FINAL TECHNICAL REPORT

Microbial Activity and Precipitation at Solution-Solution Mixing Zones in Porous Media

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A Collaborative Research Project between Montana State University, Oregon State University and the Idaho National Laboratory

Submitted to the DOE

by

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1. Executive Summary

Background. The use of biological and chemical processes that degrade or immobilize contaminants in subsurface environments is a cornerstone of remediation technology. The enhancement of biological and chemical processes *in situ*, involves the transport, displacement, distribution and mixing of one or more reactive agents. Biological and chemical reactions all require diffusive transport of solutes to reaction sites at the molecular scale and accordingly, the success of processes at the meter-scale and larger is dictated by the success of phenomena that occur at the micron-scale. However, current understanding of scaling effects on the mixing and delivery of nutrients in biogeochemically dynamic porous media systems is limited, despite the limitations this imposes on the efficiency and effectiveness of the remediation challenges at hand.

Objectives. We therefore proposed to experimentally characterize and computationally describe the growth, evolution, and distribution of microbial activity and mineral formation as well as changes in transport processes in porous media that receive two or more reactive amendments. The model system chosen for this project was based on a method for immobilizing ^{90}Sr, which involves stimulating microbial urea hydrolysis with ensuing mineral precipitation ($CaCO_3$), and co-precipitation of Sr. Studies at different laboratory scales were used to visualize and quantitatively describe the spatial relationships between amendment transport and consumption that stimulate the production of biomass and mineral phases that subsequently modify the permeability and heterogeneity of porous media. Biomass growth, activity, and mass deposition in mixing zones was investigated using two-dimensional micro-model flow cells as well as flow cells that could be analyzed using synchrotron-based x-ray tomography. Larger-scale flow-cell experiments were conducted where the spatial distribution of media properties, flow, segregation of biological activity and impact on ancillary constituents (i.e., Sr) was determined. Model simulations accompanied the experimental efforts.

Benefits and Outcomes of the Project. The research contributed towards defining the key physical, chemical, and biological processes influencing the form and mobility of DOE priority contaminants (e.g., ^{60}Co, ^{90}Sr, U) in the subsurface. The work conducted and reported herein, will in the future (i) contribute to controlling the juxtaposition of microbial activity, contaminants and amendments, (ii) promote new strategies for delivering amendments, and (iii) allow new approaches for modifying permeability and flow in porous media. We feel that the work has already translated directly to improving the efficiency of amendment based remediation strategies.

Products. The results of the project have been published in a number of peer reviewed journal articles. The abstracts and citations to those articles, given in section 2.0 below, make up the bulk of this final report.

2.0 Publications

2.1 Publication 1

2.1.1. Citation:
Gerlach, R.; Cunningham, A.B. (2010): Influence of Biofilms on Porous Media Hydrodynamics. Vafai, K. (ed.), Porous Media: Applications in Biological Systems and Biotechnology. Taylor Francis. pp. 173-230.

2.1.2. Abstract:
Microbial biofilms form in natural and engineered systems and can significantly affect the hydrodynamics in porous media. Microbial biofilms develop through the attachment and growth of microorganisms, which encase themselves in self-produced extracellular polymeric substances (EPS). Microbial biofilms are, in general, more resistant to environmental stresses, such as mechanical stress, temperature, pH, and water potential fluctuations, than planktonic cells. Biofilm growth in porous media influences porosity, permeability, dispersion, diffusion, and mass transport of reactive and nonreactive solutes. Understanding and controlling biofilm formation in porous media will maximize the potential benefit and will minimize the detrimental effects of porous media biofilms. Subsurface remediation, enhanced oil recovery, and carbon sequestration are only a few examples of beneficial porous media biofilm applications.

2.1.3. Summary of Major Results:
- Overview of processes and factors that can affect the growth and activity of microbial biofilms in porous media and how they (the biofilms) can affect the hydrodynamics in porous media.
- Table of initial and final (or lowest) permeabilities reported for experiments in which biofilm growth in porous media or fractures was promoted.

2.2 Publication 2

2.2.1. Citation:

Schultz, L.; Pitts, B.; Mitchell, A.C.; Cunningham, A.B.; Gerlach, R. (2011): Imaging Biologically-Induced Mineralization in Fully Hydrated Flow Systems. *Microscopy Today*. September 2011:10-13. doi:10.1017/S1551929511000848

2.2.2. Abstract:

No abstract

2.2.3. Summary of Major Results:

- Cover image feature, Microscopy Today, Vol. 19, Issue 5, September 2011
- Highlighted the usefulness of microscopic techniques at two different scales to better understand the process of biomineralization quantitatively and qualitatively.
- Stereomicroscopy was used to analyze the size and distribution of calcite precipitates, the overall immobilization of dissolved calcium, and the solubility of precipitates as a function of position.
- High resolution, confocal scanning laser microscopy allowed for the observation of biofilms and mineral interactions in-situ and non-destructively.
- It is implied that attached microbial communities can have significant local effects on the chemical, biological, and nucleation environment at the mineral surface.

2.3 Publication 3

2.3.1. Citation:

Ebigbo A.; Phillips, A; Gerlach, R.; Helmig, R.; Cunningham, A.B.; Class, H.; Spangler, L. (2012): Darcy-scale modeling of microbially induced carbonate mineral precipitation in sand columns. *Water Resources Research*. 48, W07519, doi:10.1029/2011WR011714

2.3.2. Abstract:

This investigation focuses on the use of microbially induced calcium carbonate precipitation (MICP) to set up subsurface hydraulic barriers to potentially increase storage security near wellbores of CO_2 storage sites. A numerical model is developed, capable of accounting for carbonate precipitation due to ureolytic bacterial activity as well as the flow of two fluid phases in the subsurface. The model is compared to experiments involving saturated flow through sand-packed columns to understand and optimize the processes involved as well as to validate the numerical model. It is then used to predict the effect of dense-phase CO_2 and CO_2-saturated water on carbonate precipitates in a porous medium

2.3.3. Summary of Major Results:

- A numerical model was developed, capable of describing microbially-induced carbonate precipitation and dissolution in porous media in the presence of water and CO_2 flow.
- The numerical model was calibrated and validated using one-dimensional column experiments.
- Relatively uniform microbially mediated precipitation was achieved in three of four MICP experiments involving water-saturated flow through 61 cm, sand-packed columns when near-injection-point calcium-medium displacement strategies were used.
- Calcium deposition efficiency may be optimized by balancing biomineralizing periods with bacterial resuscitation events. Long biomineralization periods could lead to inactivation of bacterial cells (due to cell encapsulation).

2.4 Publication 4

2.4.1. Citation:

Connolly, J.; Kaufman, M.; Rothman, A.; Gupta, R.; Redden, G.; Schuster, M.; Colwell, F.; Gerlach, R. (2013): Construction of two ureolytic model organisms for the study of microbially induced calcium carbonate precipitation. *Journal of Microbiological Methods*. 94(3):290-299. DOI: 10.1016/j.mimet.2013.06.028

2.4.2. Abstract:

Two bacterial strains, *Pseudomonas aeruginosa* MJK1 and *Escherichia coli* MJK2, were constructed that both express green fluorescent protein (GFP) and carry out ureolysis. These two novel model organisms are useful for studying bacterial carbonate mineral precipitation processes and specifically ureolysis-driven microbially induced calcium carbonate precipitation (MICP). The strains were constructed by adding plasmid-borne urease genes (ureABC, ureD and ureFG) to the strains *P. aeruginosa* AH298 and *E. coli* AF504gfp, both of which already carried unstable GFP derivatives. The ureolytic activities of the two new strains were compared to the common, non-GFP expressing, model organism *Sporosarcina pasteurii* in planktonic culture under standard laboratory growth conditions. It was found that the engineered strains exhibited a lower ureolysis rate per cell but were able to grow faster and to a higher population density under the conditions of this study. Both engineered strains were successfully grown as biofilms in capillary flow cell reactors and ureolysis-induced calcium carbonate mineral precipitation was observed microscopically. The undisturbed spatiotemporal distribution of biomass and calcium carbonate minerals were successfully resolved in 3D using confocal laser scanning microscopy. Observations of this nature were not possible previously because no obligate urease producer that expresses GFP had been available. Future observations using these organisms will allow researchers to further improve engineered application of MICP as well as study natural mineralization processes in model systems.

2.4.3. Summary of Major Results:

- Two novel model organisms were constructed to conduct pore scale ureolysis-driven MICP experiments, where organism and mineral growth can be visualized continuously and non-invasively.
- Kinetic analysis revealed that the specific ureolytic activity of MJK1 and MJK2 is not as high as for *S. pasteurii*, the most common organism used for MICP experiments.
- Differences in specific growth and ureolysis rates, along with physiological differences, make the potential use of each of the new model organisms unique.

2.5 Publication 5

2.5.1. Citation:

Lauchnor, E.G.; Schultz, L.; Mitchell, A.C.; Cunningham, A.B.; Gerlach, R. (2013): Bacterially Induced Calcium Carbonate Precipitation and Strontium Co-Precipitation under Flow Conditions in a Porous Media System. *Environmental Science and Technology*. 47(3):1557–1564. http://dx.doi.org/10.1021/es304240y

2.5.2. Abstract:

Strontium-90 is a principal radionuclide contaminant in the subsurface at several Department of Energy sites in the Western U.S., causing a threat to groundwater quality in areas such as Hanford, WA. In this work, we used laboratory-scale porous media flow cells to examine a potential remediation strategy employing co-precipitation of strontium in carbonate minerals. $CaCO_3$ precipitation and strontium co-precipitation were induced via ureolysis by *Sporosarcina pasteurii* in two-dimensional porous media reactors. An injection strategy using pulsed injection of calcium mineralization medium was tested against a continuous injection strategy. The pulsed injection strategy involved periods of lowered calcite saturation index combined with short high fluid velocity flow periods of calcium mineralization medium followed by stagnation (no-flow) periods to promote homogeneous $CaCO_3$ precipitation. By alternating the addition of mineralization and growth media the pulsed strategy promoted $CaCO_3$ precipitation while sustaining the ureolytic culture over time. Both injection strategies achieved ureolysis with subsequent $CaCO_3$ precipitation and strontium co-precipitation. The pulsed injection strategy precipitated 71−85% of calcium and 59% of strontium, while the continuous injection was less efficient and precipitated 61% of calcium and 56% of strontium. Over the 60 day operation of the pulsed reactors, ureolysis was continually observed, suggesting that the balance between growth and precipitation phases allowed for continued cell viability. Our results support the pulsed injection strategy as a viable option for ureolysis-induced strontium co-precipitation because it may reduce the likelihood of injection well accumulation caused by localized mineral plugging while Sr co-precipitation efficiency is maintained in field-scale applications.

2.5.3. Summary of Major Results:

- An injection strategy alternating the addition of $CaCO_3$-precipitating and growth media allowed for more efficient strontium co-precipitation while sustaining ureolytic activity in porous media.
- The results support the pulsed injection strategy as a viable option for ureolysis-induced strontium co-precipitation because it may reduce the likelihood of injection well accumulation caused by localized mineral plugging while Sr co-precipitation efficiency is maintained.

2.6 Publication 6

2.6.1. Citation:

Phillips, A.J.; Gerlach, R.; Lauchnor, E.; Mitchell, A.C.; Cunningham, A.B.; Spangler, L. (2013): Engineered applications of ureolytic biomineralization: a review. *Biofouling*. 29(6): p. 715-733. DOI: 10.1080/08927014.2013.796550

2.6.2. Abstract:

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.

2.6.3. Summary of Major Results:

- Literature review/overview of ureolysis-induced calcium carbonate mineral precipitation research, development and applications.
- Proposal of approaches how to spatially and temporally control precipitation by controlling the ureolytic activity of microorganisms, the reaction and transport of substrates, and the saturation conditions of carbonate minerals.

2.7 Publication 7

2.7.1. Citation:

Ziganshin, A.M.; Gerlach, R. (2013): Pathways of 2,4,6-Trinitrotoluene Transformation by Aerobic Yeasts. In Singh, S. N. (Ed): Biological Remediation of Explosive Residues. Springer International Publishing Switzerland. DOI: 10.1007/978-3-319-01083-0_14

2.7.2. Abstract:

n/a

Introduction: The production and use of various highly persistent synthetic compounds lead to environmental pollution. Among such compounds, 2,4,6-trinitrotoluene (TNT) is the one which is commonly used as an explosive. Synthesis and wide use of TNT in ammunition have resulted in the contamination of soil, air, surface water, and groundwater. TNT and its nitro group reduction products are highly toxic, potentially mutagenic and persistent contaminants which can persist in the environment for a long time. The U.S. Environmental Protection Agency has classified TNT as one of the most dangerous pollutants in the biosphere. Hence, remediation of TNT-contaminated sites is urgently warranted at places of its production and use.

Conclusions: Despite several studies on the mechanisms of TNT transformation by organisms of different evolutionary levels, the understanding of TNT biotransformation is still far from complete. The obligate aerobic yeast strains *Y. lipolytica* AN-L15 and *Geotrichum* sp. ANZ4 transform TNT via two principally different pathways: (1) aromatic ring reduction as the primary transformation pathway resulting in TNT-hydride complexes as intermediates and ultimately leading to the elimination of nitro groups from the aromatic systems as well as the possible destruction of the TNT aromatic backbone; and (2) nitro group reduction leading to the production of hydroxylamino- and amino-dinitrotoluenes. There appear to be at least two principally different pathways of nitro group elimination from the produced Meisenheimer complexes: One pathway is based on the decomposition of 3-H^--TNT with the formation of 2,4-DNT. Another pathway is based on the degradation of one of the isomers of 3,5-2H^--TNT·H^+. It is also possible that a third pathway exists that involves 1-H^--TNT as an intermediate, but due to the low amounts of 1-H^--TNT produced by the yeasts, it has not been possible to purify sufficient amounts of 1-H^--TNT to demonstrate the possible pathway. Each one of these pathways results in the release of the nitro-groups from the carbon skeleton of TNT, accompanied by the appearance of nitrite, which can be further converted enzymatically to nitrate and abiotically to nitrate and NO. The yeasts *Y. lipolytica* AN-L15 and *Geotrichum* sp. AN-Z4 were isolated from oil-polluted peat bog and petrochemical wastes, respectively. Their ability to survive under such extreme conditions, combined with their fairly unique mechanism of TNT degradation, makes these microorganisms promising for the bioremediation of soils and industrial wastes contaminated with explosives and potentially other (nitro)aromatic compounds.

2.7.3. Summary of Major Results:

- The obligate aerobic yeast strains *Y. lipolytica* AN-L15 and *Geotrichum* sp. AN-Z4 transform TNT via two principally different pathways: (1) aromatic ring reduction as the primary transformation pathway resulting in TNT-hydride complexes as intermediates and ultimately leading to the elimination of nitro groups from the aromatic systems as well as the possible destruction of the TNT aromatic backbone; and (2) nitro group reduction leading to the production of hydroxylamino- and amino-dinitrotoluenes.

- There appear to be at least two principally different pathways of nitro group elimination from the produced Meisenheimer complexes: One pathway is based on the decomposition of 3-H^--TNT with the formation of 2,4-DNT. Another pathway is based on the degradation of one of the isomers of 3,5-2H^--TNT$\cdot H^+$.
- Each one of these pathways results in the release of the nitro-groups from the carbon skeleton of TNT, accompanied by the appearance of nitrite, which can be further converted enzymatically to nitrate and abiotically to nitrate and NO.
- The yeasts were isolated from oil-polluted peat bog and petrochemical wastes, respectively. Their ability to survive under such extreme conditions, combined with their fairly unique mechanism of TNT degradation, makes these microorganisms promising for the bioremediation of soils and industrial wastes contaminated with explosives and potentially other (nitro)aromatic compounds.

2.8 Publication 8

2.8.1. Citation:

Connolly, J.; Gerlach, R. (2014): Microbially induced carbonate precipitation in the subsurface: Fundamental reaction and transport processes. Handbook of Porous Media. 3rd Edition. Taylor and Francis. (in print)

2.8.2. Abstract:

n/a
Introduction: Many microorganisms are capable of inducing carbonate mineral precipitation under certain conditions. The geologic record holds many examples of microbially produced carbonates and presently the process is being utilized to manipulate porous media properties. Applications in porous media are numerous but can generally be divided into either increasing the material strength or reducing permeability. The microbiology of such systems is discussed including metabolisms, biofilm concepts and biological reaction rates, followed by mineral precipitation fundamentals and reactive transport modeling approaches. Microbial activity can induce a cascade of physical and chemical changes, which can include the shift of carbonate equilibrium chemistry, precipitation, and changes to system hydrodynamics. Changes in hydrodynamics in turn can change the reaction and transport in biofilm-and mineral-affected porous media. Both Darcy-scale and pore-scale reactive transport concepts will be discussed and classical porous media methodology will be applied to MICP systems. In the subsurface, microorganisms have a close association with the surfaces of the porous medium they live in making them potentially sensitive to changes to those porous media surfaces. Precipitation can cause physical and chemical changes to the surfaces the microbes are attached to and, as a result, can change the reactive transport behavior in the porous medium. The aim of the chapter is to describe the major pore-scale processes associated with MICP and put them in the context of porous media reactive transport modeling approaches. First MICP is divided into biological, physical, and geochemical sub processes; their interactions are discussed at the pore-scale, and finally it is discussed how these processes and interactions affect local and system wide reactive transport. The chapter as a whole is intended to serve as an overview of the current knowledge within the MICP field in regards to pore-scale processes. The processes are represented in equation form where possible to provide mathematical relationships that are useful for modeling.

2.8.3. Summary of Major Results:

- Pore-scale behavior in MICP systems is important to understand for the accurate predictions of larger scale process.
- The biological and abiotic components of the process on their own are relatively well understood but their interactions are complex, particularly in the context of porous media systems, which leaves room for future research in the field. Qualitatively we understand that microorganisms, existing in biofilms, attached cells or as free floating planktonic cells, can increase alkalinity as a product of their metabolisms which can consequently result in carbonate mineral precipitation and porosity reduction. It is known that biologically produced

molecules affect the mineralogy of the precipitates however in a reactive transport context this information remains difficult to quantify.

- The coupled nature of the system where biomass growth and mineral precipitation affect transport, and vice versa, makes it difficult to isolate and study processes independently of each other experimentally. This makes nondestructive analytical techniques and modeling important in future research.
- This field will benefit from a multidisciplinary approach using methods such as microfluidics, confocal microscopy, X-ray microtomography and nanotomography, nuclear magnetic resonance imaging, as well as spectroscopic techniques.
- Mathematical models will be essential in order to make predictions about compounds and behavior at spatial and temporal scales currently not observable or measurable. Furthermore, cross validation between techniques is often best done through modeling.

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Major Project Participants: Dr. Robin Gerlach, Montana State University (point of contact and coordinator for the combined research activity), Dr. Andrew Mitchell (Montana State University, University of Aberystwyth), collaborating with Dr. Frederick Colwell (Oregon State University), Dr. Dorthe Wildenschild (Oregon State University), Dr. Brian Wood (Oregon State University), Dr. George Redden (Idaho National Laboratory).