



Microbially Induced Carbonate Precipitation Techniques for the Remediation of Heavy Metal and Trace Element–Polluted Soils and Water

Xue Chen · Dan Zhang · Steven L. Larson · John H. Ballard · Heather M. Knotek-Smith · Jing Nie · Nan Hu · Dexin Ding · Fengxiang X. Han 

Received: 30 November 2020 / Accepted: 2 June 2021 / Published online: 24 June 2021
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Abstract Heavy metal pollution in soil and water has been of worldwide concern due to their biotoxicity/ecotoxicity in the ecosystem, accumulation in the food chain, and persistence in the environment. Microbially induced carbonate precipitation (MICP) is known as an efficient and cost-effective biogeochemical process for the remediation of heavy metals in contaminated environment. This study reviews the mechanisms of biomineralization of urease-producing microorganisms and their biogeochemical process with various heavy metals and trace elements. Biogeochemical factors affecting the formation of carbonate biominerals were discussed. These factors included the growth and urease activity of microorganisms, calcium concentration and coexisted cations/anions, dissolved inorganic carbon, pH, redox potential, etc. In addition, the mechanisms of the biomineral morphology and its controlling factors

such as medium types, microbial species, and extracellular polymeric substance were analyzed as well. Finally, the challenge and current knowledge gap on further application of MICP in remediation of heavy metal–, trace element–, and U-polluted soils and water were presented.

Keywords Remediation · Heavy metal · Ureolytic microorganism · Morphology · Extracellular polymeric substance

1 Introduction

In recent years, with the rapid development of urbanization and the continuous increase of population, our environment has become polluted and degraded with heavy metals and radionuclides (Han et al., 2002, 2003). A number of techniques for remediation of metal-contaminated sites and waters include soil washing (Mao et al., 2016a), physical separation, stabilization, bioremediation/phytoremediation (Han & Singer, 2007; Han et al., 2004; Su et al., 2005, 2007, 2008; Sridhar et al., 2005, 2007; Shiyab et al., 2009), electric coupled phytoremediation (Mao et al., 2015, 2016b, 2018), etc. Natural materials such as humic substance and new nanomaterials were developed to remove heavy metals and radionuclides from water (Guo et al., 2015, 2016, 2018). Microbially induced carbonate precipitation as a relatively new phenomenon has been developed with potential applications

X. Chen · D. Zhang · J. Nie · F. X. Han (✉)
Department of Chemistry, Physics and Atmospheric
Sciences, Jackson State University, Jackson, MS 39217,
USA
e-mail: Fengxiang.han@jsums.edu

X. Chen · D. Zhang · N. Hu (✉) · D. Ding
School of Resource Environment and Safety Engineering,
University of South China, Hengyang 421001, China
e-mail: hn12352000@foxmail.com

S. L. Larson · J. H. Ballard · H. M. Knotek-Smith
U.S. Army Engineer Research and Development Center,
Vicksburg, MS 39180-6199, USA

in construction, dust control, soil and subsurface strengthening, pollution control, etc. This review provides a compressive description of microbially induced carbonate precipitation (MICP) to date for the potential remediation of heavy metals.

Various industrial wastes were generated from mining and metal refining, fuel, and energy production, such as atomic energy, steel production, aerospace industry, and many other industries, releasing large quantities of toxic metals discharged directly or indirectly into the environment (Bishop, 2000; Han & Singer, 2007). In 2000, the cumulative industrial age anthropogenic global production of arsenic (As), cadmium (Cd), Cr, copper (Cu), Hg, nickel (Ni), lead (Pb), and Zn was 4.53, 1.1, 105, 451, 0.64, 36, 235, and 354 million metric tons, respectively (Han & Singer, 2007). The ratios of the potential anthropogenic trace element inputs to its content in world soil in 2000 were 0.3, 1.0, 0.4, 5.6, 8.7, 0.7, 4.2, and 3.5 for As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn, respectively (Han & Singer, 2007). Most of heavy metals such as Pb, Cd, Zn, Cu, and others exceeded the standard in the soil near major urban agglomerations in China (Duan et al., 2016). The pollution caused by toxic metals in soil not only causes metal accumulation in grains which resulted in an economic loss, but also directly affects human health (Zinjarde et al., 2014). Heavy metals in the form of ions accumulate in plants and animals, affecting their health through the food chain. Once entering the food chain, they will eventually be absorbed by the human body, causing great harm to human health (Senko et al., 2002). In addition, heavy metals can enter groundwater through natural processes, causing serious groundwater pollution.

The remediation technologies mainly include physical, chemical, and bioremediation. Physical remediation techniques that have been used for metal removal are solidification, electrokinetics, in situ separation and extraction, etc. (Mulligan et al., 2001). Chemical remediation is to add modifiers and inhibitors to contaminated soil to regulate and change the chemical properties of the soil through chemical reactions such as precipitation, adsorption, antagonism, oxidation–reduction, and so on, reducing their bioavailability (Gao, 2013; Han & Singer, 2007). Bioremediation is to use plants and microorganisms to transform and extract heavy metals from soils and water with a great potential as an environmental friendly green

technology (Gao, 2013). Compared with the traditional physical and chemical remediation technologies, it has the characteristics of simple operation and management, low processing cost, and little interference to the surrounding environment and does not produce secondary pollution (Luo et al., 2015). Therefore, it is a kind of economic, effective, and non-destructive remediation technology. One important aspect of the bioremediation is to use the principle of microbial mineralization to treat heavy metals in contaminated media. In recent years, the application of biomineralization in environmental pollution control has made a significant progress. This study reviews the most current status and development of biomineralization in remediation of heavy metals and U in soils and groundwater.

2 Microbially Induced Carbonate Precipitation to Repair Heavy Metal Contamination

2.1 Carbonate-Mineralized Microorganisms

Microorganisms are ubiquitous, and microbially mediated mineralization processes are active in almost every environment on earth (López-García et al., 2005; Shen et al., 2001) and possibly in extraterrestrial systems as well (McKay et al., 1996). Biomineralization is the process in which various organisms internally or externally produce inorganic minerals (Doi & Fujino, 2013). The mineralization process has undergone nucleation, growth, etc., and is controlled by biological chemistry, the space, and the structure. The precise control of crystal morphology, size, orientation, crystal shape, and arrangement could be realized from the molecular level to the mesoscopic level (Dai & Shen, 1995; Mann, 2001). Biomineralization is divided into two types: biologically induced mineralization (BIM) and biologically controlled mineralization (BCM) based on the degree of biological control (Weiner & Dove, 2003). BIM is characterized by the fact that mineral formation depends on the environment created by microorganisms. Minerals formed by biologically induced mineralization processes generally nucleate and grow extracellularly as a result of the metabolic activity of the organism and subsequent chemical reactions involving metabolic byproducts (Frankel & Bazylinski, 2003). In the process of BCM, microorganisms highly control the nucleation and

growth of mineral particles and have nothing to do with the external environment (Bazylini & Frankel, 2003).

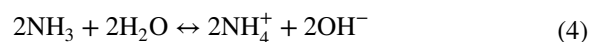
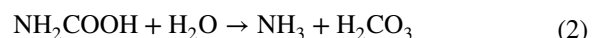
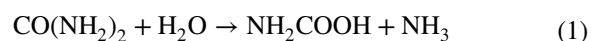
Carbonate-mineralized microorganism refers to a kind of microorganisms that produce certain enzymes to mineralize Ca^{2+} , Ba^{2+} , Cu^{2+} , and other metal ions as carbonates (Govarthanan et al., 2013). For example, microorganisms secreted metabolites that could affect biomineralization, e.g., carbonic anhydrase (CA), had the characteristic of accelerating CO_2 hydration to produce HCO_3^- , thus forming calcium carbonate deposition (Liu, 2001; Zhou et al., 2010). Cheng and Zhao (2016) used the enzyme-producing properties of *Bacillus cereus* Pb₂₁ to break down glucose to produce CO_3^{2-} , mineralizing and consolidating Pb^{2+} . The enzymes induced by the bacteria transformed lead from an extractable ionic form to a relatively stable carbonate mineral with a consolidation rate of more than 90%. In addition, a carbonate-mineralized microorganism with urease-producing properties has been extensively studied (Achal et al., 2012a; Fujita et al., 2000; Li et al., 2013a; Mugwar & Harbottle, 2016; Roman-Ross et al., 2006; Xu et al., 2015). This type of microorganism produced urease in the growth and metabolism process and decomposed the substrate to produce a large number of carbonate ions and other alkaline products (NH_4^+), promoting the pH value in the liquid phase environment, which was conducive to the formation of carbonate precipitation of metal ions (Ji et al., 2017). Most of the ureolytic microorganism were bacteria and had good immobilization effects on strontium (Sr), As, Cu, Pb, and Cd (Achal et al., 2011, 2012a, b; Kumari et al., 2014; Lauchnor et al., 2013). In addition, some calcifying ureolytic fungi exist in nature, which have the potential of biomineralization for sequestration of metals and radionuclides. Fungal metabolic activities influence alkalinity as well as Ca^{2+} concentrations, leading to induced CaCO_3 biomineralization via urea mineralization (Bindschedler et al., 2016).

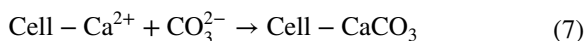
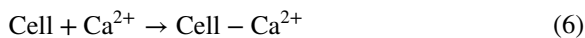
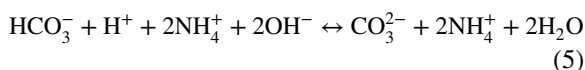
2.2 Ureolytic Microorganisms

MICP became an eco-friendly and energy-efficient technology (Xu et al., 2020). It had a potential in removing heavy metals from contaminated environment by the ureolytic microorganism. It was found that some bacteria produced urease to decompose

urea and form CO_3^{2-} , which formed insoluble carbonate directly with heavy metal ions. Xu et al. (2012) conducted consolidation tests on Pb^{2+} by using a kind of urease-producing carbonate-mineralized bacteria found in the soil and transformed it from an extractable ion to a stable PbCO_3 . Li et al. (2013a) found effective immobilization of metals, such as Cd, Ni, Cu, Pb, Co, and Zn, using *Terrabacter tumescens*, which were precipitated as CdCO_3 , NiCO_3 , CuCO_3 , PbCO_3 , CoCO_3 , and ZnCO_3 , respectively. Further, some ureolytic bacteria coprecipitated heavy metals with calcium carbonate. Studies have suggested that metal or metalloid ions replaced calcium or anions to form coprecipitates or to be absorbed on calcite to form complexes during the growth of calcium carbonate nuclei (Achal et al., 2012a; Fujita et al., 2000; Roman-Ross et al., 2006; Wang et al., 2018; Xu et al., 2015). Lauchnor et al. (2013), using ureolytic bacteria (ATCC11859), investigated a potential remediation strategy for the coprecipitation of carbonate minerals with strontium. Acid digestion verified the presence of Sr in the precipitates of the Sr-inclusive systems, and the experimental efficiency of Sr coprecipitation was 56~59%. XRD spectra suggested that these precipitates were calcite. Therefore, the fixation of metal ions by carbonate-mineralized bacteria could be divided into direct and indirect actions.

MICP-based degradation of urea provides a source of nitrogen for the microbes, increases the solution pH and alkalinity, and produces carbonate ions (Whiffin et al., 2007). Indirect immobilization of heavy metals generally requires calcium ions. When there is sufficient calcium to exceed saturation conditions, calcium carbonate (CaCO_3) precipitation can occur (Hammes & Verstraete, 2002). Equations (1)–(7) show the biochemical reactions involving ureolytic-driven MICP (Muynck et al., 2010; Qian et al., 2015; Stocks-Fischer et al., 1999).





Generally, the precipitation process is simply described as follows: positively charged Ca^{2+} ions move into the cell surface, and adsorption takes place on anions of extracellular polymeric substances (EPS) (Li et al., 2018). Changes in cell surface charge allow interaction between other bacteria with different surface charges (Li et al., 2018). The bacteria produce urease to decompose urea, leading to an increase of pH value and dissolved inorganic carbon, making a conversion of substantially soluble calcium ions to insoluble CaCO_3 precipitations (Beveridge & Fyfe, 1985; Beveridge & Murray, 1980). In addition, Dhami et al. (2017) isolated fungi with the potential for biomineralization of metals from the cave, which had the ability for calcium carbonate biomineralization and coprecipitation with other metals. This type of calcifying fungi bore immense potentials for several applications in environmental and civil engineering such as removal of heavy metals and radionuclides, calcium carbonate cements, and CO_2 sequestration.

2.3 Application of Urease-Producing Microorganism

Microbial activity is regarded as an important player in the formation of carbonate sediments and soil carbonate deposits (Rivadeneira et al., 1998). In recent years, the application of ureolytic microorganism-induced carbonate precipitation in solidifying sand soil, repairing concrete cracks, treating solid waste, and repairing heavy metal pollution has been widely studied (Achal et al., 2009; DeJong et al., 2010).

Urease-producing bacteria have been widely used in soil building restoration. Calcite production by the biomineralization processes is highly effective and may provide a useful strategy as a sealing agent for filling the gaps or cracks and fissures in any construction structures, which is environmentally innocuous (Achal et al., 2009). The precipitation of carbonates induced by ureolytic microorganisms has successfully been applied in the consolidation of soils because bioprecipitates improve the

structure, increase compressibility, and decrease the porosity and the permeability (DeJong et al., 2010). Achal et al. (2009) induced *Sporosarcina pasteurii* (MTCC 1761) mutation by ultraviolet irradiation to form phenotypic mutants (Bp M-3) with the activity of urease and the ability to produce galactose, which could be used in the remediation of cracks in building materials.

With the development of agricultural production, industrialization, and urbanization, heavy metal pollution has become an urgent environmental problem. The use of carbonate-mineralized microorganisms to restore the environment polluted with heavy metals has attracted more attention (Table 1). Achal et al. (2013) demonstrated a calcifying ureolytic bacterium (*Bacillus* sp. CS8) for the bioremediation of chromate (Cr(VI)) from chromium slag based on microbially induced carbonate precipitation. Cr(VI) mobility was found to be significantly decreased in the exchangeable fraction of Cr slag, and the Cr(VI) in the carbonate-bound state increased. The results of this study indicated that CS8 had a good effect on the immobilization of Cr(VI) from Cr slag and had a huge potential for its potential use in remediation of Cr slag contamination sites. Wang et al. (2007) found that carbonate-mineralization bacteria induced enzymatic actions in substrates and the released carbonate ions formed cadmium (Cd) carbonate in soil which reduced Cd concentration by more than 50%. Achal et al. (2012a) used *Sporosarcina ginsengisoli* CR5 for remediation of As(III) in contaminated soil. The ureolytic bacterium significantly reduced the As concentration in the exchangeable fraction of soil. Calcite production facilitated precipitation of a strong arsenic-calcite complex, thus reducing the mobility of As. Qian et al. (2011) studied the biomineralization of Cu^{2+} by carbonate-mineralization microbe. It was found that the bacteria could form mixed mineralization products dominated by $\text{Cu}_2(\text{OH})_2\text{CO}_3$ through mineralization. The effective removal rate of copper ions could reach more than 90%. Zhu et al. (2016) isolated a kind of nickel-resistant bacteria with the ability to break down urea (*Bacillus cereus* NS4) from the industrial soil. Through the treatment of *Bacillus cereus* NS4, the soluble-exchangeable fraction of Ni in soil was greatly reduced and nickel was transformed from mobile species to stable biominerals. The precipitation of nickel in the crystal lattice of CaCO_3 became immobilized in the form of NiCO_3 .

Table 1 The urease-based MICP in immobilization of heavy metals and trace elements

Elements	Microbial	Bioremediation rates, initial concentrations, and media	Reference
Cu	<i>Kocuria flava</i> CR1	95%, 340 mg/kg, soil	Achal et al. (2011)
	<i>Sporosarcina pasteurii</i>	30%, 31.8 mg/L, water	Mugwar & Harbottle, 2016)
	Carbonate-mineralization microbe	> 90%, 12.7 g/L, water	Qian et al. (2011)
Ni	<i>Sporosarcina koreensis</i> UR47	93%, 2 g/L, water	Li et al. (2013a)
	<i>Bacillus cereus</i> NS4	90.5%, 400 mg/kg, soil	Zhu et al. (2016)
Co	<i>Terrabacter tumescens</i>	90%, 2 g/L, water	Li et al. (2013a)
	<i>Sporosarcina</i> sp. R-31323 UR31	94%, 2 g/L, water	Li et al. (2013a)
Zn	<i>Sporosarcina</i> sp. R-31323 UR31	99%, 2 g/L, water	Li et al. (2013a)
	<i>Sporosarcina pasteurii</i>	> 90%, 32.7 mg/L, water	Mugwar & Harbottle, 2016)
Pb	<i>Sporosarcina koreensis</i> UR47	99%, 2 g/L, water	Li et al. (2013a)
	<i>Sporosarcina pasteurii</i>	99%, 104 mg/L, water	Mugwar & Harbottle, 2016)
	<i>Aspergillus</i> sp. UF3	34%, 10.4 g/L, water	Dhami et al. (2017)
	<i>Fusarium oxysporum</i> UF8	54%, 10.4 g/L, water	Dhami et al. (2017)
	<i>Sporosarcina pasteurii</i> ATCC 6452	> 95%, 10.4 g/L, water	Jiang et al. (2019)
	<i>Penicillium chrysogenum</i> CS1	98.8%, 200 mg/L, water; 94%, 200 mg/kg, soil	Qian et al. (2017)
Cd	<i>Enterobacter cloacae</i> KJ-46	68.1%, 7.2 mg/L, water	Kang et al. (2015)
	<i>Exiguobacterium undae</i> YR10	84%, 100 mg/kg, soil	Kumari et al. (2014)
	<i>Lysinibacillus sphaericus</i> CH-5	99%, 2 g/L, water	Kang et al. (2014)
Sr	<i>Sporosarcina pasteurii</i> ATCC11859	56–59%, 210 mg/L, water	Lauchnor et al. (2013)
	<i>Aspergillus</i> sp. UF3	48%, 4.38 g/L, water	Dhami et al. (2017)
	<i>Fusarium oxysporum</i> UF8	31%, 4.38 g/L, water	Dhami et al. (2017)
Cr	<i>Bacillus</i> sp. CS8	> 68%, 125 mg/kg, Cr slag	Achal et al. (2013)
	<i>Penicillium chrysogenum</i> CS1	95%, 100 mg/kg, soil	Qian et al. (2017)
As	<i>Sporosarcina ginsengisoli</i> CR5	96%, 10 mg/L, water	Achal et al. (2012a)

This NiCO_3 could be a CaCO_3 - NiCO_3 solid solution formed via the isomorphous substitution of Ca by Ni.

3 Biogeochemical Conditions Affecting Coprecipitation/Precipitation

Carbonate deposition with microorganisms was generally considered to be controlled by induction mechanism because the mineral type depends largely on environmental factors. The formation of carbonate in different environments was affected by microbial species and other abiotic factors (Wang, 2018). Bacterially induced CaCO_3 precipitation was regarded as a complex process which involves environmental conditions

and biotic metabolism pathway, both of which influence the initial precipitation process, as well as the overall accumulation of these deposits (Li et al., 2015b). Microbes from soils and aqueous media have been frequently reported to induce the precipitation of calcium carbonate mineral phases in both natural and laboratory settings (Lian et al., 2006). However, in order to implement MICP at the field scale, biogeochemical conditions need to be assessed to optimize the biomineralization process (Table 2).

3.1 Bacterial Growth and Urease Activity

Mortensen et al. (2011) altered the growth conditions of the bacterium *Sporosarcina pasteurii* to

Table 2 Biogeochemical conditions affecting the formation and morphology of MICP

Precipitation	Biogeochemical factors	Function
Formation	Cu, As, Pb, Sr, Cr, Cd, Zn	Affect the growth of microorganism
	Urease activity	Biological stimulation enhances urease activity
	Calcium source	Calcium chloride has a good mineralization effect
	Dissolved inorganic carbon	Promote the production of precipitation
	pH	High pH promotes the formation and stability of precipitation
	Extracellular polymeric substance	Provide nucleation site
Morphology	Growth media	Affects the morphology of carbonate precipitation produced
	Dissolved organic carbon	Hinder vaterite-to-calcite phase transition
	Microbial species	Affect the morphology of carbonate minerals
	Organic macromolecule	Hinder vaterite-to-calcite phase transition
	Extracellular polymeric substance	Hinder vaterite-to-calcite phase transition
	Precipitation rate	Low precipitation rate benefits to the uniformity of precipitation
	Sr, Pb	Affect the crystal size or morphology

investigate the effects of biogeochemical conditions on microbe-induced calcium carbonate precipitation. Several levels of water hardness and salinity were used to evaluate the growth of *S. pasteurii* and the potential to induce calcium carbonate precipitation in different environments. The result showed that ureolytic bacteria (*S. pasteurii*) were able to grow in a variety of freshwater conditions and in saline conditions equivalent to 100% seawater and induce carbonate precipitation. In addition, the presence of heavy metal ions may affect the growth of bacteria. Different microbes have different growth and metabolic capacities and resistance to heavy metals. Dhimi et al. (2017) found that different urease-producing microorganisms displayed varying toxicity tolerance to different metals (Pb and Sr). This may be due to physiological adaptation mechanisms and metal accumulation mechanisms of different microorganisms (Li et al., 2015a).

High bacterial cell concentration helps to enhance calcite deposits during the MICP process because more cells increase urease concentration for urea hydrolysis (Langdon et al., 2000). Urease is the significant enzyme indicator in carbonate precipitation. Mortensen et al. (2011) studied the effect of ammonia ions produced by urease decomposition of urea on urease activity, and the result showed that the concentration of ammonium ions had no significant effect on urease activity. In view of the application of MICP in remediation of heavy metal

pollution in groundwater, the effects of anaerobic conditions on urease activity were also studied. The result demonstrated that the urease activity was not adversely affected by anoxic conditions (Mortensen et al., 2011). If a sufficiently large ureolytic bacterium was injected into a groundwater environment with a limited oxygen supply, carbonate mineralization could still occur. It is possible that anoxic conditions may lead to lysis of cells, but there was no change in urease activity between lysed and viable cells (Mortensen et al., 2011). Cells lysed in the environment continued to exhibit similar urease activity to that of viable cells for an unknown period of time, so cell lysis would not affect carbonate mineralization as long as the enzyme was not degraded (Mortensen et al., 2011).

Ureolytic bacteria are commonly found in the subsurface. The subsurface microbiological communities may need to be stimulated or augmented to support MICP in situ (DeJong et al., 2009; Fujita et al., 2008). The research result of Chen and Achal (2019) showed that biostimulation accelerated the precipitation of CaCO_3 in soil based on urease production. Even in acid rain-treated soil, the soil showed significantly high urease activity after the biostimulation treatment. The effect of ureolytic bacteria could overcome the acidic conditions, and the high quantities of urease from ureolytic bacteria guaranteed sufficient carbonate from urea hydrolysis and prevented CaCO_3 decomposition in acidic solution (Chen & Achal, 2020).

3.2 Calcium Concentration, Dissolved Inorganic Carbon, pH, EPS, and Nucleation Site

The precipitation of CaCO_3 is governed by the following parameters: calcium concentration, dissolved inorganic carbon (DIC), pH, and the presence of nucleation sites (Hammes & Verstraete, 2002). These factors play essential roles which favor calcium carbonate precipitation during the ureolysis-driven process.

MICP by ureolytic microorganism involves a series of biochemical reactions. Apart from urease, the process requires calcium ions at a concentration that permits precipitation of carbonate, while nucleation sites with a strong affinity for cations enable the accumulation of calcium ions on cell walls (Ferris et al., 1986, 1987). For some fungi that have the ability to produce urease, Ca^{2+} -rich environments might be causing stress in fungal systems due to similar osmotic pressure and Ca^{2+} cytotoxicity to bacterial systems, leading to biomineralization as a defense tool for intracellular protection (Bindschedler et al., 2016). Different sources of calcium ions can also affect biomineralization to some extent. Selection of the appropriate calcium source is also very important in MICP-related research works, which should not affect bacterial growth and urease activity. Compared with the other calcium sources such as calcium acetate, calcium nitrate, and calcium oxalate, calcium chloride is the most effective reagent for calcium carbonate precipitation using microbial activities (Nawarathna et al., 2019). Calcium chloride showed a good mineralization effect. Thus, it is widely used in the treatment of heavy metals by microorganism-induced carbonate mineralization (Dhami et al., 2017). Zhang et al. (2015) reported that calcium acetate source provided more uniform carbonate crystals and which played an important role in improving the strength properties.

The survival and proliferation of urease-producing microorganisms lead to an increase in extracellular DIC, which would affect the solubility product of CaCO_3 and ultimately facilitate precipitation (Hammes & Verstraete, 2002). Moreover, the extent of precipitation could be limited by the maximum concentration of carbonate (CO_3^{2-}) produced by urea hydrolysis.

The release of NH_4^+ and OH^- during the hydrolysis of urea supplies a source of nitrogen to bacteria, increasing the alkalization of the medium and enabling the strain to cope with acid stress (Anbu et al., 2016). The ability of some urease-producing bacteria

to produce carbonate in acidic environment is lower than that in alkaline environment. But, they can grow at a wide range of pH conditions and hydrolyze urea, with potential applications in a variety of metal-contaminated environments. Additionally, the increase of pH can accelerate the settling rate of metal ions and promote the formation of carbonate minerals (Li et al., 2013b).

EPS plays a decisive role in the processes of biomineralization (Kawaguchi & Decho, 2002). EPS seems to cover the surface by biofilms and cell adhesion (Tsuneda et al., 2003) to capture the calcium carbonate, resulting in the formation of a homogeneous layer of calcium carbonate. Merz-Preiß and Riding (1999) showed that a biofilm was used to colonize the surface of the stones and reacted as nucleation site for extracellular calcium carbonate precipitation. The bacterial cell surface with a variety of ions could non-specifically induce mineral deposition by providing nucleation sites (Ferris et al., 1986, 1987). Especially Ca^{2+} is not likely utilized by microbial metabolic processes, but rather accumulates outside the cell (Silver et al., 1975).

For some fungi with urease production capacity, the mechanism of mineral precipitation is closely related to fungal hyphae, which may act as a nucleation center (Dhami et al., 2017). With the increase of metabolic activities, the cell walls and secreted EPS also have the ability to absorb various metals. Additionally, fungal hyphae imprint on mineral crystals confirms that fungi have a closer relationship with them and provide a much tighter framework (Gadd, 2007; Li et al., 2015a). Manoli et al. (1997) demonstrated the role of cell wall polymer chitin in binding to Ca^{2+} while Gadd (1994) found the role of cell wall glycoproteins. Recently, the cationic polysaccharide chitosan has been shown to have a positive effect on CaCO_3 crystallization and sand solidification (Nawarathna et al., 2019). Chitosan also promotes the CaCO_3 nucleation and growth as a template for the CaCO_3 crystals to nucleate due to adsorption of the Ca^{2+} ions onto the chitosan hydrogel. In contrast, without chitosan, bacterial cells themselves act as a nucleation site for CaCO_3 crystals to nucleate and growth (Nawarathna et al., 2019). Both field observation and laboratory research found that microorganisms can not only induce carbonate precipitation, but also regulate the composition, structure, and morphology of carbonate minerals (Dupraz et al., 2009).

4 Biomineral Morphology

The morphology of the precipitates produced through MICP is affected by many factors. Calcium carbonate has been reported to form different polymorphs under varying conditions as calcite (rhombic), aragonite (needlelike), and vaterite (spherical); two hydrated crystalline phases, monohydrocalcite ($\text{CaCO}_3 \cdot \text{H}_2\text{O}$) and ikaite ($\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$); and amorphous phases (ACC) (Gebauer et al., 2010; Rieger et al., 2007). All the polymorphs have unique properties varying from shape to strength (Rodriguez-Navarro et al., 2012). The formation of vaterite is rare, but elevated temperature or water rich in NaCl accelerates its transformation (Skinner & Jahren, 2007). It is desirable to precipitate calcite mineral as it is the most thermodynamically stable polymorph of CaCO_3 when compared to other less stable and less abundant polymorphic forms of CaCO_3 (aragonite and vaterite) (Boulos et al., 2014).

Despite a large number of studies on the bacterially induced CaCO_3 precipitation, the reason for the polymorph selection is not clearly understood (Rodriguez-Navarro et al., 2012). A wide range of factors has been found to play a role in determining the fate of each polymorph as composition of growth medium, temperature, pH, saturation index, dissolved organic carbon release, $[\text{Ca}^{2+}]/[\text{CO}_3^{2-}]$ ratio, and type of microbes (Dhmi et al., 2013, 2016; Gorospe et al., 2013; Rodriguez-Navarro et al., 2012). Additionally, it has been reported that specific EPS composition and structure influenced the formation of CaCO_3 crystals and controlled directly or indirectly calcite, aragonite, and vaterite selection (Chen et al., 2009; Tournay & Ngwenya, 2009).

There were significant differences for biomass yields, pH values, and urease activity when urease-producing microorganisms are cultured in different growth media or media concentration (Omeregic et al., 2019). The ionic strength of different growth media affects the ureolytic bacteria in a manner that supports or distress formation of monohydrocalcite (Bansal et al., 2016). Therefore, the composition of growth media and the conditions have been reported to affect the quantity as well as the type of carbonate precipitates (Zamarreno et al., 2009). Furthermore, the medium affects the morphology of the carbonate produced. For example, Omeregic et al. (2019) cultured ureolytic bacterial *S. pasteurii* NB28(SUTS)

and induced its production of calcium carbonate precipitation in different media to explore the influence of the medium on the morphology of the precipitation. The result shows that calcite was the product of ureolytic bacteria under most common culture medium, but a small amount of vaterite was present in its products in individual media. The same results were found in other experiments (Li et al., 2018).

Rodriguez-Navarro et al. (2012) demonstrated that the fate of calcium carbonate polymorph formed during biomineralization in a particular situation depends upon the amount of dissolved organic carbon (DOC) from bacterial activity, which hinders the vaterite-to-calcite phase transition. Under the condition of low DOC, it is conducive to the formation of calcite, while high DOC promotes the stability of vaterite.

The type of microbes affects the morphology of carbonate minerals. Cuaxinque-Flores et al. (2020) analyzed the polymorphic phases of CaCO_3 produced by two different urease-producing bacteria through SEM and XRD. The results showed that the round, microcrystalline aggregates of calcite and the spherical and disk-like shapes of vaterite were produced under the condition of separate culture, and the large double pyramidal crystals of calcite were precipitated by mixed culture. This ability to produce polymorphs of CaCO_3 of varied forms has also been reported for other species such as *Sporosarcina ureae*, *Shewanella psychrophila*, and *S. pasteurii* (Kang et al., 2014; Mugwar & Harbottle, 2016). The calcium carbonate minerals produced by ureolytic fungi also have morphological differences (Dhmi et al., 2017).

The formation of biominerals in living organisms is also related to organic macromolecules. These organic materials play an important role in the nucleation, growth, and morphology controls of the biominerals (Nawarathna et al., 2019). Chitosan is a linear polycationic biopolymer with positive charge in acidic environment, which has a certain influence on the morphology of the precipitation produced in the process of microbially induced carbonate mineralization (Jóźwiak et al., 2019; Liu et al., 2015; Nilsen-Nygaard et al., 2015). Precipitate with chitosan had both the calcite and vaterite crystals, while that without chitosan produced only calcite crystals (Nawarathna et al., 2019).

It has been suggested that different forms of CaCO_3 crystals were affected not only by the activity of bacterial strain-specific microbe urease,

but also by the EPS protein produced by different microbial strains (Hammes et al., 2003; Park et al., 2010). EPS around the cell wall is a complex biopolymer secreted by various microorganisms and consists of proteins, nucleic acids, lipids, and exopolysaccharides, which also confers a specific negative net charge capable of adsorbing nearby metal cations and plays a significant and physiology role in biomineralization (Kim et al., 2017; Lópezmoreno et al., 2014; Oppenheimershaanan et al., 2016). The EPS of different microorganisms affect the deposition process, crystal growth, crystal shape, morphology, and stability of the products (Zhou et al., 2009). In some urease-producing bacteria, the different polymorphic phases of CaCO_3 are related to the production of calcium-binding proteins (Anbu et al., 2016; Kawaguchi & Decho, 2002). EPS specifically binds Ca^{2+} inducing the formation of crystal morphology of CaCO_3 precipitation (Reddy, 2013). Studies have shown that when two strains of bacteria belonging to the same genus *Bacillus* and capable of carbonate mineralization were inoculated in the same medium, different crystal structures could be formed (Li et al., 2018). This indicates that the formation of CaCO_3 crystals is a strain-specific process, which is regulated by surface heterogeneity, the EPS, and certain protein of bacterial cells (Barabesi et al., 2007; Tournay & Ngwenya, 2009). Besides, studies have found that exopolymeric substances and biofilms play important roles in carbonate precipitation along with other enzymes (Bains et al., 2015). The existence of EPS may inhibit the conversion of vaterite into the most stable calcite (Li et al., 2018).

The morphology of the crystals also depends on the rate of precipitation. The uniformity of precipitation generation may be improved by reducing the precipitation rate, thus making the underground transport more uniform, which in turn changes the chemical equilibrium of the reaction network (Mortensen et al., 2011). The presence of heavy metal ions affects the morphology of the products in the process of MICP. In the study of the coprecipitation of strontium and calcite (Lauchnor et al., 2013), the presence of strontium had no significant effect on the precipitation rate of calcite in the urea-lysing process but inhibited the growth of calcite crystals and produced smaller crystals. The distribution of pollutants in calcite was positively correlated with

the precipitation rate of CaCO_3 (Mitchell & Ferris, 2006; Warren et al., 2001), but the initial concentration of heavy metals and the presence of other coexisting ions may affect the removal rate.

5 Uranium Remediation with MICP and Current Knowledge Gap

With the increasing number of nuclear power plants and the increasing demand for nuclear energy, uranium production has been increasing recently. The excessive exploitation of uranium resource produces a large number of mine waste residue and wastewater. Uranium contaminated soils and aquifers as an ecological threat. At present, the treatment of uranium wastewater by microorganism has been widely studied. Many microorganisms have shown specific adsorption capacity for uranium. Studies have shown that high initial U concentration increased the adsorption capacity of U(VI) by microorganisms, but the presence of Fe^{3+} and CO_3^{2-} inhibited the adsorption of uranium, leading to a decrease in the removal efficiency of U(VI) (Wei et al., 2019). The presence of K^+ , Na^+ , and Mg^{2+} slightly promoted the bacterial ability to mineralize U(VI) (Wei et al., 2019).

If the microorganisms could be used to induce carbonate precipitation in U-contaminated environment, MICP might be a cost-effective environmental friendly technology to remediate large areas of U site. However, in the process of microorganism-induced carbonate mineralization, the remediation efficiency of U sites may be controlled by biogeochemical conditions. Further researches on the biogeochemical controlling of MICP in remediating U-contaminated soils and waters and the efficiency in coupling with removing other coexisting heavy metals and anions are needed. Non-reductive biosorption of U was mainly related to the peptidoglycan, phosphate, and other functional groups on the surface of microorganisms (Liang et al., 2015; Merroun et al., 2011). Amino, carboxyl, and phosphate groups play important roles in the adsorption of U as adsorption sites (Zhang et al., 2018). The detailed mechanisms of microbe interaction with U during the adsorption with various functional groups on the cell surface in the process of MICP still require to be further explored.

Kumari et al. (2014) reviewed the ability of ureolytic microorganisms for carbonate biomineralization and applications of this process for toxic metal bioremediation. They focus on the remediation of heavy metal pollution by different microorganisms with carbonate mineralization ability. Wang et al. (2018) reviewed the progress of microbially induced carbonate precipitation and its application in the field of fixed heavy metals. In addition to summarizing the fixation effect of carbonate mineralized bacteria on various heavy metals, they also elaborated the MICP mechanism based on urease hydrolysis. The current biomineralization has focused on bacteria, including *Sporosarcina pasteurii*, *Terrabacter tumescens*, *Penicillium chrysogenum*, *Bacillus subtilis*, and *Bacillus giganteus*, and remediation of heavy metal-polluted soils (Fujita et al., 2004; Li et al., 2013a; Qian et al., 2017; Zhou et al., 2013; Zou et al., 2017). However, the MICP remediation of heavy metal pollution in groundwater was rare. In the actual field application, many carbonate-mineralized bacteria were not effective, where combined strains may be introduced to improve in situ effectiveness and efficiency. In addition, the potential of the re-release of immobilized or adsorbed heavy metals by MICP bacteria, the sensitivity of microorganisms to redox potential changes, and the change of specific toxicity as changes of oxidation state of heavy metals and U require further study due to their limitations of current bioremediation technologies (Achal et al., 2012a).

6 Conclusion

With the development of human civilization, heavy metals, trace elements, and radionuclides have become a global threat to our well-being and human health. MICP may be a potential cost-effective and environmental friendly technology in remediation of heavy metal- and radionuclide-polluted soils and waters. The biological and non-biological factors influencing carbonate mineralization and the selective formation of carbonate sediments with various crystal types are summarized in this paper. The presence of heavy metal ions may affect the growth of carbonate mineralization bacteria, reduce the yields of urease, and thus affect its efficiency in the treatment of heavy metal pollution. The anaerobic environment is not conducive to the growth of some bacteria, but urease activity is not restricted. The presence of Ca^{2+}

is crucial to the production of carbonate, and the treatment efficiency of heavy metal pollution depends largely on the amount of Ca^{2+} and the type of calcium source. Similarly, dissolved inorganic carbon, which also limits the precipitation, is another important condition for carbonate precipitation. The pH of the site also affects the treatment effect. The alkaline environment is conducive to the activity of urease and the stability of carbonate precipitation. Extracellular polymeric secretions play an important role in the adsorption of metal cations. These factors in the formation of carbonate minerals also influence the morphology of sediments.

As reviewed in this study, we found that almost all studies on the treatment of heavy metal pollution through microbially induced carbonate mineralization were based on the laboratory level with few field application in actual contaminated sites. The knowledge gap on mechanisms of MICP in reactions with heavy metals, trace elements, and U as well as biogeochemical control was not fully understood. The coexistence of multiple heavy metal ions may hinder the growth of carbonate mineralization bacteria, reducing the efficiency of the treatment of targeted heavy metals. Therefore, the synergistic effect of multiple strains with coexisting heavy metals as well as their effects on the overall efficiency needs to be optimized. Field pilot studies on application of MICP required to be further tested in order to be widely applied in the remediation of heavy metal and U-polluted sites.

Funding This study was supported by the U.S. Army Engineer Research and Development Center (W912HZ-16-2-0021), the National Natural Science Foundation of China (U1967210 and 11775106), the U.S. Nuclear Regulatory Commission (NRC-HQ-84-15-G-0042, NRC-HQ-12-G-38-0038, and NRC-HQ-84-16-G-0040), and the U.S. Department of Commerce (NOAA) (NA11SEC4810001-003499, NA16SEC4810009, NOAA Center for Coastal and Marine Ecosystems Grant # G634C22).

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