Genome-Enabled Systems Approach to Predict Immobilization of Technetium in the Subsurface – 14351

Hope Lee*, Danielle Saunders, Brady Lee*
* Pacific Northwest National Laboratory

ABSTRACT

A variety of phylogenetically diverse bacteria play an important role in immobilizing contaminants by directly reducing ^{99}Tc species to less soluble forms. Although much has been learned about the physiology and metabolic potential of single microbial species (pure cultures) that immobilize ^{99}Tc, major gaps exist in our understanding of how these and other microorganisms function in natural and contaminated ecosystems. As such, tools that integrate the chemical and biological reaction network influencing the mobility of ^{99}Tc in the subsurface need to be developed and tested. Batch chemostat studies using isolates and a defined microbial consortium for reduction of ammonium pertechnetate were used to test the use of cultivation-independent molecular tools, such as high throughput sequencing techniques, coupled to community-level metabolic models, as a method of predicting and controlling the biotic component of ^{99}Tc immobilization. Isolates and the designed consortium consisting of pure cultures of dissimilatory metal reducing bacteria isolated from Oak Ridge Field Research Center (FRC) sediments (*Geobacter sulfurreducens* strain PCA, *Geobacter daltonii* strain FRC-32, and *Anaeromyxobacter dehalogenens* sp. strain 2CP-2), from Hanford subsurface sediments (*Cellulomonas* sp. strain ES6), and from freshwater lake sediments (*Shewanella oneidensis* MR-1) were used during testing. Results for ammonium pertechnetate for isolates and the consortium, and a draft initial model and the implications for ^{99}Tc reduction and immobilization, will be discussed. Results generated will provide important information related to ^{99}Tc reduction by mixed microbial communities. The model developed will provide important information that can then be coupled to geochemical models, providing a microbial component that is often missing from these types of models.

INTRODUCTION

Technetium (^{99}Tc) has been released into the subsurface over the past three decades through fallout from weapons tests and through discharges from “active” nuclear processing plants and other facilities. ^{99}Tc has a relatively long half-life ($t_{1/2} = 2.1 \times 10^5$ years), is soluble and mobile in groundwater, and can be taken up by plants and animals. If left untreated, ^{99}Tc poses a risk to the environment and human health for thousands of years. A variety of technical, scientific, and financial challenges complicate efforts for ^{99}Tc remediation, due to the complex nature of the subsurface and associated biogeochemical cycles, regulated primarily by microbial activity.

^{99}Tc is a widespread contaminant throughout the world, is considered one of the most problematic radionuclides in the environment, and is an important contaminant at several U.S. Department of Energy sites, such as Hanford, WA, Oak Ridge, TN, Paducah, KY, and Portsmouth, OH [1]. At Hanford, more than 500 Ci of ^{99}Tc, as the pertechnetate anion (TcO_4^-), have been released to the vadose zone as part of past site operations. ^{99}Tc is mobile in predominantly oxidizing ground waters, with eventual discharge to the

Columbia River, making it one of the site's major risk-driving concerns [2]. At the gaseous diffusion plants at Paducah and Portsmouth, ^{99}Tc levels of 1000 - 3000 pCi L^{-1} have been detected in the groundwater [3]. In addition, residents near Paducah have been provided bottled drinking water because of spills and disposal operations that have contaminated local aquifers and ultimately private drinking wells [4]. At the Field Research Center (FRC) in Oak Ridge, soils and groundwater are contaminated with concentrations of up to 40,000 pCi per liter (0.02 µM) [5]. At Hanford S-SX farm, 10^8 pCi per liter (54 µM) has been detected in groundwater. The permissible concentration of ^{99}Tc in groundwater is 900 pCi per liter.

The mobility of ^{99}Tc in the geologic medium is mainly a result of redox chemistry. Under oxidizing conditions, ^{99}Tc generally exists as TcO_4^{2-}, which has limited sorption to mineral surfaces and is therefore highly mobile [6 - 9]. Under reducing conditions, TcO_4^- is reduced to the less soluble and therefore less mobile Tc(IV), which is strongly retained by geologic materials [7, 10]. ^{99}Tc immobilization can occur through direct enzymatic reduction to form hydrous TcO_2 or indirectly via abiotic electron transfer from reduced chemical species such as Fe(II) [11 - 13]. Direct enzymatic reduction of ^{99}Tc is typically associated with hydrogenase enzymes and is thought to be the predominant mechanisms for immobilization at relatively high concentrations of ^{99}Tc [14]. At environmentally relevant concentrations (10^{-8} to 10^{-11} mol per L) [15, 16], reduction is thought to be mediated by abiotic reaction with reduction products, primarily Fe(II) [11, 17, 18], although recent studies have shown enzymatic reduction at environmentally relevant conditions [9, 19].

Although much has been learned about the physiology and metabolic potential of single microbial species (pure cultures) that immobilize ^{99}Tc, major gaps exist in our understanding of how these and other microorganisms function in natural and contaminated ecosystems. As such, tools that integrate the chemical and biological reaction network influencing the mobility of ^{99}Tc in the subsurface need to be developed and tested. A first step in this development will be testing the use of cultivation-independent molecular tools, such as high throughput sequencing techniques, coupled to genome enabled community-level metabolic models, as a method of predicting and controlling the biotic component of ^{99}Tc immobilization. While other metabolic models have been tested and validated, some for contaminated subsurface environments, the proposed approach, stoichiometric metabolic modeling, simplifies the microbial community into key or sentinels metabolisms, and is not based on cultured surrogates or well-characterized single microbial members. This type of modeling has been used successfully to analyze natural and engineered microbial systems on both a pure culture and community level, and defines a network's metabolic potential based on a complete listing of the simplest, non-divisible pathways.

The work presented here represents the initial phases of developing the tools to integrate the microbial component with the chemical component related to ^{99}Tc reduction. A metal reducing microbial community will be constructed from five known metal reducing bacteria following initial experiments examining ^{99}Tc reduction with individual species, performed in batch chemostats. In addition, initial steps of developing a community-level metabolic model will be performed using genome information available from the Joint Genome Institute (JGI). An *in silico* representation of the microbial community will be developed using elementary mode analysis.

METHODS

Batch chemostat studies were performed to determine the ability of five bacteria to change the valence state of ^{99}Tc (Tc(VII) to Tc(IV)) using lactate as an electron donor, thereby forming insoluble ^{99}Tc that will precipitate out of solution. Experiments were performed with each bacterium individually; future studies will combine all five species into an engineered consortium to evaluate how communities act on ^{99}Tc immobilization and/or degradation. In addition, a draft metabolic model is proposed for the consortium using genome information available from JGI.

Chemostat Studies: Individual Species

Experiments were performed using New Brunswick CelliGen® BLU disposable bioreactors controlled using a BioFlo® 310 console (Figure 1). ^{99}Tc reduction experiments were performed initially using non-growth conditions, which were established by removing any nitrogen and phosphorus sources from the media. *Geobacter sulfurreducens* strain PCA, *Geobacter daltonii* strain FRC-32, and *Anaeromyxobacter dehalogenens* sp. strain 2CP-2, *Cellulomonas* sp. strain ES6, and *Shewanella oneidensis* MR-1 were the dissimilatory metal reducing bacteria used during the experiments. Each bacterial species was maintained in flask cultures, which were used to inoculate a 2 L culture that was harvested, washed, and resuspended in 30 µM PIPES buffer under anaerobic conditions. Cultures were pumped into the bioreactor under a stream of nitrogen to maintain anaerobic conditions in the reactor. Lactate (10 mM) was added to the cultures as an electron donor for ^{99}Tc reduction. Ammonium pertechnetate was added to achieve a final concentration of 50 µM. Once the bioreactor was set up, experiments were run for four days and samples were taken twice daily to monitor lactate, cell density, total ^{99}Tc, and ^{99}Tc speciation.

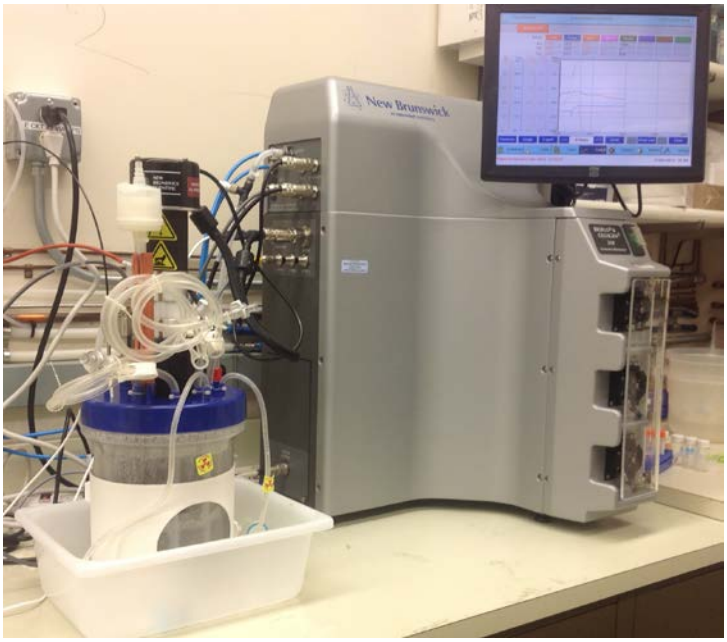

Figure 1. CelliGen BLU bioreactor and BioFlo 310 control unit.

Chemostat Studies: Consortium

Using the same setup described in the previous section, experiments will be performed with a consortium consisting of all five of the bacterial species discussed above. Each of the bugs will be grown individually, harvested and washed, and the cell density for each culture will be normalized, such that when the cultures are combined, they will each make up the same proportion of the total population at time zero. Bacterial species will be grown in their optimum growth medium to optimal cell densities (10^6) and then combined in

a minimal medium containing nitrogen and phosphorus for growth. Washed cells will be pumped into the bioreactor and experiments initiated by adding lactate and 50 µM ^{99}Tc.

Considerations for Draft Metabolic Model

The goal of the modeling task will be to indicate the influence of the biogeochemical conditions and microbial community structure and function on ^{99}Tc immobilization efficiency *in situ*. Molecular level metabolic network models will be used to describe the subsurface environments in terms of their environmental chemistry, microbial physiology, and phylogenetic diversity. The models will be decomposed into a complete set of the simplest biochemical pathways (steady state) with enzymatic fluxes occurring in physiologically reasonable directions using elementary flux mode analysis (EFMA). The flux analysis will organize and assimilate the genomic and transcriptomic data under a variety of conditions [20]. The goal is to construct a functional model of the system, both microbiologically and physiochemically, as a single ecosystem with many functions, or alternatively a simplification of the most important metabolic potentials [21, 22]. The models will be constructed to represent the central metabolism of key functional guilds important to immobilization of ^{99}Tc via direct reduction; other competing processes will be modeled in subsequent studies, including development of biomass or flow of electrons from the carbon to competing electron acceptors (e.g., O_2, SO_4, Mn(IV)). The strengths of the proposed model include (a) novel approaches for interrogating microbial ecosystems as a whole, (b) identification of central cellular metabolic pathways, and (c) an ideal method for understanding which group or central metabolic pathway is performing any defined metabolic transformation. These studies will provide a fundamental framework for integrating both theoretical and practical data generated at laboratory scale. Once demonstrated at the laboratory scale, iterative studies including field scale analyses will be incorporated into the model for validation; these studies will likely be completed independently of this work in collaboration with the Deep Vadose Zone Applied Field Research Initiative (DVZ-AFRI).

In silico community models will be built using EFMA from collected genomic and metabolic data. Techniques developed previously by the Carlson group permit flexibility in the level of *a priori* data required to build these metabolic models [23]. For example, with sufficient *a priori* information, compartmentalized metabolic models can be built where each functional guild is treated as a distinct physical entity with mass and energy transfer occurring between guilds (Figure 2A).

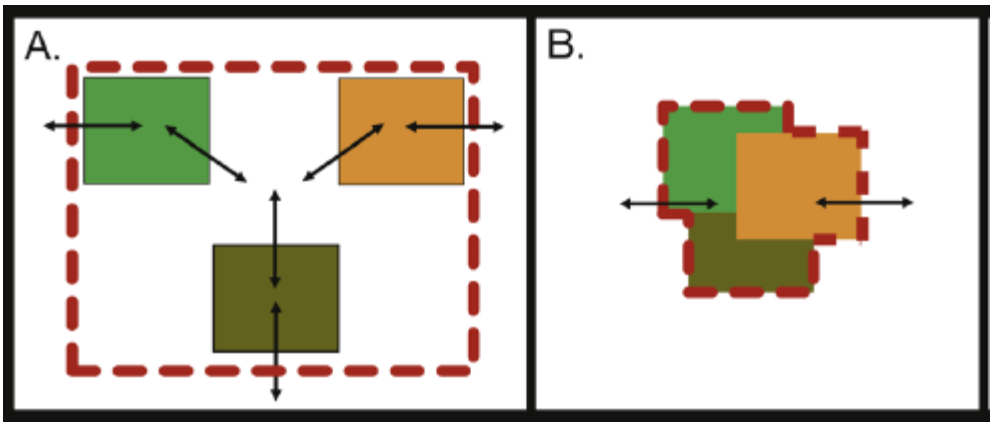

Figure 2. Multi-species modeling approaches. Schematic of (A) the concept for the compartmentalized approach, and (B) the concept for the pooled approach. Adapted from Taffs et al., 2009 [23].

Alternatively, if there is not enough information to assign genes to distinct guilds, a pooled or metagenomic community model can be constructed where the metabolic potential of the entire community is "pooled" into a single compartment (Figure 2B). We expect to find genes that allow the microbial community to exploit available energy sources and resist toxic conditions. The models will play a critical role in organizing and compiling the collected data into a testable platform. Metabolic models are invaluable for testing hypotheses. The EFMA data sets will be mined for ecologically relevant strategies such as optimal use of resources or minimization of potentially toxic byproducts. The carbon, nitrogen, energy, and electron fluxes between the community members will be modeled and tested using combinations of geochemical data sets and activity measurements. The modeling results will be compared to the experimental observations by transforming the experimental data into mathematical vectors representing either enzyme usage patterns or metabolite fluxes. The experimental data vectors will then be mapped onto the EFMA results using the Euclidean distance metric [24-26]. Small Euclidean distances represent a convergence of experimental data and theoretical predictions [27]. This type of analysis is an iterative process of prediction, comparison, and refinement.

DISCUSSION

Experiments are in progress to determine the ability of individual bacterial species to reduce ^{99}Tc when lactate is provided as the electron acceptor. The ability of each species and the engineered consortium to reduce ^{99}Tc is being tested in duplicate. Using genome information available from JGI, as well as genome information generated for *Cellulomonas* strain ES6, community-level metabolic models are being constructed to determine distribution of carbon and energy between community members and for ^{99}Tc reduction. Information in the literature related to growth, carbon conversion, and cellular energetics is being used with experimental information to develop the model. Once developed, the model will be validated with experimental data by predicting carbon and electron flow during operation of the engineered community chemostat studies.

Understanding carbon and electron flow in natural environments is important in predicting ^{99}Tc reduction, and for optimizing conditions for biological ^{99}Tc reduction. Being able to predict carbon and electron flow

in a microbial consortium is also important because, unlike an individual bacterium, sharing of carbon and electrons between cells and cell types, and competing electron acceptors, will have to be considered. While competing electron acceptors need to be considered with monocultures, partitioning of carbon is more straightforward. For a mixed microbial community, like those found in the environment, products from carbon metabolism from one species may be the carbon and/or electron source for another species.

CONCLUSIONS

Research will demonstrate the feasibility of using community-level metabolic models for predicting and possibly controlling ^{99}Tc transformation. Specifically, the research will show (a) the ability of genome information to demonstrate the phylogenetic and functional diversity of ^{99}Tc transforming microbial communities; and (b) the utility of community-level metabolic models for predicting microbial activity related to ^{99}Tc transformation using relevant geochemical conditions. Results will also provide fundamental information on the microbiological and chemical reduction of a global radionuclide contaminant in soil and groundwater, ^{99}Tc.

Demonstration of this technique is an important first step for demonstrating the effectiveness of applying community-level metabolic models for monitoring and predicting ^{99}Tc transformation; follow-on collaborative research with the DVZ-AFRI will include enrichment and monitoring of microbial communities from ^{99}Tc contaminated sites at Hanford and Paducah using high throughput molecular techniques and associated metabolic models. This phase would naturally be followed by a field-scale demonstration of the technology. Part of the field-scale demonstration would be eventual integration with models such as the Advanced Simulation Capability for Environmental Management.

REFERENCES

1. Riley, R. G. and J. M. Zachara (1992). Chemical contaminants on DOE lands and selection of contaminant mixtures for subsurface science research. D. o. Energy. Washington D. C.

2. Khaleel, R., M. D. White, et al. (2007). “Impact assessment of existing vadose zone contamination at the Hanford Site SX tank farm.” Vadose Zone Journal **6**(4): 935-945.

3. Bostick, W. D., P. E. Osborne, et al. (1995). Treatment of aqueous solutions contaminated with technetium-99 originating from uranium enrichment activities. D. o. Energy. Oak Ridge, Oak Ridge K-25 Site.

4. DOE (2007). Department of Energy Five-Year Plan FY 2008 – FY 2012. D. o. Energy, Office of Environmental Management. Washington D.C.

5. McBeth, J., G. Lear, et al. (2007). “Technetium Reduction and Reoxidation in Aquifer Sediments.” Geomicrobiology Journal **24**(3 & 4): 189-197.

6. Palmer, D. A. and R. E. Meyer (1981). “Adsorption of technetium on selected inorganic ion exchange materials and on a range of naturally occurring minerals under oxic conditions.” Journal of Inorganic & Nuclear Chemistry **43**(11): 2979-2984.

7. Lieser, K. H. and C. Bauscher (1987). “Technetium in the hydrosphere and in the geosphere. 1. Chemistry of technetium and iron in natural waters and influence of the redox potential on the sorption of technetium.” Radiochimica Acta **42**(4): 205-213.

8. Holtta, P., A. Hautojarvi, et al. (1992). “Transport and retardation of non-sorbing radionuclides in crystalline rock fractures.” Radiochimica Acta **58-9**: 285-290.

9. Wildung, R. E., S. W. Li, et al. (2004). “Technetium reduction in sediments of a shallow aquifer exhibiting dissimilatory iron reduction potential.” FEMS Microbiology Ecology **49**: 151-162.

10. Bondietti, E. A. and C. W. S. Francis. (1979). “Geologic migration potential of Tc-99 and Np-237.” Science **203**: 1337-1340.

11. Lloyd J. R., Sole V. A., et al. (2000). “Direct and Fe(II)-mediated reduction of technetium by Fe(III)-reducing bacteria.” Applied and Environmental Microbiology **66**: 3743-3749.

12. Lloyd, J. R., C. L. Harding, et al. (1997). “Tc(VII) reduction and accumulation by immobilized cells of Escherichia coli.” Biotechnology and Bioengineering **55**(3): 505-510.

13. De Luca, G., P. de Philip, et al. (2001). “Reduction of Technetium(VII) by Desulfovibrio fructosovorans Is Mediated by the Nickel-Iron Hydrogenase.” Applied and Environmental Microbiology **67**(10): 4583-4587.

14. Lloyd, J. R., J. Ridley, et al. (1999). “Reduction of Technetium by Desulfovibrio desulfuricans: Biocatalyst Characterization and Use in a Flowthrough Bioreactor.” Appl. Environ. Microbiol. **65**(6): 2691-2696.

15. Beasley, T. M. and H. V. Lorz (1986). “A review of the biological and geochemical behavior of technetium in the Marine environment.” Journal of Environmental Radioactivity **3**(1): 1-22.

16. Morris, K., J. C. Butterworth, et al. (2000). “Evidence for the Remobilization of Sellafield Waste Radionuclides in an Intertidal Salt Marsh, West Cumbria, U.K.” Estuarine, Coastal and Shelf Science **51**(5): 613-625.

17. Fredrickson, J. K., J. M. Zachara, et al. (2004). “Reduction of TcO4−by sediment-associated biogenic Fe(II).” Geochim Cosmochim Acta **68**: 3171-3187.

18. Burke, I. T., C. Boothman, et al. (2005). “Effects of progressive anoxia on the solubility of technetium in sediments.” Environmental Science & Technology **39**(11): 4109-4116.

19. Begg, J., I. T. Burke, et al. (2007). “The behaviour of technetium during microbial reduction in amended soils from Dounreay, UK.” Science of the Total Environment **373**: 297-304.

20. Hunter, P. J. and T. K. Borg (2003). “Integration from proteins to organs: the Physiome Project.” Nature Reviews Molecular Cell Biology **4**: 237-242.

21. Schuster, S. and C. Hilgetag (1994). “On elementary flux modes in biochemical reaction systems at steady state.” Journal of Biological Systems **2**(2): 165-182.

22. Schuster, S., D. Fell, et al. (2000). “A general definition of metabolic pathways useful for systematic organization and analysis of complex metabolic networks.” Nat Biotechnol **18**(3): 326-332.

23. Taffs, Reed, J. Aston, et al. (2009). “In silico approaches to study mass and energy flows in microbial consortia: a syntrophic case study.” BMC Systems Biology **3**(1): 114.

24. Carlson, R., A. Wlaschin, et al. (2005). “Kinetic Studies and Biochemical Pathway Analysis of Anaerobic Poly-(R)-3-Hydroxybutyric Acid Synthesis in Escherichia coli.” Applied and Environmental Microbiology **71**(2): 713-720.

25. Carlson, R., D. Fell, et al. (2002). “Metabolic pathway analysis of a recombinant yeast for rational strain development.” Biotechnology and Bioengineering **79**(2): 121 - 134.

26. Carlson, R. P. (2009). “Decomposition of complex microbial behaviors into resource-based stress responses.” Bioinformatics **25**(1): 90-97.

27. Segre, D., D. Vitkup, et al. (2002). “Analysis of optimality in natural and perturbed metabolic networks.” Proceedings of the National Academies of Science **99**: 15112-15117.

ACKNOWLEDGEMENTS

Funding for this work was provided through the Laboratory Directed Research and Development Program at the Pacific Northwest National Laboratory. The Pacific Northwest National Laboratory is operated by Battelle Memorial Institute for the Department of Energy (DOE) under Contract DE-AC05-76RL01830.