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Engineered applications of ureolytic biomineralization: a review

Adrienne J. Phillips[a,b]*, Robin Gerlach[a,b]*, Ellen Lauchnor[a], Andrew C. Mitchell[c], Alfred B. Cunningham[a,d] and Lee Spangler[e]

[a]*Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA;* [b]*Chemical and Biological Engineering Department, Montana State University, Bozeman, MT, USA;* [c]*Institute of Geography & Earth Sciences, Aberystwyth University, Wales, UK;* [d]*Civil Engineering Department, Montana State University, Bozeman, MT, USA;* [e]*Energy Research Institute, Montana State University, Bozeman, MT, USA*

Microbially-induced calcium carbonate ($CaCO_3$) precipitation (MICP) is a widely explored and promising technology for use in various engineering applications. In this review, $CaCO_3$ precipitation induced *via* urea hydrolysis (ureolysis) is examined for improving construction materials, cementing porous media, hydraulic control, and remediating environmental concerns. The control of MICP is explored through the manipulation of three factors: (1) the ureolytic activity (of microorganisms), (2) the reaction and transport rates of substrates, and (3) the saturation conditions of carbonate minerals. Many combinations of these factors have been researched to spatially and temporally control precipitation. This review discusses how optimization of MICP is attempted for different engineering applications in an effort to highlight the key research and development questions necessary to move MICP technologies toward commercial scale applications.

Keywords: calcium carbonate; urea hydrolysis; biofilm; MICP; mineral precipitation; mineralization

Introduction

Contrary to the commonly known detrimental effects of biofilms in industrial and medical environments, biofilms may be used for beneficial engineering applications. In particular, ureolytic biofilms or microbes which induce calcium carbonate ($CaCO_3$) precipitation (MICP) have been studied widely for beneficial use in construction materials, cementation of porous media, hydraulic control, and environmental remediation (Figure 1). A primary research focus has been controlling MICP by manipulating parameters that influence the saturation state to achieve specific engineering goals. In many cases, engineered applications depend on controlling the rate and distribution of $CaCO_3$ precipitation *in situ*, which is governed by the spatial and temporal variation in saturation state.

Several reviews addressing MICP for use in engineering, particularly, construction applications and cementation of porous media have been prepared previously. De Muynck, De Belie, et al. (2010) elegantly reviewed the role of MICP in enhancing and rehabilitating construction materials. Siddique and Chahal (2011) also reviewed MICP for use in construction materials, specifically focusing on concrete. Separately, Ivanov and Chu (2008) and DeJong et al. (2010, 2011) comprehensively highlighted the role of the biogeochemical MICP processes in soil and porous media systems. In addition, Al-Thawadi (2011) reviewed MICP for strengthening of sand. This review focuses on how the spatial and temporal control of MICP has been explored to treat construction materials, consolidate porous media, control hydraulics and remediate environmental problems.

Microbially-induced $CaCO_3$ precipitation

The involvement of microorganisms in mineral precipitation occurs *via* different mechanisms (Benzerara et al. 2011; Northup & Lavoie 2001; Fouke 2011). Firstly, biologically-controlled mineralization describes cellular activities which specifically direct the formation of the mineral, for example, the cell mediated process of exoskeleton, bone or teeth formation, or the formation of intracellular magnetite crystals by magnetotactic bacteria (Decho 2010; Benzerara et al. 2011). Secondly, biologically-influenced mineralization is the process by which passive mineral precipitation is caused through the presence of cell surfaces or organic matter such as extracellular polymeric substances (EPS) associated with biofilm (Decho 2010; Benzerara et al. 2011). Thirdly, biologically-induced mineralization is the chemical alteration of an environment by biological activity that generally results in supersaturation and precipitation of minerals (Stocks-Fischer et al. 1999; De Muynck, De Belie, et al. 2010). Often combinations of the three different processes are active at the same time in a system. For

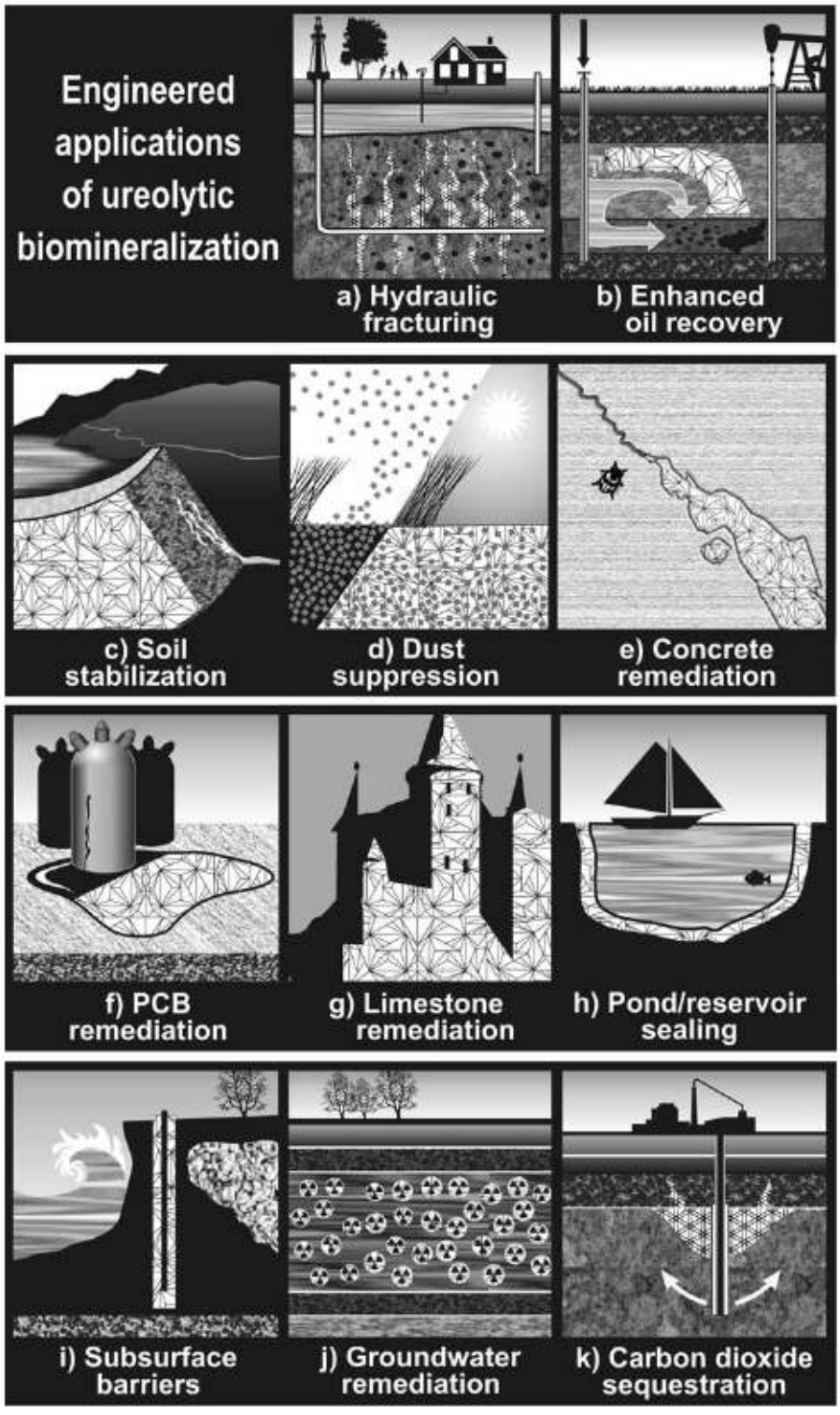

Figure 1. Proposed ureolysis-driven MICP engineering applications. White crystal hatch pattern represents $CaCO_3$. (a) Sealing subsurface hydraulic fractures (eg during well closure); (b) manipulating subsurface flow paths to improve oil recovery; (c) strengthening earthen dams or consolidating porous materials; (d) minimizing dust dispersal from surfaces; (e) sealing or remediating concrete fractures; (f) coating PCB-oil contaminated concrete resulting from leaking equipment; (g) treating or coating limestone or concrete to minimize acid erosion; (h) sealing ponds or reservoirs; (i) forming subsurface barriers to control salt water or contaminated groundwater intrusion; (j) remediating the subsurface contaminated with radionuclides or toxic metals (represented by radioactivity symbols); (k) treating fractures (in cap rocks, well bore cements, or casing/cement/formation interfaces) to mitigate leakage from geologically sequestered CO_2 injection sites.

instance, in the case of microbially-induced calcium carbonate precipitation or mineralization (MICP), where the cellular activity influences chemical conditions (saturation state) to promote mineralization, it is possible that biologically-influenced mineralization is also occurring since the cells themselves or their exudates may act as nucleation sites for $CaCO_3$ crystal formation (Stocks-Fischer et al. 1999).

MICP can occur as a byproduct of urea hydrolysis, photosynthesis, sulfate reduction, nitrate reduction, or any other metabolic activity that leads to an increase in the saturation state of calcium carbonate (DeJong et al. 2010; Benzerara et al. 2011). This review focuses on urea hydrolysis (ureolysis) to promote $CaCO_3$ precipitation. In ureolysis-driven MICP, the cellular or urease enzyme activity influences chemical conditions (the saturation state) to promote mineralization through four factors: (1) dissolved inorganic carbon (DIC) concentration, (2) pH, (3) calcium concentration, and (4) potential nucleation sites (Hammes & Verstraete 2002). The first three factors determine the saturation state, because DIC and pH influence the carbonate ion concentration or activity $\{CO_3^{2-}\}$. The fourth factor impacts the critical saturation state (S_{crit}), which is the saturation state at which nucleation (ie precipitation) occurs under the given conditions. Additionally, the species and the concentration of microbe(s), their ureolytic activity, the form of microbial growth (ie biofilm or planktonic), temperature, salinity, injection strategy (ie flow rate, treatment times), and reactant concentration (or activity) may impact the saturation conditions and the efficiency and extent of $CaCO_3$ precipitation (Harkes et al. 2010; Okwadha & Li 2010; Mortensen et al. 2011; Cuthbert et al. 2012). Carefully manipulating: (1) the ureolytic activity of microorganisms, (2) the reaction and transport rates of substrates, and (3) the saturation state may greatly influence treatment efficacy.

Ureolytic activity of microorganisms

The urease enzyme can be found in a wide variety of microorganisms (Mobley & Hausinger 1989; Hammes et al. 2003) and contributes to the ability of the cell to utilize urea as a nitrogen source (Ferris et al. 2003; Burbank et al. 2012). While urease production is quite common across a wide range of soil organisms and found in other natural environments, in the laboratory, many researchers have examined ureolytic MICP using the common soil organism *Sporosarcina pasteurii* ATCC 11859, formerly *Bacillus pasteurii* (Yoon et al. 2001). *S. pasteurii* is non-pathogenic, does not readily aggregate under most growth conditions, and produces large quantities of active intracellular urease (Ferris et al. 1996; Stocks-Fischer et al. 1999; DeJong et al. 2006). *S. pasteurii* has been isolated from soil, water, sewage, and urinal incrustations (De Muynck, De Belie, et al. 2010).

One disadvantage of studying laboratory strains is the microbial complexity of real world environments. In the context of soil stabilization, it was noted that injection of these organisms may result in non-homogeneous distribution of the microbes, or the organisms may face challenges of competition or predation from native organisms (van Veen et al. 1997; van Elsas et al. 2007; Burbank et al. 2011). As such, to maintain ureolytic populations in subsurface applications, it may be advantageous to stimulate native attached (biofilm) ureolytic populations rather than augmenting the environment with laboratory strains not adapted to the treatment environment (Fujita et al. 2010; DeJong et al. 2011; Tobler et al. 2011; Burbank et al. 2012). Also, when considering the augmentation of the subsurface with certain organisms, particularly *S. pasteurii,* described as a facultative anaerobe (Ferris et al. 1996; Tobler et al. 2011) and more recently as an obligate aerobe (Martin et al. 2012), it is important to consider the impact of electron acceptors (for example, oxygen in the case of *S. pasteurii*) on microbial growth. Although ureolytic activity itself does not depend upon oxygen (Mortensen et al. 2011), microbial growth and urease production could be limited by the availability of electron acceptor. It has been demonstrated that *S. pasteurii* cannot anaerobically synthesize *de novo* urease; therefore the active urease may be limited to the existing enzyme injected with the aerobically grown inoculum (Martin et al. 2012). To overcome challenges associated with growth-coupled urease production, stimulation of native populations, injection of electron-acceptor rich growth medium, or the injection of urease enzyme might be considered.

Additionally, mineral precipitation around cells can influence ureolytic activity by either causing cell inactivation through membrane disruption or by limiting nutrient transport to the cell (Stocks-Fischer et al. 1999; Parks 2009; Cuthbert et al. 2012). Zamarreño et al. (2009) suggest that precipitation and entombment might be a passive process, which the organisms cannot help but be involved in. Alternatively, they suggest that the precipitation protects cells for a short period of time from detrimental calcium concentrations. In an engineering application, it is important to consider that entombment may lead to reduced ureolysis and potentially limit further precipitation. To overcome inactivation and promote additional $CaCO_3$ precipitation, resuscitation or reinjection of organisms as well as additional treatments may be required to maintain an active ureolytic population and maximize precipitation (Tobler et al. 2011; Ebigbo et al. 2012).

Reaction and transport

Chemical reactions. During ureolysis-driven MICP, urease catalyzes the hydrolysis of one mole of urea to form

one mole of ammonia and one mole of carbamic acid (Equation 1), which spontaneously hydrolyses to carbonic acid and another mole of ammonia (Equation 2). Under circum-neutral conditions, the two moles of ammonia become protonated by deprotonating water to form two moles of ammonium (NH_4^+) and two moles of hydroxide ions (Equation 3). The generated hydroxide ions shift the equilibrium of DIC species towards bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) (Equations 4 and 5) (Stocks-Fischer et al. 1999; Dick et al. 2006; Mitchell et al. 2010).

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{Urease}} NH_2COOH + NH_3 \quad (1)$$

$$NH_2COOH + H_2O \xrightarrow{\text{Spontaneous}} NH_3 + H_2CO_3 \quad (2)$$

$$2NH_3 + 2H_2O \longleftrightarrow 2NH_4^+ + 2OH^- \quad (3)$$

$$H_2CO_3 \longleftrightarrow HCO_3^- + H^+ \quad (4)$$

$$HCO_3^- + H^+ + 2OH^- \longleftrightarrow CO_3^{2-} + 2H_2O \quad (5)$$

In the presence of sufficient calcium ion activity, saturation conditions become favorable for $CaCO_3$ precipitation (Equation 6).

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3(s) \quad (6)$$

Kinetics of reactions. Although urease increases urea ureolysis rates 10^{14} times over uncatalyzed rates (Mitchell & Ferris 2005), ureolysis is the rate-limiting step in MICP. The concentration of bacteria, temperature, pH, saturation conditions, and salinity have been shown to influence ureolysis kinetics (Ferris et al. 2003; Dupraz, Parmentier, et al. 2009; Tobler et al. 2011). In general, a higher concentration of cells producing urease has been shown to positively impact the rate of ureolysis, as have elevated (20 °C *vs* 10 °C) temperatures (Ferris et al. 2003; Mitchell & Ferris 2005; Tobler et al. 2011).

Several models to predict rates of ureolysis can be considered. In conditions of excess urea, a zero order model might be appropriate, where the rate of ureolysis, r_{urea}, is equal to the rate constant and not influenced by the urea concentration [urea] (Equation 7):

$$r_{urea} = \frac{[urea]}{time} = -k_{urea} \quad (7)$$

Most commonly, first order rate models are presented (Equation 8) (Ferris et al. 2003; Mitchell & Ferris 2005; Tobler et al. 2011; Cuthbert et al. 2012), where the ureolysis rate is dependent on the urea concentration:

$$r_{urea} = -k_{urea}[urea] \quad (8)$$

Ureolysis rates have also been modeled using Michaelis–Menten type expressions that include a term accounting for non-competitive inhibition by ammonium (Equation 9) (Fidaleo & Lavecchia 2003; Ebigbo et al. 2012). Here v_{max} is the maximum rate of ureolysis, K_m is the half saturation coefficient, [*P*] is the concentration of ammonium, and K_P is an inhibition constant for ammonium:

$$r_{urea} = \frac{v_{max}[urea]}{(K_m + [urea])\left(1 + \frac{[P]}{K_P}\right)} \quad (9)$$

The rates of ureolysis are dependent on a wide range of factors and have been extensively studied in MICP systems, particularly in laboratory batch systems. Simple batch studies with planktonic cells produce valuable parameters to be used in MICP models, recognizing that the same parameters may not be fully transferable when considering values associated with biofilm. Models can help develop understanding of more complex environments not easily studied in the laboratory.

Transport. In fluid systems relevant to MICP, both advective and diffusive transport occurs, and dominance of one or the other depends on the system. Advection refers to movement of a species with fluid flow. Diffusion refers to the movement of species independent from the bulk fluid movement and driven by concentration or electrostatic potential gradients. The fluid flow conditions (such as whether the flow is laminar or turbulent, axial or radial) and the fluid properties (density and viscosity) influence the advective and diffusive properties of the species transport. In MICP application, transport conditions may be complex, particularly in the case of radial flow where the fluid velocity changes with distance.

Damköhler (Da) number. The dimensionless Da number, which describes the ratio of reaction rate to transport rate, may serve as an important design tool in MICP application. In biogeochemical processes such as MICP, the reactions (particularly ureolysis) are coupled to the transport of the reactive species. In general terms, Da relates the reaction rate of a species to the advective or diffusive mass transport rate of that species (Equation 10) (Berkowitz & Zhou 1996; Dijk & Berkowitz 1998; Domenico & Schwartz 1998).

$$Da = \frac{\text{Reaction rate}}{\text{Transport rate}} \quad (10)$$

More specifically, Da depends on the kinetics of the reaction and the transport through a specific reactor (or

natural) system. For example, in a plug flow system where advective transport dominates, Da represents a ratio of the reaction rate to the advective mass transport rate of the species. When Da < 1, it does not indicate that reaction is not occurring; it does, however, imply that not all the supplied substrate is utilized and may be transported from the reaction zone. Da values >1 indicate that the reaction is limited by the transport rate for a given length scale.

In a pulsed flow system or within stagnant pore spaces, where diffusive transport is likely to dominate, Da is the ratio of reaction rate to the effective diffusion rate of the reactive species. In diffusion dominated cases, a Da < 1 indicates the reaction rate is limited by reaction kinetics rather than diffusion; however, given enough time, the reaction may proceed to completion. Alternatively, Da >1 indicates the reaction rate drives the establishment of concentration gradients of reactive species.

Da incorporates many of the factors related to reaction and transport into a single unitless number, for ease of comparison and design. The systematic analysis of Da may reveal a functional design tool (for example, predicting flow rates or pulsed treatment times) for MICP not previously explored.

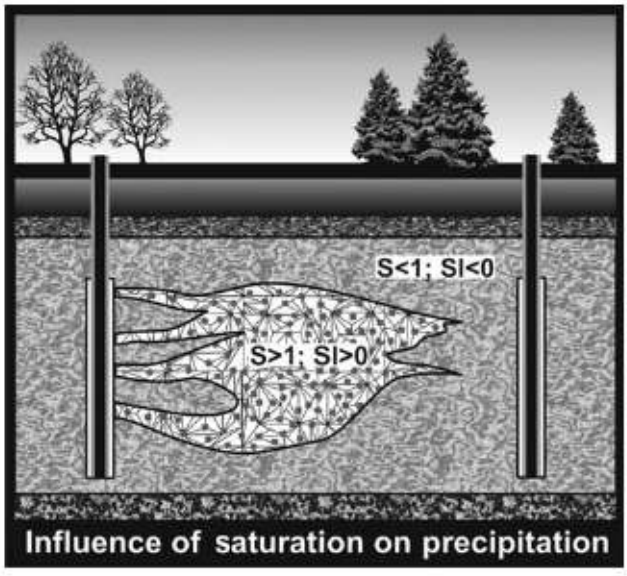

Figure 2. Influence of saturation on precipitation in a cross section of a groundwater aquifer (precipitates are represented by white crystal hatch pattern). Saturation states >1 (S > 1) and saturation indices >0 (SI > 0) indicate that precipitation is thermodynamically favored; saturation states <1 (S < 1) and saturation indices <0 (SI < 0) indicate that dissolution is favored if the mineral form is present. The saturation state can vary spatially and temporally due to reaction and transport rates which create concentration gradients (Zhang et al. 2010).

Saturation conditions

$CaCO_3$ precipitation is ultimately governed by the saturation state (S or Ω) of calcium carbonate where $\{Ca^{2+}\}$ and $\{CO_3^{2-}\}$ represent the activities of Ca^{2+} and CO_3^{2-} ions, which are approximately equal to concentration for low ionic strength conditions, and K_{so} is the temperature-dependent equilibrium solubility constant (Equation 11):

$$S \text{ or } \Omega = \frac{\{Ca^{2+}\}\{CO_3^{2-}\}}{K_{so}} \tag{11}$$

At $S = 1$, the solution is considered in equilibrium with the solid phase. If $S > 1$, the solution is considered supersaturated with respect to $CaCO_3$ and $CaCO_3$ precipitation is thermodynamically favored. If $S < 1$, the solution is considered undersaturated and dissolution of solid phase $CaCO_3$, if present, is thermodynamically favorable (Figure 2) (Stumm & Morgan 1996). The saturation index (SI) is represented as the $\log_{10}$ of the saturation state (Equation 12). When SI is positive, then the solution is supersaturated and *vice versa*. Further detailed calculations can be found in several publications of potential interest to the reader (Ferris et al. 2003; Dupraz, Parmentier, et al. 2009; Tobler et al. 2011).

$$SI = \log_{10}(S) \tag{12}$$

While the S or SI predicts whether precipitation is thermodynamically favored, it does not necessarily predict the saturation state at which precipitation begins (S_{crit}). S_{crit} or SI_{crit} are empirical values which reflect how highly supersaturated a solution must become before precipitation is observed. This critical supersaturation is related to overcoming the nucleation activation free energy barrier (Ferris et al. 2003) and is likely impacted by a variety of system parameters influencing the activity of Ca^{2+} and CO_3^{2-} ions. Saturation values in the literature for batch systems have been reported in the range of $S = 12$–436 (Ferris et al. 2003; Mitchell & Ferris 2005, 2006a; Dupraz, Parmentier, et al. 2009; Tobler et al. 2011). S_{crit} may depend on many factors, including the kinetics of ureolysis, the initial cell density, the presence of nucleation points, and the presence of organics.

Nucleation. As outlined above, it is quite possible that combinations of different biomineralization processes are active at the same time in a system. For instance, while ureolysis can increase the saturation state of the bulk environment (biologically-induced mineralization) the precipitation process itself might be initiated by the microbes serving as nucleation sites (biologically-influenced mineralization) (Figure 3a and b) (Stocks-Fischer et al. 1999; De Muynck, De Belie, et al. 2010). Once

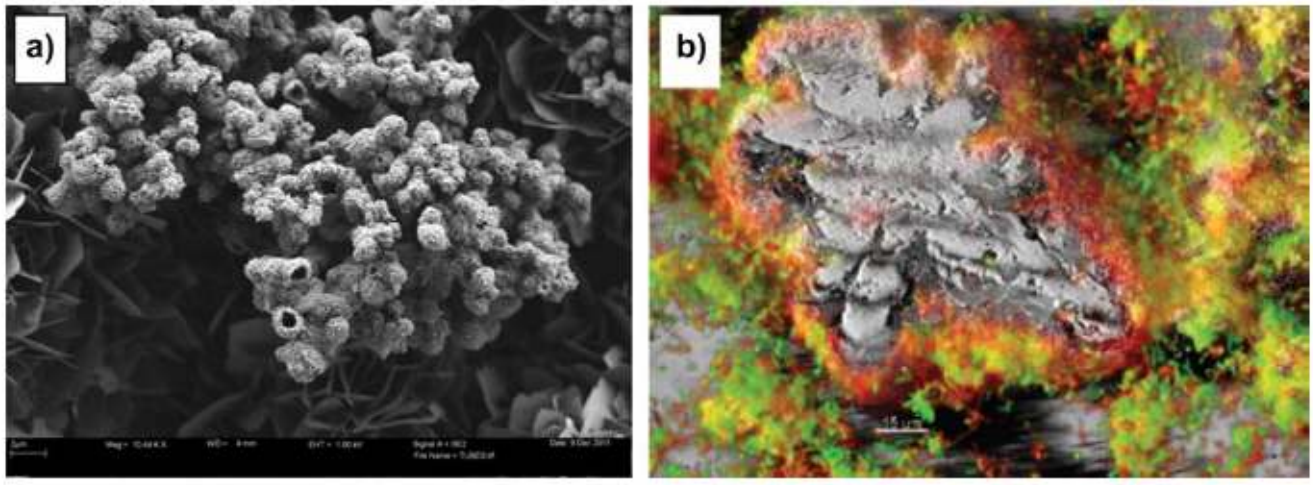

Figure 3. Images of cells associated with minerals. (a) SEM image of tube-like calcium-containing minerals (with similar diameters to bacterial cells) possibly entombing *S. pasteurii* shaped cells. Other researchers have noted similar findings *via* SEM analysis where rod shaped bacteria-like structures were observed inside and adjacent to $CaCO_3$ crystals or as rod shaped impressions in the $CaCO_3$ crystal (Stocks-Fischer et al. 1999; Fujita et al. 2004; Mitchell & Ferris 2005; De Muynck et al. 2008; Dupraz, Parmentier, et al. 2009); (b) CLSM image of bacterial cells (red and green) closely associated with $CaCO_3$ precipitates (grey). © Cambridge University Press. Reprinted with permission. From Schultz et al. (2011).

precipitation has commenced, ureolysis may maintain a high SI and cells as well as newly precipitated minerals likely act as additional templates or nucleation sites to facilitate crystal growth (Stocks-Fischer et al. 1999; Hammes 2002). While the influence of cell surfaces as nucleation sites has been widely discussed (Douglas & Beveridge 1998), Mitchell and Ferris (2006b) observed an equal S_{crit} in solutions with and without bacterial cells separated by dialysis membranes that allowed for transport of solutes between the two solutions. In addition, $CaCO_3$ nucleation has been noted in a variety of systems to be influenced by the presence of certain proteins, microbial biomolecules, EPS, other available passive substrates, heterogeneous nucleation on bottle walls or be solely occurring homogeneously in solution (Mitchell & Ferris 2006b; Dupraz, Parmentier, et al. 2009; Fouke 2011).

Mineralogy. Three primary polymorphs of $CaCO_3$ exist: calcite, vaterite, and aragonite. It is well known that surface-attached communities of microorganisms, or biofilms, secrete EPS rich in polysaccharides and other organic macromolecules. EPS and organic matter have been linked to the formation of vaterite which may be stabilized in the presence of certain organics (Braissant et al. 2003; Rodriguez-Navarro et al. 2007). Vaterite has been found as a minor, meta-stable, or transitional phase in the formation of calcite (Tourney & Ngwenya 2009). The maturation of $CaCO_3$ from vaterite to calcite may be described by the Ostwald Step Rule where metastable forms nucleate and then are replaced with more stable forms, a sequential formation in time also known as paragenesis (Morse & Casey 1988). The mechanisms of initial nucleation, which may be influenced by the microbial growth conditions, the presence of certain organics, such as EPS, or the saturation conditions of the fluid, as well as subsequent maturation are not completely understood (Morse & Casey 1988; Jiménez-López et al. 2001; Braissant et al. 2003; Zamarreño et al. 2009). Crystal size may be a factor in the efficacy of an MICP technology. $CaCO_3$ crystals precipitated *via* ureolysis-driven MICP have been observed to be generally larger and less soluble than those precipitated under the same abiotic bulk solution conditions (Mitchell & Ferris 2006b; Mitchell et al. 2013).

To summarize, $CaCO_3$ precipitation *via* ureolysis-driven MICP is initiated by creating conditions oversaturated with respect to $CaCO_3$, likely combined with the increased abundance of cell surfaces as nucleation points at the point of critical saturation and finally crystal growth on nuclei (Ferris et al. 2003).

Engineering applications

Construction materials

Biodeposition

Biodeposition refers to the deposition of MICP to protect the surface of porous materials (such as limestone, concrete, or bricks) from water intrusion. MICP treatment can decrease the ability of a material to absorb water, restore the surface, and reduce further potential weathering (Figure 1g) (Dick et al. 2006; De Muynck, De Belie, et al. 2010). For example, in reinforced concrete, pores

might allow penetration of water and ions, particularly chloride or acids, leading to deleterious corrosive effects to the embedded reinforcing steel (Dick et al. 2006; Achal et al. 2011a; De Muynck et al. 2011). In a MICP treated surface, $CaCO_3$ can clog pores and decrease water penetration through a protective calcite layer. Since De Muynck, De Belie, et al. (2010) provided a very comprehensive review of this topic, this review will discuss how the experimental conditions, particularly the promotion of ureolytic activity and application of substrates, influence treatment efficacy.

First, ureolytic *Bacillus sphaericus* isolates from calcareous sludge were found to be effective at $CaCO_3$ precipitation on limestone cubes (Dick et al. 2006). The cubes were immersed in liquid bacterial cultures to promote biofilms and then immersed in urea and calcium chloride treatments to promote $CaCO_3$ formation. It was concluded, that isolates with a highly negative zeta (ζ)-potential, an indication of electrical surface potential of cells, would more successfully colonize positive ζ-potential limestone. It was also concluded, that the high initial urea degradation rate and the high surface covering with $CaCO_3$ on the biofilm produce the most homogeneous and coherent $CaCO_3$ coating to provide protection of limestone from water intrusion (Dick et al. 2006).

De Muynck et al. (2008) performed similar biodeposition tests on concrete cubes treated with urea and calcium chloride or calcium acetate (an alternative to corrosive chloride) treatment solutions. Their study found no difference between calcium sources when examining *B. sphaericus* ureolysis-driven MICP in terms of weight gain of the samples due to precipitation or chloride penetration resistance. Additionally, they concluded that the biofilm may act as a template or primer for initial deposition of $CaCO_3$ (De Muynck et al. 2008). Secondly, De Muynck, Verbeken, et al. (2010) examined the influence of urea and calcium concentrations on MICP coating of limestone. It was reported that increasing urea and calcium concentrations and repeated treatment improved the resistance of the limestone to water absorption due to $CaCO_3$ precipitation. It was nevertheless concluded that the benefits of increased urea and calcium chloride concentration should be balanced with the detrimental impacts such as unwanted ammonium by-product formation or stone discoloration (De Muynck, Verbeken, et al. 2010). Finally, De Muynck et al. (2011) investigated the pore structure of French limestone base materials to determine the impact on the penetration depth and protective performance of *B. sphaericus* ureolysis-driven MICP deposits. More successful bacterial penetration of larger pores resulted in more deposition in stones with higher porosity.

Chunxiang et al. (2009) used *S. pasteurii*-facilitated MICP to coat cement with $CaCO_3$ biodeposits to study corrosion resistance. By altering the order of addition of calcium and urea, the researchers increased the effectiveness of the MICP deposits against water absorption and acid corrosion of the cement. They concluded that adding calcium before urea to a stationary phase bacterial culture produced a more compact $CaCO_3$ deposit because calcium influenced ureolysis activity and rates which may impact the adhesion and thickness of the $CaCO_3$ layer (Chunxiang et al. 2009). Whiffin (2004) suggested that high calcium nitrate ($Ca(NO_3)_2$) concentrations may inhibit urease activity, although mixed effects on activity were observed among environmental isolates or a microbial consortium (Hammes et al. 2003; Burbank et al. 2011). Therefore, depending on the organisms' tolerance for calcium concentrations, a balance might need to be struck between high Ca^{2+} concentrations which may inhibit ureolysis and low Ca^{2+} concentration which may not allow for the formation of sufficiently protective deposits.

Biocement

Concrete is one of the most commonly used construction materials, but it is prone to weathering and cracking. Cracks form in concrete due to aging and/or freeze thaw cycles which lead to pathways for corrosive fluid intrusion (Bang et al. 2010; Jonkers et al. 2010; Achal et al. 2011b; Wiktor & Jonkers 2011). Healing of fractures in concrete with MICP (Figure 1e) would be advantageous since other sealants may degrade over time or are environmentally toxic, whereas $CaCO_3$ may be a more benign treatment (Siddique & Chahal 2011). Here, biocement refers to the use of MICP to produce binder materials to seal fractures or improve strength and durability of cementitious materials (such as adding microbes to cement mixtures). Since this topic has been extensively reviewed by others (De Muynck, De Belie, et al. 2010; Siddique & Chahal 2011), this section will focus on investigations related to the control of MICP treatment for both concrete fracture sealing and improvement of cementitious material.

Bacteria in or applied to concrete may face challenges to their activity including small pore sizes as concrete cures, which may damage or inhibit the penetration of organisms, and the high pH, which may inhibit biological activity. Cement, or rather the water associated with cement, can have a pH of 11–13 even after it is completely cured (Bang et al. 2001; Jonkers et al. 2010). Alkaliphilic spores embedded in concrete were observed to retain culturability for <4 months presumably due to cell damage as the cement cured and pore size decreased (Jonkers et al. 2010). Given small pore sizes and high pH conditions, research has focused on the use of alkaliphilic organisms and/or methods to protect the organisms in order to maintain viability and ureolytic activity during treatment.

To protect microbial urease activity from high pH in cement, *S. pasteurii* cells were immobilized in polyurethane (PU) foam in cement fractures and treated with urea/calcium solutions. Researchers found urease activity was maintained and hypothesized that enzyme activity might be stabilized for longer periods of time when embedded in a matrix such as PU foam (Bang et al. 2001). Instead of immobilizing cells, Bachmeier et al. (2002) investigated the use of urease immobilized in PU foam, since this treatment methodology does not depend upon maintaining cell viability for ureolysis. Immobilized enzyme treatments showed decreased $CaCO_3$ precipitation rates, possibly due to diffusion limitation of either calcium or carbonate. However, increased enzyme stability was observed at elevated temperature compared to the free enzyme (Bachmeier et al. 2002). More recently, Bang et al. (2010) immobilized varying concentrations of *S. pasteurii* cells on Siran™ glass beads to fill into concrete cracks for crack remediation. Once again immobilization was speculated to have stabilized cell and urease activity from the adverse effects of the high pH of the concrete (Bang et al. 2010).

Van Tittelboom et al. (2010) studied the efficacy of silica gel supplemented with *B. sphaericus* cells injected into concrete fractures and treated with calcium chloride, calcium acetate, calcium nitrate, and urea solutions. The calcium source did not change the reduction in water absorption (all sources worked to produce deposits in fractures) indicating the possibility of using alternative calcium sources. The necessity for some protection of cells from the high pH in concrete was suggested as bacteria injected without gel failed to precipitate $CaCO_3$, although it is also possible that cells injected in the fracture without silica gel may have not attached well and thus resulted in reduced treatment efficacy (Van Tittelboom et al. 2010). Another approach to concrete fracture remediation is self-healing, where healing agents are released or activated when fractures form (Wiktor & Jonkers 2011; Wang et al. 2012). In one unique study, carrying agents including PU or silica-gel, *B. sphaericus*, and urea/calcium nitrate treatments were loaded into separate glass capillaries and embedded in mortar, which upon cracking fractured the glass capillaries allowing the carrying agents, cells, and treatment solutions to mix. The bacteria retained ureolytic and $CaCO_3$ precipitating activity after immobilization in both PU and silica, but a more homogeneous distribution of $CaCO_3$ crystals was observed in the silica gel *vs* the PU foam which was attributed to the ability of bacteria to distribute more homogeneously through the less viscous silica sol (before gelation) than PU pre-polymer (Wang et al. 2012).

Ureolytic MICP can potentially improve the strength of cement by incorporating cells into the cement mixture, although high concentrations of cells may reduce the compressive strength due to interference by the biomass with the integrity of the mortar (Ramachandran 2001). When certain cell concentrations of *Bacillus* sp., isolated from commercially available cement, were mixed into a water, cement, sand mixture and cured in urea/$CaCl_2$ treatment, the microbial cement was found to resist water uptake better and showed improved compressive strength compared to the control cement (Achal et al. 2011b). The compressive strength of fly ash or silica fume amended concrete was also found to be improved by MICP induced by *B. megaterium* (Achal, Pan, et al. 2011) and *S. pasteurii* (Chahal et al. 2012a, 2012b).

In summary, construction materials may be improved by MICP. It has been shown that increasing the number of treatment applications, changing the calcium source to avoid deleterious impacts from chloride, applying treatment to higher porosity materials, promoting biofilm growth before calcium treatment, and varying the order in which the constituents are applied (calcium before urea) can yield improvements in protective $CaCO_3$ coatings *via* the MICP process. Also, some promise was found in using ureolytic MICP to improve the strength of concrete and remediate concrete fractures, but immobilization of cells or urease enzyme in gels or PU was required to provide protection from high pH activity inhibition or damage to cells during cement curing. Immobilization in turn may lead to diffusion limitations and potentially reduced precipitation. These studies demonstrate the importance of protecting the urease activity by either promoting cells to attach to the surface or immobilizing them.

Cementation of porous media

Ureolysis-driven MICP to alter or improve the mechanical properties of unconsolidated porous media has been extensively investigated. This method has been proposed to suppress dust (Figure 1d), reduce permeability in granular media, improve soils, stabilize slopes (Figure 1c), and strengthen liquefiable soils (Gollapudi et al. 1995; Ferris et al. 1996; Whiffin et al. 2007; Bang et al. 2011; Burbank et al. 2011). $CaCO_3$ crystals precipitated during MICP can bridge gaps between the grains in porous media to bind them together; precipitation can also reduce the pore throat size, porosity, and permeability, and increase the stiffness and strength of the porous media matrix (DeJong et al. 2010). Much of the work to date has been performed to improve the efficiency of precipitation, maximize the extent of the treatments, and balance chemical use to reduce costs for field application. In engineering applications such as sand consolidation or soil strengthening, it is preferable to precipitate $CaCO_3$ homogeneously over distance and use as little reactant volume as possible for economic reasons (Harkes et al. 2010; van Paassen et al. 2010). While preferential plugging may be effective in some engineer-

ing applications, non-homogeneous bacterial distribution and non-homogeneous precipitation may have the disadvantage of near-injection-point plugging where substrates are abundant, limiting the spatial extent of the treatment (Cunningham et al. 2007; Gerlach & Cunningham 2011; Mortensen et al. 2011). Proposed strategies for controlling precipitation include promoting the spatial distribution of ureolytic activity of cells or biofilm, manipulating the transport and reaction rates of the reactive species and promoting favorable saturation conditions in specific regions.

Sand consolidation

Whiffin et al. (2007) described a sand stabilization treatment method (BioGrout), which followed *S. pasteurii* inoculation with a calcium chloride solution to increase bacterial adhesion to the sand before MICP treatment. This treatment sequence achieved significant strength improvement and porosity reduction in sand packed columns. Although non-uniform precipitation was observed along the length of the column, it was reasoned that a more homogeneous distribution could be achieved by shifting the balance of supply and conversion (ie Da) by increasing flow rates or lowering conversion rates to achieve higher reactant infiltration (Whiffin et al. 2007).

Following this initial Biogrout work, Harkes et al. (2010) altered the ionic strength and flow rates, again influencing the reaction kinetics and transport rates related to Da, to study the impact on ureolytic bacterial distribution in sand to prevent near-injection-point clogging. Bacterial attachment was found to be positively influenced by increased salinity or ionic strength of the transporting fluids, which could be due to a decrease in the electrostatic repulsion forces between the cells and the porous media surfaces (Scholl et al. 1990; Foppen & Schijven 2006). However, the increase in ionic strength might also promote attachment of cells near the injection point and limit the spatial extent of the treatment. So, by altering the transport rate (increasing flow) of low ionic strength solutions Harkes et al. (2010) observed a more homogeneous distribution of bacteria, but cautioned against the loss of attachment and activity when low ionic strength solutions are used. Transport of bacteria through the matrix of a porous medium is a complex function of the size and surface properties of the cell, electrical interactions, the flow rate and the chemistry of the transport fluid as well as the pore size distribution of the porous medium (Jenneman et al. 1985; Scholl et al. 1990; Bouwer et al. 2000; Mitchell & Santamarina 2005; Harkes et al. 2010). A balance between ionic strength and transport could help promote more homogeneous cell and ultimately ureolytic activity distribution.

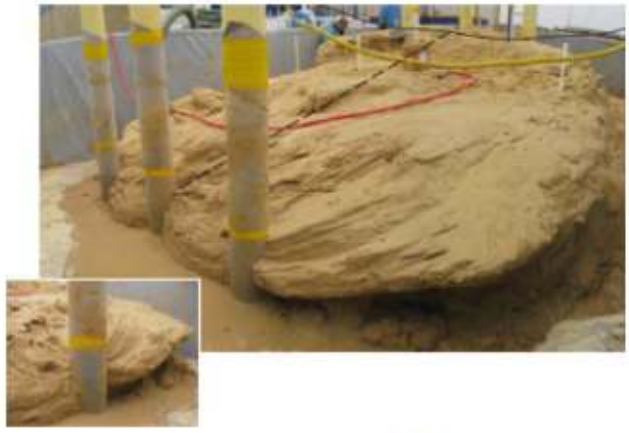

Figure 4. Image of a cemented sand body from a large scale Biogrout experiment. Reprinted from van Passen et al. (2010), with permission from ASCE.

Much of the work presented has been performed on a smaller scale in a laboratory-controlled environment, yet in 2010 van Paassen et al. embarked on a scaled-up demonstration of MICP in 100 m^3 of sand to determine the ground improvement abilities and the extent of precipitation. Similar to the injection strategies developed by Whiffin et al. (2007) and Harkes et al. (2010), the sand was inoculated with *S. pasteurii* cells, cementation solution followed to promote bacterial cell adhesion and then urea and calcium solutions were injected 10 times over 16 days. As much as 40 m^3 of the 100 m^3 sand reactor were cemented *via* MICP with a visible wedge shape between the injection and extraction wells (Figure 4) (van Paassen et al. 2010).

Liquefiable soils

Other researchers have also examined scaled-up ureolysis-driven MICP. Burbank et al. (2011) studied field-scale ureolysis-driven MICP to strengthen liquefiable soils. Liquefiable soils are loose granular soil deposits generally found in saturated conditions, which may undergo a decrease in shear strength when subject to seismic waves and contribute to man-made structure failure during earthquakes (Burbank et al. 2011). Soils on the shore of the Snake River (USA) were subjected to ureolytic biomineralization treatments, which yielded soils cemented with ~1% by weight $CaCO_3$ in the near surface and 1.8–2.4% calcite below 90 cm (Burbank et al. 2011). This was less precipitation than observed in laboratory enriched samples, which was attributed to the lower technical quality of the calcium source in the field study. Their findings also suggested higher concentrations of $CaCO_3$ formed away from the injection point rather than closer to the injection point. Researchers attributed this to either (1) eluviation where fine-grained materials or

$CaCO_3$ particles may have been transported downward with the infiltrating water, or (2) increased ureolysis and possibly delayed subsequent precipitation occurring in the deeper soil profile.

Subsurface barriers

In certain coastal areas, salt water intrusion into freshwater aquifers during groundwater extraction has become a major problem. The problem is often addressed by creating underground dams or increasing artificial recharge of fresh water to prevent migration of salt-laden water into freshwater aquifers. Subsurface MICP barriers may be an alternative to these methods (Figure 1i) (Rusu et al. 2011). Due to salt water intrusion into ground water, MICP must be able to occur in saline conditions to be applied in these environments. Mortensen et al. (2011) assessed the influence of various environmental factors on ureolysis-driven MICP to determine suitable *in situ* environments. First, they observed that short term ureolytic activity did not appear to be inhibited by anaerobic conditions after cells were cultured aerobically, which agrees with findings by Parks (2009), Tobler et al. (2011), and Martin et al. (2012). Secondly, they found full and half-strength seawater enhanced $CaCO_3$ precipitation rates, possibly due to increased alkalinity and cation availability (Mortensen et al. 2011). Finally, the authors note that manipulating the reaction and transport rates by inhibiting precipitation with increased ammonium concentrations or by controlling flow rates is important in achieving homogeneous distribution of MICP. These results demonstrate the potential of ureolysis-driven MICP for developing subsurface barriers to prevent salt water intrusion.

Aquaculture: impermeable crusts

One promising engineering application of ureolysis-driven MICP is the preparation of crusts to control seepage from aquaculture ponds or reservoirs into underlying soils or sands (Figure 1h). Stabnikov et al. (2011) used the halotolerant, alkaliphilic *Bacillus* sp. VS1 isolate to seal a sand-lined model pond. Successive percolation treatments with high concentrations of urea and calcium solutions resulted in a nearly impermeable crust on the surface of the sand, which markedly reduced the seepage rate, taking sand to the same permeability range as well compacted clay (Figure 5).

Dust suppression

Bang et al. (2011), showed the potential for using ureolysis-driven MICP to suppress dust (Figure 1d). Dust poses problems to human health and is traditionally suppressed by means of chemical application or watering

Figure 5. Photograph of an ~1 mm thick crust of calcite on a sand surface. Reprinted from Stabnikov et al. (2011), with permission from Elsevier.

down which may be difficult to maintain or may use environmentally problematic chemicals. Ureolysis-driven MICP is proposed as an alternative to consolidate dust particles. *S. pasteurii* cells or urease and urea/calcium chloride treatment solutions were sprayed over sand samples, which were then subjected to wind erosion tests. Bang et al. (2011) found MICP dust control to be very effective, but its efficiency was subject to the soil type and grain size distribution, as well as environmental conditions such as humidity and temperature.

In summary, ureolysis-driven MICP has been explored for several engineered applications involving porous media, including consolidating sand or soils, creating subsurface barriers, sealing aquaculture ponds and suppressing dust. These applications are often controlled by manipulating the transport and reaction rates to either promote homogeneous deposition or controlled deposition in selective areas. In MICP application to porous media, a complex set of factors, including environmental conditions may greatly influence the results of the treatment.

Hydraulic control and environmental remediation

Radionuclide and metal remediation

Radionuclide remediation. The US Department of Energy faces environmental remediation challenges such as the long-term management of the Hanford site in Washington, USA, where groundwater is contaminated with radionuclides (Warren et al. 2001; Fujita et al. 2004, 2008, 2010; Wu et al. 2011). Traditional treatment methods such as pump and treat have been found ineffective at the site to remediate or prevent migration of mobile radionuclide groundwater contaminants (Fujita et al. 2008). Therefore, years of research have evolved methods to stimulate ureolytic subsurface organisms to

promote $CaCO_3$ precipitation which in turn promotes co-precipitation and solid phase capture of some of these contaminants, in particular strontium-90, a uranium fission by-product (Figure 1j). In subsurface environments saturated with respect to $CaCO_3$ minerals, the co-precipitation forms a long-term immobilization mechanism while the ^{90}Sr decays (Mitchell & Ferris 2006a; Fujita et al. 2010; Wu et al. 2011).

Control of strontium co-precipitation in the subsurface has been widely researched by studying the rates of ureolysis and precipitation. Warren et al. (2001) demonstrated that 95% of total strontium was captured in the solid phase during batch ureolysis-driven MICP experiments. Further studies demonstrated that *S. pasteurii* in artificial groundwater media exhibited higher rates of ureolysis at slightly elevated temperatures, strontium co-precipitation increased with increasing $CaCO_3$ precipitation rates, and higher ureolysis rates could reduce the time to reach critical saturation (S_{crit}) which is important since the greatest $CaCO_3$ precipitation rates were observed near S_{crit} (Ferris et al. 2003; Fujita et al. 2004; Mitchell & Ferris 2005).

Since augmentation of subsurface environments with microbes may not be ideal or feasible, Fujita et al. (2008) investigated the potential of enriching native ureolytic organisms *in situ* in the Eastern Snake River Plain Aquifer (Idaho, USA) for the purpose of remediating groundwater by co-precipitating strontium. The authors suggest that multiple treatments with low concentrations of a carbon source (molasses) to stimulate the subsurface community followed by the injection of urea can promote ureolytic subsurface populations (Fujita et al. 2008). Another microbial enrichment test was performed with groundwater and sediment samples from wells at the Hanford site in Washington, USA (Fujita et al. 2010). Urea stimulated sediment samples showed specific ureolytic activity 2–4 orders of magnitude higher compared to groundwater samples, leading researchers to hypothesize that greater activity was associated with attached (or biofilm) communities compared to planktonic cells (Fujita et al. 2010).

Metal remediation. Toxic metal (eg copper, arsenic, and chromium) contamination in soil or groundwater has been attributed to mining and smelting as well as other industrial activities. Toxic metal contamination is linked to human health problems and current remediation efforts can be costly and relatively ineffective. Traditional remediation efforts include phytoremediation, removing, or covering the soils with clean soil, on-site chemical leaching of contaminants or bioremediation with toxic metal-tolerant bacterial species (Achal et al. 2012). However, these treatment methods may not be long-term solutions. For example, in bioremediation many bacterial species can decrease the solubility and thus immobilize metals by changing their redox state. However, future changes in oxidation-reduction potential could lead to remobilization; therefore, an alternate remediation method is $CaCO_3$-based co-precipitation.

It was previously shown that chromate can be associated with $CaCO_3$ in co-precipitated form (Hua et al. 2007), also Achal et al. (2012) isolated *Sporosarcina ginsengisoli* CR5, an arsenic-tolerant, urease-positive bacterium and researched its MICP potential to remediate arsenic contaminated soils. Although growth of the organism was slowed in the presence of arsenic, significant arsenic was removed from aqueous solution during ureolytic MICP (Achal et al. 2012). Another study focused on remediation of copper *via* the MICP process by the copper-tolerant, ureolytic organism, *Kocuria flava* CR1. Copper bioremediation studies were performed with *K. flava* in urea and calcium containing batch with copper concentrations up to $1000\,mg\,l^{-1}$ (Achal, Pan, et al. 2011). The authors reported a positive correlation between higher urease production and higher copper removal from aqueous solutions (Achal, Pan, et al. 2011).

In elevated concentrations, metals may be toxic to organisms involved in remediation. Kurmaç (2009) evaluated the impact of varying concentrations of lead, cadmium, chromium, zinc, copper, and nickel to ureolysis-driven MICP treatment technology in synthetic wastewater amended with urea and calcium chloride. They found the impact of metal toxicity on microbial substrate degradation, as measured by the reduction in biochemical oxygen demand (BOD), increased in the following order: Cd (II) > Cu(II) > Pb(II) > Cr(VI) > Ni(II) > Zn(II) (Kurmaç 2009). In the application of MICP, metal toxicity may be a limiting factor in treatment efficacy, but isolation of metal-tolerant ureolytic organisms from contaminated environments may improve the treatment potential.

Polychlorinated biphenyl containment

Additional recalcitrant contaminants threatening environmental and human health are polychlorinated biphenyls (PCBs), which can contaminate concrete surfaces when PCB-containing oil leaks from equipment. Methods of removing PCB-contaminated oil include solvent washing, hydroblasting, or sandblasting followed by encapsulation in epoxy coating. Epoxy coating may be ineffective due to resurfacing of the oil over time (Okwadha & Li 2011). An alternative to epoxy coating is the use of ureolysis-driven MICP to produce a coating to seal PCB-contaminated concrete (Figure 1f). By applying *S. pasteurii* cultures and urea/calcium treatment to the surface of PCB-coated cement cylinders, surficial PCB-containing oils were encapsulated. No leaching through the MICP coating was observed and permeability was reduced by 1–5 orders of magnitude (Okwadha & Li 2011).

Table 1. Summary of control parameters and ranges used to promote microbially induced calcium carbonate precipitation (MICP).

Control variable	Range	General assessment of success	Relevant references
		Ureolytic activity of organisms	
Inoculation concentration	0.03–2.88 OD_{600} 10^5–10^9 cfu ml^{-1}	Greater bacterial concentrations = faster rates of ureolysis & produce larger and less soluble crystals	Mitchell and Ferris (2006b), Harkes et al. (2010), Okwadha and Li (2010), Tobler et al. (2011)
Microorganism	*S. pasteurii, B. lentus, B. megaterium, B. sphaericus, S. ginsengisoli, K. flava, B. pseudofirmus, B. cohnii, B. alkalinitrilicus,* native organisms or enzyme	Biofilm communities may have higher activity; stimulation of native ureolytic organisms desirable; strain should have strong urease production, should not be pathogenic or genetically modified if bioaugmentation is necessary	Bachmeier et al. (2002), Hammes et al. (2003), Whiffin (2004), Dick et al. (2006), Fujita et al. (2008, 2010), Achal, Pan, et al. (2011), Burbank et al. (2011), Tobler et al. (2011), Wiktor and Jonkers (2011), Achal et al. (2012), Cuthbert et al. (2012)
Carbon source	BHI, NB, Yeast extract, Tryptic soy broth, Peptone, Acetate, Lactose mother or Corn starch liquor, Molasses	Alternate carbon sources may improve economic feasibility for field over lab grade reagents; injection of a carbon source prior to urea may stimulate subsurface attached communities	Stocks-Fischer et al. (1999), Fujita et al. (2008), Achal et al. (2009, 2011a), Mitchell et al. (2010), van Paassen et al. (2010), Burbank et al. (2011), Mortensen et al. (2011), Tobler et al. (2011)
		Reaction and transport	
Flow conditions	Constant flow Examples: 0.7 pore volumes per day; 0.35–12 $l h^{-1}$	Constant low flow rates may lead to non-homogeneous $CaCO_3$ distribution/injection point plugging; higher injection rates = more even distribution of bacteria, homogenous $CaCO_3$ distribution, minimize injection point cementation (ie $Da \ll 1$)	Whiffin et al. (2007), Harkes et al. (2010), Cunningham et al. (2011), Mortensen et al. (2011), Schultz et al. (2011), Tobler et al. (2012)
	Pulse or percolation flow Example: static intervals between treatments	Growth treatments may be used to overcome mortality due to $CaCO_3$ entombment & supply electron acceptors; pulsed flow may give more homogeneous $CaCO_3$ precipitation	DeJong et al. (2006), De Muynck, Verbeken, et al. (2010), van Paassen et al. (2010), Burbank et al. (2011), Cunningham et al. (2011), Mortensen et al. (2011), Stabnikov et al. (2011), Al Qabany et al. (2012), Ebigbo et al. (2012), Tobler et al. (2012), Lauchnor et al. (2013), Phillips et al. (2013)
Viscosity	Low (ie close to water) to high (PU)	Low viscosity fluids can penetrate smaller fractures/ treatment areas with less pumping pressure; bacterial distribution may be more homogenous in less viscous solutions	Cunningham et al. (2011), Wang et al. (2012)
Temperature	10–60 °C	Increased ureolysis rates observed at higher temperature; urease enzyme can withstand even higher temperatures than mesophilic ureolytically active cells, particularly if immobilized	Bachmeier et al. (2002), Ferris et al. (2003), Mitchell and Ferris (2005, 2006a), Dupraz, Parmentier, et al. (2009), Tobler et al. (2011), Bang et al. (2011)
Salinity	0.36–100 $g l^{-1}$	Increased salinity may increase alkalinity, ureolysis rates, and bacterial adsorption; increased salinity may increase time delay to S_{crit}; phosphates may decrease precipitation rates	Ferris et al. (2003), Dupraz , Menez, et al. (2009), Dupraz, Parmentier, et al. (2009), Harkes et al. (2010), Mortensen et al. (2011), Rusu et al. (2011), Stabnikov et al. (2011)

(*Continued*)

Table 1. (*Continued*)

Control variable	Range	General assessment of success	Relevant references
		Saturation conditions	
pH	4.5–13	Peak enzyme activity = pH 8.0; optimal *S. pasteurii* growth = pH 8.5; extreme (low and high) pH environments may be detrimental to activity; once formed $CaCO_3$ is resilient to acid attack when pH > 1.5 (at certain time scales); pH increases after ureolysis are followed by pH decrease due to $CaCO_3$ precipitation	Gollapudi et al. (1995), Stocks-Fischer et al. (1999), Bang et al. (2001), Achal et al. (2009), Chunxiang et al. (2009), Dupraz , Menez, et al. (2009), Dupraz, Parmentier, et al. (2009), Bang et al. (2010), Okwadha and Li (2010), Tobler et al. (2011)
Urea/calcium concentration	Urea: 6 mM–1.5 M Calcium: 25 μM–1.25 M	Equimolar urea/Ca^{2+} ratio may be optimal since greater reactant concentration = higher kinetic rates (but only to a certain point) & to balance reagents for reaction but to not add extra unwanted chemicals to environment; calcium nitrate or acetate alternatives to calcium chloride	Warren et al. (2001), Ferris et al. (2003), De Muynck et al. (2008), Chunxiang et al. (2009), Dupraz, Parmentier, et al. (2009), De Muynck, Verbeken, et al. (2010), Okwadha and Li (2010), Van Tittelboom et al. (2010), Burbank et al. (2011), Cunningham et al. (2011), Mortensen et al. (2011), Stabnikov et al. (2011), Tobler et al. (2011), Lauchnor et al. (2013)
Saturation state (Ω, S or S_{crit})	12–436	Saturation state and critical saturation state influence spatial & temporal precipitation of $CaCO_3$	Ferris et al. (2003), Mitchell and Ferris (2005, 2006b), Dupraz, Parmentier, et al. (2009), Tobler et al. (2011)

Carbon dioxide sequestration

With atmospheric carbon dioxide (CO_2) concentrations on the increase, mitigation strategies are being explored widely. One proposed mechanism for reducing emissions is the capture and storage of CO_2 in deep geologic reservoirs, such as deep saline aquifers. The efficacy of this mitigation method depends on preventing potential CO_2 leakage either back to the surface or into overlying aquifers. Possible reasons for leakage may include: (1) decreased well bore integrity, possibly due to the corrosive effect of supercritical CO_2, also known as carbonation, or fractures in those well cements; or (2) areas of increased cap rock permeability (Huerta et al. 2008; Barlet-Gouédard et al. 2009; Wigand et al. 2009; Carey et al. 2010). Traditional well repair methods include the use of cements (such as fine cement); however, these may be of higher viscosity than the aqueous solutions used to promote MICP. Higher viscosity fluids may not adequately penetrate small pore spaces and potentially not seal microfractures where low viscosity supercritical CO_2 could find leakage pathways. As such, MICP may be an effective tool to seal fractures or high permeability leakage zones in the context of CO_2 sequestration (Figure 1k), and may also be effective in helping to reliably abandon wells after fossil fuel extraction (Figure 1a).

Three proposed methods to which ureolysis-driven MICP have been suggested to contribute to *in situ* CO_2 leakage mitigation are formation trapping, solubility trapping, and mineral trapping (Dupraz, Menez, et al. 2009; Mitchell et al. 2010). MICP may reduce permeability to mitigate leakage potential (formation trapping). Also, the storage of CO_2 might be enhanced by ureolysis-driven MICP by increasing the dissolved CO_2 (as carbonate or bicarbonate) in the subsurface formation water (solubility trapping). Finally, ureolysis-driven MICP might enhance the precipitation of dissolved CO_2 in carbonate minerals (mineral trapping) (Mitchell et al. 2010).

Formation trapping. Engineered MICP has been proposed to protect well cements from supercritical CO_2, plug microfractures in the near well environment and reduce permeability in cap rock (Mitchell et al. 2010; Phillips et al. 2013). In these applications, the spatial extent and temporal efficiency of precipitation must be controlled. Experiments under atmospheric conditions have led to evolved injection strategies to promote more uniform spatial distribution of $CaCO_3$. Pulse flow, with brief fluid injection followed by batch biomineralization periods, rather than continuous flow injections precipitated less $CaCO_3$ near the influent in sand column reactors. Additionally, reducing the SI near the injection point during periods of active biomineralization reduced near-injection-point plugging (Cunningham et al. 2009, 2011; Schultz et al. 2011; Ebigbo et al. 2012). Recently,

these injection strategies have been used to seal hydraulic fractures in 70 cm diameter sandstone cores under ambient (Phillips et al. 2013) and high pressure (Phillips et al. personal communication) conditions.

Solubility and mineral trapping. Spore and biofilm-forming *Bacillus* species are resistant to high pressures and supercritical CO_2 (Mitchell et al. 2008, 2009). Accordingly, Mitchell et al. (2010) studied S. *pasteurii*, for ureolysis-driven $CaCO_3$ precipitation with a range of initial ^{13}C–CO_2 head pressures and urea concentration in artificial groundwater. Precipitated $CaCO_3$ was heavily enriched in ^{13}C–CO_2 and the fraction of ^{13}C–CO_2 increased with increasing headspace pressure and urea concentrations, suggesting that ureolysis enhanced the amount of carbonate in the $CaCO_3$ derived from headspace CO_2 (g) (mineral trapping). Dupraz, Menez, et al. (2009) also studied *S. pasteurii* in artificial groundwater to determine the transformation of CO_2 into a solid carbonate phase (mineral trapping) under different temperature and salinity conditions (relevant to subsurface saline aquifer conditions) with different partial pressures of CO_2. While no temperature dependence of $CaCO_3$ precipitation rates was found in their studies, it was observed that increased salinities increased alkinalization and ureolysis rates, but created a delay in time before $CaCO_3$ precipitation began (Dupraz, Menez, et al. 2009). Finally, Mitchell et al. (2010) also demonstrated that as pH increases, the DIC increases and headspace CO_2 (g) decreases (solubility trapping). It was concluded that ureolysis-driven MICP in the subsurface can potentially increase the security of long-term CO_2 storage. Ongoing research suggests ureolysis-driven MICP also occurs at high pressures (>73 bar) and those derived minerals are relatively stable under the time scales tested when subjected to supercritical CO_2 exposure (Mitchell et al. 2013).

In summary, control of ureolysis-driven MICP for remediating subsurface environments of strontium contaminated groundwater, toxic metal contaminated soils and groundwater, PCB-contaminated concrete or improving security of geologically sequestered CO_2 has been widely explored. Research has focused on methods to maintain ureolytic activity and understand the transport and reaction rates of urea and calcium, which influence $CaCO_3$ saturation conditions (Table 1). Ureolysis-driven MICP may effectively treat a wide variety of engineering challenges, but care should be taken to consider the maintenance of ureolytic activity (viability of organisms) under adverse contaminant exposure.

Summary

Much of the literature surrounding ureolysis-driven MICP focuses on controlling the wide-range of parameters that influence precipitation. The range of variables and the optimum values determined for specific MICP applications indicate that there is not one 'recipe' for controlling MICP in engineered applications. The success of MICP treatment depends on the ability to precipitate $CaCO_3$ at appropriate locations and times. Ureolysis-driven MICP is controlled by three main parameters, (1) the ureolytic activity (of microorganisms), (2) the reaction and transport rates of the substrates, and (3) the saturation conditions of carbonate minerals (Table 1).

First, organisms or enzyme are either injected or stimulated to provide the catalyst for ureolysis and cells may act as nucleation sites for precipitation to occur. Several challenges surround maintaining the ureolytic activity of microorganisms, such as adverse environmental conditions (eg high pH or toxic metals), electron acceptor (eg oxygen) limitations, entombment in calcium carbonate, and nutrient diffusion limitations causing cell inactivation after entombment.

Second, the reaction rates and the transport rates of reactants are manipulated, for example, by changing the flow conditions (eg velocity) or reagent concentrations. Factors such as fluid salinity and temperature can influence the rates of ureolysis and mineral precipitation. Flow rate and fluid viscosities can influence the transport conditions. Exploring the dimensionless Da, which is the ratio of reaction rate to transport rate, as a tool in MICP design under various conditions may provide valuable insight for controlling ureolysis and precipitation and ultimately the success of MICP engineered applications.

Finally, whether calcium carbonate has the thermodynamic propensity to precipitate is governed by the saturation conditions, and the location and timing of precipitation can be influenced by the presence of nucleation sites. The S or SI is determined by the activity of Ca_2^+ and CO_3^{2-} and S_{crit} or SI_{crit} are empirical values which reflect how highly supersaturated a solution must become before precipitation is observed. S_{crit} can be influenced by a variety of factors including but not limited to ureolysis kinetics and the availability of nucleation sites.

A wide range of factors can impact the saturation state to promote precipitation of $CaCO_3$ in engineered MICP technologies (Table 1). Since controlling saturation conditions and precipitation in time and space is a multi-factored reactive transport challenge, modeling has become an essential tool to optimize injection and treatment strategies. Current models, carefully interpreted and calibrated, explore promotion of favorable saturation state and predict treatment efficacy while decreasing the need for labor-intensive laboratory experiments (Ebigbo et al. 2010, 2012; Zhang & Klapper 2010; Barkouki et al. 2011; Fauriel & Laloui 2011).

Improving the economic and environmental feasibility of ureolysis-driven MICP treatment must be consid-

ered in the transition from laboratory to field-relevant scale engineered MICP technologies. There is an economic limitation to the use of laboratory grade nutrient sources in field applications and alternate nutrient sources such as inexpensive industrial wastewater, lactose mother liquor (dairy industry), and corn steep liquor (starch industry) may offer a possibility of cheaper nutrient sources (Achal et al. 2009, 2011a; Mitchell et al. 2010). Additionally, large volumes of reactant and the production of bacterial cultures for injection (if necessary) may make certain engineered applications of MICP economically challenging compared to traditional treatments. Optimizing treatment strategies may reduce cost by minimizing unnecessary injection or the excessive use of amendments. Unwanted by-products from ureolysis such as NH_4^+, have to be considered and controlled at least in certain prospective applications. NH_4^+ is undesirable, since groundwater aquifer health may be harmed, stone discolored, or subsurface communities changed by metabolic competition (eg outcompeting bioaugmented organisms) due to NH_4^+ salts or conversion products (De Muynck, De Belie, et al. 2010; Tobler et al. 2011). While promising and effective treatment strategies using MICP have been demonstrated, additional research is necessary in order to improve economic feasibility, define optimal treatment strategies and reduce unwanted by-products.

Outlook

With the wide variety of ureolysis-driven MICP applications being researched and developed around the world, there remain a number of technology development challenges and thus research opportunities. In order to improve the potential for successful MICP application, additional strategies have to be developed through further research including, but not limited to: (1) investigating the potential of biofilm-based MICP approaches compared to suspended cell-based approaches, specifically differences in ureolysis and mineral precipitation kinetics, mineralogy, mineral reactivity and stability between attached and planktonic cultures; (2) determining the optimal substrate balance (eg urea and calcium) for various MICP applications with the goal of optimizing $CaCO_3$ precipitation efficiency, which may increase economic feasibility and reduce production of unwanted byproducts; (3) investigating nano- to micro-scale mineral nucleation processes and determining the effects on subsequent mineral growth, morphology, and stability at larger scales; (4) improving mathematical models describing MICP processes in porous media by developing quantitative descriptions of fundamental processes at the micro- and macro-scale (eg ureolysis and growth kinetics, precipitation kinetics, crystal growth, and microbe-mineral interactions) as well as integrating these process descriptions into Darcy-scale models for large-scale application design; (5) experimenting at larger scales, which, together with the developed models, will allow for the evaluation of the importance of transport processes in controlling MICP for engineered field application; (6) developing *in situ* monitoring technologies (such as geophysical methods) that allow assessment of success in field applications; and (7) evaluating long-term stability of MICP treatments compared to conventional (eg cement-based) technologies.

It is evident that the implementation of MICP-based technologies on a field scale requires the expertise of many disciplines, and multi-disciplinary research and development teams will be necessary. This review summarizes the research results across many proposed engineered applications in an effort to inspire researchers to address the key research and development questions necessary to move MICP technologies toward commercial scale applications.

In conclusion, ureolysis-driven MICP has been suggested for a wide variety of engineered treatments including modification of construction materials, cementing porous media, hydraulic control, and remediating environmental contaminants (Figure 1). A majority of the literature focuses on promoting ureolytic activity, understanding the reaction and transport conditions, and ultimately manipulating the saturation state to achieve the desired timing and location of $CaCO_3$ precipitation. Many potential applications of ureolysis-driven MICP exist, including those discussed in this review and other applications such as stabilizing building foundations or slopes; minimizing erosion, stabilizing grounds prior to tunneling; sealing tunnel seepage; strengthening earthen dams and dikes; strengthening dunes to protect shorelines or prevent desertification; as well as removing calcium from waste streams (Hammes 2002; DeJong et al. 2010, 2011). A diverse, multi-disciplinary research effort including field demonstrations, modeling, and elucidation of the fundamental mechanisms of ureolysis-driven MICP has and will continue to aid in the effort of transitioning MICP-based technologies from the laboratory to the field.

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