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Mineral Formation by Microorganisms

Concepts and Applications

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Key Applications of Biomineralization

Arda Akyel, Micah Coburn, Adrienne J. Phillips, and Robin Gerlach

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Abstract Biomineralization is a natural process with significant potential for use in various engineering applications. Engineered biomineralization has been researched intensively, primarily to develop methods to control mineral formation by microorganisms to enable various technologies. Engineered microbial mineral formation processes have developed from theory and a proof-of-principle vision to a technology being applied in the marketplace. Biological manufacturing methods, such as engineered mineral precipitation, can significantly reduce energy-intensive cement manufacturing activities and contribute to resource and climate conservation.

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This chapter provides an overview of key applications of biomineralization, already realized or currently under development, categorized as aboveground, near-ground, or belowground. Aboveground applications consist of biological building products with potential to replace energy-intensive materials, such as cement. Ground-level applications consist of surface and near-surface stabilization applications which can increase soil stability or treat toxic chemical contamination. Belowground applications include enhanced oil recovery as well as the sealing of leakage pathways around wells. Applications in construction, soil stabilization, and the sealing of leaky wells have advanced the furthest, and some of them have been commercialized; other technologies are on the verge to commercialization. Also described in this chapter are the many metabolic pathways used by microorganisms, which can result in mineral precipitation, where urea hydrolysis-induced calcium carbonate precipitation is the technology likely used most frequently in full-scale applications. Much research and development work remains to be performed in this field, and additional applications for biomineralization will be developed in the construction, environmental, biotechnology, and medical fields. This chapter reviews in some detail existing research and development activities with a focus on parameters important for engineered biomineralization applications.

1 Introduction

Engineered biomineralization has been researched intensively for approximately two decades with a significant uptick in the past 10 years. The controlled mineral formation by microorganisms has enabled technologies which were not available previously. As a result, numerous research articles and literature reviews have been written over the past decade, which summarize the research along with established or potential applications (Bang et al. 2010; Cunningham et al. 2011; El Mountassir et al. 2018; Krajewska 2018; Phillips et al. 2013a; Phillips et al. 2018; Stocks-Fischer et al. 1999). The prior chapters of this book mostly focused on fundamental science and modeling related to understanding mineral formation by microorganisms. This chapter reviews key applications that have been realized or are currently under development. Key applications are categorized as (1) aboveground, (2) near-ground, and (3) belowground in this chapter. Aboveground applications consist of biological building products with potential to reduce energy-intensive materials. Ground-level applications consist of surface and near-surface stabilization applications which can increase soil stability or treat toxic chemical contamination. Belowground applications include enhanced oil recovery as well as the sealing of leakage pathways around wells.

1.1 Chemistry and Pathways

Biomineralization reactions can generally be classified into three categories: biologically controlled mineralization, biologically induced mineralization, and biologically influenced mineralization (Dupraz et al. 2009a; Phillips et al. 2013a). Biologically induced mineralization, the precipitation of minerals as by-products of microbial metabolism, is the most frequently used approach in application development; specifically, ureolysis-induced (aka ureolytic) biomineralization of calcium carbonates (explained in detail below) has been used most frequently in research and application development.

There are various types of minerals that can be produced by microorganisms, e.g., carbonates, iron oxides, silicates, and gypsum (Cecchi et al. 2018; Miot et al. 2009a, b; Skorupa et al. 2019). At this point carbonate minerals, such as calcium carbonate, appear to be the most frequently used minerals produced for engineered biomineralization applications. A prerequisite for the formation of minerals is that saturation for the mineral of interest is exceeded locally and at least temporally, and microbiological activities can influence saturation and promote mineral precipitation. For calcium carbonate ($CaCO_3$) mineralization, the saturation state (S) depends on Ca^{2+} and $CO_3{}^{2-}$ concentrations as well as temperature, ionic strength, and other parameters that affect the solubility “constants” (K_{SO}) (Eq. 1) (Phillips et al. 2013a). When S is greater than 1, a system is considered supersaturated and precipitation is thermodynamically favorable (Stumm and Morgan 2013). Supersaturation is required for precipitation, but supersaturation does not guarantee precipitation (Connolly and Gerlach 2015) because compounds such as organics (proteins, organic acids, chelators, etc.) can inhibit precipitation reactions (Aggarwal et al. 2013; Arp et al. 2001; Bentov et al. 2010):

$$S = \frac{\left\{Ca^{2+}\right\}\left\{CO_3^{2-}\right\}}{K_{SO}} \tag{1}$$

Microbially catalyzed reactions that increase alkalinity and thus usually carbonate and bicarbonate concentrations are summarized in Table 1. Microorganisms can be responsible for carbonate generation using urea hydrolysis, nitrate reduction, sulfate reduction, photosynthesis, asparaginase hydrolysis, and iron reduction among other mechanisms. In the presence of certain cations, such as calcium (Ca^{2+}), this increase in carbonate alkalinity can induce the precipitation of carbonate minerals such as calcium carbonate ($CaCO_3$) (Eq. 2) (Connolly and Gerlach 2015; Phillips et al. 2013a):

$$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3 \tag{2}$$

For each of the reactions listed in Table 1, microorganisms generate, directly or indirectly, inorganic carbon in the form of $HCO_3{}^{-}$ or $CO_3{}^{2-}$, thus increasing alkalinity (Eq. 3). Depending on the prevailing pH, bicarbonate ($HCO_3{}^{-}$), and/or

Table 1 Microbially catalyzed reactions that increase alkalinity and thus carbonate and bicarbonate concentrations with potential for engineered biomineralization applications

Biomineralization type	Reaction	Microorganisms	References
Urea hydrolysis	$CO(NH_2)_2$ (urea) + $H_2O \rightarrow NH_2COOH + NH_3$ NH_2COOH (carbamic acid) + $H_2O \rightarrow NH_3 + H_2CO_3$ $2NH_3 + H_2O + H_2CO_3 \leftrightarrow 2NH_4^+ + OH^- + \mathbf{HCO_3}^-$	*Sporosarcina pasteurii*, *Bacillus sphaericus*	Connolly and Gerlach (2015), De Muynck et al. (2011), Phillips et al. (2013a)
Asparaginase hydrolysis	$C_4H_8N_2O_3$ (asparagine) + $H_2O \rightarrow C_4H_6NO_4^- + NH_4^+$ $C_4H_6NO_4^-$ (aspartate) + $H_2O \rightarrow C_3H_7NO_2$ (alanine) + $\mathbf{HCO_3}^-$	*Bacillus megaterium*	Lee and Park (2018), Li et al. (2015)
Iron reduction	$8FeO(OH) + CH_3COO^- + 15H^+ \rightarrow 8Fe^{2+} + 2\mathbf{HCO_3}^- + 12H_2O$	*Geobacter* sp., *Shewanella* sp.	Connolly and Gerlach (2015), Li et al. (2019)
Photosynthesis	$\mathbf{HCO_3}^- \rightarrow CO_2 + OH^-$ (CO_2 fixation) $\mathbf{HCO_3}^- + OH^- \leftrightarrow \mathbf{CO_3}^{2-}$	Cyanobacteria, algae	Arp et al. (2001)
Oxidation of organic Ca-salts (e.g., Ca-acetate)	$Ca(C_4H_6O_4) + 4O_2 \rightarrow Ca\mathbf{CO_3} + 3CO_2 + 3H_2O$	*Bacillus pseudofirmus*	Jonkers et al. (2010), Sharma et al. (2017), van Paassen et al. (2010b)
Nitrate reduction (e.g., with acetate as electron donor)	$5CH_3COO^- + 8NO_3^- + 3H^+ \rightarrow 10\mathbf{HCO_3}^- + 4\ N_2 + 4H_2O$	*Pseudomonas calcis*	Boquet et al. (1973), Connolly and Gerlach (2015), van Paassen et al. (2010b)
Sulfate reduction (e.g., with acetate as electron donor)	$CH_3COO^- + SO_4^{2-} \rightarrow 2\mathbf{HCO_3}^- + HS^-$	Sulfate-reducing bacteria (SRB)	Connolly and Gerlach (2015), Van Lith et al. (2003), van Paassen et al. (2010b)

carbonate (CO_3^{2-}) ion concentrations increase while hydroxyl ion (OH^-) production and proton (H^+) consumption occurs:

$$\text{Alkalinity} \approx HCO_3^- + 2CO_3^{2-} + OH^- - H^+ \quad (3)$$

Calcium carbonate can precipitate in various forms, with calcite, aragonite, and vaterite being the most common forms observed (Krajewska 2018; Mitchell and Ferris 2006a). Transition from less crystalline phases, such as vaterite, to more crystalline phases, such as calcite, occurs through Ostwald ripening during which more thermodynamically stable mineral phases form from less stable intermediates (Connolly and Gerlach 2015; Tourney and Ngwenya 2009; Xiao et al. 2010).

There are many metabolic pathways used by microorganisms, which can result in calcium carbonate formation. Urea hydrolysis is likely the most frequently used pathway in engineering research and development, followed by nitrate reduction, but other metabolisms such as sulfate reduction, iron reduction, photosynthesis, and asparagine hydrolysis are also possible pathways (Connolly and Gerlach 2015; Phillips et al. 2013a). Ureolytic biomineralization is somewhat unique among these metabolisms since it can act independently of growth and thus provides engineers with the opportunity to control mineral formation by adjusting urea and calcium concentrations and amounts while being able to rely on a fairly easily controlled catalyst: the enzyme urease. Urease is a ubiquitous enzyme, essential in nitrogen metabolism, and has functions in nitrogen provision, detoxification, and organismal defense (Feder et al. 2020; Krajewska 2009a; Lauchnor et al. 2015). Urease is found in many different organisms, including bacteria, plants, and fungi (Feder et al. 2020; Krajewska 2009a; Lauchnor et al. 2015).

1.2 Parameters Affecting Mineral Formation by Microorganisms

Environmental parameters, such as temperature and pH, as well as the chemical environment influence microbial growth and activity (Mortensen et al. 2011; Qabany et al. 2012); the same factors can also influence mineral formation. Hence, each biomineralization application at each location will require a certain level of optimization to successfully adapt to the existing conditions and control saturation. In this section we focus on describing the influence of temperature and pH value on microbial activity, saturation conditions, and thus mineral formation since these two parameters are some of the most important in the development and application of engineering applications.

Temperature is important since it affects microbial and enzyme activity; temperature can also affect the saturation state of a solution since solubility "constants" are indeed temperature-dependent; temperature can also affect mineralogy and morphology of precipitates (Feder et al. 2020; Ferris et al. 2004; Skorupa et al. 2019). As

Table 2 Carbonic acid diprotic dissociation and calcium carbonate precipitation

Reaction	Equation	pKa[a]
Carbonic acid dissociation	$H_2CO_3 + OH^- \leftrightarrow HCO_3^- + H_2O$	6.3
Bicarbonate dissociation	$HCO_3^- + OH^- \leftrightarrow CO_3^{2-} + H_2O$	10.3
Calcium carbonate precipitation	$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3$ (biocement)	–

[a]Note that pKa values are also dependent on temperatures and ionic strength; here the pKa values are provided for ~20 °C and low ionic strength

outlined in Table 1, there are several metabolisms that can promote carbonate mineral precipitation. Some of these reactions are microbial growth-dependent, while others simply rely on the activity of enzymes produced by microbes but might not be essential for growth. Microbial growth and enzyme activity usually increase with temperature until they peak and decrease again with further increasing temperature because enzymes and other essential cell components become damaged at higher temperatures. In general, the temperature range for effective microbial growth is narrower than the temperature range for acceptable enzyme activity. For example, the ureolytic bacterium, *Sporosarcina pasteurii*, can grow at temperatures from >0 °C to around 40 °C, while the enzyme urease produced by *S. pasteurii* or derived from plants is capable of hydrolyzing urea at temperatures of up to ~75 °C for at least several minutes (Feder et al. 2020; Skorupa et al. 2019).

Appropriate pH values are also critical; microbial and enzymatic activities, for instance, are sensitive to pH since both high and low pH values can inhibit or permanently denature enzymes, and other essential microbial functions (Dupraz et al. 2009b; Fidaleo and Lavecchia 2003; Krajewska 2016; Lauchnor et al. 2015; Qin and Cabral 1994). With regard to calcium carbonate precipitates, pH values indirectly influence the saturation state since carbonate concentrations are strongly dependent on pH values with increasing fractions of the dissolved inorganic carbon being present as carbonate (CO_3^{2-}) at higher pH values (Table 2).

Negatively charged bacterial surfaces or extracellular polymeric substances (EPS) can act as nucleation sites for mineral formation by accumulating calcium or other multivalent cations in close proximity to each other, thus effectively increasing their local concentrations (Mitchell and Ferris 2006b). In addition, bacterial cell wall functional groups such as hydroxyl and phosphate groups can become negatively charged at high pH values (Phillips et al. 2013a; Rodriguez-Navarro et al. 2003; Sharma et al. 2017). The resulting, increased number of nucleation points could enable more and/or larger crystal formation events (Rivadeneyra et al. 1996; Sharma et al. 2017). Indeed, when mineral formation was examined microscopically, minerals were observed mainly associated with the bacteria initially, and bacteria were observed inside mineral precipitates later on, indicating that bacteria acted as nucleation sites (Zambare et al. 2020).

2 Key Applications

In the remainder of this chapter, current and envisioned biomineralization technologies are summarized. The most mature technologies can be separated roughly into three categories: (1) aboveground, building, and construction materials, (2) near ground-surface and stabilization applications, and (3) subsurface applications (Fig. 1).

The building and construction materials section covers the possibility of using biomineralization to create building materials, to remediate existing structures and the possibility of adding self-healing properties to existing or new building materials. The stabilization applications section discusses the expanding research and field demonstrations of utilizing microorganisms and enzymes to stabilize or immobilize soil, dust, and toxic mine tailings as an alternative to more traditional methods. Finally, engineering applications related to the deeper subsurface are highlighted. Biomineralization technologies for deeper subsurface applications, e.g., leaky well sealing, have been developed over the past decades and have reached commercial sector application.

Different applications have different requirements with respect to time available for implementation, desired permeability, strength, toughness, etc. Thus, depending on the application, implementation strategies are likely to be different. Some applications may require the relatively rapid formation of strong bonds, e.g., for building materials (bricks, foundations, etc.), while other applications, like self-healing concrete, require long-lasting biomineralization potential that can ramp up rapidly when needed to fill cracks in a structure once present (Seifan et al. 2016). Hence, it is important to understand how to control biomineralization to address the requirements inherent in these different applications.

Economics are one of the greatest hurdles in enabling the widespread use of biomineralization as an alternative to materials, such as cement which is well-established and relatively inexpensive. While biomineralization technologies are at

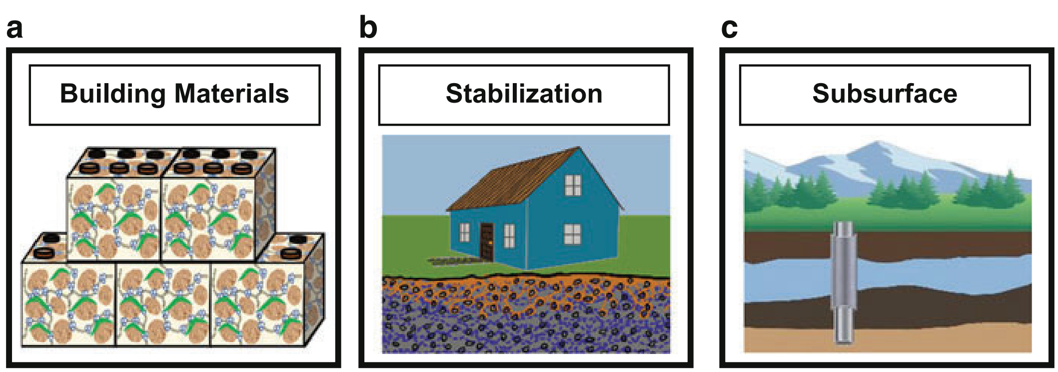

Fig. 1 Schematic overview of the most technologically advanced applications of microbial biomineralization. The remainder of this chapter summarizes the state of the technology as well as recent research and development activities in these three main topic areas: (**a**) building and construction materials, (**b**) ground stabilization applications, and (**c**) subsurface applications

this point more expensive, they have proven to be competitive due to their success rate, reliability, and relative ease of implementation. Ureolytic biocementation could indeed prove to be a greener (i.e., lower carbon emission) alternative since it largely avoids high-temperature processes with exception of the production of urea, which is often produced through the combination of the Haber-Bosch process (Haber 1905) for ammonia production and the Bosch-Meiser process for ammonia to urea conversion (Bosch and Meiser 1922). Depending on the source of ammonia and CO_2 for these processes, a significant amount of energy for heat and pressurization might be necessary. Other mineralization mechanisms, as outlined above, are also providing possible strategies, but ureolysis-induced calcium carbonate precipitation is currently the most commonly used strategy for larger-scale applications.

2.1 Building and Construction Materials

Cement production requires extensive, though well-established, high-temperature processing and currently contributes 6–7% of the annual anthropogenic carbon emissions (Abdul-Wahab et al. 2016; Achal et al. 2015). Portland cement is used to produce concrete, likely the most commonly used building material in the world. Concrete is a mixture of cement, aggregate (e.g., sand or gravel) and water, and overall, concrete is a durable, low-cost construction material with more than 10 billion tons used annually around the globe (Abdul-Wahab et al. 2016; Brown et al. 2014). Concrete is known for its high compressive strength but is characterized by a relatively low tensile strength. Thus, in many applications steel must be used to reinforce concrete. Under ideal conditions concrete prevents steel reinforcements from corrosion. However, cracks can form when concrete structures age or are mechanically damaged and experience freeze-thaw cycles or other environmental stresses. The cracking of concrete is a worldwide problem, and, in a 2006 report, it was estimated that the annual cost for repair, protection, and strengthening of concrete structures amounts to between $18 and $21 billion in the USA alone (Emmons and Sordy 2006). Indeed, some large concrete structures such as bridges could be impractical to replace or will be extremely costly to repair (Gardner et al. 2018). Specifically, cracks in cement can provide pathways for corrosive substances, such as oxygen and water into the structures, which can lead to corrosion of the steel reinforcements. These damages can affect mechanical properties and durability of the concrete structure, consequently reducing the useful life of concrete (Achal et al. 2015).

Biological building materials are considered an alternative to concrete, and biocementation is considered a viable strategy for maintenance and repair of existing concrete structures (Khodadadi et al. 2017; Van Tittelboom et al. 2010). These potentially more environmentally friendly and sustainable technologies do not require energy-intensive Portland cement production. Instead, biocement requires two main constituents: (1) microorganisms (or enzymes) to initiate the biomineralization reactions and (2) aqueous solutions that provide the ingredients and proper conditions to enable the mineral precipitation. Biocement can be produced in place, using low-energy methods while using local materials such as existing sand and gravel; however, a need still exists for somewhat energy-intensive raw materials, such as $CaCl_2$ (ice-melt) and urea (fertilizer) if ureolysis-induced biomineralization is used. Both urea and $CaCl_2$, productions require energy, and comprehensive life cycle analyses and techno-economic analyses (LCAs and TEAs) comparing traditional Portland cement and biocement are, at this point, not available.

2.1.1 Biological Bricks, Grout, and Mortar

2.1.1.1 Biomineralized Bricks

Bricks and precast concrete parts are the most-used building materials in the world (Brown et al. 2014; Wong et al. 2018). They are part of our everyday world (Fig. 2) and are designed to have great compressive strength, which is achieved through extensive manufacturing at temperatures above 1000 °C. The company, bioMASON, is one of the first to produce biologically produced masonry products. Their current products can be used for exterior cladding, paving, flooring, and on walls. These products contain approximately 85% by-product from granite quarrying and 15% calcium carbonate which is produced using ureolysis-induced calcium carbonate precipitation, thus decreasing the overall energy consumption. bioMASON's bioLITH tiles can be manufactured within approximately 72 h and

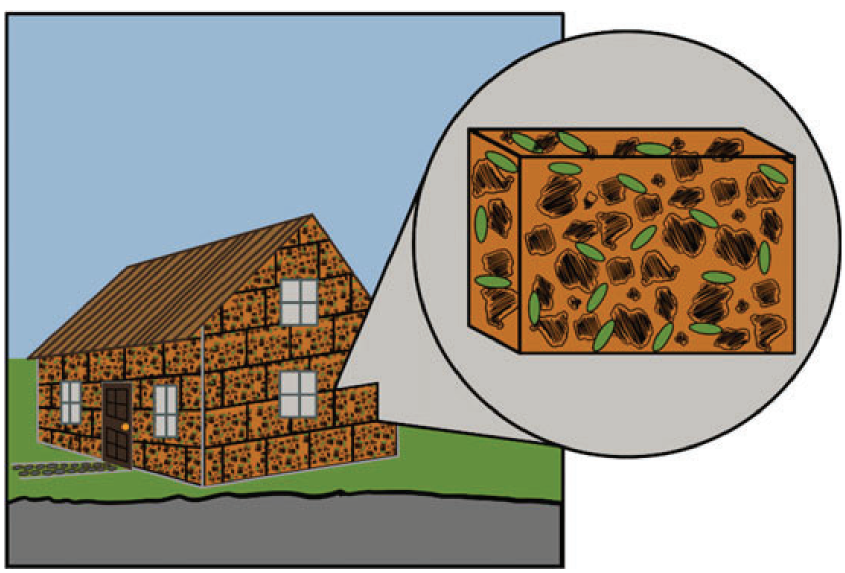

Fig. 2 Eco-manufactured modular building materials (MBMs) are a new paradigm for sustainable construction. Bricks (orange), mortar (black lines), and grouts (not shown) can be produced through biomineralization processes. Biologically produced building materials can potentially overcome several limitations inherent to conventional cement manufacturing and usage, such as high-energy manufacturing processes and lack of recyclability

are reported to require only 3.5% of the manufacturing energy of traditional engineered stone (bioMASON 2020). bioMASON reports that this energy reduction is achieved by avoiding the heat treatment necessary for traditional clay brick manufacturing (bioMASON 2020). bioMASON also reports that their products are lighter than natural stone, exceed performance in terms of CO_2 emissions, and possess higher compressive strength.

In a recent study, Heveran et al. (2020) engineered a living building material (LBM) using cyanobacteria to make biologically produced bricks. Cyanobacteria were placed in a sand-gelatin scaffold, and the bacteria increased the stiffness through photosynthesis-driven biomineralization (cf. Table 1). The resulting products did not only provide strength but also demonstrated the ability to self-heal when deformed. Self-healing requires long-term viability of microorganisms. This study suggests that lower temperatures and increased relative humidity (RH) increase cell survival with approximately 9% and 14% of cells being recoverable after 30 days at 4 °C at 50% and 100% RH; lower recoveries were observed at ambient conditions (22 °C and 24% RH) where cells were viable until day 7. The long-term viability of microbes must be improved further so that LBMs can sustain their structural and biological functions for the lifetime of structures (cf. "Concrete Remediation and Self-Healing Cement" section).

2.1.1.2 Biological Grout and Mortar

Grout and mortar are other materials commonly used in the construction industry. Grout and mortar are used for filling gaps between tiles and bricks among other uses. The difference between grout and mortar is that grout generally is being applied to fill and seal gaps between, e.g., tiles, but provides minimal structural support. Mortar is generally applied between bricks or under tiles and provides certain structural support during and after curing while also acting as a glue. Hence, grout must be able to penetrate and ultimately seal small gaps, while mortar must be viscous enough to support not only its own weight but also that of masonry placed above it. Most grouts and mortar are Portland cement-based, but synthetic grouts, such as acrylamide-, lignosulfonate-, and polyurethane-based grouts, are also in use; each of these compounds has its own environmental footprint (Achal and Kawasaki 2016). Biomineral-based grouts and mortar, which are under development, have potential to reduce the environmental footprint relative to the traditional grouts and mortars.

Literature regarding the use of biomineralization to produce a biogrout for building materials, e.g., tiling, is limited at this point. However, biogrouts have been demonstrated by multiple groups to have potential for concrete repair and self-healing applications. As mentioned above, grouts must readily fill gaps, voids, and cracks, often with the goal of preventing the entry of water after curing. Hence, tile grouts generally require lower viscosities than mortar. *[Note: The terms "grout," "grouting,"* etc. *are also used in the context of soil stabilization; the application of biomineralization-based grouting for soil stabilization is discussed in the next section ("b. Stabilization Applications")]*. The biomineralization technology is water-based; thus, low viscosity is one of its inherent characteristics. Minerals are generally precipitated through a biologically catalyzed, chemical reaction, allowing for the grout to develop in place. Biomineralization-based grouts might have advantages over traditional grouts since the microbes and enzymes are smaller than traditional cementitious grout particles and less viscous than, e.g., synthetic grouts, and thus might more efficiently penetrate small gaps before precipitating and forming bonds. Indeed, it is suggested that biogrout can be less expensive than chemical grouting while achieving unconfined compressive strengths comparable to traditionally used products (Achal and Kawasaki 2016; Li et al. 2015).

As mentioned above, mortar, in contrast to grout, needs to perform a structural function. Even during application, limited structural support needs to be provided to building components. For instance, mortar must maintain the spacing between masonry, such as bricks even during curing. Currently, there are no published works that specifically address the challenge of providing higher viscosity, mortar-like products using biomineralization methods. Furthermore, upon curing, significant structural strength (compressive strength) needs to be ensured along with other properties, such as fire resistance. Fire resistance of biobricks and biomortar is predicted to be comparable to traditional cement-based bricks and mortar since $CaCO_3$ is generally stable up to approximately 600 °C after which decomposition of $CaCO_3$ to CO_2 and CaO occurs at increasing rates (Abdel-Gawwad 2017). This decomposition also occurs in traditional cements and can result in decreases in compressive strength and other properties (Abdel-Gawwad 2017). The incorporation of organics into biobricks, biogrout, and biomortar must also be considered. While organics can increase viscosity and potentially elasticity, biomass generally begins decomposing around 250 °C and decomposes completely at temperatures around 500 °C (Abdel-Gawwad 2017). The effect of the loss of organics from the created biocement, grout, mortar, or bricks has not been investigated in detail.

2.1.2 Limestone Remediation

Many historic structures around the world, from the Great Sphinx to old churches to the Lincoln memorial, were constructed using limestone. Unfortunately, these structures are subject to weathering, exacerbated by the generally decreasing pH of rainwater, due to increasing atmospheric CO_2 concentrations and other acidic gases in the atmosphere (Nazel 2016; Villa et al. 2020). These carbonate-based stones and structures are vulnerable to gradual dissolution, leading to increased porosity, the accelerated entry of water, more rapid dissolution, increased freeze-thaw damage, and associated decreasing mechanical integrity (Marvasi et al. 2020; Nazel 2016; Tiano et al. 1999).

Limestone remediation is often focused on preventing the entry of rain- and meltwater, the restoration of mechanical integrity, and the protection of the weakened inner structure by establishing protective surface layers, filling existing pores, and reducing the porosity of deteriorated limestone (Fig. 3) (Nazel 2016). Existing remediation methods often include the use of chemicals, such as fluorinated polymer

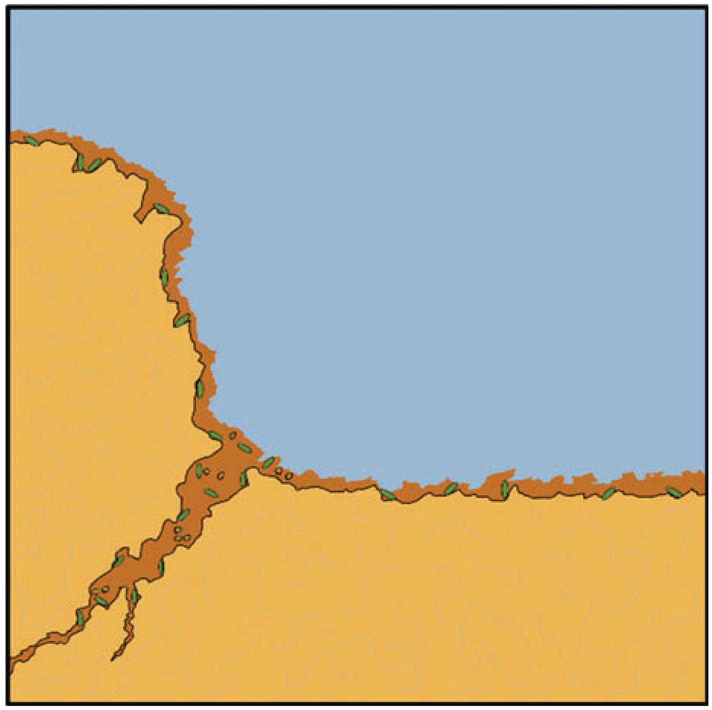

Fig. 3 Schematic indicating the potential for biomineralization-based approaches to protect limestone structures (light orange) by filling existing cracks and cover the limestone surface with biocement (dark orange with green organisms) to minimize the entry of acidic rainwater that would lead to limestone deterioration

coatings (Marvasi et al. 2020; Sadat-Shojai and Ershad-Langroudi 2009). Biomineralization could be an ecological alternative since biomineralization mimics the natural stone formation process (Marvasi et al. 2020) and is generally based on the use of low viscosity, aqueous fluids, which can readily penetrate pores and can be applied directly onto the surfaces using low-cost approaches, such as spraying (Castanier et al. 2000; Marvasi et al. 2020; Perito et al. 2014). As outlined in Table 1, several biomineralization approaches result in the production of carbonate minerals and have potential for the restoration of limestone structures. The use of alkalizing, calcium carbonate-precipitating microbial cultures could also combat the development of autochthonous acidifying microbial cultures, which can locally contribute to increases in porosity and decreasing mechanical strength due to localized acidification and associated dissolution of calcium carbonates (Castanier et al. 2000).

There are still several limitations to employing biomineralization-based approaches to building restoration. The use of microorganisms can result in a certain level of skepticism in the general population. The development of stains or off-color in the precipitates due to metabolic by-products associated with the biological production of calcium carbonates is also of concern. Furthermore, the possibility of subsequent growth of other microbes (e.g., airborne fungi) is a concern; fungi or other microorganisms, commonly referred to as "black molds," can cause damage or defacement to building materials. Mold growth could be stimulated by decaying bacteria, added enzymes or nutrients, or metabolic by-products generated during the biomineralization treatment (Nazel 2016). While Tiano et al. (1999) were unable to detect a color change on limestone, due to deposited biominerals, with the naked eye, it could be detected quantitatively using a chromameter (Tiano et al. 1999). The addition of pigments has been suggested, which could alleviate the potential aesthetic issue of color differences (Castanier et al. 2000; Nazel 2016); in addition, a reduction of the amount of organics included in the treatment solutions or the use of enzymes instead of microbes could decrease potential worries regarding the use of living microbes and reduce the concern regarding subsequent growth of fungi or other microbes on the remediated surfaces. A remaining limitation is that mineral precipitation occurs predominantly on the surface of microporous structures, on which microbes or enzymes would be deposited (De Muynck et al. 2011; Marvasi et al. 2020). The use of smaller microbes (e.g., starved bacteria or spores), which transport more readily into and through porous media (Bouwer et al. 2000; Cunningham et al. 2007; Gerlach 2001), or the use of (much smaller sized) enzymes has potential to alleviate these limitations (De Muynck et al. 2011; Krajewska 2009a).

2.1.3 Concrete Remediation and Self-Healing Cement

As noted above, defects, such as tiny cracks or gaps, are one of the biggest problems in cement and concrete longevity because these defects can drastically reduce the life of the structure by causing corrosion of the cement, concrete, or their reinforcements. These defects can occur through quality issues during implementation, temperature fluctuations (freeze-thaw cycles), vibrations (earthquakes, traffic, construction), chemical corrosion, or other processes causing cosmetic or structural damage. Biologically induced mineral precipitation has been proposed for the remediation of cement and concrete as well as for the development of self-healing materials (Achal et al. 2015; Phillips et al. 2013a). In principle, biomineral-precipitating solutions and enzymes (or organisms) can either be applied once a defect has been discovered (remediation) or can be designed to be activated once a defect occurs (self-healing) (Fig. 4).

Establishing conditions appropriate for biomineralization to occur in cementitious materials remains a challenge. Cement, in contrast to limestone, is a very high pH environment with pH values as high as 13 combined with often low oxygen availability (Lee and Park 2018). Both these parameters can pose challenges for some microbes and enzymes because extreme pH values and low oxygen availability can negatively influence the survival and activity of microbes and enzymes. While the pH of water in cement generally decreases over time due to carbonation of the cement (Papadakis et al. 1989), it can remain a challenge to reliably provide pH values around 10 or lower, which are more amenable to supporting microbial growth and the activity of enzymes such as urease (Fidaleo and Lavecchia 2003; Lauchnor et al. 2015). Some organisms have been demonstrated to have high salt and pH tolerance while being ureolytically active, but there remains a need to identify more high pH-tolerant organisms and enzymes (Skorupa et al. 2019).

Treating cracked or cracking cement materials using surface-applied treatments is fairly straightforward unless cosmetics are of concern (as discussed above for many

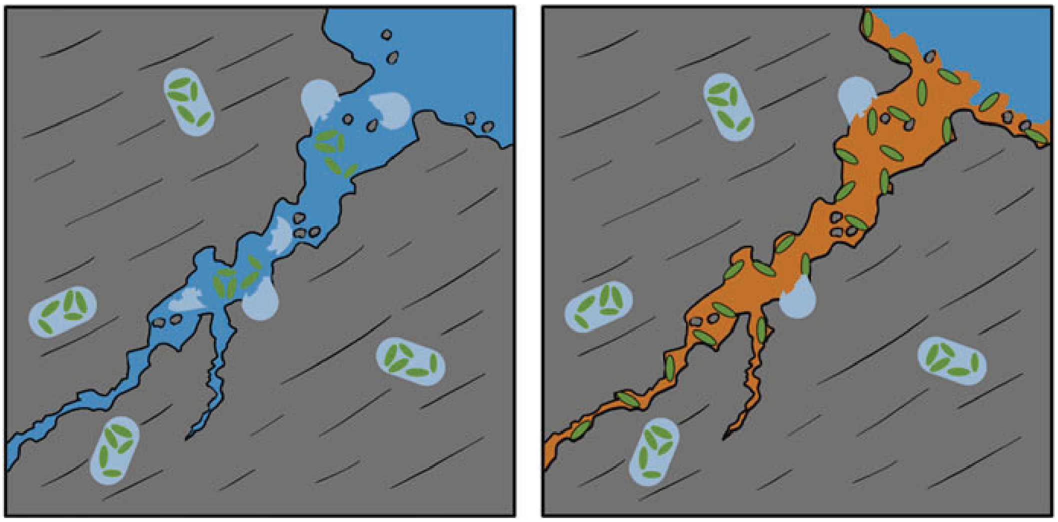

Fig. 4 Biomineralization-driven concrete remediation and self-healing. Existing cracks can be filled with biocement (orange) or self-healing cement can be designed, which contains microbes in the form of cells or endospores as well as chemicals that promote precipitation once damage occurs. Biomineral-precipitating bacteria or enzymes are envisioned to become active once cracks occur in the cement and reseal the developing fractures

limestone remediation projects). In one laboratory study, cement specimens were immersed in suspensions containing *Bacillus sphaericus*, and the resulting biomineral formation on the surface reduced subsequent water absorption by 65–90% and improved the resistance to freeze-thaw cycle damages (De Muynck et al. 2008). Biomineralization-based concrete repair approaches are suggested to be suitable for sealing cracks up to 2 mm in width (Achal et al. 2015; Wiktor and Jonkers 2016; Wiktor and Jonkers 2011). When crack healing was compared with control samples, significant differences were observed after 100 days of immersion in water (Wiktor and Jonkers 2011). In field trials, it was demonstrated that bacterial biomineralization systems can be used to create concrete with self-healing properties in applications such as irrigation canals and parking garages (Wiktor and Jonkers 2016). It has also been reported that this approach could be used to potentially recycle concrete and other building materials (Wiktor and Jonkers 2016). If post-cement damage treatment is pursued, active bacterial cultures or enzymes can be used, and oxygen, or other electron acceptor limitations, discussed below, is often not a concern.

The principal feasibility of using ureolysis-induced calcium carbonate precipitation by *Sporosarcina pasteurii* and other *Bacillus* sp. has been demonstrated by many researchers (see, e.g., reviews by Arias et al. 2017; Joshi et al. 2017; Krajewska 2009b; Phillips et al. 2013a). The feasibility of creating self-healing cements has also been demonstrated in principle (Erşan et al. 2016b; Wang et al. 2014a; Zhang et al. 2020). In cement structures cracks occur due to changes in temperature, pressure, and mechanical stresses (Jonkers et al. 2010), and most cracks start small. Thus, even slow precipitation, e.g., promoted by metabolic biomineralization, might be sufficient to seal the initially small apertures and prevent them from becoming larger (Alazhari et al. 2018). Studies have demonstrated *B. pseudofirmus*, *B. subtilis*, and *B. alkalinitrilicus* are capable of self-healing cracks of <0.8 mm in concrete (Basilisk 2020; Jonkers et al. 2010). In these and other studies, the cracks were effectively sealed in the presence of water (Sharma et al. 2017). However, it is less clear whether bacteria or their spores can survive long enough and resuscitate quickly enough to reliably seal cracks inside cement or concrete under the wide range of conditions expected depending on season and climate (Bang et al. 2010; Mitchell et al. 2019; Sharma et al. 2017).

If self-healing of cement through biomineralization is desired for extended periods of time, the self-healing agents have to be incorporated into the cement or concrete (Wang et al. 2014b) and have to remain active, or need to be activated, inside the cement or concrete over the service life of the structure once damage occurs. It has been suggested that encapsulation of bacteria, their spores, and their incorporation into cement mixtures can increase spore survival (Alazhari et al. 2018; Wang et al. 2014b). Indeed, it has been proposed that enzymes or bacteria could retain sufficient self-healing potential for years to seal cracks that might develop (Sharma et al. 2017; Van Tittelboom et al. 2010). In addition, for self-healing to occur, sufficient calcium, electron donor, and acceptor are necessary; however, high concentrations of material other than cement and aggregate (sand, gravel, etc.) may have detrimental effects on the mechanical properties of the concrete (Alazhari et al. 2018). Thus, trade-offs between the amount of additional materials to be incorporated into concrete to ensure self-healing and the required mechanical properties of concrete need to be considered.

Once resuscitation of bacteria needs to occur, electron acceptors (e.g., oxygen), electron donors, and a carbon source need to be available. Limitations in the availability of any one of these can affect the rate of activation of bacteria, enzymatic activity, and thus the speed of self-healing. It has been proposed to incorporate capsules into cements, containing oxygen-releasing compounds, such as calcium peroxide (CaO_2), combined with electron donors, a carbon source, and an additional source of calcium, such as calcium lactate. The capsules would release these compounds upon crack formation and would promote microbial resuscitation, growth, activity, and ultimately $CaCO_3$ precipitation (Lee and Park 2018).

The existence of bacterial strains capable of growth in the absence of oxygen and calcium carbonate precipitation has also been demonstrated. In laboratory studies anaerobic growth of and calcium carbonate precipitation by, both, ureolytic and non-ureolytic organisms have been observed, such as fermenters, denitrifiers, sulfate reducers, and iron reducers (Hamdan et al. 2017; Skorupa et al. 2019; van Paassen et al. 2010b). Until now, only nitrate reduction seems to have emerged as a possibly viable alternative to urea hydrolysis (Erşan et al. 2016a, b), but other methods for concrete self-repair are being developed (Zhang et al. 2020).

Basilisk, a biotechnology startup company in the Netherlands (https://www.basiliskconcrete.com), focuses on next-generation cement additives. Basilisk produces admixtures for concrete self-repair and concrete remediation. Basilisk uses a metabolically driven biomineralization reaction, using bacterial cultures consisting of several *Bacillus* species. Basilisk indeed uses calcium lactate as the source of calcium and encapsulates spores and calcium lactate into concrete materials for long-lasting performance. The technology is protected by several patents (Jonkers 2011; Jonkers 2009; Jonkers and Mors 2016; Wiktor and Jonkers 2014) and is designed to reduce maintenance requirements, extend service life, and protect concrete reinforcements. The *Bacillus* sp. spores used are estimated to survive up to 200 years as long as appropriate pH, temperature, oxygen, moisture, and nutrients are provided (Jonkers et al. 2010). Basilisk's concrete repair admixtures can be mixed and applied using conventional sprayers resulting in the formation of limestone. While promising, it is unclear at this point how long the potential for self-healing will remain active.

A similar approach for self-healing was used as part of the UK's first site trial of self-healing concrete (Davies et al. 2018). In a collaboration between academic (Cardiff University) and industrial (Costain) engineers, five concrete panels for a highway upgrading project were tested as part of the "Materials for Life" project. Researchers created five different concrete panels, one of them had bacteria in the cement mixture. When load-displacement curves were analyzed, the panel with bacteria exhibited a displacement of about 5.4 mm, while the other panels exhibited 9.2 mm displacement on average. Results after 6 months were sufficiently positive for the bacterially mediated self-healing process, but longer-term assessments have to be performed to verify applicability of reducing or removing the requirement for inspection, maintenance, and repair of concrete structures (Davies et al. 2018).

2.2 *Stabilization Applications*

2.2.1 Soil Stabilization

Soil is a complex mixture containing minerals, liquids, gases, organic matter, and organisms that help create a dynamic environment in which many biological, chemical, or physical processes are taking place. Soils can be highly heterogeneous, and inconsistent soil properties can cause structural damage or, worse, put human lives at risk. Soil stabilization is designed to provide consistent properties, and it is common practice for many construction and engineering processes including highway, building, and airfield construction, supporting earthen dams or embankments, irrigation networks, or preventing wind and water erosion. Regardless of the application, soil stabilization involves improving the mechanical properties of the soil by either mechanical or chemical means to achieve the properties needed, e.g., high strength and durability. Many different methods for soil stabilization (aka soil grouting) are available including biomineralization-based methods (Kalkan 2020; Mujah et al. 2017; van Paassen 2011).

Biomineralization can be applied to soils or other porous media to increase stability or enhance mechanical properties; if used for those applications, it is often referred to as biogrouting or biocementation of soils (*Note that the use of biomineralization as a potential tile grout was discussed above in the in the "Biological Bricks, Grout and Mortar" section and was also referred to as*

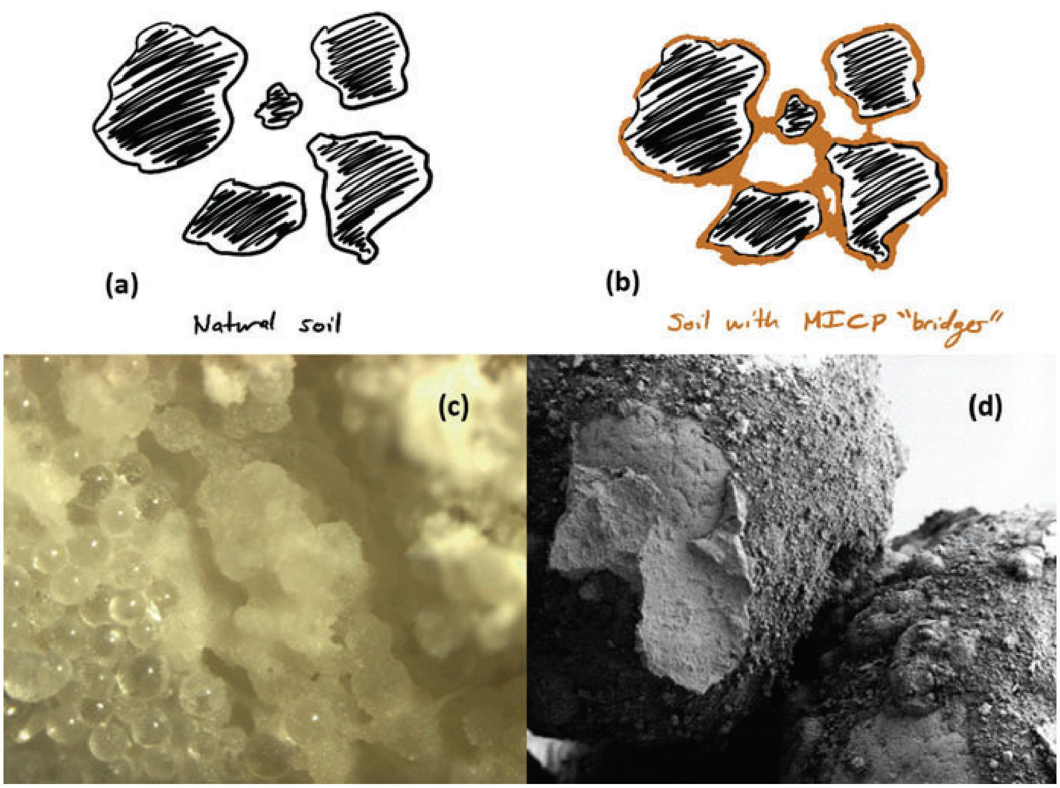

Fig. 5 Schematic representation of biomineralization-based soil stabilization or "biogrouting." (**a**) Soil particles not biomineralized represent unstable soils prone to soil liquefaction; (**b**) soil particles with mineral "bridges" (orange) between them. These bridging connections stabilize soils and make them less prone to liquefaction. (**c**) Stereoscope image showing glass beads cemented together by ureolysis-induced calcium carbonate precipitates; (**d**) SEM image showing sand grains connected by microbial ureolysis-produced calcium carbonate precipitates (**c**, **d**: Montana State University, unpublished data)

biogrout). As summarized in this section, biocementation has been applied at large scales and continues to be investigated as an alternative to the more traditional mechanical or chemical means of soil stabilization. Many of the current stabilization methods are reliant on mechanical means or materials with a significant energy and environmental footprint (Behnood 2018; Mujah et al. 2017). One of the most common methods of biomineralizing soil is microbially induced calcium carbonate precipitation (MICP) treatment, which allows soil particles to be bound together by microbially precipitated calcium carbonate, providing a stable connection (or "bridge") between the particles while maintaining sufficient permeability for water to infiltrate (Kalkan 2020; Mujah et al. 2017) (Fig. 5).

MICP-based soil stabilization can principally be accomplished in two ways: biostimulation and bioaugmentation. Biostimulation is achieved by providing nutrients to indigenous bacteria in order to stimulate growth and precipitation, which can be beneficial in order to adapt to certain local environmental conditions such as temperature or regulatory restrictions. Bioaugmentation is the method of supplementing the soil with exogenous bacteria and has been researched more extensively for soil stabilization than biostimulation. Bioaugmentation is useful when hoping to achieve desired stabilization parameters quickly, and usually pre-cultivated organisms are used with well-known characteristics, such as *Sporosarcina pasteurii*. The ability to improve soil onsite using biocementation could significantly reduce the costs of soil improvement due to a decrease in costs associated with manufacturing and transporting materials, such as cement, bentonite, or other materials, used in more traditional soil stabilization methods (Gowthaman et al. 2019; Kalkan 2020; Mujah et al. 2017). Additional benefits might be achieved when biostimulation is utilized instead of bioaugmentation, because the materials and energy needed for pre-cultivating and transporting the bacteria are eliminated. In addition, more uniform precipitation may be achieved in the presence of native bacteria instead of injecting bacteria or enzymes into the soil and creating gradients of bacterial or enzyme concentrations, which in turn often result in nonhomogeneous mineral precipitation, though this risk can be alleviated through the use of well-designed injection strategies (Barkouki et al. 2011; Ebigbo et al. 2012; Hommel et al. 2016; Hommel et al. 2015). In addition, there is a risk of ecological impacts from introducing large amounts of non-native bacteria (Gomez et al. 2019), and introducing non-native organisms might also face regulatory restrictions. Overall, biostimulation may have the advantage of relying on organisms that have adapted to the local environment, including—but not limited to—adaptation to the prevailing dominant electron acceptor(s), but biostimulation-based implementations might be slower, at least initially, than bioaugmentation-based approaches (Gat et al. 2016; Phillips et al. 2013a).

Research by Gat et al. (2016) on the potential for biostimulation in arid and low nutrient environments, such as in coastal liquefiable sand, showed promise. This study investigated simple carbon sources (molasses and yeast extract), which are rich in organic carbon, and found that mineralization can be achieved even when only using molasses. This could be beneficial for future research and applications aimed at achieving soil stabilization using relatively common and inexpensive carbon sources, and reducing additional costs associated with bioaugmentation. One important note from this research is the recommendation of promoting slow increases in pH when attempting biostimulation because rapid pH increases can cause significant alterations to the native soil community (Gat et al. 2016).

Dhami et al. (2017) performed a comparison between bioaugmentation and biostimulation using soil samples from the Margaret River region in Australia. In the bioaugmented treatments, both *S. pasteurii* and *Bacillus cereus* were used for urease production and carbonic anhydrase production, respectively. Biostimulation and bioaugmentation promoting ureolysis were found to be more effective than bioaugmentation with carbonic anhydrase producers. It was also demonstrated that when high amounts of nutrients are present in the soil—either naturally occurring or through injection—both ureolytic and carbonic anhydrase augmentations were found to be effective, and the carbonate crystals from MICP grew to larger sizes than under low nutrient conditions. In addition, *S. pasteurii* and *B. cereus* were able to survive alongside indigenous organisms in high nutrient conditions, but the native bacterial populations experienced significant changes regardless of the treatment method. A key takeaway from these results was that the choice of biomineralization method may largely depend on the organic carbon content of the soil and the time available for the treatment. Biostimulation seems to be very useful in carbon-rich environments, while bioaugmentation paired with supplemental nutrients may be favored in low nutrient soils (Dhami et al. 2017).

Gomez et al. (2019) compared the differences in biocementation between bioaugmentation with *S. pasteurii* and biostimulation of sandy soils using column experiments. Gradients of cementation developed throughout the columns and final calcite content between 0.5% and 5.3% by mass were observed. Near the inlet the soil was highly cemented, resulting in cone penetration resistances increasing by over 500% (up to 32.1 MPa) in the treated soils, and the shear wave velocities increased by 600% (up to 967 m/s). In addition, the biostimulated columns contained larger calcite crystals than the columns bioaugmented with *S. pasteurii*, while *S. pasteurii*-bioaugmented columns produced more overall crystals of smaller size (Gomez et al. 2019). Differences in amount, distribution, and crystal size of calcium carbonate could have an influence on the mechanical properties of porous media. This along with other work demonstrates strategies for achieving appropriate soil improvements depending on how soil stabilization is performed, therefore allowing customization based on the needs of the project and the regulatory framework.

Cost uncertainty is still one of the limitations of biomineralization for soil stabilization because so much of the research has been done on the laboratory scale using analytical-grade reagents. Using high purity reagents could be cost prohibitive for full-scale applications due to the amounts of urea and calcium chloride needed. Omoregie et al. (2019) compared the feasibility of using technical-grade reagents with laboratory-grade reagents along with using tap water or deionized water when preparing cementation solutions. Surface strength generally increased with increasing cementation solution concentrations, regardless of whether analytical- or technical-grade chemicals and water were used. Samples treated with the highest concentrations (1.0 M) achieved surface strength values of at least 4826 kPa when measured from the top surface (the handheld pocket penetrometer used to measure this had a maximum range of 4826 kPa). The surface strengths varied for the samples treated with 0.25 M, 0.5 M, and 0.75 M without a clear relationship between surface strength and type or concentration of cementation solution. Post-treatment XRD analyses revealed nearly identical calcium carbonate content and polymorph composition with ~93% calcite and ~ 7% vaterite for both the analytical-grade and technical-grade chemicals (Omoregie et al. 2019). These results along with the cost analysis indicate that technical-grade media may serve as a cost-effective alternative for biomineralization-based soil stabilization (Omoregie et al. 2019).

As described in Behnood (2018), one of the most common soil stabilization practices is chemical grouting. However, chemical grouting can be expensive, and it can be difficult to treat large volumes of soil due to the fairly high viscosity requiring high injection pressures and the viscosity increase over time (Mujah et al. 2017). Similar issues have been encountered with soil biocementation; rapid precipitation and premature clogging of the injection site can occur if the bacterial suspension and the cementation solutions are injected simultaneously. Ebigbo et al. (2012) demonstrated that injection site clogging can be avoided and a much more uniform distribution of calcium carbonate can be achieved through an injection strategy, which includes rapid injection of bacteria, followed by a bacteria- and urea-free rinse, a rapid injection of a calcium and urea mixture, another calcium-free rinse, and a rest period to allow for precipitation. Mujah et al. (2017) reported a similar strategy, during which the bacterial suspension was injected first, followed by the calcium-urea solution to achieve a more homogenous soil treatment.

Another difference between chemical methods and biomineralization is the speed of the cementation process. Depending on numerous environmental factors such as temperature, concentrations of nutrients, pH, etc. the microbes and enzymes involved in the biocementation process can exhibit varying levels of activity which can make biocementation a more complex and slower process than chemical grouting. However, biomineralization-based grouting of soils might provide a sustainable alternative to chemical grouting due to lower toxicity, lower energy requirements, and tunable rates of solidification depending on bacterial (or enzyme) activity and supply of biocementation agents (Mujah et al. 2017; Reddy et al. 2013).

However, there are potential drawbacks with a biomineralization approach to soil stabilization. When the process involves ureolysis, the production of ammonia is a concern, especially when it has the potential to leach into groundwater. There are strategies to alleviate this potential threat through treatment of the process water and potentially utilizing the produced ammonium as fertilizer for local agriculture (Mujah et al. 2017). The effects of adding nutrients to soil and groundwater are also not always readily predictable but may contribute to the growth of unwanted microbes (Reddy et al. 2013).

Fly ash, a waste product from coal combustion, has not only been repurposed for building materials, it is also used in many applications including stabilization of soil and embankments. While the load bearing capacity of fly ash is generally lower than soil, ureolytic biomineralization using *S. pasteurii* stabilized fly ash and increased compressive strength by 25–390% (Yadav et al. 2020). In specific, fly ash samples were treated with the same number of bacteria and varying concentrations of urea and $CaCl_2$ between 0.1 M and 0.25 M. Relationships between fly ash particle size and bacteria size were determined to be important for the movement of the bacteria, with a particle size 50–400μm being the most favorable for bacterial transport (Yadav et al. 2020). The strength of untreated fly ash was 37.19 kPa compared to 183.31 kPa for the 0.1 M urea and 0.25 M $CaCl_2$ mixture. The other concentrations resulted in strength values ranging from 46.47 kPa to 171.61 kPa. The strength increase was attributed to higher amounts of calcium carbonate and a more uniform distribution of calcite throughout the fly ash. Significant reductions in permeability (by about one order of magnitude) were observed after some of the treatments compared to the untreated fly ash (Yadav et al. 2020).

Maintaining permeability during biomineralization treatments is important, since it allows for continued injections at relatively low injection pressures and increases the treatable volume of soil, fly ash, or similar (Yadav et al. 2020). van Paassen (2011) and Ebigbo et al. (2012) specifically discussed the importance of maintaining permeability to achieve homogeneous distributions of $CaCO_3$ precipitates and how biomineralization can provide significant advantages over traditional chemical grouting techniques using resins, gels, or cement, which often drastically affect soil permeability. Ebigbo et al. (2012) developed an injection method (described in some detail above) and demonstrated experimentally and through mathematical modeling that this easily implemented method allows for a fairly homogeneous distribution of precipitated calcium carbonate. van Paassen's work (2010a and 2011) involved significant laboratory testing on sand columns and eventually a 100 m^3-scale demonstration in a testbed. It was found that approximately 43 m^3 of the sand were successfully cemented and had compressive strengths of up to 12 MPa. The cementation patterns on the 100 m^3 scale followed the patterns of the fluid flow through the soil but were unfortunately not homogenous (van Paassen et al. 2010a; van Paassen 2011). Subsequent laboratory and field tests were performed by van Paassen et al. to demonstrate the feasibility of stabilizing gravel beds with biogrout. Borehole collapse during horizontal directional drilling is a common problem, so several laboratory experiments, a mesoscale experiment in a 3 m^3 container of gravel and a field demonstration, were performed in the Netherlands. Gravel bed stabilization was successful, and up to 6% of the total dry weight of field samples were described to be calcium carbonate (van Paassen 2011).

Although biomineralization for the purpose of soil stabilization has primarily been performed using urea hydrolysis-induced calcium carbonate precipitation, there is emerging research into other microbially induced methods as well. Specifically, nitrate reduction-induced calcium carbonate precipitation may be a promising alternative and is often referred to as MIDP (microbially induced desaturation and precipitation). Initial successes on the laboratory and field scale have been demonstrated (van Paassen et al. 2010b; Wang et al. 2020a, b; Zeng et al. 2021). Nitrate reduction promotes calcium carbonate precipitation according to the reaction outlined in Table 1 with nitrogen gas being the major by-product. In contrast to the ammonium produced during urea hydrolysis, the nitrogen gas would not require removal and can result in desaturation of the soil, which can be desirable when trying to reduce the liquefaction potential of soils (Fig. 6). Both urea-hydrolyzing and nitrate-reducing microorganisms are ubiquitous in soils, and the addition of substrates such as calcium acetate and calcium nitrate will readily stimulate growth of denitrifying microbes. As outlined in Table 1, the activity of denitrifiers will increase carbonate alkalinity and promote the precipitation of calcium carbonate. A potential drawback of this method can include slower than urea hydrolysis-induced calcium carbonate precipitation because denitrification is a growth-dependent process while urea hydrolysis may be growth-independent. Furthermore, the produced nitrogen gas can impact the permeability and thus the calcium carbonate distribution through MIDP treatment. In addition, nitrite, nitrous oxide, and nitric oxide are all potential unwanted by-products, which can accumulate if nitrate reduction is incomplete, and thus would also require treatment of the process water and potentially soil gases (van Paassen 2011).

Wang et al. (2020b) recently summarized the potential for nitrate reduction-induced MIDP to stabilize soils that are prone to liquefaction in an effort to reduce

Fig. 6 Left, untreated loose soil can cause foundations to fail and structures to be damaged. Right, soil stabilized using engineered biomineralization

the risk to structures built on these types of soils. There are a number of research and development projects that have focused on the potential of using the combined desaturation of and calcium carbonate precipitation in porous media to improve soil properties (O'Donnell et al. 2017a, b; Pham et al. 2018). Media optimization demonstrated that denitrification and $CaCO_3$ precipitation rates were maximal with only small amounts of toxic by-products accumulating when the molar carbon to nitrogen ratio was 1:1.6 (Pham et al. 2018). Based on measurements of pore water pressure, volumetric strain and cyclic shear resistance behavior soils treated with MIDP were found to be at a significantly lower risk for liquefaction (He et al. 2014). Even when the saturation only decreased by 5%, shear strength was significantly increased (He et al. 2014). Wang et al. (2020b) reported details of work demonstrating that even a single MIDP treatment can reduce the saturation of soils to 80%; however, the saturation and mechanical properties achieved through MIDP appeared to vary depending on the type of soil and number of applications (Wang et al. 2020b). In summary, biomineralization-based soil stabilization technologies are beginning to mature to full-scale applications, and their potential is beginning to be recognized by contractors and customers. It is expected that full-scale applications of biomineralization-based biogrouting technologies will become common over the next decade.

2.2.2 Mine Tailings and Bioremediation

Remediation of mine tailings is another promising application of biomineralization. Mine tailings are often problematic because of the danger of collapse and the potential of releasing metals such as Cu, Pb, Zn, Cd, Cr, Se, and As, thus posing a risk to human and environmental health. Heavy metals can leach through rain- or

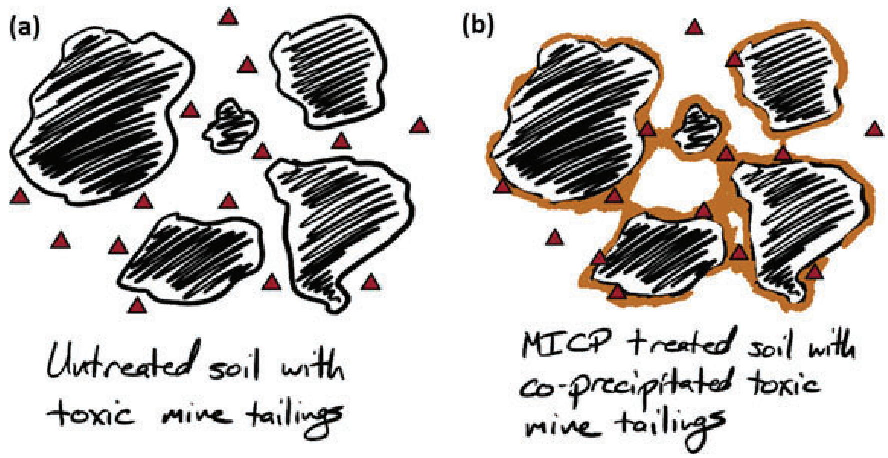

Fig. 7 (**a**) Untreated soil in the presence of toxic metals (red triangles) from mine tailings, (**b**) the biomineralization process can coprecipitate toxic metals initially present in the solution while also providing additional surface area for sorption

snowmelt waters. Immobilizing or otherwise shielding heavy metals from exposure to water can reduce the metal leaching rate (Reddy et al. 2013; Yang et al. 2016). There are a number of more traditional treatment methods that can be applied, including phytoremediation, thermal treatments, excavation, electro reclamation, and capping (Reddy et al. 2013; Yang et al. 2016). Biomineralization of soils or mine tailings loaded with heavy metals, as demonstrated by Yang et al. (2016), has been proposed as a treatment considering how stable MICP precipitates can be in multiple geological settings (Fig. 7).

Yang et al. (2016) isolated the urease-producing bacterium, *Bacillus firmus*, from acidic copper mine tailings at the Xinjiang Copper Industry Co. Ltd. in China. *B. firmus* XP8 exhibited high urease activity and resistance to high metal concentrations. XP8 cells were grown overnight and exposed to the contaminated soil for 7 days; uninoculated control samples maintained a pH of 5.6, while the pH of inoculated mine tailings rose from 5.6 to 7.7 within 7 days. The original soil had a pH of 2.7 because it had not yet been amended with the nutrient media. Soil pH is an important factor when considering this method of metal immobilization because higher pH encourages precipitation by affecting the solubility of metal hydroxides, carbonates, and phosphates (Yang et al. 2016). Because the bioavailability of a metal can influence its resulting toxicity, sample analysis was performed for five different fractions of metals (soluble-exchangeable, carbonate-bound, Fe/Mn oxide-bound, organic/sulfide-bound, and "residual fraction") to estimate bioavailability. The metal content of each fraction was divided by the total amount of that specific metal found in the mine tailing soil; this ratio was termed the distribution coefficient. Carbonate-bound metals increased after MICP treatment, while the soluble-exchangeable fractions decreased (Yang et al. 2016) demonstrating the ability of biomineralization-based technologies to reduce the bioavailability and mobility of toxic metals.

Zhao et al. (2017) found significant potential for MICP to be a sustainable and efficient method for treating water contaminated with heavy metals. Zhao et al. (2017) observed a cadmium removal efficiency of ~53% (10–4.7 mg/L) after 3 hours, and 61% after 48 hours using MICP promoted by a *Bacillus* sp. isolated from mine soil. Mugwar and Harbottle (2016) investigated the MICP-based remediation potential for cadmium, zinc, lead, and copper using *S. pasteurii*. They observed that, when the medium was amended with urea, microbial activity continued even in the presence of metal concentrations that were higher than previously determined minimum inhibitory concentrations (MICs). Their research suggests that even though microbial activity might be inhibited in the presence of heavy metals, with urea present there is sufficient ureolytic activity to promote the coprecipitation of heavy metals using MICP. As the metals coprecipitate, the toxicity of the solution is reduced and enables increased hydrolysis and precipitation. These results indicate that MICP remediation is possible even at metal concentrations significantly higher than estimated based on toxicity data as long as a large enough proportion of the initial microbial population survives, commences urea hydrolysis, and induces calcium carbonate precipitation. Li et al. (2013) performed a similar study and found that a strain of *S. pasteurii* was able to achieve greater than 90% removal of cadmium, zinc, copper, and lead within 2 h. Results of control treatments were not reported by Li et al. (2013); thus, differences in the rates and extent of metal removal compared to other studies could be due to abiotic processes (Li et al. 2013; Mugwar and Harbottle 2016).

Kumari et al. (2014) compared the ability of the ureolytic bacterium *Exiguobacterium undae* to remove cadmium from soils at 10 °C and 25 °C. After the original sample of soil was air-dried and autoclaved, 100 mg of $CdSO_4$ was added per 1 kg of soil followed by addition of an overnight culture of *E. undae*. Forty-five percent of Cd^{2+} were immobilized within 1 h at both temperatures, and after 5 h the immobilized Cd^{2+} reached 79% and 84% for 10 °C and 25 °C, respectively. After 2 weeks, the more bioavailable, soluble-exchangeable fractions of cadmium had been reduced by about 97% relative to untreated controls at both temperatures. Over the same 2-week period, the more stable, carbonate-bound form of cadmium in the soil increased approximately threefold to 71.4 mg kg^{-1} and 67.8 mg kg^{-1} for the 25 °C and 10 °C MICP treatments relative to untreated controls (Kumari et al. 2014).

As mentioned at the beginning of this section, the stabilization of mine tailings is also important. Gowthaman et al. (2019) evaluated the effectiveness of stabilizing soil slopes in cold subarctic regions by isolating *Lysinibacillus xylanilyticus*, an indigenous, ureolytic bacterium from soil samples taken from Onuma, Hokkaido, Japan. The bacterium was tested for growth and urease activity at temperatures ranging from −10 °C to 50 °C to address the potential of MICP for the treatment of mine waste tailings in colder regions. Most work, so far, has only been performed within the temperature range of 25 °C–60 °C (Gowthaman et al. 2019; Kumari et al. 2014). Little growth of *L. xylanilyticus* was observed above 40 °C, and urease activity appeared to be insignificant below 5 °C and above 25 °C, but relatively stable between these temperatures (Gowthaman et al. 2019). Soil stabilization

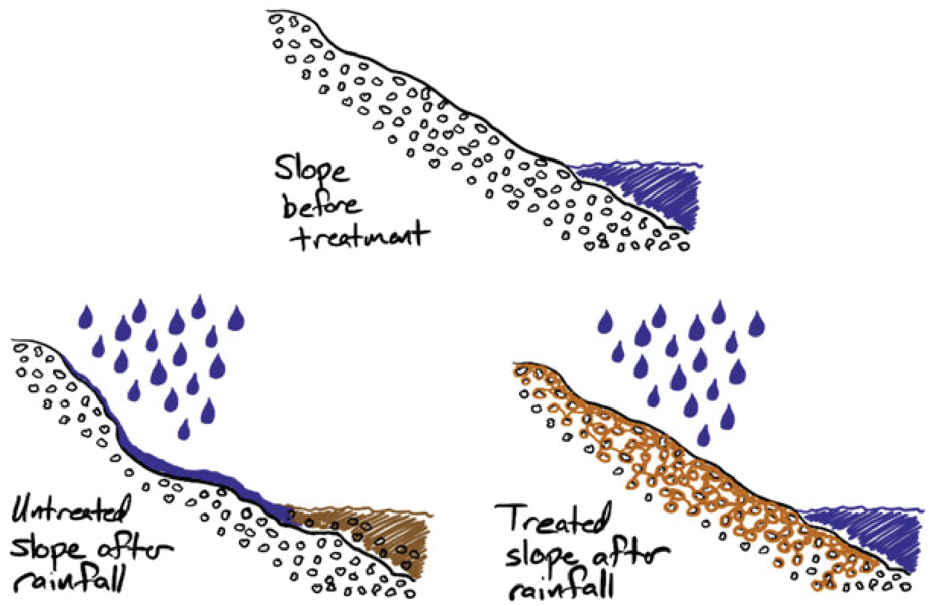

Fig. 8 Biomineralization treatments can stabilize slopes reducing erosion during runoff, snowmelt, etc.

experiments were performed with three types of sand with average particle sizes (D_{50}) of 1.6 mm, 0.87 mm, and 0.2 mm as well as with soil samples; the sand fraction of the soil had an average particle size (D_{50}) of 0.23 mm. In column experiments the MICP-treated sand was found to have a UCS (uniaxial compressive strength, estimated using a needle penetrometer) about 3.75 times higher than untreated sands. The authors concluded that the fine particles present in the slope soil—about 12% of this soil consisted of particles with a grain size of less than 125μm—were beneficial in providing support for the calcium carbonate bridging between larger sand grains. It was also noted by Gowthaman et al. (2019) that very fine particulate soils may have limited permeability that could limit infiltration of bacteria and cementation solutions into the soil, a challenge discussed above in the context of fly ash stabilization in the "Soil Stabilization" section. Recognizing that fine particles can aid in stabilization but can become problematic at higher percentages within the soil should be an important consideration in future biomineralization technology scale-up.

Gowthaman et al. (2019) also tested samples modeled as a physical slope instead of a column (Fig. 8). More than 80% of the soil was successfully stabilized with a 3–4 cm layer of cemented soil at the surface, while the soil below that layer was not successfully stabilized. The UCS of the slope surface was determined to be between 2 and 8 MPa with the higher strength values being obtained from the lower areas of the slope. This difference was attributed to the injection method, which likely caused the bacteria being transported preferentially toward the bottom of the slope sample.

2.2.3 Dust Suppression

Another problem being addressed by utilizing biomineralization is dust suppression and erosion control. Among other contributors to erosion, wildfires are becoming more frequent, and the altered chemical and biological makeup of burned soil can lead to problems with erosion, water quality, flood control, and general ecosystem health (Hodges and Lingwall 2020). Current technologies have not been able to provide a cost-effective method for treating large areas affected by forest fires in order to prevent erosion (Hodges and Lingwall 2020). Hodges and Lingwall (2020) explored the effects of wind and water on burned soils that were treated using MICP; both biostimulation and bioaugmentation were evaluated. *S. pasteurii* and different concentrations of biomineralization solutions, which consisted of urea, nutrient broth, ammonium chloride, and $CaCl_2$, were applied to soil samples (Fig. 9). In addition, some soil samples were treated with only the urea-broth solution with bacteria and no added calcium or with only the urea and Ca^{2+} solutions but no added bacteria to compare potential benefits of bioaugmentation vs. biostimulation. Erosion tests were performed at 10, 20, and 30 mph simulated wind speed on burned and unburned soils subjected to the above treatments (Hodges and Lingwall 2020). Successful treatments created a water-permeable crust that stabilized the soils through an increase in soil strength by 25–50 kPa. These MICP treatment methods were generally not effective against mass loss at higher wind speeds, but even a single application resulted in some stabilization and a thin crust layer which could play an important role in protecting the soil for long enough to allow new plant growth to develop and further stabilize the soil. At lower wind speeds, these biomineralization methods were more successful and proved effective for dust control for both burned and unburned soil. Similar to the smaller particles discussed above in the “Mine Tailings and Bioremediation” section, ash particles could be improving the stabilization of the soils by promoting calcium carbonate bridging between larger sand grains. In addition, it was found that there were beneficial effects through treatment with only urea solutions, only calcium solutions, or without the addition of bacteria. The reasons for these improvements have not

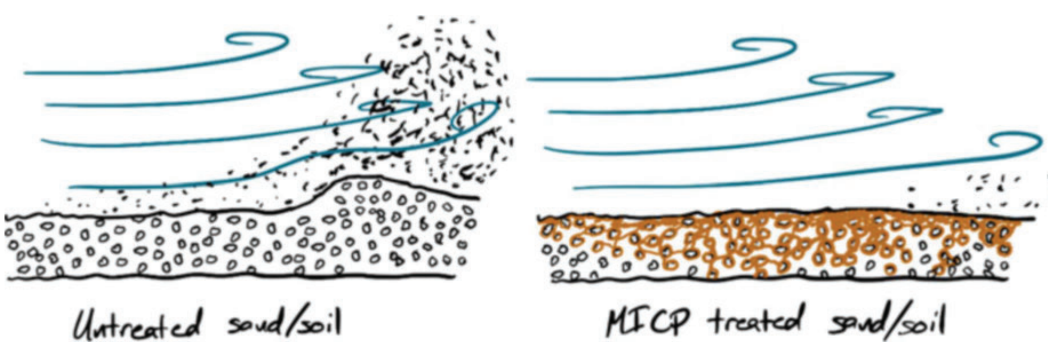

Fig. 9 Schematic representation of dust suppression using engineered biomineralization treatments. Microbially produced mineral precipitates cement together fine particles that would otherwise be eroded as dust during wind and weather events

been explored in depth, and further tests are being performed (Hodges and Lingwall 2020).

Similar to burned soils, sandy soils can also contribute to dust pollution, and many regions could benefit from dust suppression. Almajed et al. (2020) explored the use of enzymatically induced calcium carbonate precipitation (EICP) treatment to manage wind-caused erosion of desert sand. Multiple concentrations and combinations of urea, calcium chloride, sodium alginate (SA, a biopolymer), and powdered milk were applied to sand samples; EICP was promoted using jack bean urease. Multiple treatments were evaluated, including different molar urea to calcium chloride ratios (1:0.67 and 1:1.25); SA solutions of 0.5%, 1.0%, and 2.0%; and treatments with only $CaCl_2$, along with samples treated with only water or untreated samples (Almajed et al. 2020). All treatments, except the untreated controls and water-only treatments, formed a crust, and the resistance to wind erosion increased after curing the samples for 7 days. The erosion resistance remained effective after 28 days with erosion rates (percent of mass loss), staying below 0.01% for all treated samples. This represented a significant improvement over the erosion rate of the control and water-treated samples which were around 97% and 94%, respectively (Almajed et al. 2020). Similar to previous studies, the points of contact between soil particles were stabilized by the presence of calcium carbonate, which contributed to erosion resistance. An additional factor contributing to the wind resistance in the SA treated soils was the formation of alginate hydrogels created as divalent calcium ions replaced sodium ions within the sodium alginate. While the developed hydrogel increased the soils' ability to resist erosion and retain water, it also reduced the permeability of the soil making it more difficult for EICP treatment solutions to penetrate deeper into the soil; as a result, thinner crusts were observed in the SA-treated soils (Almajed et al. 2020).

Research and development are continuing with the goal of increasing the effectiveness and usefulness of biomineralization for multiple stabilization applications, including soil stabilization, stabilization, and immobilization of metals in mine tailings and other bioremediation applications, as well as in dust suppression. The use of microorganisms and enzymes to promote mineral precipitation offers potential benefits, including being more environmentally friendly, reducing costs, and energy usage. However, there is still limited use of this technology on the larger scale, and further research and development is needed.

2.3 Subsurface Applications

The earth's subsurface contains many important resources, such as water, minerals, oil, and gas. The subsurface is also used for the storage of natural gas and other compounds, such as wastes, wastewater, and CO_2 (Baines and Worden 2004; Bauer et al. 2013; Ferguson 2015). In addition, the subsurface is used for the purification of useful products, such as drinking water. A concern exists that contaminants from natural or anthropogenic sources can enter the subsurface where they can pose a risk to human and environmental health. Technologies exist to inject or extract compounds into or out of the subsurface, and engineering applications, such as oil production, deep subsurface mining, groundwater management, and subsurface remediation, are common (Montana Emergent Technologies 2020; National Research Council 2005; Yudhowijoyo et al. 2018).

Leakage pathways around wells or in their vicinity can be problematic for any subsurface application since they can lead to loss of injected or stored fluids as well as result in inefficient recovery. However, safe injection into, storage in, and recovery from the subsurface are essential, and ensuring wellbore integrity is in turn essential for environmental and economic reasons. Wellbore integrity can be defined as the "application of technical, operational and organizational solutions to reduce risk of uncontrolled release of formation fluids throughout the life cycle of a well," and wellbore integrity concerns are often related to the development of leakage pathways (NORSOK Standard 2013). Leakage pathways can often be sealed using cement injections ("cement squeezes") with Portland cement remaining the most commonly used wellbore integrity remediation agent (Kirkland et al. 2020; Yudhowijoyo et al. 2018). Unfortunately, in some cases leakage pathways consist of very small aperture fractures or delaminations that can be difficult to seal because the low injectivity of these small aperture leakage pathways might require injection pressures higher than permissible during wellbore remediation, due to concerns regarding damage to the well or the formation. Thus, cement squeezing might be ineffective or even impossible because the fairly large cement particles simply might be too big to effectively enter and seal small apertures (Phillips et al. 2018). An advantage of biomineralization-based methods is that the minerals are formed in situ by microbes from aqueous solutions. Microbes are only a few micrometers in size and can therefore access areas inaccessible to regular cement (Cunningham et al. 2011; Kirkland et al. 2019; Phillips et al. 2013a; Phillips et al. 2018).

Enhanced oil recovery, wellbore sealing, and secure subsurface CO_2 storage are some of the applications, which have been demonstrated on the field scale using engineered biomineralization technology (Cunningham et al. 2011; Hommel et al. 2020; Mitchell et al. 2010; Montana Emergent Technologies 2020; Phillips et al. 2013b).

2.3.1 Finite Resource Recovery

Enhanced oil recovery (EOR) operations are designed to increase the recovery of finite oil and gas resources from existing reservoirs (Alvarado and Manrique 2010). Enhanced oil recovery is generally achieved through a process called "sweeping," during which fluids are injected into oil-bearing formations to push remaining oil out of the formation. Often water is injected into one well to "push" oil toward another well, which is pumping ("pulling") the oil out of the ground (Thomas 2008). The process becomes challenging if high permeability zones exist, through which the injected water escapes. These zones are often referred to as "thief zones" and transport injected fluids at rates faster than the oil-bearing formation, therefore

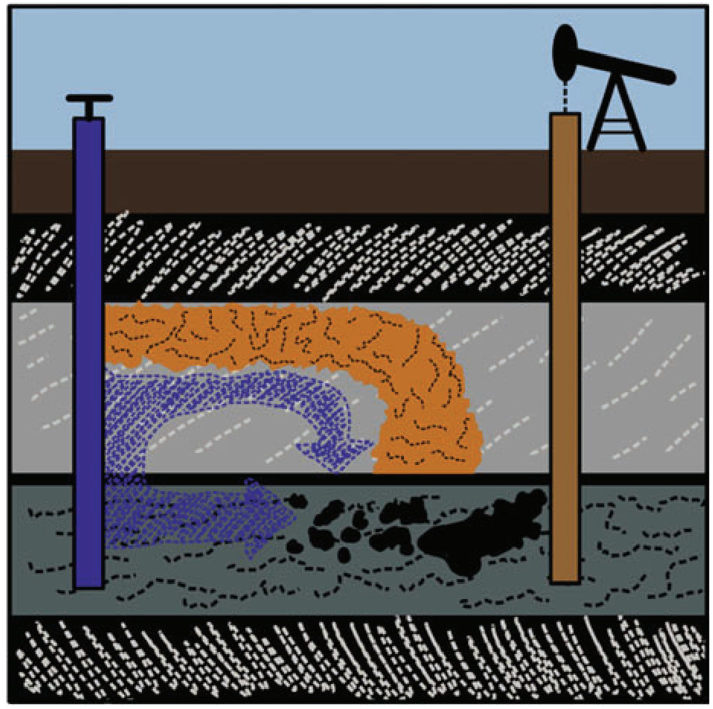

Fig. 10 Enhanced oil recovery enabled through microbial mineral formation. Biominerals (orange) block fractures or high permeability thief zones, thus directing the flow of sweeping fluids such as water or CO_2 (blue) through resource-rich zones, enhancing the recovery of oil (black)

reducing the net recovery of resources (Kirkland et al. 2020; Sen 2008). Even though having higher permeability, these thief zones still consist of formations with fairly small pores or fractures (Fig. 10). It can be challenging to reduce the permeability of these zones reliably using cement injection since cement cures over a finite amount of time and it is necessary to control where cementing occurs.

Biocementation fluids have viscosities similar to water and can be transported with the injection water into thief zones where the enzymes or bacteria adhere to the pore or fracture surfaces. Subsequent injection of biocementation fluids (e.g., urea- and calcium-containing solutions) promotes precipitation of minerals (e.g., calcium carbonate) in the areas where bacteria or enzymes are present, which reduces permeability (Ebigbo et al. 2012; Kirkland et al. 2020; Sen 2008). When permeability is reduced sufficiently in the thief zone, injected sweeping fluids will travel through oil-bearing zones for additional resource recovery as demonstrated by Kirkland et al. (2020).

Hydraulically fractured horizontal oil and gas wells in unconventional formations accounted for $<5\%$ of wells drilled in 2006 but $>75\%$ by 2016; these wells are now responsible for approximately 50% of US oil and gas production (EIA 2016; EIA 2020). However, these unconventional wells can exhibit rapid decline in production (as much as 60–80% after the first year) (Thomas 2008). Currently, wells drilled and hydraulically fractured to extract oil and gas may recover only 10% of the fossil fuels present in the formation because only the oil and gas close to a fracture can be extracted reliably due to the very limited fluid mobility in shale rocks (Thomas 2008). Thus, significant amounts of producible (but not accessed) reserves remain in the reservoir. One way to recover more oil and gas from these wells is by blocking old fractures with diverting agents and refracturing the formation to open new fractures in the reservoir. Existing diverting agents are difficult to control in wells that may be several miles deep, but biomineralization may represent a new diverting agent technology that could be used to improve the success of refracturing to enhance the recovery of oil and gas from declining wells. However, additional research including an expansion of the temperature range, in which biomineralization could be used, and demonstrations at the reservoir scale are needed to assess the actual feasibility in the subsurface.

2.3.2 Wellbore Integrity

Oil and gas wells have been drilled for more than a century, and there are about 2.3 million abandoned and more than 900,000 active wells in the USA alone (EIA 2020; Townsend-Small et al. 2016). Many wells develop leaks, especially as they age (Boothroyd et al. 2016; Dusseault et al. 2000). Wells develop leaks because subsurface pressures and temperatures vary, resulting in contractions and expansions, shrinkage or cracking in the cement over time, and through ground movement from earthquakes or the drilling of nearby wells. Leaking wells are a problem for the oil and gas industry in several ways. First, the lost hydrocarbons represent lost revenue. Second, lost hydrocarbons are a source of air and water pollution in the vicinity of leaking wells and may even pose acute risks to human life in the case of dangerous gas buildup (Boothroyd et al. 2016; McKenzie et al. 2012). Third, even perceived pollution in the vicinity of an oil or gas well can cause negative public perception that is difficult to overcome and can cause financial harm. Repairing leaky wells potentially thousands of feet below surface can be expensive and is often unsuccessful (Bagal et al. 2016; Montana Emergent Technologies 2020).

Phillips et al. (2018) described the use of biomineralization to remediate a leakage pathway (channel) 310 m belowground located in the well cement at a well in Alabama. It was observed that MICP treatment using conventional oil field subsurface fluid delivery technologies (packer, tubing string, and a slickline deployed bailer) was successful in sealing the compromised wellbore cement. The authors injected urea-calcium solutions and microbial suspensions (*Sporosarcina pasteurii*). Injectivity decreased with the number of MICP treatments. A decrease in the pressure decay after shut-in, a measure of improved wellbore integrity, was also observed. The authors also observed a substantial deposition of precipitated solids in the original flow channel when comparing the pre- and post-MICP treatment cement bond logs suggesting the biomineralization treatment sealed the channel and could be used to remediate leakage pathways in oil and gas wells (Fig. 11).

Montana Emergent Technologies (MET) has trademarked a process called BioSqueeze, which uses ureolysis-induced calcium carbonate precipitation to seal difficult to seal wells (Montana Emergent Technologies 2020). Much of the required R&D was conducted in collaboration with Montana State University and has been published in various peer-reviewed journals (Kirkland et al. 2021a, b; Kirkland et al. 2019; Kirkland et al. 2020; Phillips et al. 2018). So far, MET has successfully

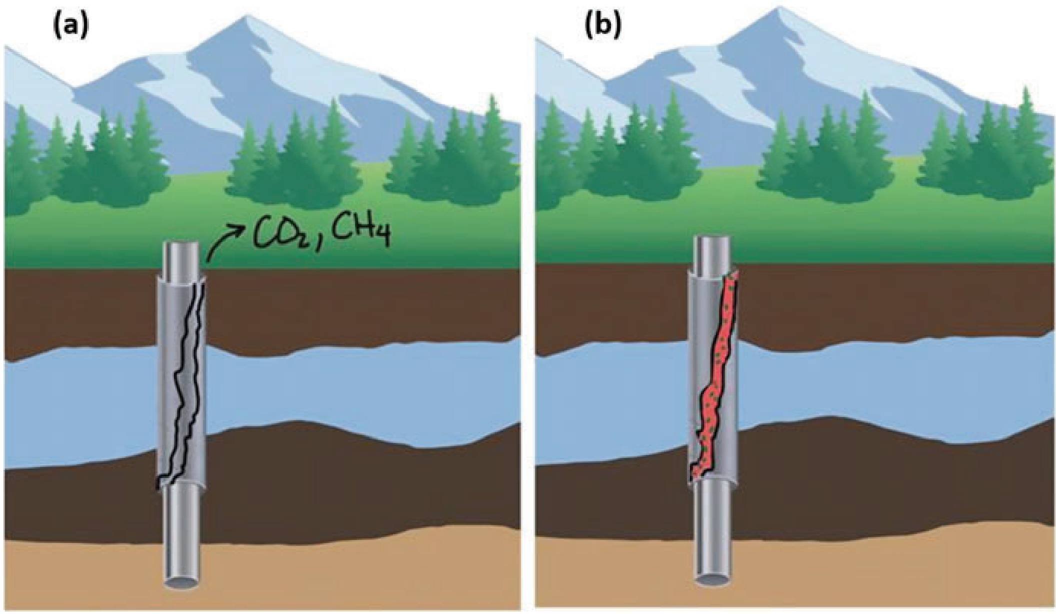

Fig. 11 (**a**) When wellbore integrity is disturbed, gas can leak through small apertures such as delaminations or fractures. (**b**) Microbial biomineralization has been demonstrated to be capable of sealing these small apertures and preventing the leakage of gases

completed more than 30 commercial scale BioSqueeze treatments often resulting in residual annular pressures of less than 1 psi.

Other envisioned applications are related to geologic carbon sequestration (GCS) and geothermal well drilling and operation. Concerns over global warming have stimulated a concerted effort to limit CO_2 emissions (IPCC 2014; IPCC 2018). Geologic carbon sequestration (GCS) has been proposed as one part ("wedge") in the battle of tackling the reduction of CO_2 emissions (Pacala and Socolow 2004). Carbon capture is suitable for large point sources, such as large fossil power plants and cement plants. CO_2 can be captured at the source and injected into the deep subsurface. One challenge with GCS is storage security, meaning the injected CO_2 must remain safely underground for at least decades, if not centuries (IPCC 2014; Kudryavtsev et al. 2012). Wellbore and caprock integrity are crucial to the success of GCS, and methods to seal potential leakage pathways around wells in aquifers containing CO_2 using ureolysis-induced calcium carbonate precipitation are under development (Kirkland et al. 2021a, b; Phillips et al. 2013b). Recently, Kirkland et al. (2021a, b) performed a biomineralization treatment of a compromised wellbore cement 300 m belowground under conditions that simulated the low pH that might be found in carbon sequestration storage environments. It was observed that biomineralization treatment reduced injectivity by 94% and that mineralization could be promoted in CO_2-affected brines. However, the authors concluded that additional research is required to assess the long-term seal integrity and ensure storage of CO_2 in GCS (Kirkland et al. 2021a, b).

Geothermal energy is gaining popularity since it represents a low carbon emission source for heat and electricity generation. The drilling of geothermal wells is expensive, and a major cause of nonproductive drilling time is the loss of drilling fluids, aka lost circulation (Alsaba et al. 2014; Denninger et al. 2015; Mansour et al. 2019; Marbun 2013). The loss of drilling fluids occurs when fractures or other high permeability zones are encountered during drilling. If circulation is lost, lost circulation materials (LCMs), such as sawdust, mica, graphite, calcium carbonate, nylon fibers, mylar, or walnut shells, are often injected at high rates to stop losses of drilling fluids (Boukadi et al. 2004; Nayberg 1987). Existing LCMs are not readily immobilized in the formation or can degrade or erode over time, potentially requiring continuous addition throughout the drilling process. Biomineralization of LCMs could result in immobilization and create a more durable seal against the loss of drilling fluids. The use of biomineralization to enhance LCM performance is in the early stages of technology development, and to the authors' knowledge, no field demonstrations have been performed yet.

2.4 Other Potential Applications of Biomineralization

Biomineralization has also been demonstrated to be useful in areas such as art. Limestone and rock have long been used in the creation of sculptures. Recently, a group of interdisciplinary engineering and art undergraduate students at Montana State University used ureolysis-induced calcium carbonate biomineralization to design and sculpt an approximately 0.9 m × 0.3 m × 0.3 m large replica of the Bridger Mountain range as well as an approximately 1 cm × 5 cm × 7 cm replica of the MSU mascot, the Bobcat, using biomineralization approaches (Fig. 12) (Troyer et al. 2017). The Bridger Mountain range is located just outside of Bozeman (MT, USA) and served as the inspiration for the biomineralized replica. As described in Troyer et al. (2017), the project was supported through a design contest, "Engineers Make a World of Difference," sponsored by the Norm Asbjornson College of Engineering at Montana State University to promote the spirit of discovery and imagination for the engineering students. Loose sand was treated with microbes and urea-calcium solutions until a solid cohesive block was formed roughly representing the outline of part of the Bridger Mountain range. Subsequent carving and sculpting of the relief resulted in a replica of the Bridger mountains. In the development of the

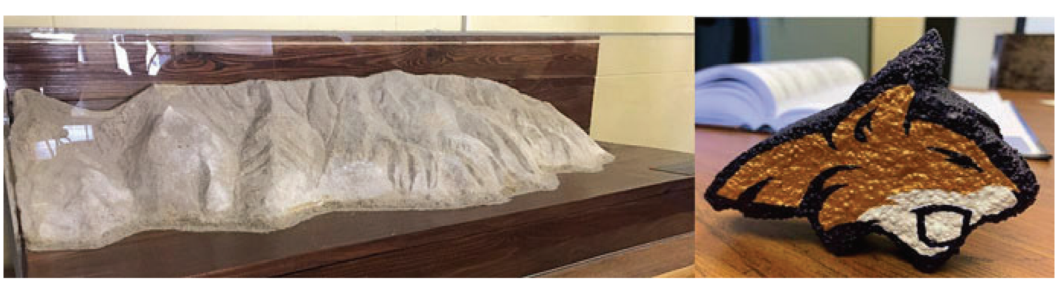

Fig. 12 (Left) 0.9 m × 0.3 m × 0.3 m biomineralized replica of the Bridger Mountain range. (Right) Biomineralized replica of the Montana State University mascot, the Bobcat

Bobcat mascot replica, ureolytic biomineralization techniques were used to biocement loose sand inside a Bobcat-shaped cookie cutter, which was subsequently painted in Montana State University colors.

2.5 Conclusions and Outlook

Biomineralization is a natural process with significant potential for engineering applications. While this book focused on various minerals and various processes related to the precipitation and dissolution of minerals through microbial activity, this chapter focused on developed and currently developing applications. A review is presented of parameters that influence biomineralization for engineering applications associated with development of novel construction materials, soil stabilization to mitigate hazards to public health, and subsurface applications such as improving wellbore integrity. By far, the most frequently utilized mineral is calcium carbonate, and urea hydrolysis is the most frequently researched microbial metabolism to initiate mineral precipitation. Applications in construction, soil stabilization, and the sealing of leaky wells have advanced the furthest, and some of them have been commercialized. Other technologies are on the verge to full-scale application and thus commercialization. Much research and development work remains to be performed in this field, and additional applications for biomineralization will be developed in the construction, environmental, biotechnology, and medical fields.

These research and development activities will contribute to the development of environmentally friendly methods and products as well as economic competitiveness. Biological manufacturing methods, such as engineered mineral precipitation, can significantly reduce energy-intensive cement manufacturing activities and contribute to resource and climate conservation. The world is expected to add more than two trillion square feet of new building space by 2060, which is equivalent to adding another New York City every month for the next 40 years (UN Environment 2017). Microbially induced calcium carbonate precipitation has the potential to reduce the net carbon footprint of building and construction materials, but work remains to develop this technology for the wide range of applications that cement currently dominates (Davies et al. 2018). In addition, biomineralization has been demonstrated to be useful in areas such as art. There are also applications where biomineralization does not directly compete with traditional cement, including the coprecipitation of certain groundwater contaminants such as strontium (Lauchnor et al. 2013; Mitchell and Ferris 2006a) and restoring the integrity of wells with ultrafine leaks. Engineered microbial mineral formation has grown from a theory and proof-of-principle vision to a technology being applied in the marketplace.

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