

An Overview of Factors Influencing Microbially Induced Carbonate Precipitation for Its Field Implementation



Surabhi Jain

Abstract Naturally prevailing biological processes viz. biomineralization, biofilm formation, bioaccumulation, production of extracellular polymeric substance (EPS), biodegradation, biotransformation, biosorption, biogas generation, in the surface and subsurface environments can be adapted for altering the nature of geomaterials. Among them, biomineralization is gaining huge attention due to its widespread application. This chapter reviews the role of bacteria and different metabolic pathways involved in the carbonate biomineral precipitation along with its feasibility to implement in the field. Field-scale implementation by augmentation or stimulation and challenges faced during the MICP application process has been reviewed. The different abiotic and biotic factors affecting the mineralization process are critically discussed with the requirement of future research. The chapter further sheds light on the perspective of MICP and its successful implementation in large commercial scale.

Keywords Bacteria · Biomineralization · Building material · Microbial calcite · Urease

1 Introduction to Biomineralization

Biomineralization is a widespread phenomenon of forming minerals by different phyla of the organism and its metabolic activity. The existence of biominerals is all over in the environment, spanning across the involvement of the entire six taxonomic of biological kingdoms. The properties of mineral such as morphology, polymorph, compositions and crystallinity controlled by organisms separate this phenomenon from the abiotic mineral formation. Among different biochemical process, biomineralization process has gained significant popularity in the fields of biotechnology, earth science, environmental, chemical and geotechnical engineering owing to its

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widespread application (Achal et al. 2015; Anbu et al. 2016; Dejong et al. 2013; Dhimi et al. 2013). The major three mechanisms involved in the biomineralization process are biologically controlled, biologically influenced and biologically induced mineralization.

In biologically controlled mineralization, microbial activity control over the nucleation, growth, location of deposition of mineral, mineral's nature such as size, shape, etc. However, the degree of control is pertaining to the microbial species and its biological functions. The biologically controlled mineralization mechanism is divided into three parts i.e., biologically controlled extra, inter and intracellularly mineralization process pertaining to the location of mineralization site (Weiner and Dove 2003). In some cases, the biomineral formation starts inside the cell and further pushed the end product outside of the microbial cell. In this paragraph, an overview of the mentioned three processes involved in biologically controlled mineralization has been presented. In biologically controlled extracellular mineralization, the location of mineralization is a macromolecular matrix, outside the organic cell. The macromolecular matrix is the composition of different metabolite products such as proteins, polysaccharides, etc. Organisms transfer the cations and constituents through the membrane and into the surrounding region by ion diffusion or the cation-loaded vesicles export and break down by precursor compounds at the organic matrix. The biologically controlled inter-cellular mineralization is not much popular and mostly occur in a community of single cellular organism. The epidermis, outer layer of cell act as isolating the location of mineralization. Literature has shown that the epidermis of the individual organic cell controls the nucleation and nature of biomineral. But, in biologically controlled intra-cellular mineralization, a particular space inside the cell is closed from the external environment for mineralization site. Once space and vesicles are formed inside the cell, the ions will transport to the site and a stage of supersaturation is reached. In this mechanism, the organism has a high control on the composition of biominerals and the ligands also act as the nucleation of biomineral. The structure and polymorphism of minerals are highly controlled by the specific species involved (Konhauser 2007). This process is not sensitive to the outside environment because the cell wall acts as a barrier to diffuse any external ions or chemicals to the mineralization site. Also, the precipitated minerals are thermodynamically unstable and unfavourable due to the isolation of biominerals from the external environment.

In biologically influenced mineralization, passive mineral precipitation occurs by the interaction of organic matter present in microbial cell surface and the geochemical environment (Phillips et al. 2013). The organic matter is the extracellular polymeric substances (EPS) associated with microbial biofilms which are commonly present in many natural and engineered surfaces. Decho (2010) reviewed how different properties of EPS inhibit, accelerate, modify the calcium carbonate precipitation in the different geochemical environment. The evidence shows that the EPS serve as initial nucleation sites for biomineralization process.

In biologically induced mineralization (BIM), microorganisms amend geochemical reactions in the nearby environment by their metabolic activities such as discharge of metabolic wastes (OH^- , HCO_3^- , Fe^{2+}) and/or altering the redox state

(oxidation of Fe^{2+} or Mn^{2+}) which in turn resulting in extracellular biomineral growth (Lowenstam and Weiner 1989; Stocks-Fischer et al. 1999). In contrast to BCM, the BIM process is uncontrolled by the microbial cell over the type and nature of mineral precipitated (Fortin et al. 1997; Weiner and Dove 2003). Hence, the heterogeneity of BIM mostly depends on the external environmental condition, in which the minerals are formed. The precipitated minerals via the BIM process has identical crystallochemical features of abiotic precipitation, as governed by the similar geochemical reactions (Fortin et al. 1997). The biologically induced mineralization can be mimetic and assist to overcome various bioengineering applications.

2 Introduction to Microbially Induced Calcium Carbonate Precipitation

In nature, nearly sixty-four different minerals such as phosphorites, carbonates, silicates, iron and manganese oxides, sulfide minerals, and amorphous silica have been produced using variety of metabolic pathways of microorganisms (Knoll 2003). Numerous researchers are currently investigating the precipitation mechanisms of minerals other than the existing sixty-four varieties of biomineral, as enlisted via the biologically induced or controlled or influenced process. Until early 1980, the field of biomineralization was known as calcification due to the abundant generation of calcium-containing biominerals. In nature, 50% of known minerals are calcium-bearing because the massive presence of calcium ion in most of the soil or aquatic system and the divalent calcium ion perform huge functions in cellular metabolism process (Lowenstam and Weiner 1989; Simkiss and Wilbur 1989; Berridge et al. 1998; Dhami et al. 2013). The calcium bearing minerals can be calcium- phosphate, carbonate, oxalate and etc. Among all calcium bearing minerals, microbially induced calcium carbonate precipitation (MICP) process have gained special attention due to its effectiveness in precipitating the huge deposits in various harsh or extreme aquatic and soil conditions with a wide variety of microorganisms involved (Ehrlich 1998; Castanier et al. 1999; Dhami et al. 2013). Figure 1 depicts four different images of the biomineralization of calcium carbonate in natural habitat.

3 Pathways Involved in Microbially Induced Carbonate Precipitation

A wide variety of prokaryotic and eukaryotic microorganisms facilitate the synthesizing process of calcium carbonate-based biominerals by either autotrophic or heterotrophic pathways. A flowchart of different pathways involved in MICP is presented in Fig. 2.

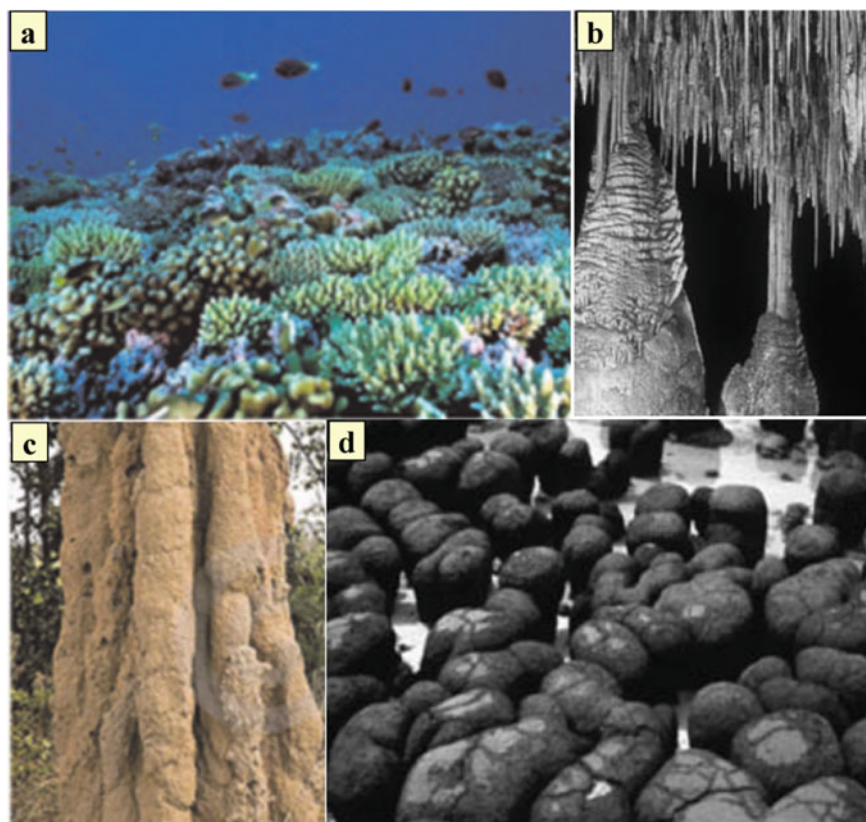


Fig. 1 Biominalization of calcium carbonate in nature **a** Corals **b** Speleothems in the Carlsbad Caverns, New Mexico **c** Anthills **d** Stromatolites exposed at low tide, Hamelin Pool, Western Australia (Konhauser 2007; Dhami et al. 2013)

3.1 Autotrophic Pathways

Photosynthesis and methane oxidation are the two metabolic process involved in the autotrophic pathways, as discussed in this section. In the photosynthetic process, the microorganisms use gaseous or dissolved CO_2 as a carbon source, exchange the bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions and produce carbonate minerals. The carbonic anhydrase in the cell–matrix catalyzes the dissociation of HCO_3^- into CO_2 and OH^- , which lead to an increase of pH in the system. High alkaline environment and presence of free divalent cations such as calcium assist to form bicarbonate mineral in the surrounding microenvironment. The process of photosynthetic are also described in the equation form in Eqs. 1 and 2 (Castanier et al. 1999; Dhami et al. 2014; Achal et al. 2015). Cyanobacteria, Purple photosynthetic bacteria and microalgae are the main photosynthetic microorganisms responsible for carbonate precipitation.

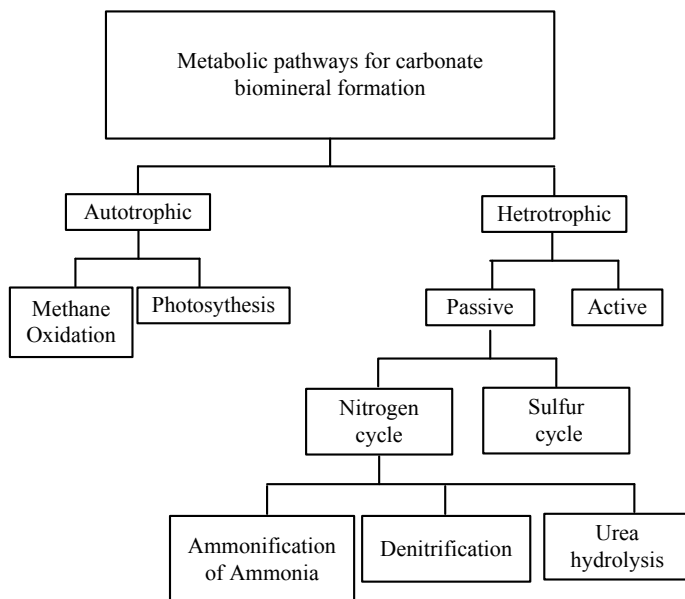
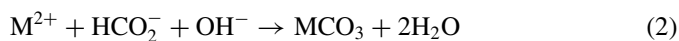
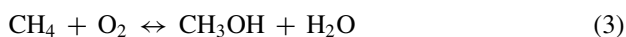
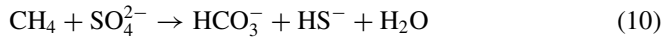
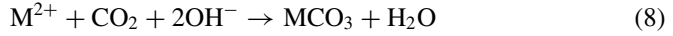
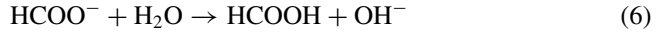


Fig. 2 Flowchart for Metabolic pathways involved in microbially induced carbonate precipitation



The Methane oxidation process which is involved in carbonate mineral formation is largely driven by the methane-oxidizing bacteria in all aerobic, anoxic and anaerobic conditions. In aerobic or anoxic conditions, methane is converted to methanol by methane mono-oxygenase activity and further forms formate by another cell enzymatic activity, as shown in Eqs. 3 and 4. Subsequently, formate is in equilibrium with formic acid and oxidizes to CO_2 , as shown in Eqs. 5–7. Further, carbonate is generated from CO_2 and carbonate mineral forms around the microbial cell in the alkaline environment, as in Eq. 8 (Castro-Alonso et al. 2019; Ersan 2019). Similarly, in the anaerobic condition, bicarbonate ions and carbonate mineral forms by the anaerobic methane oxidation process. But in this, sulphate act as an electron acceptor not the oxygen, as shown in Eqs. 9 and 10.





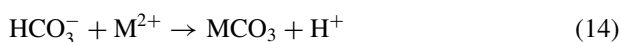
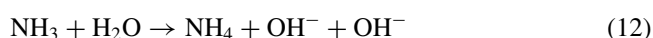
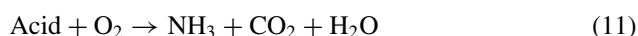
In the photosynthesis process, continuous exposure of sunlight and inorganic carbon such as CO_2 is required for carbonate precipitation. Hence, it is not a feasible pathway to apply in the field of construction engineering or building materials such as bioconcrete, biobrick, etc. (Seifan et al. 2016). In autotrophic pathways, a continuous supply of inorganic carbon and calcium ion is required to precipitate a significant amount, i.e., nearly $60 \text{ kg}\cdot\text{m}^{-3}$ of biomineral for efficient soil reinforcement (Whiffin et al. 2007; van Paassen et al. 2010a). However, by supplying the enormous amount of carbon and cementation solution cause a higher rate of precipitation and hinder the flow of cementation liquid to a great distance. Hence, the autotrophic pathways are not suitable for subsurface soil modification. Very few studies have shown the potential of methane oxidation process for bicarbonate precipitation (Stadnitskaia et al. 2008; Ganendra et al. 2014; Ganendra 2015; Meister et al. 2018; Caesar et al. 2019) and the results of Ganendra (2015) concluded that methane oxidation is a more environmentally friendly approach in terms of bioconcrete application. However, there are no real field studies to utilize the mechanism which is a challenge for future researchers and industrialist.

3.2 Heterotrophic Pathways

Bio-carbonate precipitation takes place by active or passive ways in heterotrophic metabolic pathways. In the active precipitation, during the activation of cell ionic pumps, the carbonate ions are formed by the exchange of ionic species through the cell membrane, and subsequent precipitation of corresponding carbonate minerals take place around the cell surface (Castanier et al. 1999; Konhauser 2007). Though active precipitation occurs often in nature, implementing it for engineering application is impracticable. The passive carbonate biomineral precipitation occurs due to environmental, and chemical modification by the metabolic activity of microorganisms. In many conditions, the active precipitation is followed by a passive one, and the nature, size, and growth of biominerals alter simultaneously (Castanier et al. 1999). The two heterotrophic pathways often involved during passive carbonate biomineral precipitation are sulphur reduction and nitrogen cycle.

3.2.1 Nitrogen Cycle

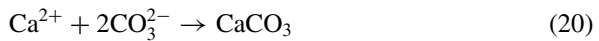
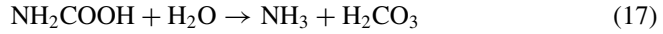
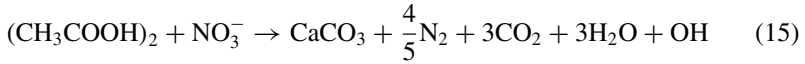
In the nitrogen cycle, three different reactions; amino acids ammonification, nitrate reduction, and urea or uric acid degradation lead to mineralize carbonate-based biominerals. A wide variety of aerobiosis metabolize amino acid, produce CO_2 and ammonia which further get hydrolyzed to ammonium and hydroxide ions, as in Eqs. 11 and 12. The products OH^- and CO_2 forms bicarbonate ions in the alkaline environment around the microbial cell. Further, the supersaturation of bicarbonate ions and free divalent cations favour the precipitation of carbonate mineral as shown in the Eqs. 13 and 14 (Castanier et al. 1999; Zhu and Dittrich 2016).



Myxococcus xanthus, *Alcanivorax borkumensis* are the aerobiosis reported to survive in liquid and solid matrix, utilize the ammonification process and lead to form different polyforms of carbonate mineral (Rodriguez-Navarro et al. 2003; Chekroun et al. 2004; Jimenez-Lopez et al. 2007; Krause et al. 2018). Interestingly, it also precipitates a phosphate mineral of uranium i.e., meta-autunite in the presence of uranium and immobilizes the radioactive waste uranium in a system (Turick and Berry 2016).

In the denitrification process, the anaerobiosis or microaerophily form carbonate biomineral in the presence of organic matter, nitrate and divalent cations. The microbes oxidize the organic matter by using NO_3^- as an electron acceptor and generate NO_2 , CO_2 , and OH^- . The generation of hydroxyl ions creates an alkaline environment and in the presence of soluble calcium ions, calcium carbonate biominerals form. The denitrification process is also explained in Eq. 15.

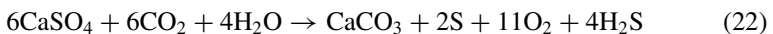
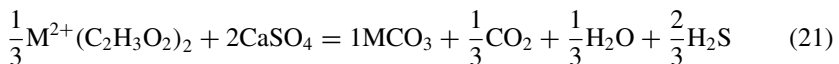
In the urea hydrolysis process, one mole of urea is hydrolyzed to generate one mole of ammonia and carbamic acid due to the microbial urease enzymatic reactions (Eq. 16). The one mole of carbamic acid further hydrolyzes and produces an additional one mole of ammonia and carbonic acid, Eq. 17 (Hammes et al. 2003). These products equilibrate in water and form bicarbonate ion, as mentioned in Eq. 18. Also, the interaction of ammonia and water results in the formation of ammonium ion and hydroxyl ion, which induces a favourable alkaline environment for the formation of carbonate with $\Delta G = -27 \text{ kJ}\cdot\text{mole}^{-1}$, Eq. 19 (Achal and Pan 2011). Subsequently, the over-saturation of calcium ions in the solution produces calcium carbonate biomineral with a solubility product of 3.8×10^{-9} , Eq. 20 (Burbank et al. 2012).



Microbes not only help to modify the environment by physiological activities but also play an important role in the mineral nucleation and growth. Divalent cations and negatively charged cell wall interaction may change the overall ionic charge of the cell wall (Stocks-Fischer et al. 1999; Dhami et al. 2013). As a consequence, bacteria act as a nucleus, reduce the energy barrier, and increase biomineral crystal size (Chahal et al. 2011). Sometimes, the EPS and its functional group also modify the ionic charge of the substance, acts as a nucleation site, and influence the biomineralization process (Konhauser 2007; Wu and Zeng 2017).

3.2.2 Sulphur Cycle

In the anoxic or anaerobic sulphur cycle, sulfate-reducing bacteria undergoes the process of sulphate reduction and generates bicarbonate ions and hydrogen sulphide. The further precipitation of carbonate mineral depends on the consumption of hydrogen sulphide. The discharge of hydrogen sulphide and/or oxidation of hydrogen sulphide to sulphur by phototrophic anaerobic sulphide bacteria increase the pH, which in turn assists carbonate mineral precipitation (Eq. 15). However, in contrast, the autotrophic aerobic bacteria can oxidize the hydrogen sulphide to sulphate ions, eventually producing sulphuric acid. The production of acid decreases the pH of the system, inhibiting any further mineral formation (Castanier et al. 1999). Some of the sulfate-reducing bacteria such as *Desulfovibrio* sp. remove the sulfates from gypsum ($\text{CaSO}_4 \cdot \text{H}_2\text{O}$) by a dissolution, diffusion and calcium carbonate precipitation, as shown in Eq. 16 (Perito and Mastromei 2011).



Even though different heterotrophic biogeochemical pathways are involved in the formation of calcium carbonate biominerals, all are not equally effective for soil modification, bioconcrete, bio-brick formation and other engineering application. The basic four criteria to evaluate the suitability of the above-mentioned pathways for an effective soil modification are (1) solubility, (2) requirement of cementation reagents, (3) rate of carbonate biomineral formation and, (4) generation of secondary products (van Paassen et al. 2010a; Montoya 2012).

The poor solubility of sulphate with calcium during sulphate reduction and oxygen in the water during ammonification makes this process infeasible for ground modification (van Paassen et al. 2010a). Though during denitrification, a substrate such as acetate, citrate, and nitrate are reasonably soluble with calcium, cementation substrates used for the urea hydrolysis process are highly soluble compare to the substrates used for all other metabolic cycles (Zabozlaev et al. 2007). In terms of the requirement of cementation reagents to produce per gram of calcium carbonate biomineral, all the biochemical processes need nearly equal amount of chemical substrates. Concerning the rate of biomineral precipitation, the denitrification and urea hydrolysis processes have nearly similar rate of precipitation for equal amount of biomass concentration (van Paassen et al. 2010a). However, the rate of precipitation can be manipulated by altering the biochemical condition according to the engineers need (Montoya 2012). In addition to this, invariably, in all these processes, secondary by-products produced by the biogeochemical reactions can hinder the reaction rate and cementation properties. In denitrification and sulphate reduction processes, the toxic by-products such as N_2 , CO_2 , H_2S lower the permeability locally, obstructing the flow of cementation reagents and, in turn, inhibiting the biomass growth. Some intermediate harmful compounds like nitrite or nitrous oxide can also be generated in the denitrification process (van Paassen et al. 2010b). The urea hydrolysis process generates ammonium chloride but it can be removed from the site or can be utilized as a fertilizer in-situ (Mujah et al. 2017).

The MICP process has gained significant popularity in the fields of biotechnology, earth science, environmental, chemical and geotechnical engineering owing to its widespread application (Achal et al. 2015; Anbu et al. 2016; Dejong et al. 2013; Dhami et al. 2013). The application includes solid-phase capture of contaminants such as radionuclide and heavy metals (Warren et al. 2001; Achal et al. 2013; Jain et al. 2019b; Kumari et al. 2014; Jiang et al. 2019), wastewater treatment (Hammes et al. 2003), carbon sequestration (Mitchell et al. 2010; Okyay and Rodrigues 2015), etc. Besides this, it has a huge potential in the field of construction materials such as enhancing the geotechnical properties such as strength, permeability, liquefaction potential of geomaterials, crack repair of the monument, improving the strength and durability of cement and brick (Sarda et al. 2009; Achal et al. 2011; Bernardi et al. 2014; Manzur et al. 2017).

4 In Situ Implementation of the MICP Process

Several studies have been carried out to modify the geomaterials via the biocementation process in the laboratory. However, up-scaling it for in-situ implementation is still in progress. At first, in 2004, a field study was performed to modify the Rotter dam port area in the Netherlands, which shows high-quality performance i.e., long term reduction in permeability (Hongzhi 2007; Mujah et al. 2017). In 2010, van Paassen (2010b) experimented to improve the strength of 1–100 m³ of sand via the biocementation process in the laboratory. Though there was a drastic improvement in the strength of geomaterial after biocementation, a large degree of variation was recorded in the strength enhancement at different locations of the sand column. The method of injection of microbes and cementation reagents, obstruction of the flow of microbes into the higher depths due to the biomineral precipitation in the soil surface zone and, the non-homogeneity of CaCO₃ distribution in the intended zone were the major reasons for the strength variation in the sand tank. A similar observation was reported while conducting a biocementation experiment using 0.5 × 0.5 × 0.15 m of soil zone to simulate the real field conditions by Martinez et al. (2013). Further, for the first time in 2015, Gomez et al. (2015) conducted a field-scale MICP experiment to control the erosion of loose sand and stabilize the surface soil for vegetation.

Biocalcis is an MICP implementation method and validated by doing different laboratory and pilot studies (Filet et al. 2012). Later, this method has successfully used in the south of France to treat sand-silt geomaterial on a large scale (Esnault-Filet et al. 2016). In recent time, researchers are attempting to stabilize the surface and subsurface geomaterial by stimulating the native ureolytic microorganisms via MICP process (Gomez et al. 2017; Wang et al. 2019). Ghasemi and Montoya (2020) have applied *S. pasteurii* and MICP on a coastal sandy slope using surface spraying method to stabilize the soil in sunny and rainy days and concluded its feasibility. Similarly, Hodges and Lingwall (2020) proposed a short term erosion control method in South Dakota by doing seeded MICP method, which is beneficial for vegetation.

However, in the case of field implementation, the technique demands a fundamental understanding of the associated biogeochemical process under prevailing subsoil environmental conditions (Mortensen et al. 2011; Ng et al. 2012; Cheng et al. 2017). Furthermore, many biotic and abiotic factors such as pH, temperature, type of microorganism, bacterial concentration, the concentration of cementation reagents, salt concentration, etc. alter the nature of precipitated biominerals and hence determine the efficacy of MICP (De Muyncka et al. 2010; Okwadha and Li 2010; Mortensen et al. 2011; Ng et al. 2012; Mitchell et al. 2018; Mujah et al. 2019). Given this, the following section presents the factors which affect the efficacy of the biomineralization and biocementation process in detail.

5 Factors Influencing MICP

5.1 Influence of Microbial Factor

5.1.1 Bacteria Type

A broad range of ureolytic microbes have been isolated from aquatic, sediment surface and subsurface regions where the environmental conditions varied from mild to highly adverse such as high salinity, extreme temperatures, etc. (Rivadeneira et al. 1998, 2004; Fujita et al. 2000; Chahalet et al. 2011). Different types of bacterial strain such as *B. megaterium*, *B. subtilis*, *P. Vulgaris*, *B. sphaericus*, *B. thuringiensis*, *S. pasteurii*, *S. ginsengisoli*, *Kocuria flava*, and species of *Spolactobacillus* have been utilized for modifying the engineering properties of geomaterial via biocementation and in brick or concrete and monument crack repair (Nemati et al. 2005; Baskar et al. 2006; Lian et al. 2006; Kucharski et al. 2008; Chen et al. 2009; Achal et al. 2011; Anbu et al. 2016; Mitchell et al. 2018). Among all the species, alkaliphilic *S. pasteurii* is found to have maximum urease activity and rate of biomineral precipitation, hence mostly preferred for enhancing the geotechnical properties of geomaterials (Dejong et al. 2006; Sarda et al. 2009; Whiffin et al. 2007; Richardson et al. 2014; Phillips et al. 2016). The study of Stocks-Fischer et al. (1999) demonstrated that at an alkaline environment with a pH value of 9, nearly 98% of precipitation occurs microbially, followed by 54% of precipitation when treated by the chemical process, under similar environmental conditions. For enhancing the ability of enzymatic activity and biomineral precipitation rate, a mutant strain of *S. pasteurii* was also developed by Achal et al. (2009). The urease enzyme kinetics, rate of biomineral precipitation, polymorphic and morphological nature of calcium carbonate biomineral varies with the bacterial strains used (Dick et al. 2006; Ng et al. 2012), which in turn may have a considerable impact on the application process. In denitrification, different isolated and exsitu microbial cultures were used for soil reinforcement via MICP process (Ersan et al. 2015; Hamdan et al. 2017; van Paassen et al. 2010b; Pham et al. 2016). However, to date, none of the studies emphasized the effect of the type of microbes (native and/or ex-situ bacteria) for implementing the MICP process in real conditions. It is essential to make a few standard protocols to understand the efficacy of microorganisms for MICP process.

5.1.2 Biomass Concentration

The MICP study also revealed that in the biomineralization process, microbial cell serves as a nucleation site and reduce the energy barrier for biomineral formation (Stocks-Fischer et al. 1999; Anbu et al. 2016). Also, their concentration has a substantial influence on the rate of ureolysis or denitrification and biomineralization (Fujita et al. 2010). Researchers have assessed the biomass concentration by colony forming units (cfu) and demonstrated the modification in soil strengthening, surface treatment

of concrete, mortar (Stocks-Fischer et al. 1999; Achal et al. 2014; Bang et al. 2001; De Muynck et al. 2008a). Many reports have presented the microbial concentration in terms of optical density (OD) at 600 nm wavelength and conducted calcification study on soil consolidation and surface remediation of brick, concrete (De Muynck et al. 2010; Mortensen et al. 2011; Abo-El-Enein et al. 2012). In literature, bench-scale experimental studies illustrated a linear correlation between the cell concentration and rate of ureolysis (Okwadha and Li 2010; Lauchnor et al. 2015). An increase in the biomass population improves enzymatic activity and enhances the rate of CaCO_3 precipitation (Nemati et al. 2005; Okwadha and Li 2010). Nearly 30% of increment was shown in the rate of mineralization by increasing the biomass concentration from 1×10^6 to 1×10^8 cells ml^{-1} of *S. pasteurii* (Okwadha and Li 2010). A similar correlation between cell concentration and the rate of mineralization was observed in a sand column experiment conducted by Zhao et al. (2014). An increase of nearly 1.5–3% in the specific urease activity and amount of CaCO_3 precipitation was noticed by increasing the cell density, OD_{600} from 0.3 to 1.5, which lead to providing a higher strength of 2.22 MPa for the *S. pasteurii*. Recently, a study conducted by Mujah et al. (2019) assessed the effect of biomass concentration on the strength of biocemented geomaterial by investigating the morphological characteristics of precipitated biomineral at different biomass concentrations. However, the effect of biomass concentration on the nature of biomineral and its effect on the MICP process needs to be studied further by considering other biotic and abiotic conditions.

5.2 Influence of Chemical Factor on Biomineralization

5.2.1 Nutrient and Culture Media

Microorganism requires a sufficient amount of nutrients for its survival, growth, metabolic, and enzymatic activity during the span of biocementation. Also, a minimal quantity of media is required to continue the metabolic process while adding the microbes in cement mortars or soil. The basic nutrients required for microorganisms are carbon, nitrogen, potassium, calcium, etc. (Ng et al. 2012; Kadhim and Zheng 2016). And, for MICP experiments, the culture media mostly used was complex media such as yeast extract and beef extract which contains the above mentioned nutrients. The lack of nutrients can hinder the survival and growth of microbes, which in turn limits the enzymatic activity and biomineralization process. Hence, it is essential to study the effect of culture media on the mineralization efficiency in greater detail for real field application.

5.2.2 Type of Cementation Reagents

Not only nutrients, the type of chemical reagents i.e., urea and calcium source also have an impact on the cementation during microbial ureolysis. In this process, the provided urea is also utilized as a nitrogen and energy source by the ureolytic microorganisms (Mobley and Hausinger 1989; Achal et al. 2009). Previous researchers revealed that the type of calcium source used in the experiments such as calcium sulphate, calcium nitrate, calcium chloride, calcium lactate, calcium diglutamate etc. and its effect on the kinetics and nature of biomineral formed (Achal and Pan 2014; Wang et al. 2012; Tittelboom et al. 2010; Xu and Yao 2014; Xu et al. 2014). The results revealed that calcium source has a direct impact on the morphology and crystallinity of carbonate biomineral which further lead to varying the bond formation between the biomineral and cement or soil matrix. It influence the recovery of flexural strength, enhancement in the compressive strength of the biomodified specimen (Xu and Yao 2014; Xu et al. 2014; De Muynck et al. 2008b; Jonkers 2011). Most of the studies hitherto have utilized urea and calcium chloride or calcium chloride dihydrate for an effective biocementation process (Nemati et al. 2005; Dejong et al. 2006; Whiffin et al. 2007; Zhao et al. 2014; Achal and Pan 2014). In line with this, it is worth mentioning that a few researchers are currently attempting to utilize different waste products as a source of microbial nutrient and chemical reagents (Achal et al. 2009; Cheng et al. 2014; Chen et al. 2019). However, the effect of type of chemical reagents used, its cost issues and environmental concern need to be investigated in detail for large scale application.

5.2.3 The Concentration of Chemical Reagents

Besides the type of calcium source, the quantity of the chemicals also alters the biomodification process. One mole of urea and one mole of calcium chloride can produce one mole of calcium carbonate. Increasing the urea concentration up to a certain limit, enhance the rate of urea hydrolysis (k_{urea}). However, beyond the threshold value, the k_{urea} value was observed to reach its maximum and become constant while following the Michaelis–Menten relationship (Lauchnor et al. 2015). Okwadha and Li (2010) reported that with the increase in calcium concentration to 250 from 25 mM, the amount of CaCO_3 precipitation enhanced by over 100% at 1×10^8 cells·ml⁻¹. The importance of calcium ion on the urease activity was also substantiated in the study of Hammes et al. (2003). However, in the study by Stocks-Fischer et al. (1999), increasing the calcium concentration up to 50 mM in the cementation media did not impart any influence on the rate of ureolysis and rate of carbonate precipitation. In general, it could be inferred that the concentration of chemical reagents has a direct effect on the rate and amount of biomineral precipitation up to a limiting value (Nemati et al. 2005; De Muynck et al. 2010; Okwadha and Li 2010). After that, the increase in solution salinity may hinder survival and microbial growth, thereby limiting the urease activity and biomineral precipitation (Mitchell and Santamarina 2005; Nemati et al. 2005).

The amount of precipitation is having mutually opposite effects on biostrengthening and bioclogging, as we discuss the engineering aspects of the geomaterials in terms of strength and permeability, respectively. The reduction in permeability is directly proportional to the amount of calcium carbonate precipitated during the MICP process. A higher concentration of cementation reagents increase the rate and quantity of biomineral precipitation and enhance the permeability reduction (Whiffin et al. 2007; Qabany and Soga 2013; Stabnikov et al. 2013; Chu et al. 2014; Li 2014; Gao et al. 2019). The experimental studies conducted by Chu et al. (2014) provided a relationship (Eq. 23) to correlate the amount of precipitation and permeability of round sand. In similar lines, by compiling the available literature data, Gao et al. (2019) fitted an (Eq. 24), to assess the permeability from the amount of biomineral precipitated for the geomaterial having D_{50} of 0.165–0.420 mm.

$$k = 507 - 403 \times (ABP) \times 10^{-7} \quad (23)$$

$$k = 3 \times (ABP)^{-0.743} \times 10^{-5} \quad (24)$$

where k is the permeability in ms^{-1} , and ABP is the amount of biomineral precipitated.

The studies showed a two order of magnitude reduction in permeability with 15% of mineral precipitation. Though the literature adopted different methodologies to quantify the bioclogging, the results obtained in terms of the reduction in the permeability was almost similar. Further, it is worth mentioning that the variation in the concentration of chemical reagents significantly alters the rate, quantity, and distribution pattern of the bioprecipitation. This can impart a profound impact on the permeability properties, as reported by Gao et al. (2019) when the authors carried out flow-through experiments on biotreated sand columns.

As observed in the permeability studies, the increase in cementation reagents concentration and the subsequent rise in mineral precipitation can significantly improve the strength of biocemented geomaterial (Chu et al. 2014; Zhao et al. 2014). However, the observations from analyzing the permeability modifications upon treatment cannot be directly borrowed in the present case. For instance, a biomineral precipitation percentage of less than 3.5 didn't affect the strength gain of the treated geomaterial to a noticeable fraction (Whiffin et al. 2007). This is quite intuitive as too little percentage of precipitation may fail in effectively bridging the particles. However, after surpassing the initial buffering range, the further increase in cementation percentage could manage to establish a linear relationship with the corresponding increase in strength. This was evident from the study conducted by Zhao et al. (2014), where the authors come up with an equation relating to unconfined compressive strength (UCS in kPa) and biocementation percentage (Eq. 25). However, the study observed that after a threshold amount of cementation reagent concentration, the further addition of cementation reagents mostly utilized, thereby negatively impacting upon the treatment efficiency.

$$UCS = 366 \times ABP \quad (25)$$

Many of the researchers have reported a significant leap in the strength aspect of the soil when treated at lower cementation reagent concentration (Qabany et al. 2012; Qabany and Soga 2013; Cheng et al. 2014). This appears contradicting to the discussions made before, but have to do more with the pattern of mineral formation. At higher cementation reagents concentration, an abundant amount of mineral deposits randomly in the soil voids due to the faster rate of precipitation. On the other hand, at low concentration, the precipitated CaCO_3 crystals get homogeneously deposited at the particle contacts, which in turn can form a more uniform network of cementitious bridging throughout the soil matrix. The difference in behaviour at higher and lower solution concentrations is attributed to the fact that the variation in the cementation reagent concentration alters the supersaturation condition and the rate of nucleation, which in turn affecting the size of the biomineral precipitated (Al-Thawadi and Cord Ruwisch 2012). The observations were substantiated in the studies by Cheng et al. (2014), where higher strength achievement was reported for the biocemented sand at a lower chemical concentration of nearly 10 mM calcium sourced from seawater. However, the treatment demanded repeated injection of the stabilizing solution to achieve the required strength. The recent research in this area is focussing on the morphology of biomineral precipitation and the impact of cementation solution strength upon the same (Mujah et al. 2019). However, the research is still in its initial stages and demands further efforts to delineate its influence on the MICP process.

5.3 *Effect of Environmental Factors on the MICP Process*

The following section provides a brief review of the studies about the effect of environmental factors such as temperature, pH, and oxygen availability on the efficiency of the biomineralization and biocementation process.

5.3.1 **Temperature**

The temperature affects the growth, survival, metabolic, and urease activity of ureolytic microorganisms, which further influence the kinetics of biomineral precipitation (Cheng et al. 2017). For soil reinforcement, it is highly impractical to control or maintain a constant temperature in the field. Also, the field soil temperature varies with latitude, altitude, type of soil, and its depth, water content, proximity to industrial or agricultural sites, etc. (Jacobson 2005). Keeping this in view, selecting a pure (single) or a mixture of native ureolytic microbes is the best-suited option to overcome the effect of temperature on microbial metabolic activity and biocementation. Also, Selection of an ex-situ calcite forming bacteria, which can survive and grow in the intended soil zone temperature is one alternative approach to avoid the potential setbacks. In some condition, the temperature of cementation reagents is

higher than the soil temperature, which in turn also influences the microbial activity (Jacobson 2005). Though the native ureolytic microbes can survive in a wide range of temperatures, the temperature plays a vital role in the activity of urease enzyme-like other enzymatic reactions (Anbu et al. 2016). Most of the studies have proven that a temperature of 20–37 °C is optimum for the urease activity (Okwadha and Li 2010; Dhama et al. 2013). With an increase in temperature from 10 to 20 °C, the rate of urea hydrolysis (k_{urea}) increased nearly 5–10 times (Mitchell and Ferris 2005).

In contrast, some of the studies have shown that the ambient range of temperature lies at ≈ 60 °C for significant urease activity (Liang et al. 2005). An increase of temperature from 20 to 50 °C enhanced the urease activity, and the rate of CaCO_3 formation, which in turn alter the morphological characteristics of mineral precipitated (Nemati and Voordouw 2003). Beyond a temperature of 55–60 °C, the mineral precipitation was ceased due to the hindrance of microbial growth and survival (Rebata-Landa 2007; Dhama et al. 2014). Though the increase in temperature enhanced the rate of mineral precipitation, at the higher temperature of 50 °C, the crystal size was observed to be very small ranging from 2–5 μm when compared to the crystal size of 15–20 μm precipitated at 25 °C of treatment. The large variation in the size of the biomineral influence the contact points between soil grains and the strength of biocemented geomaterial (Cheng et al. 2014). The longer retention time of ureolytic activity, which induces a bigger size of CaCO_3 crystal and, in turn causing effective bonding between the biomineral and geomaterial was the major reason for the high strength gain at a lower temperature (Cheng et al. 2014, Peng and Liu 2019). Hence, the effect of temperature on MICP to repair the cracks, cement mortar, soil enhancement needs to be further studied for an efficient application in extreme temperature conditions.

5.3.2 pH

Like all other enzymes, most of the studies have reported that the activity of the urease enzyme is optimum in the pH value of 7.5–8.0 (Stocks-Fischer et al. 1999; Arunachalam et al. 2010; Gorospe et al. 2013). However, the carbonate biomineral precipitation starts at a pH value of 8.7–9.5 (Stocks-Fischer et al. 1999; Ferris et al. 2003). Though an increase in pH results in urease activity reduction, a substantial activity was still visible at a high pH of 9. During MICP, the formation of hydroxyl ions induce an alkaline environment and facilitate the carbonate mineral precipitation kinetics (Ferris et al. 2003; Rebata-Landa 2007; Dejong et al. 2010). Hence, the selected microbes should be alkaliphilic, which can perform its metabolic, enzymatic activity in high pH. Subsequently, the production of carbon dioxide due to ureolysis and aerobic respiration acts as a buffer for pH rise during biomineralization. Not only the urease activity but also the system pH affects the biomineral dissolution (Loewenthal and Marais 1978). For example, cement, concrete and mortar have a high pH ranging from 11.5 and 13.5 reduce the growth or survival of some ureolytic bacteria during initial curing period (Sookie et al. 2014; Sahoo et al. 2016). Also,

sometimes the high pH inhibits the growth and endospore forms due to adverse environmental condition. Researchers have investigated the optimal pH range for optimal growth of different ureolytic bacteria such as *B. subtilis*, *B. cereus*, *B. pasteurii*, *B. megaterium* and *B. sphaericus* in various growth media (Wu et al. 2012; Sookie et al. 2014; Sahoo et al. 2016; Schwantes-Cezaro et al. 2019). In soil reinforcement, the pH of the pore solution varies widely in geomaterial based on their origin, type of weathering, mineralogical variation, environmental condition, etc., which further influence the biomineral kinetics and biocementation process (Cheng et al. 2014). Furthermore, during biocementation, the pore solution pH can influence microbial adhesion and transport through porous media, which in turn affects the uniformity of biomineral distribution. However, very few studies were carried out to comprehend the effect of pH on adhesion, transportation of microbes, the kinetics of biomineral formation, and variation in the nature of precipitated biomineral and its effect on biocementation (Cheng et al. 2014). The effect of pH is a complex process as it have a direct impact on microbial growth, enzymatic activity, calcite solubility and nature of biomineral formed. Also, the pH of the system is changing throughout MICP process.

5.3.3 Oxygen Availability

The concentration of oxygen varies with soil depth, treatment zone in crack repairs, mortar specimen. Hence, it is essential to understand the MICP process under different prevailing aerobic, anaerobic, and anoxic conditions (Jain and Arnepalli 2019a). Few studies have investigated the effect of oxygen availability on the microbial growth, urease activity and soil reinforcement (Mortensen et al. 2011; Martin et al. 2012; Li et al. 2017; Jain and Arnepalli 2019b). Keeping this in view, there is a high demand to comprehend the effect of oxygen concentration on MICP process for a successful implementation of the MICP process in the real field application.

5.4 Relative Size of Bacteria and Testing Specimen

Microorganisms are abundantly present in the zone of the soil surface and subsurface (Mitchell and Santamarina 2005). The bacterial size mostly varies from 0.5 to 3.0 μm and sometimes possesses a filament length of 100 μm . Figure 3 illustrates the comparative size of microbes and geomaterial (Kadhim and Zheng 2016). The size of the pores, i.e., 50–400 μm of geomaterial, is favourable for effortless transportation and movement of bacteria and cementation reagents during the biomineralization process (Rebata-Landa 2007). Small pore size, which mainly depends on the soil composition, can obstruct the flow of microbes and lead to hinder the microbial activity in the intended zone (Karatas 2008). For example, a large amount of silt or clay present in coarse-grained geomaterial inhibits the biomineralization process due to the obstruction of microbial movement. On the other hand, in geomaterials

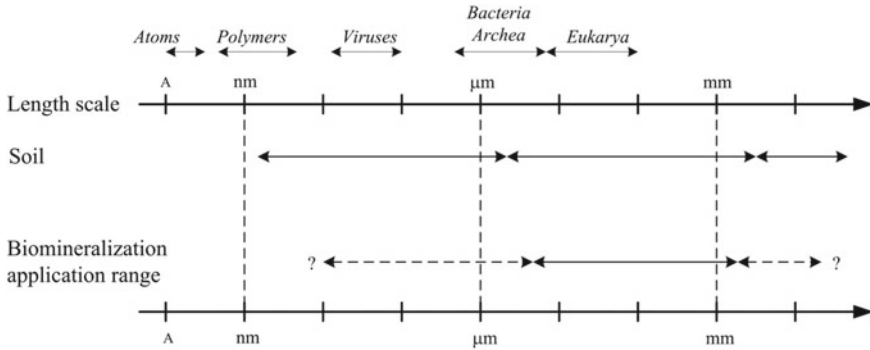


Fig. 3 Variation in the size of microorganisms and type of geomaterial

having large pore sizes, the microbes could be detached and flushed away from the targeted zone, in turn making the process inefficient. Higher bacterial concentration, i.e., more than 1×10^8 cells-ml⁻¹, also can cause a space limitation during the MICP treatment (Kadhim and Zheng 2016). Given this, an effective biocementation process needs geometrical compatibility between the size of bacteria and pores of geomaterial (Harkes et al. 2010). However, the premixing of ureolytic microbes, distributing the microbes by electro-kinetics, or utilizing urease enzyme instead of microbes are the alternatives to perform the MICP process in silt, clay, or clayey size geomaterial (Nemati et al. 2005; Keykha et al. 2014). Recently, Jain and Arnepalli (2020) have investigated the effect of solution chemistry on the attachment of microbes on the sand for an adequate attachment of microbes to implement MICP process. Similarly, in case of concrete and monument crack repair, the flow of bacteria to the intended zone is a concern and compatibility between the size of bacteria and specimen is required.

5.5 Effect of Type of Material

The type and nature such as morphology, mineralogy, the chemical composition of the soil, concrete, and mortar used to affect the survival of microbes, the kinetics of mineral precipitation, the behaviour of biomineral, bond formation between the biomineral and specimen used. It has a further impact on the efficiency of MICP for soil enhancement, crack repair, strengthening of concrete or brick. For example, an increment of 0.85–0.95 of maximum dry density enhanced the shear strength ratio from 41 to 164% for biocemented residual soil (Ng et al. 2012). The degree of reduction in permeability was also enhanced by increasing the soil density as the condition demands lesser biomineral quantity in filling the soil pores. Additionally, biocementation was found to be more effective in well-graded sand compared to uniformly graded sand because the intact packing offered by the well-graded soil

could form more effective bridging between the particle contact points (Cheng et al. 2017). Apart from the soil type, the degree of saturation of the soil can have a significant influence on the efficiency of the MICP process (Cheng et al. 2013). Most of the initial experimental studies in the field of MICP were performed at fully saturated condition, because of attaining a uniform distribution of the mineral precipitation (Dejong et al. 2006; Whiffin et al. 2007). However, this assumption was kept at stake with the findings that followed. For instance, the experimental results of biocementation at different degree of saturation had shown that higher soil strength with less amount of CaCO_3 precipitation could be achieved at a lower degree of saturation (20%) compared to fully saturated condition (Cheng and Cord-Ruwisch 2012; Cheng et al. 2013). The morphological studies of biocemented geomaterial further confirmed that the degree of saturation could significantly alter and confine the distribution pattern and position of precipitated biomineral during the MICP process. However, more research is needed to be done to assess the effect of the type of material on calcite bond formation and biomodification process.

6 An Outlook Regarding Bio-carbonate Precipitation

Study of biomineralization or MICP is an interdisciplinary field with the involvement of biotechnology, earth science, geology, environmental, chemical and geotechnical engineering. The huge potential of the process is hindered by four factors i.e., environmental concern, cost, sustainability or long term effect, and complex reaction, which need to be addressed for possible large scale application.

Implementing any novel ideas in field is a concern pertaining to the environment. In this regard, the by-product generation such as ammonium, nitrate, nitrous oxide is toxic to the ecosystem. Also, the byproduct can react with other chemicals present and generate hazardous products for building materials and the environment. To overcome this, the generated byproduct should be utilized such as ammonium chloride as a fertilizer. However, more detailed research is required in this regard for an efficient MICP accomplishment. MICP is a complex biochemical reaction and the microbial and enzymatic activity is highly depended on the prevailing environmental conditions. Also, the presence of other native microbes, the chemical composition of the testing specimen affects the mineralization process. Hence, the complex nature of MICP process makes it difficult to use in commercial purpose. The nutrients used for growth or increasing the microbial concentration is costly i.e., 60% of the total operating costs (Kristiansen 2001). Similarly, the lab grade cementation reagents are costly and bear huge cost of the total operation. However, researchers are making an effort to utilize different waste such as corn steep liquor, lactose mother, pig urine, seawater as a nutrient, urea and calcium source (Achal et al. 2009; Mitchell et al. 2010; Chen et al. 2019). Ivanov and Chu (2008) have reported that microbial grouting is more economical compare to any chemical grouting in terms of raw materials used. As, with all this, the long term effect of MICP on biomodification is still a question because carbonate biomineral is highly soluble in a highly acidic

environment. However, it can be applied for temporary treatment such as surface erosion control. The durability of MICP pertaining to freeze and thaw, different pH, salinity and presence of other adverse conditions need to be studied further. Also, a suitable injection strategy method is needed for successful MICP treatment pertaining to different applications. (Harkes et al. 2010; Jain and Arnepalli 2020).

7 Conclusion

The biomineral precipitation or biomineralization is mimicked for different engineering applications. This chapter reviews the process of MICP and its application in the field of construction engineering and building materials. Further, the various influencing biotic and abiotic factors on MICP have been critically reviewed and the requirement of further research on this regard is highlighted. Though the MICP process has different merits, there is a high demand to extend the research on optimizing the MICP process for successful implementation in the field.

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