Sequestration of Radionuclides and Metal Contaminants through Microbially-Induced Carbonate Precipitation

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ABSTRACT
Microbially induced carbonate precipitation (MICP), an emerging technology for soil improvement, also may be used to sequester (biminerализе) radionuclides and metal contaminants (e.g., Sr⁹⁰⁺⁺, Cd²⁺) in groundwater, a significant problem at some U.S. Department of Energy sites. Previous work using the bacterium Bacillus pasteurii suggests that in-situ sequestration of these contaminants can be achieved through MICP via hydrolytic ureolysis. However, ureolysis produces harmful byproducts, such as ammonium, and requires aerobic conditions. Biomineralization through bacterial denitrification offers a promising alternative for in-situ remediation in these cases. In contrast to ureolysis, highly ubiquitous denitrifying bacteria, including Pseudomonas denitrificans, are capable of MICP without the production of harmful byproducts. Biomineralization of metal contaminants through the stimulation of native denitrifying bacteria may provide a more sustainable means of remediating groundwater impacted by radionuclides and metal contaminants than hydrolytic ureolysis.

RÉSUMÉ
La précipitation de carbonate par activité bactérienne (MICP), une technologie émergente pour l’amélioration des sols peut être utilisée pour séquestrer des radionucléides ou contaminants métalliques (⁹⁰Sr⁺⁺, Cd²⁺) dans les eaux souterraines, un sérieux problème sur certains sites du Ministère de l’Energie des USA. Des travaux basés sur l’usage de la bactérie Bacillus pasteurii suggèrent que séquestrer in-situ ces contaminants peut être accomplie par MICP via l’uréolyse hydrolytique. Cependant, l’uréolyse génère des sous-produits nuisibles, telle l’ammoniaque, et exige un milieu aérobie. La biominéralisation par dénitrification bactérienne est dans ce cas une alternative prometteuse pour l’assainissement in-situ. Par contraste avec l’uréolyse, les bactéries dénitrifiantes omniprésentes (Pseudomonas denitrificans inclus) provoquent la MICP sans sous-produits nuisibles. La biominéralisation par stimulation des bactéries dénitrifiantes natives serait un moyen plus viable que l’uréolyse hydrolytique pour l’assainissement des eaux souterraines contaminées par radionucléides et contaminants métalliques.

1. INTRODUCTION

1.1 Background

Groundwater has historically been and continues to be an important source of freshwater on earth, as well as an integral component of the hydrologic cycle. Recent estimates place approximately 22% (8,400,000 km³) of Earth’s freshwater distribution in the subsurface, where a large portion of this total figure can be readily accessed via aquifers (Christopherson, 2009). Unfortunately, groundwater is susceptible to contamination through wells, unlined waste storage units, run-off, and surface waterways. The contamination of aquifers has become a growing concern in many areas and, therefore, has been the focus of recent research efforts to develop novel and effective remediation methods.

One such novel approach is microbial biomineralization of radionuclides and metal contaminants through in-situ remediation of contaminated aquifers (Mitchell and Ferris, 2005; Colwell et al., 2003; Fujita et al., 2000; Smith et al., 2004). Poor waste-disposal practice has resulted in the release of low-level radioactive waste and metal contaminants (e.g., Sr⁹⁰⁺⁺, Cd²⁺, Co⁶⁰⁺⁺) into the vadose zone and groundwater at many U.S. Department of Energy (DOE) weapons-production sites. These toxic waste products are a legacy of DOE chemical synthesis and nuclear waste facilities in locations such as Hanford, WA (100-N area) and the Idaho Nuclear Technology and Engineering Center.
Microorganisms, bacteria in particular, have long been associated with carbonate minerals and are thought to play a fundamental role in carbon cycling on the geologic timescale (Shock 2009; Vasconcelos et al., 1995). The complex interactions between microorganisms and minerals have been well-documented through the efforts of researchers attempting to understand the formation, dissolution, and alteration of minerals by microorganisms on geologic and engineering timescales (Ehrlich 2002; Drevet 2008; Karatas 2008; Phoenix and Konhauser, 2008; Shock, 2009). Indeed, the geologic record is replete with examples of carbonate deposits, such as the White Cliffs in Dover, England and the Bahamas Banks that are associated with microbially driven processes. Microbial biomineralization is a microbially mediated mineral precipitation process that results in the formation of mineral deposits found in the natural environment, including calcium-carbonate (CaCO$_3$) minerals such as calcite. Calcite is the most thermodynamically stable polymorph of CaCO$_3$ and the primary product in many microbially induced calcium carbonates. Biologically mediated precipitation of CaCO$_3$ minerals can occur when carbonate ions (CO$_3^{2-}$) are formed in the presence of calcium ions (Ca$^{2+}$) under alkaline conditions (Eq. 1).

$$\text{Ca}^{2+} + \text{HCO}_3^- + \text{OH}^- = \text{CaCO}_3(s) + \text{H}_2\text{O} \quad [1]$$

In principle, the geochemical conditions conducive to carbonate precipitation are not unique to any specific microorganism; rather, carbonate precipitation can occur when carbonate (CO$_3^{2-}$) forms in the vicinity of suitable cations under alkaline conditions. Microbially induced carbonate precipitation (MICP) relies on the byproducts of bacterial metabolism (e.g., CO$_2$, alkalinity) to facilitate the formation of carbonate ions, which precipitate in the presence of divalent cations. Biomineralization of radionuclide and contaminant metals into calcite is essentially a competitive co-precipitation reaction in which suitable divalent cations are incorporated into the calcite lattice (Eq. 2).

$$^{90}\text{Sr}^{2+} + \text{OH}^- + \text{HCO}_3^- = \text{SrCO}_3(s) + \text{H}_2\text{O} \quad [2]$$

These cations and radionuclides are thought to be integrated into the calcite structure via substitution of calcium ions in the microenvironment of the mineral precipitate, forming low-strontium carbonate minerals. In theory, these minerals should similarly resist dissolution since strontium carbonate (SrCO$_3$) is at least as equally insoluble as CaCO$_3$ ($K_{sp}$calcite $\approx$10$^{-9}$, $K_{sp}$-strontianite $\approx$10$^{-10}$, at STP). Incorporation of divalent ions into the calcite structure appears to slow their transport and possibly immobilize them within the calcite structure (Mitchell and Ferris, 2005; Colwell et al., 2003; Fujita et al., 2004; Fujita et al., 2000; Smith et al., 2004). If proven effective, immobilization of these contaminants through MICP may provide a sustainable and cost-effective in-situ remediation scheme for radionuclide and metal contaminated sites.

2. MICROBIALLY INDUCED CARBONATE PRECIPITATION

2.1 Bacterial Ureolysis

Recent work conducted by Smith et al., (2004), Fujita et al., (2004) and others on artificial groundwater designed to mimic contaminated groundwater beneath a DOE research facility provides some insight into the feasibility of MICP for radionuclide and metal contaminant sequestration. The artificial groundwater samples were formulated to simulate those found in contaminated areas of the Snake River Plain Aquifer (SRPA), a highly productive aquifer with a storage capacity of nearly 1.23E11 m$^3$ located in south-eastern Idaho. The SRPA underlies an area of interest near the Idaho National Engineering and Environmental Laboratory (INEEL), where radionuclides and contaminant metals have been detected in the groundwater and vadose zone. Although the researchers’ area of interest focused on the SRPA, the overall context and applicability of their studies presumably apply to other similarly contaminated DOE sites.

The primary contaminant of interest in these studies was the divalent radionuclide $^{90}\text{Sr}^{2+}$. Incorporation of $^{90}\text{Sr}^{2+}$ into calcite through bacterial ureolysis was carried out using Bacillus pasteurii, a heterotrophic bacterium capable of ureolytic hydrolysis. Ureolysis, the ammonification (transformation of organic nitrogen into ammonia) of urea, results in the production of ammonium ($\text{NH}_4^+$), dissolved organic carbon in the form of bicarbonate (HCO$_3^-$), and an increase in alkalinity (shown by OH$^-$ in Eq. 3).

$$\text{NH}_2\text{CONH}_2 + 3\text{H}_2\text{O} = 2\text{NH}_4^+ + \text{HCO}_3^- + \text{OH}^- \quad [3]$$

This process is associated with high rates of carbonate precipitation, but is severely limited in low-oxygen environments typical of subsurface aqueous environments, since B. pasteurii carries out aerobic respiration during ureolysis. Upon formation of a sufficiently saturated solution with respect to calcite, a precipitation reaction incorporates $^{90}\text{Sr}^{2+}$ into the calcite structure (Eq. 4).

$$\text{Sr}^{2+} + \text{HCO}_3^- + \text{OH}^- = \text{SrCO}_3(s) + \text{H}_2\text{O} \quad [4]$$

In carefully controlled bench-scale experiments, researchers have successfully demonstrated the co-
precipitation of Sr\(^{2+}\) into calcite through bacterial ureolysis (Mitchell and Ferris, 2005; Colwell et al., 2003; Fujita et al., 2004; Fujita et al., 2000; Smith et al., 2004). Although the total amounts of Sr incorporated into calcite varied considerably, the overall trend of these experiments indicate that a predominately Ca-calcite product is formed with Sr-ions partitioned between calcite and artificial groundwater. As such, treatment of radionuclide-contaminated groundwater using MICP will likely require considerable ureolysis and precipitation of Ca-calcite to immobilize the contaminants in sufficiently large quantities.

Large-scale application of MICP through bacterial ureolysis may pose significant environmental hazards since one of the primary byproducts is ammonium (NH\(_4^+\)). Ammonium is considered a toxic substance with exceptionally high water solubility (31% w/w as NH\(_3\) at 25\(^\circ\)C) and, therefore, has the potential to profoundly impair groundwater quality if left unmitigated. Although the U.S. Environmental Protection Agency (EPA) has not established a maximum contaminant level (MCL) for ammonium in drinking water, the EPA has issued a lifetime exposure advisory of 30 mg/L for ammonia. Furthermore, the 1998 European Union Council Directive 98/83/EC regarding water quality for human consumption recommends a drinking water standard of 0.5 mg/L, a measure adopted by several European nations.

Several studies have suggested that ammonium ions produced by the ureolysis reaction may exchange with radionuclides and metal contaminants sorbed to subsurface minerals, which may enhance the availability of these contaminants for co-precipitation into calcite (Colwell et al., 2003; Fujita et al., 2004; Fujita et al., 2000; Mitchell and Ferris, 2005; Smith et al., 2004). Although the ion-exchange concept may be theoretically feasible, none of the studies offered either bench-scale or field-scale data supporting the idea that ammonium, a water-soluble polyatomic ion, may sorb to minerals in exchange for radionuclides and metal contaminants. Furthermore, the inherent geochemical variability of natural systems may introduce many uncertainties in the potential sorption behavior of water soluble contaminants such as ammonium. For example, it has been reported that different radionuclides exhibit varying retention (and release) times and transport behavior in similar soil or rock-deposits (Beasley et al., 1998). Often times, some simplifying assumptions are required regarding transport and soil/rock retention of contaminants, but these factors may be secondary to highly pH-dependent mechanisms governing chemical equilibria of ammonium speciation. For example, it is likely that at the higher pH values characteristic of bacterial ureolysis a sizable fraction of ammonium may shift to ammonia and volatilize, thereby limiting the possibility of ion exchange phenomenon for this fraction. Furthermore, the generation of ammonium will have a range of negative environmental effects that include: the potential for large nitrogenous oxygen demand, reactions with chlorine, and the potential for eutrophication of surface waters. Thus, ureolysis introduces a new contaminant while displacing another.

2.2 Denitrification: An Alternative to Bacterial Ureolysis

A more environmentally acceptable bioremediation alternative to bacterial ureolysis may be bacterial denitrification, which can be carried out by many types of bacteria, including the well-studied Pseudomonas denitrificans. P. denitrificans is a gram-negative facultative anaerobe capable of reducing calcite precipitation without the production of toxic byproducts (Ehrlich, 2002; Karatas et al., 2008; van Passen et al., 2010). Recent developments in bio-soils research suggest soil cementation through denitrification is gaining ground over previously studied candidate processes, such as bacterial ureolysis (DeJong et al., 2008; Karatas, 2008; van Passen et al, 2008; van Passen et al, 2010).

As noted above, the geochemical conditions conducive to carbonate precipitation are not unique to any specific microorganism. The initial steps of calcite precipitation by bacterial denitrification involve the production of alkalinity and carbon dioxide (CO\(_2\)) when nitrate (NO\(_3^-\)) serves as an electron acceptor. The nitrate is ultimately reduced to nitrogen gas (N\(_2\)), driven by bacterial metabolism of an electron donor (e.g., acetate, CH\(_3\)COO\(^-\)). In terms of the amount of carbonate yield per mole of substrate consumed, denitrification produces 2 moles of CO\(_3^{2-}\) per mole of acetate (and 1.6 mole NO\(_3^-\)), whereas ureolysis produces only 1 mole of CO\(_3^{2-}\) per mole of urea (Eqs. 5 & 3, respectively).

\[
\frac{8}{5} \text{NO}_3^- + \text{CH}_3\text{COO}^- \rightarrow \frac{4}{5} \text{N}_2 + 2 \text{CO}_2 + \frac{13}{5} \text{HO}^- + \frac{1}{2} \text{H}_2\text{O} \quad [5]
\]

As illustrated in Eq. 5, denitrification has a distinct advantage over ureolysis in that the major byproduct, nitrogen gas, is completely inert and non-toxic. The remaining chemical processes in bacterial denitrification (Eqs. 6 & 7) involve the speciation of chemical constituents conducive to calcite precipitation similar to that described above for ureolytic hydrolysis under alkaline conditions in the presence of calcium ions. The rate of carbonate precipitation through denitrification is not limited by the lack of oxygen normally observed in the subsurface since the denitrifiers use nitrate as their electron acceptor instead of oxygen.

\[
\text{CO}_2 + \text{H}_2\text{O} = \text{HCO}_3^- + \text{H}^+ \quad [6]
\]

\[
\text{Ca}^{2+} + \text{HCO}_3^- + \text{OH}^- = \text{CaCO}_3(s) + \text{H}_2\text{O} \quad [7]
\]

The highly ubiquitous bacteria of genera Pseudomonas have been identified in the SRPA (Fujita et al., 2000; Ehrlich, 2002). Assuming an appropriate native strain, it seems perfectly plausible that denitrification may be employed for the effective treatment of aquifer contaminants through MICP with minor solution amendment; perhaps only an exogenous electron donor is required if nitrate already is present. Denitrification can occur in a broad range of low NO\(_3^-\) concentrations. For
example, Lee and Rittmann (2003) conducted autotrophic denitrification experiments using a hollow-fiber, membrane-biofilm reactor (MBIR) in which denitrification readily occurred at input NO₃ concentrations as low as 12.5 mg/L (0.201 mM), while concentrations in the MBIR were as low as 0.1 mg/L. It should also be noted that high nitrate concentrations (>25 mM) have been observed to inhibit denitrification in bench-scale experiments (Karatas, 2008).

Based on the reported composition of artificial groundwater designed to mimic the contaminated SRPA, pre-existing nitrate concentrations (~0.080 mM NO₃) may be minimally sufficient for denitrification. MICP without the addition of exogenous NO₃ would be desirable, as it avoids costs and the constraints associated with a regulated water pollutant.

As previously noted, autotrophic denitrification also may be achieved by using an MBIR. In the presence of suitable divalent cations and sufficiently high levels of aqueous CO₂, an MBIR promotes calcite precipitation by producing the necessary alkalinity and raising the pH (Lee and Rittmann, 2003; Tang et al., 2010). Furthermore the MBIR has the potential for almost 100% utilization of the electron donor, hydrogen gas (Lee and Rittmann, 2003).

3. Conclusion and Suggestions for Future Work

Co-precipitation of strontium along with calcium in calcite appears to be a viable option for in-situ remediation of radionuclide-contaminated aquifers. Co-precipitation via denitrification has the potential to be a preferred method for in-situ remediation of radionuclide and metal contaminants since: (1) denitrification is cost-effective, particularly when nearly 100% utilization of electron donor is possible; (2) denitrification does not produce toxic byproducts, such as ammonium from urea; (3) denitrification has a greater CO₂: yield per mole of substrate than ureolysis; (4) denitrification can proceed under anoxic conditions typical of subsurface environments; (5) denitrification does not require the addition of potentially toxic organics such as urea; and (6) denitrification does not require the addition of a water-soluble, organic electron donor to a freshwater aquifer if hydrogen gas is used.

Exploratory work could be undertaken to determine/confirm the feasibility of denitrification for in-situ biomineralization of radionuclides and metal contaminants in artificial SRPA groundwater. If preliminary results prove promising, further efforts could involve direct comparisons between hydrolytic ureolysis and denitrification for contaminant biomineralization, where parameters of interest may include relative rates of co-precipitation, cost per unit(s) of contaminant removed and net environmental impact of proposed remediation.

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