCO_3 and it appears as a transitional phase before getting converted into calcite [42]. The process of calcite precipitation involves: (a) formation of amorphous carbonate (b) intermediate transition to vaterite and (c) final conversion to stable calcite [43, 44].

Calcium sources are also found to influence the polymorph formation in MICP. Calcium chloride led to the precipitation of rhombohedral shaped calcite [45, 46]. Calcium acetate formed lettuce shaped vaterite while other sources like calcium lactate and calcium gluconate formed spherical shaped vaterite [47]. Among these, calcium chloride is found to be more stable source and is observed in most biocalcification processes [48]. The changes in polymorphs caused by various calcium sources are presented in Figure 2. Polymorph selection is reported to be affected by several parameters including, nature of bacteria, the growth medium, type of EPS produced by bacteria, etc. [49, 50, 13]. In all instances, the precipitation is closely associated with the bacterial cells, since cells act as the nucleation site for carbonate precipitation.

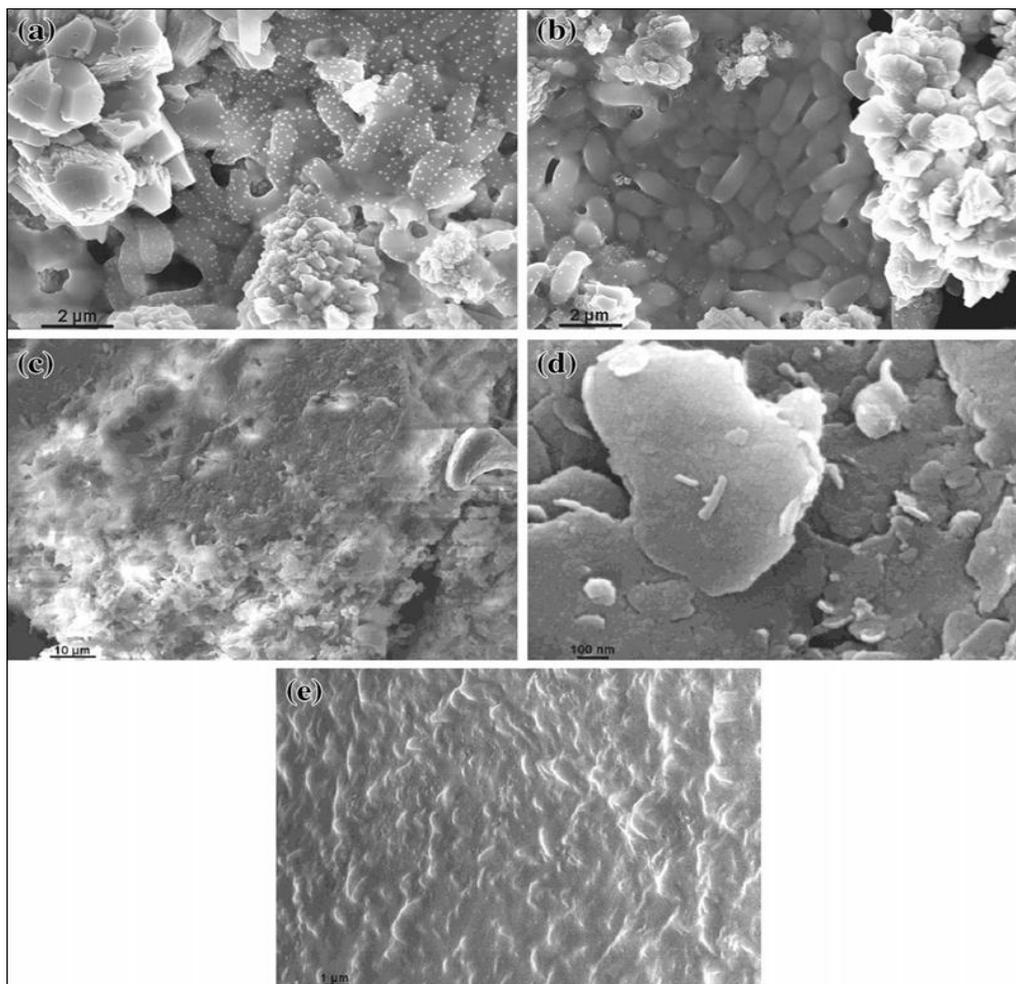


Figure 2: Scanning electron micrographs showing calcium carbonate crystals along with rod shaped *Bacillus sp. CR2* cells in media containing: a) calcium chloride, b) calcium nitrate, c) calcium acetate, d) calcium oxide and e) control. Image source: Achal and Pan (2014) [48].

BIOCALCIFICATION BY VARIOUS MICROBES FROM DIFFERENT SOURCES

Studies on exploring biocalcifying microbial strains have been on the ascending phase and reports from diverse geographic locations continue to surge. The amount of urease produced by the bacteria had a pivotal role to play in the carbonate precipitation process. The bacteria obtained from various sources have been screened for urease activity and their subsequent precipitation process. Zamarreno et al.[51] reported freshwater strains of *Pseudomonas* and *Acinetobacter* obtained from a calcified branch in a stream in Somerset, England. *Sporosarcina sp.*, *Bacillus sp.* and *Brevundimonas sp.* isolated from Beidaihe marine sediment in China were found to produce calcium carbonate crystals [52]. Sulfur oxidizing bacteria belonging to the phyla *Aquificae*, isolated from hot springs in Yunnan province, China were found to be associated with CaCO_3 precipitation [53]. Dhami et al. [50] reported a strain of *Bacillus megaterium*, isolated from

calcerous soil from Andhra Pradesh, India, which were able to produce significantly high amount of urease (Table 1) and calcite precipitates. Proportions of various metabolisms that lead to microbial carbonate precipitations are given in Figure 3 [54].

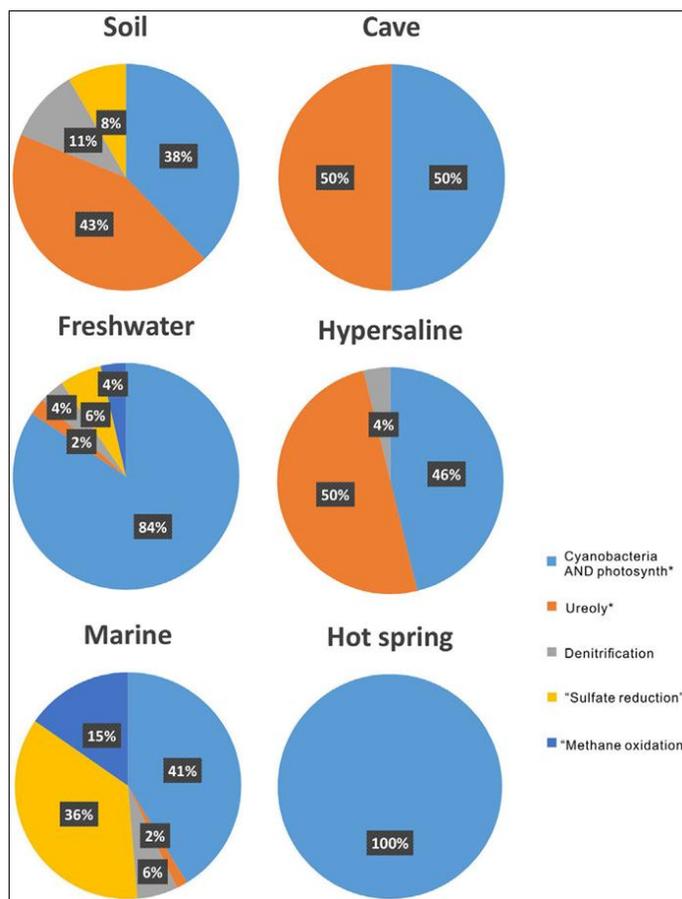


Figure 3: Graphs representing the various metabolisms associated with MICP process in various environments. Image source: Zhu and Dittrich (2016) [54].

Table 1: Urease activity of various bacteria isolated from different sources and the amount of CaCO₃ precipitated by them

Bacteria	Isolation source	Urease activity(U/ mL)	Amount of CaCO ₃ precipitate	Reference
<i>Bacillus sp.</i> CT2	Cement	575.87	NA	33
<i>Bacillus sp.</i> CT5	Cement	670.71	NA	33
<i>Sporosarcina pasteurii</i>	Mutant strain	550	NA	34
<i>Bacillus sp.</i> CR2	Mine tailing soil	432	2.32 mg/cell mass (mg)	48
<i>Bacillus megaterium</i> SS3	Calcareous soil	690	1.87 g/L	50
<i>B. thuringiensis</i>	Calcareous soil	620	1.67 g/L	50

*NA- Data not available

APPLICATIONS OF MICP

Owing to its eco-friendly attribute, MICP has been employed in diverse engineering and biotechnological applications. The cost associated with implementing MICP has proven to be cheaper than the existing technologies. The application of MICP in biocementation, heavy metal removal, radionucleotide removal, etc. have been widely studied and discussed. Over the recent years, in addition to these existing applications, MICP has gained importance in other domains as well (Figure 4).

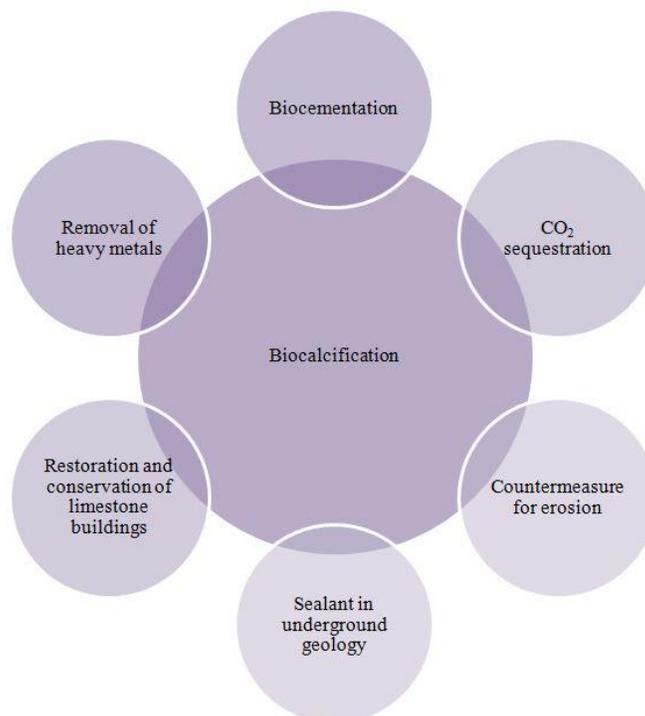


Figure 4: Application of MICP in various engineering realms.

Biocementation

Cement has become an indispensable part of any construction material and it acts as the binder of soil particles in concrete. Manufacturing of cement leads to CO₂ emission and it is reported that the cement producing industries contribute to 5-7% [55] of global CO₂ emissions which is roughly equated as 900kg of CO₂ per ton of cement produced [56]. As an effort to curb CO₂ emissions and the ever increase need to increase the strength of concretes, several attempts are made to replace cements with other materials. But the impact of the chemicals used thereby on the environment and the cost involved makes it necessary to look for other alternatives. Biogrouting methods are being carried out to evaluate their potential in improving the strength of concrete [57]. MICP has been applied to increase the concrete strength, reduce its water absorption capacity and repairing of cracks in concrete structures. Three bacterial isolates belonging to *Bacillus megaterium*, *Bacillus*

licheniformis and *Bacillus flexus* isolated from alkaline soils of a cement factory showed calcite precipitation and were effective in increasing the compressive strength of concrete after 28 days [58]. The bacterial strains were also found to be helpful in self healing of concrete cracks. Sarda et al. [59] reported that the concrete specimens cured in bacterial growth media (14%) had lesser rate of water absorption as compared to the cubes cured in water (25%). It is interpreted that the pores of the specimens are blocked by the carbonate precipitates which does not allow water to penetrate. Achal et al. (2013) [60] reported the effect of *Bacillus sp.* in increasing the durability and self healing efficiency of cementitious materials. It was observed that the rate of chloride permeability was altered from moderate to very low and the compressive strength was increased by 40%. Cracks upto 27.2mm were found to be healed by microbial precipitation process. In terms of cost comparison, the report by Ivanov and Chu [61], biogrouting costs around 0.5-9 USD/m³ of soil whereas chemical grouting costs 2–72 USD/m³ of soil. Hence, biocementation has proven to be a viable alternative to the existing technologies.

Removal of heavy metals (Bioremediation of underground water)

Heavy metal contamination of ground water as a result of rapid industrialization is on an alarming rise. The heavy metal contaminants from the solid wastes discharged by factories percolate into the underground aquifers, thereby affecting the water column [62]. Metals such as copper, lead, cadmium, chromium, zinc, mercury, arsenic, nickel have been identified as the dominant contaminants [63, 64]. These heavy metal contaminations cause serious health hazard to the living organisms, since they directly affect the food chain [65]. Various conventional techniques such as electrochemical treatment, filtration, ion exchange, etc. are not successful in removing the metals completely [66 – 68]. In addition, the aforementioned methods are expensive and they consume high amount of energy for their functioning [69]. Low cost biological alternative for removal of heavy metals were identified in the form of biosorbents, wherein microbial biomass were used to scavenge the heavy metals [70]. Various microbes including *Bacillus sphaericus* (Bacteria) [71], *Tetraselmis suecica* (alga) [72], *Mucor rouxii* (fungus) [73] and various mixed cultures of bacteria were used for bioremediation purposes [74]. However, these biosorbents take a longer time for the remediation process and the heavy metals get back to the environment after some point of time. In the recent years, MICP has been a viable alternative strategy for heavy metal removal, since it is cost effective and eco-friendly. The carbonate precipitating microbes converts the heavy metals into their carbonate form and causes it to precipitate. Achal et al. (2011) [75] reported that the strain *Kocuria flava* CR1 was able to remove 97% of copper from the environment. A strain of *Terrabacter tumescens* was found to remediate 99% of cadmium from soil wastewater. A near

100% removal of lead by *Sporosarcina koreensis* UR47 was reported, wherein lead is immobilized by the calcite produced and hence prevents it from releasing back to the environment [76]. Similarly, strains of *Sporosarcina ginsengisoli* CR5 was able to remove 96.3% of arsenic after 7 days of growth [77]. Hammes et al. [78] investigated the use of MICP for removing calcium ions from industrial wastewater and it was found that excess of 90% calcium removal was achieved in the process.

CO₂ sequestration

Global warming is on an alarming rise over the last decade, causing serious threats to mankind. A well known predominant causative factor for global warming is increase in level of the greenhouse gases, particularly CO₂ [79]. Automobile and industrial emissions, cement production, deforestation, etc have been the vital cause for the increase in atmospheric CO₂ [80]. There is an average increase of 3.8ppm over the last three years [81]. The primary aim of the climate change mitigation process is to reduce the anthropogenic emissions of CO₂. Carbon sequestration is a part of the mitigation process which involves capturing and long term storage of atmospheric CO₂. Chemical conversion of CO₂ is the conventional method that is being used for years, in which CO₂ is converted to carbonates forms such as calcite, vaterite, etc. [82, 27]. But, the process is rather slow. As an alternative, researchers have suggested the use of MICP to sequester atmospheric CO₂. Okyay and Rodrigues [83] studied the role of biotic and abiotic factors that influence carbonate precipitation and CO₂ sequestration. It was found that the pH, growth medium and urea concentration were the defining factors. An optimized medium considering these parameters yielded an increase upto 148.9% of uptake through calcification. Role of carbonic anhydrase in sequestering CO₂ were studied by Ramanan et al. [84], wherein crude and purified enzymes were evaluated for the ability to sequester CO₂ and precipitate carbonates. It was reported that the purified enzyme deposited 15 times more carbonate than the crude enzyme. Cho et al. [85] studied the effects of surfactants like sodium dodecyl sulfate (SDS), Triton X-100, and cetyltrimethylammonium chloride (CTAC) on the CO₂ biomineralization by *Sporosarcina pasteurii* and *Bacillus megaterium*. The study suggested that each of the surfactants had a different impact on biomineralization. In *S. Pasteurii*, there was a decrease in headspace CO₂ content, while in *B. megaterium* CO₂ removal increased by 8% and 16% upon using CTAC and triton X-100 respectively. This also suggested the species specific response to the surfactants and the corresponding biomineralization process.

Sealant in underground geology

In recent years, MICP has found its application in underground geology wherein it is used as sealants in wellbores. These underground structures are predominantly used for carbon sequestration, storage of nuclear wastes, etc. Hence, any leakage in these structures could be hazardous [86, 87]. Under controlled conditions, it is learnt at the field level that MICP can be used to seal fractures which could potentially cause subsurface leakages. The advantage of using MICP is its low viscosity on account of its aqueous nature. Field level studies have been carried out to study the fracture sealing potential through MICP in a 24.4 cm (9.625 inch) diameter well located on the Gorgas Steam Generation facility near Jasper, Alabama, USA, which was a part of carbon storage project [88]. The field test was successful as complete biomineralization was observed at 340.7 m below the ground level. Around 6 bacterial inoculations (*Sporosarcina pasteurii*, ATCC 11859) and 24 calcium injections were given during the sealing process. Complete biomineralization was inferred by the rejection of any further fluid injections. The efficiency of MICP in sealing fine apertures in sealing fine aperture fractures in crystalline rocks which act as geological containers for storing nuclear wastes were studied [89]. *Sporosarcina pasteurii* was used as the source of MICP and a combination of MICP and colloidal silica (filler) was also tried out in order to enhance the availability for nucleation sites for biomineralization to occur. The flow time of bacteria was increased in order to attain a better distribution in the fracture. Use of MICP alone showed significant results showing precipitates across the fracture with thicknesses ranging from 0.52mm to 0.18mm. This study reemphasized the role of MICP in subsurface engineering applications.

Restoration and conservation of limestone buildings

Limestone monuments are omnipresent, right from the Milan cathedral in Italy to the Taj Mahal in India. Limestone, composed of calcite and small portions of aragonite has been predominantly used for construction of such huge structures primarily due to its durability which can help them exist for ages [90]. However, the presence of pores in limestone surface makes them susceptible to environmental pollutants and harsh weathering conditions [91]. Various physical, chemical and biological factors contribute to the progressive deterioration of limestone monuments [92, 93]. Several attempts are being made to restore and conserve the monuments which carry historical significance. Inorganic and organic methods have been tried as a part of the remediation process, but they have not been completely satisfactory since they involve utilisation of huge amounts of solvents and in certain cases, the color and texture of the structure are lost [94]. In addition, chemical based restorations involving resins proved to be expensive. These limitations have made the researchers to turn towards bio-based remediation of limestone buildings. MICP is currently

used across the globe as an efficient alternative for restoring ancient limestone monuments. Le-metayar et al. [95] studied the ability of calcite producing bacteria for providing surface protecting coating on limestone monuments. Biocalcifying bacterial strains were applied on the tower of Saint Médard church in France. Periodical monitoring indicated the formation of calcite layers on the surface of the tower and it was sustained for a significant amount of time, thereby indicating that MICP is a viable choice for limestone restoration. Bacterial cell wall fraction of *Bacillus subtilis* was tested for its remediation property against limestone structures at S. Maria of Angera's Cathedral, Italy [96]. Calcite precipitation was observed on the walls of the Cathedral and the water absorption was found to be reduced by 16.7%, signifying the application of biomineralization based remediation.

RECENT DEVELOPMENTS AND PROCESS IMPROVEMENTS

MICP has been gaining global attention and its application in a wide range of civil, environmental and geotechnical related processes are ever increasing. Microbes capable of precipitating carbonates under controlled conditions are being continuously explored and isolated. Several factors influence the microbes' ability to form carbonate crystals including temperature, cell concentration, urea and calcium concentration, pH, etc. [97-100] and the changes in these parameters have a significant impact on the precipitation process. Studies are being conducted to improve the quantity and accelerate the precipitation process. This section describes some of the recent developments and optimization processes that have been made in improving MICP process and addressing the issues surrounding it.

Alternative bacterial growth medium

The usage of expensive commercial grade laboratory nutrient medium for field application has posed a major challenge in commercialization of MICP. As a measure to address this issue, the prospects of using cheaper alternative sources of growth medium are examined. Yoosathaporn et al. [101] investigated the use of an agriculture waste in the form of effluent from chicken manure bio-gas plant effluent from chicken manure (CME) bio-gas plant as growth medium for carbonate precipitation by *Bacillus pasteurii*. It was observed that the specific urease activity increased upon using CME- urea medium and the cost per litre was found out to be 0.71 USD which is 88.6% lesser than commercially available medium. The compressive strength of cement cubes prepared with bacterial cells grown in CME-urea medium had increase by 30.27% when compared to control. Usage of corn steep liquor (CSL), an industrial by-product rich in proteins has been tried for cultivating biocalcifying strains of *Sporosarcina pasteurii* [102]. While the growth and urease activity of *S. Pasteurii* were comparable with other growth mediums like nutrient broth and yeast

extract, the amount of calcite precipitated in CSL medium was significantly higher. The compressive strength of cement mortar cubes prepared with CSL medium showed an increase upto 30%. The usage of another industrial by- product from dairy industry, lactose mother liquor (LML), was explored for the suitability as an alternative growth medium [103]. Though no significant increase was observed in terms of bacterial growth, urease activity and compressive strength, the results were comparable with other commercial growth mediums. It suggests that LML can be used as a potential low cost alternative for culturing biocalcifying strains.

Optimization of bacterial growth parameters

Nature of bacteria and the cell concentration impacts urease production and the subsequent carbonate precipitation. Though species specific, the bacterial growth medium has a profound influence on MICP. Raut *et al.* [104] developed an optimized medium (OptU) for enhanced urease production. OptU media resulted in significant increase of urease activity in *Bacillus pasteurii* and the compressive strength of bricks increased by 83.9% when compared to results from nutrient broth medium. The water absorption also reduced by 48.9% upon using optU medium. Kakelar *et al.* [105] employed central composite design based on response surface methodology to optimize three vital parameters for increased carbonate precipitation in *Sporosarcina pasteurii* grown at different concentrations of yeast extract/ sodium acetate. The statistically predicted values significantly correlated to the experimental values with a correlation coefficient of 0.973. The study revealed that yeast concentration (9.69 g/L), NH₄ concentration (9.69 g/L) and incubation time (60h) were the defining parameters. A strength of 795 kPa was obtained on cemented column post optimization of the growth parameters even with usage of poorly graded soil. Optimization of various parameters including incubation period, temperature, pH and urea concentration for biocalcifying *Sporosarcina pasteurii* has been reported [106]. Urease activity of the bacterial strain under temperatures ranging between 20–45 °C, pH between 6.0–8.5, incubation period upto 96h and urea concentration between 2–10% were examined. Optimal conditions for high urease activity were temperature 25–30 °C, pH 6.5–8.0, 24 h incubation and 6–8% (w/v) urea concentration. Role of pH and aeration in carbonate precipitation by the strains of *Bacillus licheniformis* and *Bacillus sphaericus* has been reported [107]. A pH of 12 resulted in increasing the precipitation by 6.3 folds, while increasing the aeration in the bioreactor from 0.5 to 4.5 standard liter per minute (SLPM) resulted in 4.2 times higher carbonates. It was also observed that the pH played a pivotal role in determining the polymorphs. Calcite was the predominant polymorph at pH 12 while vaterite was abundantly found at pH less than 10, but were transformed to calcite upon increasing the pH.

LIMITATIONS AND FUTURE PERSPECTIVES

Given its broad range of applications in remediation of environment, civil, geotechnology, etc., MICP has its own limitations and shortfalls. MICP is a time consuming process as well, since it is governed by microbial activities which are highly susceptible to environmental changes. It is observed that ammonium ions that are released during ureolysis may form nitrates and react with calcite, resulting in the formation of calcium nitrate. Calcium nitrates can cause deterioration of civil structures over a period of time. The release ammonia and nitrate are as such hazardous to human health. One of the major obstacles in implementing MICP at the commercial level is the high cost associated with it due to the usage of commercial bacterial growth mediums. However, as mentioned above several groups of researchers are working on utilizing industrial by-products as media supplements to address this issue. Uncontrolled bacterial growth over a period of time is a vital shortfall as well. Scaling up of bacterial culture from the field to the industrial level is a great challenge considering the construction and maintenance of huge bioreactors.

MICP has evolved as a highly potential, solvent, chemicals- free and in-situ remediation process. Its applications are on the rise and diligent efforts are being taken to overcome its shortfalls. Further exploration of various bacterial strains from different geographic locations for their biocalcifying potential and the mechanisms involved shall improve our acumen in this research avenue. A coordinated effort to optimize the various factors influencing MICP from researchers of various disciplines including microbiology, biotechnology, geology, chemistry, etc. shall ensure that commercialization of MICP sees its daylight in the near future.

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