

**COUPLING OF BIOTIC AND ABIOTIC
ARSENITE OXIDATION IN SOIL**

by

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ABSTRACT

Arsenic (As) is a redox-active metalloid whose toxicity and environmental mobility depend on oxidation state. Recent instances of human As poisoning from drinking water motivated this investigation of the biogeochemical processes governing As mobility in soil. Arsenite [As(III)], the more toxic and mobile form of As, can be oxidized to arsenate [As(V)] by both minerals and microbes in soils. Previous studies showed that manganese (Mn) oxide minerals can oxidize As(III) and sorb As(V). Additionally, numerous soil bacteria have been shown to oxidize As(III) as a detoxification process. These and other types of mineral and microbial oxidants coexist in soils and their activity is intricately linked due to the biogeochemical heterogeneity of soils. In this study, it was found that the rate of As(III) oxidation was enhanced when heterotrophic As(III)-oxidizing soil bacteria isolates and a model Mn oxide mineral were mixed in microbe-mineral batch experiments. Soil column experiments, testing the rates of biotic versus abiotic As(III) oxidation, showed that biological activity was required for As(III) oxidation to proceed. The As(III) oxidation kinetics presented here, from multiple levels of biogeochemical complexity and a range of time scales, contribute to understanding coupled microbe-mineral processes necessary for environmental remediation of this toxic element.

Chapter 1

INTRODUCTION

1.1 The Arsenic Problem

Understanding arsenic (As) cycling in groundwater, surface water, and soil is essential for controlling human exposure. Arsenic is a toxic element, which is widespread in soils and groundwater as a result of human activities and natural geologic occurrence (Nordstrom, 2002; Frankenberger, 2002). Today, one of the most serious instances of As contamination is in Bangladesh, where human exposure is extensive due to drinking water contaminated with geogenic As (World Health Organization (WHO), 2001). In the United States, As is a contaminant in Superfund sites, landfills, and it is present in numerous public groundwater sources (Delemos et al., 2006; U.S. Environmental Protection Agency (EPA), 1999). Many Delaware soils have detectable levels of As as a result of pesticide and poultry litter application to agricultural soils (Sparks et al., 2007).

In soil, most inorganic As exists as either H_3AsO_3 , hereafter referred to as arsenite or As(III), or the oxyanions H_2AsO_4^- and HAsO_4^{2-} , referred to as arsenate or As(V), whose protonation is pH-dependent (Cullen and Reimer, 1989; Le, 2002). Arsenite is the more mobile form due to its neutral charge whereas As(V) is charged at typical soil pH and can sorb to positively charged mineral surfaces similar to phosphate (Cullen and Reimer, 1989; Hering and Kneebone, 2002). In aerobic and unsaturated soils, As(V) is more prevalent and thermodynamically favorable whereas

As(III) is prevalent in anaerobic, saturated soils (Cullen and Reimer, 1989; Oremland and Stolz, 2003). These differences in mobility and speciation depending on environmental conditions affect human exposure pathways such as drinking water purification (US EPA, 2000).

In addition to mobility, As toxicity is governed by its oxidation state (Cullen and Reimer, 1989; Le, 2002). The reduced form, As(III), is more toxic than As(V) because it inhibits dehydrogenases and other essential cellular proteins by reacting with thiol and sulfhydryl groups (National Research Council (NRC), 2001; Frankenberger, 2002). The toxicity of As(V) arises from replacing phosphorus in biological molecules like adenosine triphosphate (ATP) (NRC, 2001; Summers and Silver, 1978). In humans, ingestion of As can lead to arsenicosis (As poisoning) and long-term exposure has been linked to cancers of the skin, lungs, and urinary system (Oremland and Stolz, 2005). Interestingly, the toxic nature of As has a beneficial use as a component in chemotherapy drugs and it has been hypothesized some bacteria may be able to grow by substituting As for phosphorus in biological molecules such as DNA (Shen et al., 1997; Wolfe-Simon et al., 2010).

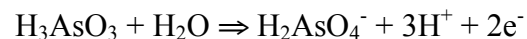
Given the aforementioned differences in the environmental behavior of As(III) and As(V), it is clear that biogeochemical electron transfer (redox) reactions control the fate, transport, and hazards of As in the environment. There are two main pathways for As reduction and oxidation in soils: biotic (mediated by microorganisms) and abiotic (mediated by minerals). The specific mechanisms, relative importance of these different pathways, and how they affect each other is essential for understanding As in the environment. Therefore, this study focuses on As(III) oxidation because it is a pathway for sequestering and reducing the toxicity of As in the environment.

Mineral oxidation is considered to be the predominant abiotic As(III) oxidation reaction in soils due to sluggish rates of oxidation by oxygen (O₂) (Oscarson et al., 1981; Eary and Schramke, 1990). Two of the most important soil mineral groups that oxidize As are manganese (Mn) oxides and hydroxides (hereafter referred to as Mn oxides) and iron (Fe) oxides and hydroxides (Scott and Morgan, 1995; Fendorf et al., 1997; Nesbitt et al., 1998; Tournassat et al., 2002; Ginder-Vogel et al., 2009). Manganese oxide minerals are ubiquitous in soils and have significant chemical influence in the environment as a result of their oxidative behavior, typically large surface area, and high adsorption capacity (Post, 1999). Even though they occur in low abundances, Mn oxides are considered “highly chemically active” because they drive rapid oxidation and cation exchange reactions (Oscarson et al., 1981; Oscarson et al., 1983; Post, 1999; Tebo et al., 2004; Zhu et al., 2009).

Arsenite oxidation by Mn oxides involves several steps starting with sorption of As(III) onto the mineral surface followed by electron exchange including Mn(IV) reduction, As(III) oxidation, and desorption of the reaction products (Scott and Morgan, 1995; Nesbitt et al., 1998; Tournassat et al., 2002; Zhu et al., 2009; Lafferty et al., 2010a). Arsenate can be sorbed in strong ligand-exchange bonds including monodentate and bidentate inner-sphere complexes (Nesbitt et al., 1998; Zhu et al., 2009). It has been observed that As(III) oxidation can be inhibited by Mn oxide surface passivation, which reduces oxidation rates (Parikh et al., 2010; Lafferty et al., 2010a, 2010b). Passivation occurs when Mn(II), Mn(III) and As(V) products sorb to the Mn oxide surface and block reactive sites. Even though As(III) oxidation by Mn oxides can be slowed by passivation, the formation of Mn oxides is a dynamic

process with new mineral being formed via abiotic and biotic pathways in soil (Tebo et al., 2004; Toner et al., 2006).

Despite its toxicity, the capacity to oxidize and reduce As has been noted in microorganisms isolated from contaminated environments (i.e. Macur et al., 2004; Jackson et al., 2005; Achour et al., 2007; Hamamura et al., 2008). In bacteria, As is oxidized and reduced as either a means of detoxification or a source of energy (Rosen, 2002; Oremland and Stolz, 2003; Silver and Phung, 2005). The As(III) oxidation reaction by heterotrophic As(III)-oxidizing bacteria can be summarized as (Turner, 1949):



Although this reaction produces two electrons, heterotrophic bacteria appear to gain minimal energy from this reaction therefore the process is considered to be a detoxification mechanism (Turner, 1949; Anderson et al., 2003; Macur et al., 2004).

There are however, some autotrophic As(III)-oxidizing bacteria with the ability to use As(III) electron transfer for energy (Oremland and Stolz, 2003; Silver and Phung, 2005; Garcia-Dominguez et al., 2008).

There is a growing body of molecular biology and field studies on As(III) oxidizing bacteria (i.e. Scott and Morgan, 1995; Oremland and Stolz, 2003; Parikh et al., 2010). Degenerate primers for As(III) oxidase genes (*aroA*, *asoA*, and *aoxB*), developed by Inskeep et al. (2007), have been utilized on environmental samples from contaminated environments and may indicate the presence of As(III) oxidizing bacteria (i.e. Achour et al., 2007; Duquesne et al., 2008; Cai et al., 2009; Clingenpeel et al., 2009). Quemeneur et al. (2008) also developed a primer set which amplifies *aoxB* genes as a biomarker of As(III) oxidation. Primers and polymerase chain

reaction (PCR) analysis can indicate the presence of genes in an environmental sample but they are not direct evidence that microbial As(III) oxidation is occurring (Madsen, 2008). This limitation of molecular techniques requires complementary field and experimental approaches for a full understanding of *in situ* activity of As(III) oxidizing bacteria.

Given these mineral and microbial pathways for As(III) oxidation in soil, experimental research must address biogeochemical complexity to be relevant to the heterogeneous soil environment. Recent research on As(III) oxidation has tended to focus on the dynamics of one or the other pathway, instead of the reaction at the mineral and microbial interface (Scott and Morgan, 1995; Oremland and Stolz, 2003; Silver and Phung, 2005). Biogeochemical kinetic studies have been carried out with other redox-active metals such as chromium (Cr) and uranium (U), which shows the potential benefit of kinetic techniques addressing both mineral and microbial redox pathways for As (Tokunaga et al., 2003; Gu et al., 2005; Bank et al., 2007).

1.2 Objectives

This study explores the coupling and kinetics of mineral and microbial As(III) oxidation at different spatial and temporal scales to simulate natural soil environments where As(III)-oxidizing bacteria and minerals coexist. The specific research objectives of this project were as follows:

- Characterize the kinetics of As(III) oxidation in mixed microbe-mineral batch experiments using hydrous Mn oxide and heterotrophic As(III)-oxidizing bacteria: *Agrobacterium tumefaciens* and *Pseudomonas fluorescens*.
- Investigate the rate and mechanisms of biotic and abiotic As(III) oxidation in a Delaware soil using sterile and non-sterile column experiments.

There is no prior published research integrating bacterial and mineral As(III) oxidation across temporal and spatial scales, making the complementary batch and column experiments presented here both foundational for future studies and a novel area of research. Overall, this project provides qualitative and quantitative kinetic information about the interactions between mineral and microbial As redox reactions in soil. The rates of these reactions in heterogeneous systems may elucidate whether they are additive, antagonistic, or do not affect each other. The big picture impact of this study is the contribution to our understanding of kinetics and biogeochemistry controlling As fate and transport and its impact on public health.

1.3 References

- Achour, A.R., Bauda, P., Billard, P. 2007. Diversity of arsenite transporter genes from arsenic-resistant soil bacteria. *Research in Microbiology*. 158: 128-137.
- Anderson, G.L., Love, M., Zeider, B.K. 2003. Metabolic energy from arsenite oxidation in *Alcaligenes faecalis*. *J. Phys. IV France*. 103: 49-52.
- Bank, T.L., Vishnivetskaya, T.A., Jardine, P.M., Ginder-Vogel, M.A., Fendorf, S., Baldwin, M.E. 2007. Elucidating biogeochemical reduction of chromate via carbon amendments and soil sterilization. *Geomicrobiology Journal* 24: 125-132.
- Cai, L., Guanghui, L., Rensing, C., Wang, G. 2009. Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils. *BMC Microbiology*. 9: 4.
- Clingenpeel, S.R., D'Imperio, S., Oduro, H., Druschel, G.K., McDermott, T.R. 2009. Cloning and in situ expression studies of *Hydrogenobaculum* arsenite oxidase genes. *Applied and Environmental Microbiology*. 75: 3362-3365.
- Cullen, W.R., Reimer, K.J. 1989. Arsenic Speciation in the Environment. *Chemical Reviews*. 89: 713-764.

- Delemos, J.L., Bostick, B.C., Renshaw, C.E., Strup, S., Feng, X. 2006. Landfill-stimulated iron reduction and arsenic release at the Coakley Superfund Site (NH). *Environmental Science and Technology*. 40: 67-73.
- Duquesne, K., Lieutaud, A., Ratouchniak, J., Muller, D., Lett, M., Bonnefoy, V. 2008. Arsenite oxidation by a chemoautotrophic moderately acidophilic *Thiomonas* sp.: from the strain isolation to the gene study. *Environmental Microbiology*. 10: 228-237.
- Eary, L.E., Schramke, J.A. 1990. Rates of inorganic oxidation reactions involving dissolved oxygen. *In* *Chemical Modeling of Aqueous Systems II*. American Chemical Society. Washington, DC. 379-396.
- Fendorf, S., Eich, M.J., Grossl, P., Sparks, D.L. 1997. Arsenate and chromate retention mechanisms on goethite. 1. Surface structure. *Environmental Science and Technology*. 31: 315-320.
- Frankenberger, W.T., (ed.) 2002. *Environmental Chemistry of Arsenic*. Marcel Dekker. New York, NY. 391 p.
- Garcia-Dominguez, E., Mumford, A., Rhine, E.D., Paschal, A., Young, L. 2008. Novel autotrophic arsenite-oxidizing bacteria isolated from soil and sediments. *FEMS Microbiological Ecology*, 66: 401-410.
- Ginder-Vogel, M., Landrot, G., Fischel, J.S., Sparks, D.L. 2009. Quantification of rapid environmental redox processes with quick-scanning x-ray absorption spectroscopy (Q-XAS). *Proceedings of the National Academy of Sciences*. 106: 16124-16128.
- Gu, B., Wu, W., Ginder-Vogel, M.A., Yan, H., Fields, M.W., Zhou, J., Fendorf, S., Criddle, C.S., Jardine, P.M. 2005. Bioreduction of uranium in a contaminated soil column. *Environmental Science and Technology*. 39: 4841-4847.
- Hamamura, N., Macur, R.E., Korf, S., Ackerman, G., Taylor, W.P., Kozubal, M., Reysenbach, A.L., Inskeep, W.P. 2008. Linking microbial oxidation of arsenic with detection and phylogenetic analysis of arsenite oxidase genes in diverse geothermal environments. *Environmental Microbiology*. 11: 421-431.
- Hering, J.G., Kneebone, P.E. Biogeochemical controls on arsenic occurrence and mobility in water supplies. *In* Frankenberger, W.T., (ed.) 2002. *Environmental Chemistry of Arsenic*. Marcel Dekker. New York, NY. 155-181.

- Inskeep, W.P., Macur, R.E., Hamamura, N., Warelou, T.P., Ward, S.A., Santini, J.M. 2007. Detection, diversity and expression of aerobic bacterial arsenite oxidase genes. *Environmental Microbiology*. 9: 934-943.
- Jackson, C.R., Harrison, K.G., Dugas, S.L. 2005. Enumeration and characterization of culturable arsenate resistant bacteria in a large estuary. *Systematic and Applied Microbiology*. 28: 727-734.
- Lafferty, B.J., Ginder-Vogel, M., Sparks, D.L. 2010a. Arsenite oxidation by a poorly crystalline manganese-oxide: 1. Stirred-flow experiments. *Environmental Science and Technology* 44: 8460-8466.
- Lafferty, B.J., Ginder-Vogel, M., Zhu, M., Livi, K.J.T., Sparks, D.L. 2010b. Arsenite oxidation by a poorly crystalline manganese-oxide: 2. Results from x-ray absorption spectroscopy and x-ray diffraction. *Environmental Science and Technology* 44: 8467-8472.
- Landrot, G., Ginder-Vogel, M., Sparks, D.L. 2010. Kinetics of chromium(III) oxidation by manganese(IV) oxides using quick scanning x-ray absorption fine structure spectroscopy. *Environmental Science and Technology*. 44: 143-149.
- Le, X.C. Arsenic speciation in the environment and humans. *In* Frankenberger, W.T., (ed.) 2002. *Environmental Chemistry of Arsenic*. Marcel Dekker. New York, NY. 95-116.
- Macur, R.E., Jackson, C.R., Botero, L.M., McDermott, T.R., Inskeep, W.P. 2004. Bacterial populations associated with the oxidation and reduction of arsenic in an unsaturated soil. *Environmental Science and Technology*. 38: 104-111.
- Madsen, E.L. 2008. *Environmental Microbiology: From genomes to biogeochemistry*. Blackwell Publishing. Malden, MA. 479 p.
- National Research Council. 2001. *Arsenic in drinking water: 2001 update*. National Academy Press. Washington DC.
<http://www.nap.edu/openbook.php?isbn=0309076293> (May 11, 2011).
- Nesbitt, H.W., Canning, G.W., Bancroft, G.M. 1998. XPS study of reductive dissolution of 7A-birnessite by H_3AsO_3 , with constraints on reaction mechanism. *Geochimica et Cosmochimica Acta*. 62: 2097-2110.
- Nordstrom, D.K. 2002. Worldwide occurrences of arsenic in ground water. *Science*. 296: 2143-2144.
- Oremland, R.S., Stolz, J.F. 2003. The ecology of arsenic. *Science*. 300: 939-944.

- Oremland, R.S., Stolz, J.F. 2005. Arsenic, microbes and contaminated aquifers. *Trends in Microbiology*. 13: 45-49.
- Oscarson, D.W., Huang, P.M., Liaw, W.K. 1981. Role of manganese in the oxidation of arsenite by freshwater lake sediments. *Clays and Clay Minerals*. 29: 219-225.
- Oscarson, D.W., Huang, P.M., Hammer, U.T. 1983. Oxidation and sorption of arsenite by manganese dioxide as influenced by surface coatings of iron and aluminum oxides and calcium carbonate. *Water, Air, and Soil Pollution*. 20: 233-244.
- Parikh, S.J., Lafferty, B.J., Meade, T.G., Sparks, D.L. 2010. Evaluating environmental influences on As(III) oxidation kinetics by a poorly crystalline Mn-oxide. *Environmental Science and Technology* 44: 3772-3778.
- Post, J.E. 1999. Manganese oxide minerals: crystal structures and economic and environmental significance. *Proceedings of the National Academy of Sciences*. 96: 3447-3454.
- Quemeneur, M., Heinrich-Salmeron, A., Muller, D., Lievreumont, D., Jauzerin, M., Bertin, P.N., Garrido, F., Joulain, C. 2008. Diversity surveys and evolutionary relationships of *aoxB* genes in aerobic arsenite-oxidizing bacteria. *Applied and Environmental Microbiology*. 74: 4567-4573.
- Rosen, B.P. 2002. Biochemistry of arsenic detoxification. *FEBS Letters*. 529: 86-92.
- Scott, M.J., Morgan, J.J. 1995. Reactions at oxide surfaces. 1. Oxidation of As(III) by synthetic birnessite. *Environmental Science and Technology*. 29: 1898-1905.
- Shen, Z., Chen, D., Ni J., Li, X., Xiong, S., Qiu, Q., Zhu, J, Tang, W., Sun, G., Yang, K., Chen, Y., Zhou, L., Fang, Z., Wang, Y., Ma, J., Zhang, P., Zhang, T., Chen, S., Chen, Z., Wang, Z. 1997. Use of arsenic trioxide (As₂O₃) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. *Blood*. 89: 3354-3360.
- Silver, S., Phung, L.T. 2005. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. *Applied and Environmental Microbiology*. 71: 599-608.
- Sparks, D.L., Sims, J.T., Seiter, J., Gardner, S. 2007. Fate and transport of arsenic in Delaware soils: assessing potential impacts on water quality. University of Delaware Report Submitted to DNREC, DWR, Watershed Assessment Branch.

- Summers, A.O., Silver, S. 1978. Microbial transformations of metals. *Annual Review of Microbiology*. 32: 637-672.
- Tebo, B.M., Bargar, J.R., Clement, B.G., Dick, G.J., Murray, K.J., Parker, D., Verity, R., Webb, S.M. 2004. Biogenic manganese oxides: properties and mechanisms of formation. *Annual Reviews in Earth and Planetary Science*. 32: 287-328.
- Tokunaga, T.K., Wan, J., Hazen, T.C., Schwartz, E., Firestone, M.K., Sutton, S.R., Newville, M., Olson, K.R., Lanzirrotti, A., Rao, W. 2003. Distribution of chromium contamination and microbial activity in soil aggregates. *Journal of Environmental Quality*. 32: 541-549.
- Toner, B., Manceau, A., Webb, S.M., Sposito, G. 2006. Zinc sorption to biogenic hexagonal-birnessite particles within a hydrated bacterial biofilm. *Geochimica et Cosmochimica Acta*. 70: 27-43.
- Tournassat, C., Charlet, L., Bosbach, D., Manceau, A. 2002. Arsenic(III) oxidation by birnessite and precipitation of manganese(II) arsenate. *Environmental Science and Technology*. 36: 493-500.
- Turner, A.W. 1949. Bacterial oxidation of arsenite. *Nature*. 164: 76-77.
- United States Environmental Protection Agency 1999. A review of contaminant occurrence in public water systems. EPA 816-R-99-006.
http://www.epa.gov/ogwdw000/occur/nov99_lo.pdf (February 5, 2010).
- United States Environmental Protection Agency 2000. Technologies and costs for removal of arsenic from drinking water. EPA 815-R-00-028 Cincinnati.
http://www.epa.gov/ogwdw000/arsenic/pdfs/treatments_and_costs.pdf (February 5, 2010).
- Wolfe-Simon, F., Blum, J.S., Kulp, T.R., Gordon, G.W., Hoefft, S.E., Pett-Ridge, J., Stolz, J.F., Webb, S.M., Weber, P.K., Davies, P.C.W., Anbar, A.D., Oremland, R.S. 2010. A bacterium that can grow by using arsenic instead of phosphorus. *Science*. 332: 1163-1166.
- World Health Organization. 2001. Arsenic in Drinking Water: Fact Sheet 201.
<http://www.who.int/mediacentre/factsheets/fs210/en/print.html> (May 11, 2010).
- Zhu, M., Paul, K.W., Kubicki, J.D., Sparks, D.L. 2009. Quantum chemical study of arsenic (III, V) adsorption on Mn-oxides: implications for arsenic(III) oxidation. *Environmental Science and Technology*. 43: 6655-6661.

Chapter 2

ARSENITE OXIDATION KINETICS AT THE MICROBE-MANGANESE OXIDE INTERFACE

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2.2 Abstract

Arsenic (As) is a redox-active metalloid whose toxicity and mobility in soil depend on oxidation state. Arsenite [As(III)] can be oxidized to arsenate [As(V)] by both minerals and microbes in soils however, the coupling of these abiotic and biotic processes is not well understood. Previous studies have shown that manganese (Mn) oxide minerals can oxidize As(III) and sorb As(V) and that soil bacteria can also oxidize As(III) via a detoxification mechanism. In this study, the time dependency of As(III) oxidation by two heterotrophic soil bacteria, *Agrobacterium tumefaciens* and *Pseudomonas fluorescens*, and a poorly crystalline Mn oxide mineral, δ -MnO₂, was determined using batch experiments. Arsenate (AsV) appearance, sorption, and desorption in batch experiments with combinations of these components were evaluated. The heterotrophic As-oxidizing bacteria enhanced As oxidation on δ -MnO₂, suggesting that mineral-only systems may underestimate the oxidative capacity

of complex natural systems and emphasizing the importance of microbe-mineral redox processes in the environmental mobility of this toxic metalloid.

2.3 Introduction

With millions of people being poisoned by arsenic (As) laden drinking and irrigation water in Southeast Asia, the question of how As is mobilized in the environment is both pertinent and urgent (Smith et al., 2000; Bhattacharya et al., 2007). Arsenic mobility and toxicity in soil depend on oxidation state and the biogeochemical processes that drive electron transfer (redox) in soil (Nordstrom, 2002; Borch et al., 2010). The oxidized form, As(V), whose behavior is similar to phosphate in soils, is less mobile and less toxic than the reduced form, As(III) (Duker et al., 2005). Therefore, an investigation of the rates and mechanisms of biogeochemical As(III) oxidation addresses remediation and risk assessment for this massive human health issue.

One pathway for As(III) oxidation in soil is via mineral oxidants. Manganese (Mn) oxides are common soil minerals with a great impact on this reaction because of their capacity to oxidize and sorb heavy metals and metalloids (Post, 1999). The reactivity of Mn oxide minerals, many of which are poorly crystalline and biogenic, is a function of high surface areas and low points of zero charge (pzc) at typical soil pH (Tebo et al., 2004; Post, 1999). Arsenite oxidation on Mn oxides results in formation and sorption of As(V) as inner-sphere ligand complexes and production of Mn(II) and Mn(III) by direct reduction and disproportionation (Lafferty et al., 2010a, 2010b; Zhu et al., 2009). Laboratory studies have shown rapid kinetics of As(III) oxidation by Mn oxide minerals accompanied by product [As(V), Mn(II), Mn(III)] sorption, both of which may be slowed by mineral surface passivation.

Passivation is the process in which reaction products block reactive sites on a mineral surface and thereby inhibit the oxidation reaction (Lafferty et al., 2010a and 2010b; Nesbitt et al., 1998; Tournassat, et al., 2002; Scott and Morgan, 1995). It has also been noted that oxidation enhances As removal from solution because more total As is sorbed by reacting Mn oxides with As(III) than with As(V) (Oscarson et al., 1983; Manning et al., 2002; Parikh et al., 2008).

Despite its toxicity, the capacity to oxidize and reduce As has been noted in many microorganisms from the bacteria and archaea domains of life. Arsenic oxidation by bacteria may occur through chemolithoautotrophic mechanisms, in which bacteria gain energy from electrons transferred, or detoxification mechanisms for heterotrophic bacteria, which cannot grow solely on As (Mukhopadhyay et al., 2002; Oremland and Stoltz, 2003; Anderson et al., 2003; Rhine et al., 2005; Garcia-Dominguez et al., 2008). An As oxidase enzyme and corresponding genes (*aro*, *aso*, and *aox*) have been identified and it is suspected that other enzymes and/or mechanisms could also be involved in As oxidation for some bacteria (Anderson et al., 1992; Ellis et al., 2001; Silver and Phung, 2005; Kashyap et al., 2006; Inskeep et al., 2007). In addition to isolates of As-oxidizing bacteria, primers for As oxidase genes have been used to identify As-oxidizing bacteria in contaminated soils, providing evidence that microbial As(III) oxidation may be widespread (Achour et al., 2007; Inskeep et al., 2007; Quemeneur et al., 2008).

The prevalence of these biotic and abiotic As oxidation pathways in soil environments raises the questions of how and at what rate As oxidation proceeds when oxidizing agents are coupled together. Both processes have temporal variability: As sorption and oxidation mechanisms on poorly crystalline Mn oxides change over

time and As oxidizing bacteria exhibit a variety of oxidation rates, yet little is known about what happens in the case of mixtures of biotic and abiotic oxidants (Anderson et al., 1992; Lafferty et al., 2010a; Macur et al., 2004). Therefore, studying the time-dependency of mixed microbe-mineral systems connects prior research on biotic oxidation, abiotic oxidation, and sorption processes and better simulates the natural environment. The objectives of this study were to characterize the kinetics of As(III) oxidation in mixed microbe-mineral batch experiments using a model poorly crystalline Mn oxide mineral, δ -MnO₂, and model heterotrophic soil bacteria, *Pseudomonas fluorescens* and *Agrobacterium tumefaciens*. The potentially competitive, complementary, or non-existent effects of the bacteria and mineral components on the overall apparent rate of As(III) oxidation and As(V) sorption links single-oxidant studies with a more complex system.

2.4 Materials and Methods

2.4.1 δ -MnO₂

Poorly crystalline Mn oxide, δ -MnO₂, was used because of its similarity to natural biogenic Mn oxides, which are characterized by higher surface area and reactivity than crystalline Mn oxides (Villalobos et al., 2003). δ -MnO₂ was synthesized following established procedures (Lafferty et al., 2010a, b; Ginder-Vogel et al., 2009). Synthesis began by adding a solution containing Mn(II)(NO₃)₂·4H₂O and 18.2 MΩ deionized (DI) water to a solution containing KMn(VII)O₄ and NaOH with a final Mn(II):Mn(VII):OH⁻ ratio of 3:2:4 by mass. The solution was then stirred for at least 12 hours until the KMn(VII)O₄ was completely reacted. The mixture was centrifuged at 10,000 x g for 15 minutes, supernatant decanted, replaced with DI

water, and resuspended by sonication. This washing step was repeated three times. Following washing, the mineral was dialysed for at least 48 hours or until the conductivity of dialysis water remained unchanged for at least 12 hours. Each batch of δ -MnO₂ was used within three weeks of synthesis.

2.4.2 As(III) and As(V) solutions

All As solutions were made using NaAsO₂ and 18.2 M Ω DI water, and concentrations were verified with high performance liquid chromatography with inductively-coupled plasma mass spectrometry (HPLC-ICP/MS) using the method described in section 2.4.6. Batch experiments were conducted at a concentration of 65-77 μ M As(III) and no added As(V) (concentration below detection).

2.4.3 Bacterial growth

All microbe-mineral and singular microbe batch experiments included washed resting cell suspensions of bacteria in late exponential growth phase and no carbon source in the media (Macur et al., 2004). Isolates of the heterotrophic bacteria species *Agrobacterium tumefaciens* and *Pseudomonas fluorescens* were obtained from Dr. Richard Macur (Montana State University) and cryogenically preserved (-80 °C). Bacteria from the -80 °C reserve were used to inoculate starter plates, consisting of Petri dishes with nutrient rich agar (R2A) that did not contain As. Defined growth media, modified from the media used to isolate these bacteria, consisted of sterilized As(III) (75 μ M), 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (5 mM, pH 7.2), glucose (20 mM), NH₄NO₃ (1.25 mM), CaSO₄ (2 mM), MgCl₂ (2 mM), KH₂PO₄ (0.064 mM), KOH (1.25 mM), FeCl₂ (5 μ M), micronutrients and vitamins (Macur et

al., 2004). The same media without As(III) or glucose was used in batch kinetics experiments.

Liquid cultures, agitated on a rotary shaker at 100 rpm, consisted of defined growth media with 75 μM As(III) in sterile plastic Erlenmeyer flasks inoculated with single colonies from plates or starter liquid cultures. Growth was monitored by optical density (OD) at 650 nm using a spectrophotometer (Spectronic 21, Bausch & Lomb). When liquid cultures reached late exponential phase (16-24 hours depending on the strain) cells were rinsed three times in defined media lacking glucose and As(III) at 8,000 $\times g$ for 10 minutes, preserved at 4 $^{\circ}\text{C}$, and used in batch experiments within 24 hours.

Cell count and protein content were correlated to OD using direct cell counts on a Zeiss AxioImager microscope (phase contrast) and a protein assay. Protein was measured using the Bradford Assay method in which frozen resting cell cultures were centrifuged, digested with HCl, neutralized with NaOH, and diluted with NaPO_4 (Bradford et al., 1976; Mukhopadhyay et al., 1999). This solution was then assayed with a Coomassie blue dye reagent and OD was measured at 595 nm. Protein was determined using a standard curve with bovine serum albumin (BSA).

2.4.4 Batch As(III) oxidation and As(V) sorption kinetics experiments

Batch experiments were conducted to determine the apparent rate of As(III) oxidation and As(V) sorption. All experiments were carried out in triplicate replicates in sterile plastic Erlenmeyer flasks using 100 mL of defined media buffered with MOPS (5 mM at pH 7.2), pristine $\delta\text{-MnO}_2$ (0.05 g L^{-1}), and resting cell cultures of *A. tumefaciens* (9.8 $\mu\text{g protein mL}^{-1}$) and *P. fluorescens* (6.9 $\mu\text{g protein mL}^{-1}$) harvested in late exponential growth phase. The reaction was initiated by adding

As(III) (NaAsO₂) to achieve a final concentration of 75 μM for mixed microbe-mineral experiments or 0.3-140 μM As(III) for *P. fluorescens*-only experiments. Experiments were initiated at two microbe-mineral mixing times: several minutes after mixing the cells and mineral and 24 hours after mixing. During the experiments, 1 mL samples were removed periodically from the flasks, filtered through 0.22 μm filters (Cole-Parmer, NY membrane) to stop the reaction, and preserved at 4 °C until As speciation analysis.

Sorbed As was calculated using the following mass balance equation:

$$[(C_{0AsTotal}V_{0AsTotal}) - (C_{fAs(III)}V_{fAs(III)} + C_{fAs(V)}V_{fAs(V)})] / (C_{0AsTotal}V_{0AsTotal}) = q*100\%$$

where $C_{0AsTotal}$ and $V_{0AsTotal}$ are the initial total As in μM and volume in L, $C_{fAs(III)}V_{fAs(III)} + C_{fAs(V)}V_{fAs(V)}$ are final (300 or 320 minutes) values, and q is the amount of sorption. It was observed that all sorbed As was associated with the mineral phase because sorption by bacteria was not detected.

The apparent first order rate constant (k_{obs}) for the δ-MnO₂-only and mixed microbe-mineral batch experiments was described by the rate equation (Tournassat et al., 2002):

$$-d[As(III)] / dt = k_{obs}t$$

and k_{obs} was calculated using the integrated form:

$$\ln ([As(III)]_t / [As(III)]_0) = - k_{obs}t$$

for the first 80 minutes of reaction. Half-life ($t_{1/2}$) for the apparent first order reactions was calculated using the equation:

$$t_{1/2} = \ln 2 / k_{obs}$$

These kinetic calculations reflect apparent rates and do not imply any reaction mechanism. k_{obs} and $t_{1/2}$ were calculated for each experiment in order to quantitatively compare results from the various batch kinetic experiments.

Sterile control experiments were conducted with killed cells to check whether As(III) oxidation requires living bacteria. Cultures were sterilized by autoclaving twice (45 min, 121 °C). Oxidation batch experiments were carried out using the killed cells with the same method described above.

2.4.5 Batch desorption experiments

The purpose of replenishment desorption experiments was to investigate the stability of sorbed As(V) on δ -MnO₂ and microbial reactants as a function of pH. Previous studies reported lower As(V) sorption on oxide minerals at lower pH (O'Reilly et al., 2001; Amirbahman, et al., 2006). Sorption microbe-mineral batch experiments (as described above) were reacted until steady-state (250 minutes), centrifuged at 10,000 x g (15 minutes), and decanted. Desorption experiments were started by resuspending the paste in 100 mL of defined media buffered with 2-(*N*-morpholino)ethanesulfonic acid (MES) at pH 5.8. The solutions were briefly vortexed to mix and reacted for 250 minutes. Samples were filtered through 0.22 μm filters (Cole-Parmer, NY membrane) and preserved at 4 °C until As speciation analysis.

The amount of sorbed As was calculated based on percent of sorption (see above equation) and $\mu\text{moles As}_{\text{sorbed}}$. The ratio of $\text{As}_{\text{sorbed}}$ to $\text{As}_{\text{desorbed}}$ was reported as a percentage of sorbed As that was desorbed by the replenishment technique.

2.4.6 HPLC-ICP/MS analysis

Dissolved inorganic As species As(III) and As(V) were monitored over time in all experiments. As_{Total} was calculated from the sum of As(III) and As(V) and sorbed As was calculated as described above. Dissolved concentrations of As were determined by HPLC-ICP/MS (HPLC: Agilent 1200; ICP/MS: Agilent 7500cx) following the protocol described in Parikh et al. (2010).

2.4.7 FESEM analysis

Batch reaction products including δ -MnO₂-only, cell-only, and mixed microbe- δ -MnO₂ samples were imaged by field-emission scanning electron microscopy (FESEM). Samples were filtered onto 0.22 μ m filter papers (Nucleopore, PC membrane) and attached to a Gatan sample holder with a thin layer of TFMTM Tissue Freezing Medium (Electron Microscopy Sciences) mixed with graphite powder (Polysciences, Inc.). The samples were plunged into liquid nitrogen slush and transferred into the Gatan Alto 2500 cryo-stage. Surface ice was removed through a 10 minute sublimation at -90 °C. Samples were re-cooled to -125 °C and sputter coated with a 2-3 nm layer of gold-palladium. Samples were imaged in a Hitachi S-4700 FESEM at -125 °C using a 3.0 kV accelerating voltage and a working distance of 6.8 and 7.8 mm.

2.5 Results and Discussion

2.5.1 As(III) oxidation in mixed microbe-Mn oxide experiments

Disappearance of As(III) and appearance of As(V) was rapid in all batch experiments (**Fig. 2.1**).

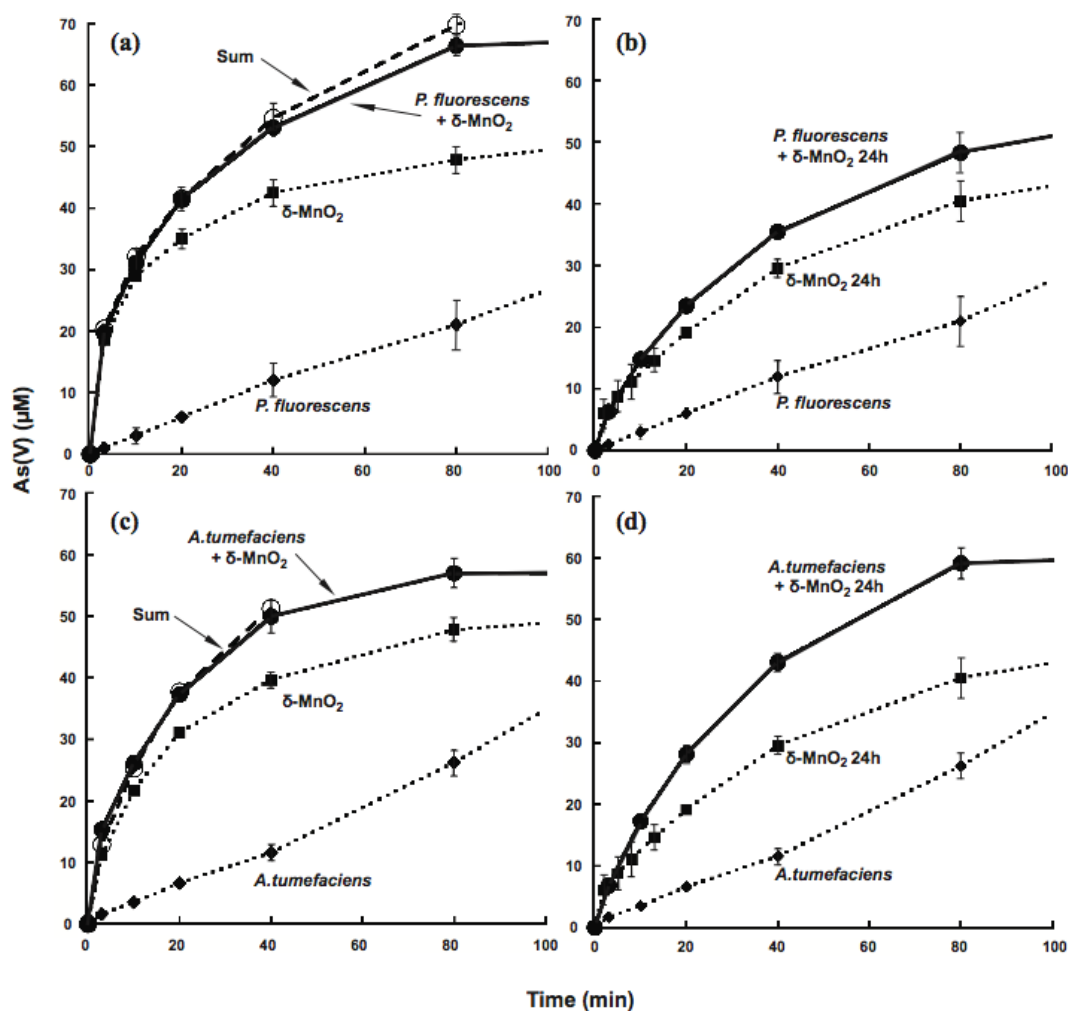


Figure 2.1 Appearance of As(V) in solution over time for singular and dual mixtures of $\delta\text{-MnO}_2$ with *P. fluorescens* and *A. tumefaciens*. (a) *P. fluorescens* and $\delta\text{-MnO}_2$ mix and sum of single-oxidizer experiments. (b) *P. fluorescens* and $\delta\text{-MnO}_2$ 24 hour mix. (c) *A. tumefaciens* and $\delta\text{-MnO}_2$ mix and sum. (d) *A. tumefaciens* and $\delta\text{-MnO}_2$ 24 hour mix.

Agrobacterium tumefaciens and *P. fluorescens* oxidized As(III) at similar rates of $3.2 \times 10^{-2} \mu\text{mol As min}^{-1} \text{mg protein}^{-1}$ and $3.9 \times 10^{-2} \mu\text{mol As min}^{-1} \text{mg protein}^{-1}$,

respectively, and killed cell cultures did not oxidize As(III) indicating that oxidation required viable cells. To reduce the effects of different protein contents and achieve similar rates, cell protein concentrations in mixed experiments were normalized to 6.9 $\mu\text{g protein mL}^{-1}$ *P. fluorescens* and 9.8 $\mu\text{g protein mL}^{-1}$ *A. tumefaciens*. In the absence of any mineral at those concentrations, *P. fluorescens* and *A. tumefaciens* oxidized As(III) at 0.2647 $\mu\text{mol As min}^{-1}$ and 0.3176 $\mu\text{mol As min}^{-1}$. All of the As was accounted for by mass balance indicating that no As was retained in the cells. The model mineral, $\delta\text{-MnO}_2$, oxidized As(III) in two phases with a rapid initial rate followed by a slower approach to steady state at around 250 minutes.

Four types of mixed microbe-mineral experiments were conducted using the model bacteria, $\delta\text{-MnO}_2$, and either a short (several minutes) microbe-mineral mixing time or a 24 h mixing time prior to starting the reaction. The short and 24h mixing times were selected to compare the effect of microbe-mineral interactions at varying equilibration times. Time series results (**Fig. 2.1**) include the following four experiments: *P. fluorescens* (6.9 $\mu\text{g protein mL}^{-1}$) with $\delta\text{-MnO}_2$ (0.05 g L^{-1}) mixed immediately before addition of As(III) (**Fig. 2.1a**), *P. fluorescens* with $\delta\text{-MnO}_2$ coincubated for 24h before As(III) addition (**Fig. 2.1b**), *A. tumefaciens* (9.8 $\mu\text{g protein mL}^{-1}$) with $\delta\text{-MnO}_2$ (0.05 g L^{-1}) short mix (**Fig. 2.1c**), and *A. tumefaciens* with $\delta\text{-MnO}_2$ 24h mix (**Fig. 2.1d**). Also shown in **Fig. 2.1**, is As(V) appearance in singular mineral-only or cell suspension-only experiments and the sum of those singular experiments. The initial rate of As(V) appearance in mixed microbe-mineral experiments had an initial rapid period followed by a slow approach to steady state. Even though both mixed and singular systems had this bi-phasic kinetic behavior, the magnitude of

As(V) in solution was greater for mixed microbe-mineral experiments than for singular systems with only mineral or cell suspension.

The apparent rate of As(V) appearance in solution in microbe-mineral experiments is within the error of the sum of mineral and microbial components in singular experiments (**Fig. 2.1a** and **2.1c**). This was determined by adding data points for single oxidizer experiments (*P. fluorescens* or *A. tumefaciens* alone plus δ -MnO₂ alone) at each sampling time and comparing those values with the microbe-mineral results (thick dashed lines in **Fig. 2.1a** and **2.1c**). The same trend was observed for the 24h equilibrated experiments (data not shown). The sums of single oxidizer As(V) curves were equivalent to results for dual mineral-microbe batch experiments indicating that the cells and mineral components were working independently and not influencing each other's oxidation rate over time.

As(III) oxidation rates in mixed microbe-mineral and singular δ -MnO₂ experiments were fit to a 1st order kinetic model (**Fig. 2.2**).

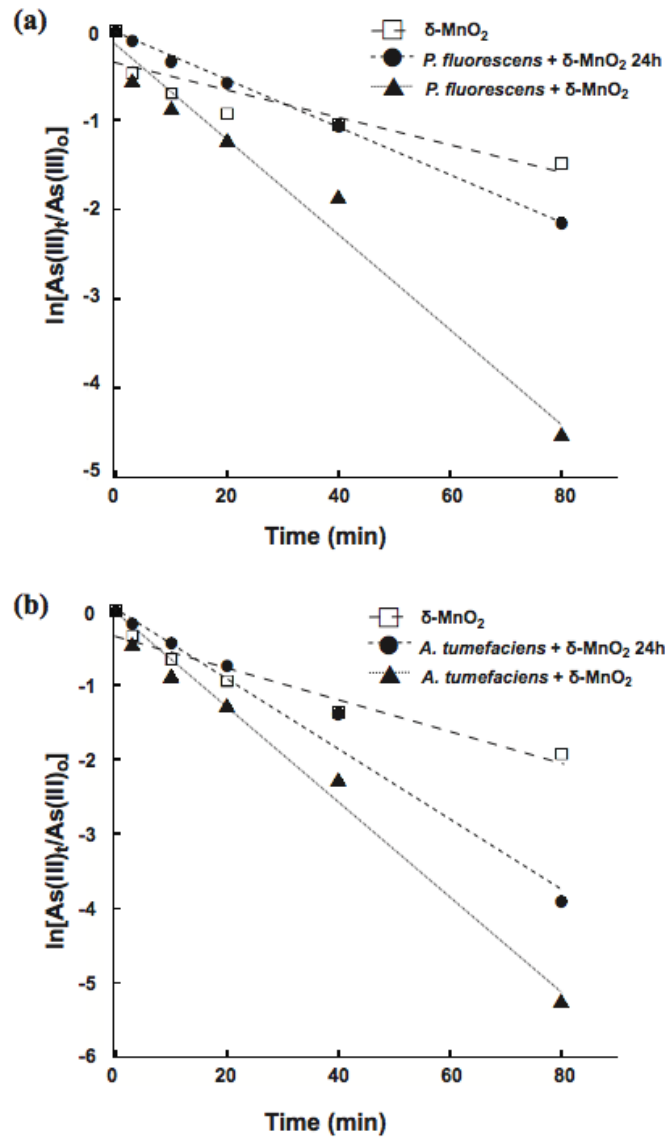


Figure 2.2 Apparent 1st order kinetics for singular and dual mixtures of δ -MnO₂ with *P. fluorescens* (a) and *A. tumefaciens* (b). k_{obs} and R^2 values are included in Table 2.1.

Apparent kinetics of δ -MnO₂ experiments were comparable to published results with differences arising from mineral crystallinity, surface area, pH, and electrolyte

concentration (Manning et al., 2002; Tournassat et al., 2002; Oscarson et al., 1983). Rate constants (k_{obs}) were higher and half-lives were shorter in mixed experiments than in the δ -MnO₂ experiments (**Table 2.1**).

Table 2.1 Apparent 1st order kinetics for δ -MnO₂-only and mixed microbe- δ -MnO₂ batch experiments. Experiments with *A. tumefaciens* and *P. fluorescens* mixed with δ -MnO₂ exhibit a more rapid oxidation rate than δ -MnO₂ alone. Subscripts indicate different batches of δ -MnO₂.

| | k_{obs} (h ⁻¹) | Half-life (h) | R ² |
|--|--|------------------|----------------|
| <i>A. tumefaciens</i> + δ -MnO ₂ (2) | 3.77 | 0.185 | 0.990 |
| <i>P. fluorescens</i> + δ -MnO ₂ (1) | 3.13 | 0.221 | 0.976 |
| <i>A. tumefaciens</i> + δ -MnO ₂ (2) 24h | 2.84 | 0.244 | 0.975 |
| <i>P. fluorescens</i> + δ -MnO ₂ (2) 24h | 1.58 | 0.438 | 0.999 |
| δ -MnO ₂ (2) | 1.33 | 0.523 | 0.918 |
| δ -MnO ₂ (1) | 0.90 | 0.770 | 0.803 |

The rates of 24 hour mixed experiments were lower than those with a shorter mixing time but still faster than experiments with only δ -MnO₂. Overall, As(III) oxidation was most rapid when microbial and mineral oxidants were mixed.

2.5.2 As sorption and desorption

A decrease of As from solution (15.2-24.5%) was observed in all experiments with δ -MnO₂ by steady state at 250 min reaction time (**Table 2.2**).

Table 2.2 Sorption and desorption results from mineral-only and mixed microbe- δ -MnO₂ batch experiments. Desorption was conducted with the replenishment technique by decreasing the pH from 7.2 to 5.8. Subscripts indicate different batches of δ -MnO₂.

| | Sorbed As (μ mol) | Sorbed As (%) | Desorbed As (μ mol) | Desorbed As (%) |
|---|---------------------------|------------------|-----------------------------|--------------------|
| δ -MnO ₂ (1) | 1.59 | 21.3 | 0.926 | 58.3 |
| <i>P.fluorescens</i> + δ -MnO ₂ (1) 24h | 1.20 | 17.1 | 0.729 | 61.0 |
| <i>P.fluorescens</i> + δ -MnO ₂ (1) | 1.12 | 16.1 | 0.724 | 64.5 |
| δ -MnO ₂ (2) | 1.83 | 24.5 | 0.857 | 46.7 |
| <i>A.tumefaciens</i> + δ -MnO ₂ (2) 24h | 1.14 | 15.2 | 0.729 | 64.0 |
| <i>A.tumefaciens</i> + δ -MnO ₂ (2) | 1.20 | 16.1 | 0.757 | 63.0 |

Some of the range of As sorption could be explained by natural variation by batch of δ -MnO₂. This variation was probably an effect of age (0 to 3 weeks) and crystallinity of the mineral. Increased crystallinity and decreased number of reactive sites on the mineral was expected to reduce the amount of As sorbed (Tournassat et al., 2002). Because of this variability in δ -MnO₂-only experiments, sorption in mixed batch experiments must be compared only with the corresponding batch of δ -MnO₂ as indicated with subscripts in **Table 2.2**.

Sorption in mixed microbe-mineral experiments differed slightly depending on the strain of bacteria (**Table 2.2**). More As was sorbed in δ -MnO₂ batch experiments without (21.3-24.5%) bacteria than with *A. tumefaciens* (16.1% and 15.2%) or *P.fluorescens* (16.1% and 17.1%). Reduced sorption may have been a result of mineral surface passivation by extracellular polymeric substances (EPS), which have phosphate functional groups that may compete with As sorption (Huang et al., 2011). Passivation of δ -MnO₂ and reduced As(III) oxidation have been shown to occur in the presence of these specific bacteria using attenuated total reflectance (ATF) Fourier transform infrared (FTIR) spectroscopy (Parikh et al., 2010). Rapid

oxidation of As(III) by the bacteria may also prevent As(III) interaction with the mineral phase and thereby reduce sorption.

Desorption experiments, conducted with a replenishment technique, evaluated the stability of sorbed As when pH was decreased from 7.2 to 5.8 (**Table 2.2**). Desorption can also be induced by a change in electrolyte or competitive cation concentration, but those factors were not investigated for this system (Lafferty et al., 2011). In experiments with *A. tumefaciens*, more As was desorbed from mixed microbe-mineral experiments (63.0% and 64.0%) than from δ -MnO₂ alone (46.7%). Similarly, more As was desorbed from the mixed *P. fluorescens* and δ -MnO₂ experiments (64.5% and 61.0%) than from δ -MnO₂ alone (58.3%). This indicates that, at pH 5.8, the As sorbed in the δ -MnO₂-bacteria system is more easily removed than the As sorbed in on δ -MnO₂ alone. While the microscopic and kinetic evidence to not suggest that this microbe-mineral interaction affects As(III)-oxidation, the higher desorption in mixed δ -MnO₂-bacteria experiments suggests that the presence of cells affects As desorption from δ -MnO₂.

2.5.3 Influence of cell-mineral interactions on As(III) oxidation

The more rapid As(III) oxidation in mixed microbe-mineral experiments compared with the mineral alone was the first line of evidence that the mineral and the cells are not interacting in a way that inhibits As(III) oxidation (**Fig. 2.1**). Additional evidence was apparent from SEM imaging (**Fig. 2.3**),

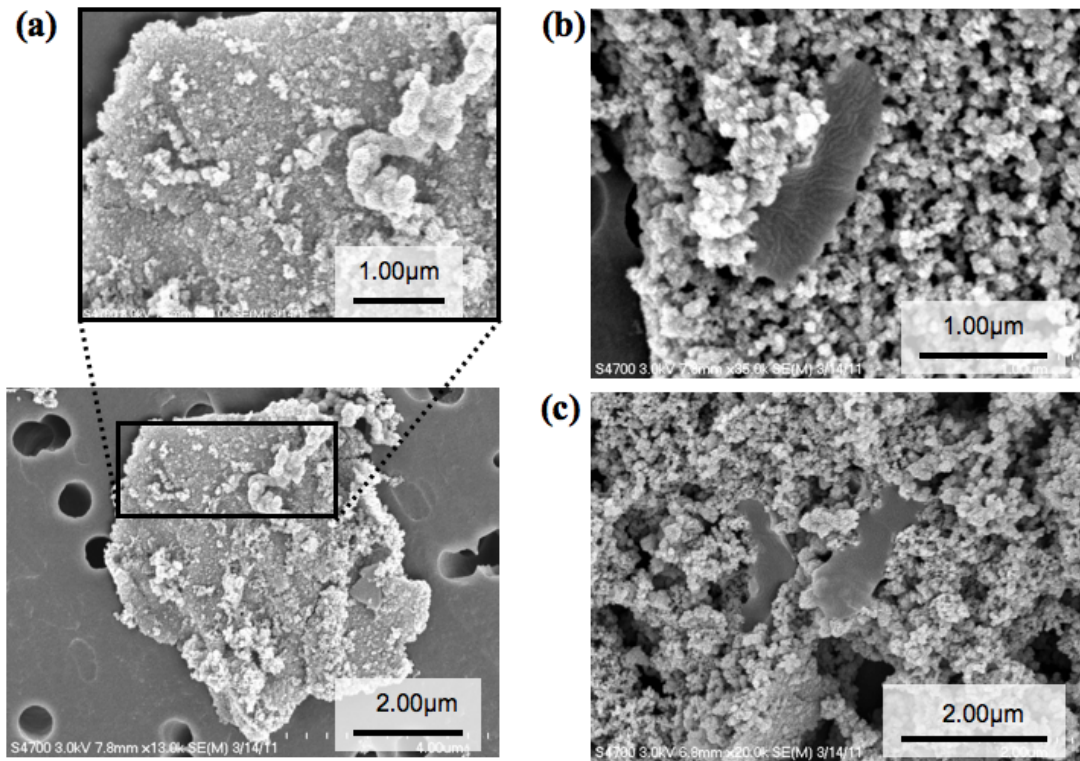


Figure 2.3 Field emission scanning electron microscopy (FESEM) images of singular (a) and dual mixtures of δ -MnO₂ with *P. fluorescens* (b) and *A. tumefaciens* (c). No difference in cell-mineral associations at different mixing times, cell morphology, or mineral surface were observed. Cells appeared smooth and not strongly associated with the mineral phase.

which showed minimal macroscopic association between cells and mineral phases. In **Fig. 2.3**, the cells and mineral appear to be discrete with neither component coating or blocking the other. This does not eliminate the possibility of some EPS material coating the mineral because EPS could be invisible with the image resolution. Further investigation into the presence of EPS may benefit from FTIR studies as in Huang et al., 2011.

Pseudomonas fluorescens was evaluated in singular batch experiments for its initial As(III) oxidation rate at 6.9 $\mu\text{g protein mL}^{-1}$ and a range of As(III) concentrations from 0.3 μM to 140 μM . The fastest oxidation of As(III) by *P. fluorescens* was at a concentration of 7.1 μM As(III), 10x lower than the concentration used in mixed microbe-mineral batch experiments [75 μM As(III)] (Table 2.3).

Table 2.3 Initial As(III) oxidation rate for *P. fluorescens* (6.9 $\mu\text{g protein mL}^{-1}$)

| Initial As(III) (μM) | Initial Oxidation Rate ($\mu\text{M As(III) min}^{-1}$) | R ² |
|--------------------------------------|--|----------------|
| 140 | 0.127 | 0.997 |
| 67 | 0.165 | 0.999 |
| 37 | 0.210 | 0.980 |
| 16 | 0.303 | 0.988 |
| 7.1 | 0.427 | 0.992 |
| 1.4 | 0.156 | 0.934 |
| 0.30 | 0.040 | 0.912 |

This result indicates that the additive effect of *P. fluorescens* on the rate of As(III) oxidation by δ -MnO₂ may be even greater at lower substrate concentrations. The reason why *P. fluorescens* oxidizes As(III) faster at lower concentrations is unknown but it could be a result of competing or inhibitory enzymatic pathways or the toxicity of high substrate concentrations (Marangoni, 2003).

In summary, the experiments presented here show that *P. fluorescens* and *A. tumefaciens* cells enhance As(III) oxidation in a dual system and have some influence on mineral-surface reactions including As sorption. However, passivation of the mineral surface in the mixed microbe-mineral experiments does occur according to the bi-phasic behavior of As(V) appearance in solution. Kinetic and microscopic evidence indicates that passivation was likely to have been a combined result of

Mn(II), Mn(III), and As(V) sorption with some surface inhibition by cells or EPS possible (Lafferty et al., 2010a; Zhu et al., 2009).

2.5.4 Implications for As mobility in the environment

This study supports previous work showing that poorly crystalline Mn oxide minerals can rapidly oxidize As(III) and remove As from solution (Manning et al., 2002, Tournassat et al., 2002, Ginder-Vogel et al., 2009; Lafferty et al., 2010a and b). Rapid abiotic As(III) oxidation can be accelerated in the presence of the As(III)-oxidizing bacteria studied here with up to 3.5 times shorter half-lives in mixed microbe-mineral batch experiments (**Table 2.1**). Coupled biotic-abiotic As(III) oxidation experiments bridge prior work on separate mineral and microbial As oxidation studies with a more complex and heterogeneous system (i.e. Oscarson et al., 1983; Anderson et al., 1992; Macur et al., 2004). Similar effects on the rate of As(III) oxidation by As-oxidizing bacteria in the presence of Mn oxide minerals are expected in the environment. However, the initial rapid rates of As(III) oxidation observed here may over-estimate the rate that could be achieved in soils which have aged minerals, organic coatings on the minerals, and lower concentrations of As(III) oxidizing cells warranting further study on coupled biotic and abiotic systems and environmental As mobility.

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bacteria isolated used here. This research was funded in part by two fellowships: the Delaware Environmental Institute Fellowship (Unidel Foundation) and the EPA Science to Achieve Results (STAR) graduate fellowship.

2.7 References

- Achour, A.R.; Bauda, P.; Billard, P. Diversity of arsenite transporter genes from arsenic-resistant soil bacteria. *Research in Microbiology* **2007**, *158*, 128-137.
- Amirbahman, A.; Kent, D.B.; Curtis, G.P.; Davis, J.A. Kinetics of sorption and abiotic oxidation of arsenic(III) by aquifer materials. *Geochimica et Cosmochimica Acta* **2006**, *70*, 533-547.
- Anderson, G.L.; Love, M.; Zeider, B.K. Metabolic energy from arsenite oxidation in *Alcaligenes faecalis*. *J. Phys. IV France* **2003**, 49-52.
- Anderson, G.L.; Williams, J.; Hille, R. The purification and characterization of arsenite oxidase from *Alcaligenes faecalis*, a molybdenum-containing hydroxylase. *J. Biol. Chem.* **1992**, *267* (33), 23674-23682.
- Bhattacharya, P.; Welch, A.H.; Stollenwerk, K.G.; McLaughlin, M.J.; Bundschuh, J.; Panauallah, G. Arsenic in the environment: Biology and Chemistry. *Science of the Total Environment* **2007**, *379*, 109-120.
- Borch, T.; Kretzschmar, R.; Kappler, A.; Van Cappellen, P.; Ginder-Vogel, M.; Voegelin, A.; Campbell, K. Biogeochemical redox processes and their impact on contaminant dynamics. *Environ. Sci. Technol.* **2010**, *44*, 15-23.
- Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **1976**, *72*, 248-254.
- Duker, A.A.; Carranza E.J.M.; Hale, M. Arsenic geochemistry and health. *Environment International* **2005**, *31*, 631-641.
- Ellis, P.J.; Conrads, T.; Hille, R.; Kuhn, P. Crystal structure of the 100 kDa arsenite oxidase from *Alcaligenes faecalis* in two crystal forms at 1.64 Å and 2.03 Å. *Structure*, **2001**, *9*, 125-132.

- Garcia-Dominguez, E.; Mumford, A.; Rhine, E.D.; Paschal, A.; Young, L. Novel autotrophic arsenite-oxidizing bacteria isolated from soil and sediments. *FEMS Microbiol. Ecol.*, **2008**, *66*, 401-410.
- Ginder-Vogel, M.; Landrot, G.; Fischel, J.S.; Sparks, D.L. Quantification of rapid environmental redox processes with quick-scanning x-ray absorption spectroscopy (Q-XAS). *Proc. Natl. Acad. Sci.* **2009**, *106* (38), 16124-16128.
- Huang, J-H.; Elzinga, E.J.; Brechbuehl, Y.; Voegelin, A; Kretzschmar, R. Impacts of *Shewanella putrefaciens* strain CN-32 cells and extracellular polymeric substances on the sorption of As(V) and As(III) on Fe(III)-(hydr)oxides. *Environ. Sci. Technol.* **2011**, *45*, 2804-2810.
- Inskeep, W.P.; Macur, R.E.; Hamamura, N.; Warelow, T.P.; Ward, S.A.; Santini, J.M. Detection, diversity and expression of aerobic bacterial arsenite oxidase genes. *Environmental Microbiology* **2007**, *9* (4), 934-943.
- Kashyap, D.R.; Botero, L.M.; Franck, W.L.; Hasset, D.J.; McDermott, T.R. Complex regulation of arsenite oxidation in *Agrobacterium tumefaciens*. *Journal of Bacteriology* **2006**, *188* (3), 1081-1088.
- Lafferty, B.J.; Ginder-Vogel, M.; Sparks, D.L. Arsenite oxidation by a poorly crystalline manganese-oxide: 1. Stirred-flow experiments. *Environ. Sci. Technol.* **2010a**, *44* (22), 8460-8466.
- Lafferty, B.J.; Ginder-Vogel, M.; Zhu, M.; Livi, K.J.T.; Sparks, D.L. Arsenite oxidation by a poorly crystalline manganese-oxide: 1. Results from x-ray absorption spectroscopy and x-ray diffraction. *Environ. Sci. Technol.* **2010b**, *44* (22), 8467-8472.
- Lafferty, B.J.; Ginder-Vogel, M.; Sparks, D.L. Desorption of As from a poorly crystalline manganese oxide. Paper in press, **2011**.
- Macur, R.E.; Jackson, C.R.; Botero, L.M.; McDermott, T.R.; Inskeep, W.P. Bacterial populations associated with the oxidation and reduction of arsenic in an unsaturated soil. *Environ. Sci. Technol.* **2004**, *38*, 104-111.
- Manning, B.A.; Fendorf, S.E.; Bostick, B.; Suarez, D.L. Arsenic(III) oxidation and arsenic(V) adsorption reactions on synthetic birnessite. *Environ. Sci. Technol.* **2002**, *36*, 976-981.
- Marangoni, A.G. *Enzyme Kinetics: A Modern Approach*. John Wiley & Sons. Hoboken, NJ, **2003**, pp. 44-78.

- Mukhopadhyay, B.; Johnson, E.F.; Ascano, M. Conditions for vigorous growth on sulfide and reactor-scale cultivation protocols for the thermophilic green sulfur bacterium *Chlorobium tepidum*. *Appl. Environ. Microbio.* **1999**, *65* (1), 301-306.
- Mukhopadhyay, R.; Rosen, B.P.; Phung, L.T.; Silver, S. Microbial arsenic: from geocycles to genes and enzymes. *FEMS Microbiology Reviews* **2002**, *26*, 311-325.
- Nesbitt, H.W.; Canning, G.W.; Bancroft, G.M. XPS study of reductive dissolution of 7A-birnessite by H₃AsO₃, with constraints on reaction mechanism. *Geochimica et Cosmochimica Acta* **1998**, *62* (12), 2097-2110.
- Nordstrom, D.K. Worldwide occurrences of arsenic in ground water. *Science* **2002**, *296*, 2143-2144.
- Oremland, R.S.; Stolz, J.F. The ecology of arsenic. *Science* **2003**, *300*, 939-944.
- O'Reilly, S.E.; Strawn, D.G., Sparks, D.L. Residence time effects of arsenate adsorption/desorption mechanisms on goethite. *Soil Sci. Soc. Am. J.* **2001**, *65*, 67-77.
- Oscarson, D.W.; Huang, P.M.; Liaw, W.K.; Hammer, U.T. Kinetics of oxidation of arsenite by various manganese dioxides. *Soil Sci. Soc. Am. J.* **1983**, *47*, 644-648.
- Parikh, S.J.; Lafferty, B.J.; Meade, T.G.; Sparks, D.L. Evaluating environmental influences on As(III) oxidation kinetics by a poorly crystalline Mn-oxide. *Environ. Sci. Technol.* **2010**, *44* (10), 3772-3778.
- Parikh, S.J.; Lafferty, B.J.; Sparks, D.L. An ATR-FTIR spectroscopic approach for measuring rapid kinetics at the mineral/water interface. *Journal of Colloid and Interface Science* **2008**, *320*, 177-185.
- Post, J.E. Manganese oxide minerals: Crystal structures and economic and environmental significance. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3447-3454.
- Quemeneur, M.; Heinrich-Salmeron, A.; Muller, D.; Lievreumont, D.; Jauzerin, M.; Bertin, P.N.; Garrido, F.; Joulain, C. Diversity surveys and evolutionary relationships of aoxB genes in aerobic arsenite-oxidizing bacteria. *Applied and Environmental Microbiology* **2008**, *74*, 4567-4573.

- Rhine, E.D.; Garcia-Dominguez, E.; Phelps, C.D.; Young, L.Y. Environmental microbes can speciate and cycle arsenic. *Environ. Sci. Technol.* **2005**, *39*, 9569-9573.
- Scott, M.J.; Morgan, J.J. Reactions at oxide surfaces. 1. Oxidation of As(III) by synthetic birnessite. *Environ. Sci. Technol.* **1995**, *29*, 1898-1905.
- Silver, S.; Phung, L.T. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. *Appl. Environ. Microbio.* **2005**, *71* (2), 599-608.
- Smith, A.H.; Lingas, E.O.; Rahman, M. Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bulletin of the World Health Organization* **2000**, *78* (9), 1093-1103.
- Tebo, B.M.; Bargar, J.R.; Clement, B.G.; Dick, G.J.; Murray, K.J.; Parker, D.; Verity, R.; Webb, S.M. Biogenic manganese oxides: Properties and mechanisms of formation. *Annu. Rev. Earth Planet. Sci.* **2004**, *32*, 287-328.
- Tournassat, C.; Charlet, L.; Bosbach, D.; Manceau, A. Arsenic(III) oxidation by birnessite and precipitation of manganese(II) arsenate. *Environ. Sci. Technol.* **2002**, *36*, 493-500.
- Villalobos, M.; Toner, B.; Bargar, J.; Sposito, G. Characterization of the manganese oxide produced by *Pseudomonas putida* strain MnB1. *Geochimica et Cosmochimica Acta* **2003**, *67* (14), 2649-2662.
- Zhu, M.; Paul, K.W.; Kubicki, J.D.; Sparks, D.L. Quantum chemical study of arsenic (III, V) adsorption on Mn-oxides: Implications for arsenic(III) oxidation. *Environ. Sci. Technol.* **2009**, *43*, 6655-6661.

Chapter 3

TIME DEPENDENCY OF BIOTIC AND ABIOTIC ARSENIC OXIDATION IN A DELAWARE SOIL

3.1 Authors

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3.2 Abstract

Massive arsenic (As) poisoning occurring in Southeast Asia has motivated this research and its focus on the intricate biogeochemical processes controlling As mobility in soil-water interfaces. The oxidation of As(III) to As(V) results in a less mobile and less toxic phase of As and is therefore a natural sequestration and detoxification pathway. Laboratory experiments with manganese (Mn) oxides and As-oxidizing microorganisms have shown that both of these common soil components can

oxidize As(III) and Mn oxides can also sorb As. However, whether these two pathways for As oxidation in soil are competitive, additive, or interact at all remains poorly understood. Here, we report a series of soil column experiments addressing biological and abiotic As oxidation in a Delaware agricultural soil. The oxidation of As(III) was found to be at least indirectly affected by biological activity in all experiments. These results indicate the importance of coupled microbiological-abiotic processes in As oxidation and emphasize the need for addressing biogeochemical and temporal complexity of soil processes in experimental studies.

3.3 Introduction

Reduction and oxidation (redox) reactions control the mobility, bioavailability, toxicity, and speciation of arsenic (As) in soils (Borch et al., 2010). The reduced form of As, As(III), is more toxic and mobile than the oxidized form, As(V), which makes As oxidation an important reaction for detoxification and natural sequestration of As (Le, 2002; Nordstrom, 2002). Arsenic oxidation involves integrated biological and geological reactions at a range of spatial and temporal scales, making it a challenge to develop environmentally relevant experiments (Inskeep et al., 2002). It has been shown that both mineral components, such as manganese (Mn) oxide minerals, and diverse soil microorganisms can oxidize As in soils yet the interplay between these components is not well characterized (Oscarson et al., 1983; Scott and Morgan, 1995; Oremland and Stolz, 2005).

Approaches that address the biogeochemical complexity of As(III) oxidation include experiments using a simplified soil system, analyzing environmental samples, and integrative modeling (Macur et al., 2004, Polizzotto et al., 2008; Bachate et al., 2009; Lafferty et al., 2010). A promising experimental technique is the soil

column method for determining *in situ* mineral oxidation and sorption including native microbial activity, physical heterogeneity, and flow-through conditions (Tokunaga et al., 2003; Tokunaga et al., 2004; Gu et al., 2005; Bank et al., 2007; Tufano et al., 2008; Tufano and Fendorf, 2008; Sun et al., 2009). The flow-through conditions involve a soil-solution phase continuously being replenished and carrying away reaction products, which is advantageous for preventing reverse reactions and extended soil-solution reaction time. Another advantage is the ability to capture the activity of native bacteria, which have not been cultivated in isolation (Kirk et al., 2004).

Two strategies for understanding coupled As(III) oxidation processes in column experiments are abiotic controls and molecular biological analyses. Abiotic control experiments, lacking cell activity, can be used to distinguish mineral- from biogeochemical-driven reaction pathways. To achieve a good non-biological analogue, soil sterilization must not change mineralogy or soil chemistry, so methods such as gamma (γ) irradiation are preferred (Trevors, 1996; McNamara et al., 2003). Molecular analysis of the microbial community and polymerase chain reaction (PCR) primers for As oxidase genes provide information on the biological components of soil column experiments. The *aox* gene, isolated from heterotrophic As-oxidizing soil bacteria, codes for an As oxidase enzyme and is thought to signify As oxidation activity (Ellis et al., 2001; Muller et al., 2003). Primers developed for *aoxB* have been used successfully in environmental samples and indicate that biotic As(III) oxidation may be widespread (Inskeep et al., 2007; Quemeneur et al., 2008).

While the column experiment technique has been used before for other trace element studies, to date there is no published research addressing mineral and

microbial As(III) oxidation along with molecular biological analysis of native bacteria (Tokunaga et al., 2004; Bank et al., 2007; Tufano and Fendorf, 2008). The objective of this study was to evaluate coupled biogeochemical and abiotic As(III) oxidation in a Delaware agricultural soil using column experiments. Using a soil with low background As could provide information on the capability of the native microbial community to respond to a large addition of As. Temporal geochemical data on As speciation in soil and column effluent are the main components of this study. By addressing the time-dependency and interplay of mineral (abiotic) versus biogeochemical pathways for As(III) oxidation in a heterogeneous environment, this study contributes to explaining the environmental cycle of this toxic element.

3.4 Materials and Methods

3.4.1 Soil and As(III) solutions

Soil samples were collected at 5-10 cm depth from the Matapeake silt loam soil at the University of Delaware Farm, Newark, DE. Aliquots of soil samples were air-dried before soil analysis and sterilization. The soil had 13.5 % sand, 64.5 % silt, 22 % clay. The cation exchange capacity (CEC) was 16.34 cmol kg⁻¹, pH was 6.2, organic matter (OM) content by loss on ignition was 2.6 %. Air-dried soil was acid-digested with microwave heating following the protocol for EPA 3051 soil analysis and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) for elemental content (US EPA, 1995). Total Fe was 15476 mg kg⁻¹, total Mn was 400.08 mg kg⁻¹, total Pb was 76.37 mg kg⁻¹, and the total As concentration in the soil was 9.9 mg kg⁻¹.

Soil columns (described below) were amended with sterile As(III)-spiked media with and without additional carbon sources. The concentration of As(III) used in all columns was $200 \pm 20 \mu\text{M}$ added as NaAsO_2 , and As(V) was below detection. Organic matter (OM)-amended media included 5 mg L^{-1} yeast extract, 10 mg L^{-1} tryptic soy broth, and 500 mg L^{-1} NaCl. Carbon-free media included 500 mg L^{-1} NaCl. All media had a defined solution of trace metals added at the following concentrations: 0.100 mg L^{-1} $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.120 mg L^{-1} $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.070 mg L^{-1} ZnCl_2 , 0.060 mg L^{-1} H_3BO_3 , 0.025 mg L^{-1} $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 0.015 mg L^{-1} $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 0.025 mg L^{-1} $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, and 0.001 mg L^{-1} 12M HCl. No buffer was added and the pH of all media was 6.5-7. Media solutions were sterilized by autoclave and $0.22 \mu\text{m}$ filtration.

3.4.2 Soil sterilization

Gamma (γ)-irradiation is considered an effective soil sterilization method with minimal effects on soil chemistry and has been used in previous column type experiments (McNamara et al., 2003; Bank et al., 2007). Air-dried soil samples were sterilized by autoclaving three times or by γ -irradiation. For the γ -irradiation technique, soil was packed into a polypropylene bottle and irradiated with a total dosage of 25,000 Gy using a Sheperd Mark 1 γ -irradiator (J.D. Sheperd & Associates, CA). Soil from both sterilization techniques was amended with As(III) media in column experiments to test the effects of biological activity on As(III) oxidation. Sterilization was verified using viable plate counts.

3.4.3 As(III) oxidation column experiments

Soil columns were packed immediately after sampling to preserve the activity of native bacteria in sterile 20 mL plastic syringes capped with rubber stoppers (Fig. 3.1).



Figure 3.1 A column packed with quartz sand. The influent solution was injected by a peristaltic pump at the bottom of the column and collected from the top. The effluent was sampled daily throughout the experiments.

All columns were assembled in a sterile aerobic environment at ambient room temperature. Fresh soil was gently crushed, picked for large ($\gg 200 \mu\text{m}$) particles, such as gravel, and mixed with sterile coarse quartz sand in a 30:70 soil to sand ratio by mass. The quartz sand was added to increase the porosity of the columns and prevent development of preferential flow paths.

A peristaltic pump was used to flow As(III) solutions through the columns. Columns were first injected with NaCl solution lacking As(III) for 24h before beginning the As treatment. The flow rate was $2 \pm 0.5 \text{ ml h}^{-1}$ (3.9 pore volumes day^{-1}) for all experiments. Pore volume of the columns was calculated by taking the mass of a dry column, filling it with water, and then taking the mass of the water-saturated column. The difference was the porosity of the columns. Flow rate was translated to pore volumes by converting the volume of flow to column pore volume.

Aerobic conditions in the columns were verified by monitoring of Fe(II) and Fe_{Total} in the effluent solution using the Ferrozine spectrophotometric method (Stookey, 1970). Samples of the effluent were taken daily, filtered with a $0.22 \mu\text{m}$ nylon filter, and refrigerated. Experiments were run for 21 days and residual solids collected at the end of the experiments were preserved at $-80 \text{ }^\circ\text{C}$ for molecular biology analysis.

Arsenic speciation [As(III) and As(V)] was determined by high performance liquid-chromatography (HPLC) inductively coupled plasma mass spectrometry (ICP/MS) in the influent and effluent solutions following the protocol described in Parikh et al. (2010). Column soil from the beginning and the end of experiments was air dried and digested with the EPA 3051 protocol. Total As in the

soil was quantified by inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of the digestate.

3.4.4 As(III) oxidation batch experiments

Batch experiments were performed using sterile Matapeake silt loam soil and heterotrophic As(III)-oxidizing bacteria, *Pseudomonas fluorescens* (isolated by Macur et al., 2004). All experiments were carried out in triplicate replicates in sterile plastic Erlenmeyer flasks using 100 mL of defined medium, resting cell cultures of *P. fluorescens* (6.8×10^7 cells mL⁻¹) harvested in late exponential growth phase, and 1.0 g L⁻¹ sterile soil for mixed soil-cell experiments. The defined media consisted of sterilized As(III) (75 µM), 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (5 mM, pH 7.2), glucose (20 mM), NH₄NO₃ (1.25 mM), CaSO₄ (2 mM), MgCl₂ (2 mM), KH₂PO₄ (0.064 mM), KOH (1.25 mM), FeCl₂ (5 µM), micronutrients and vitamins (Macur et al., 2004). The reaction was initiated by adding As(III) (NaAsO₂) to achieve a final concentration of 75 µM. During the experiments, 1 mL samples were removed periodically from the flasks, filtered through 0.22 µm filters to stop the reaction, and preserved at 4 °C until As speciation analysis by HPLC-ICP/MS.

3.5 Results and Discussion

3.5.1 Time-dependency of As(III) oxidation in soil columns

As(III) oxidation capability of sterile and non-sterile soil columns differed dramatically. In the sterile columns, minimal As(V) was eluted whereas the non-sterile columns eluted mostly As(V) by the end of the experiments (**Fig. 3.2**). The delay in appearance of effluent As can be attributed to the 24h NaCl solution flush and a period of interaction between the As and the soil matrix.

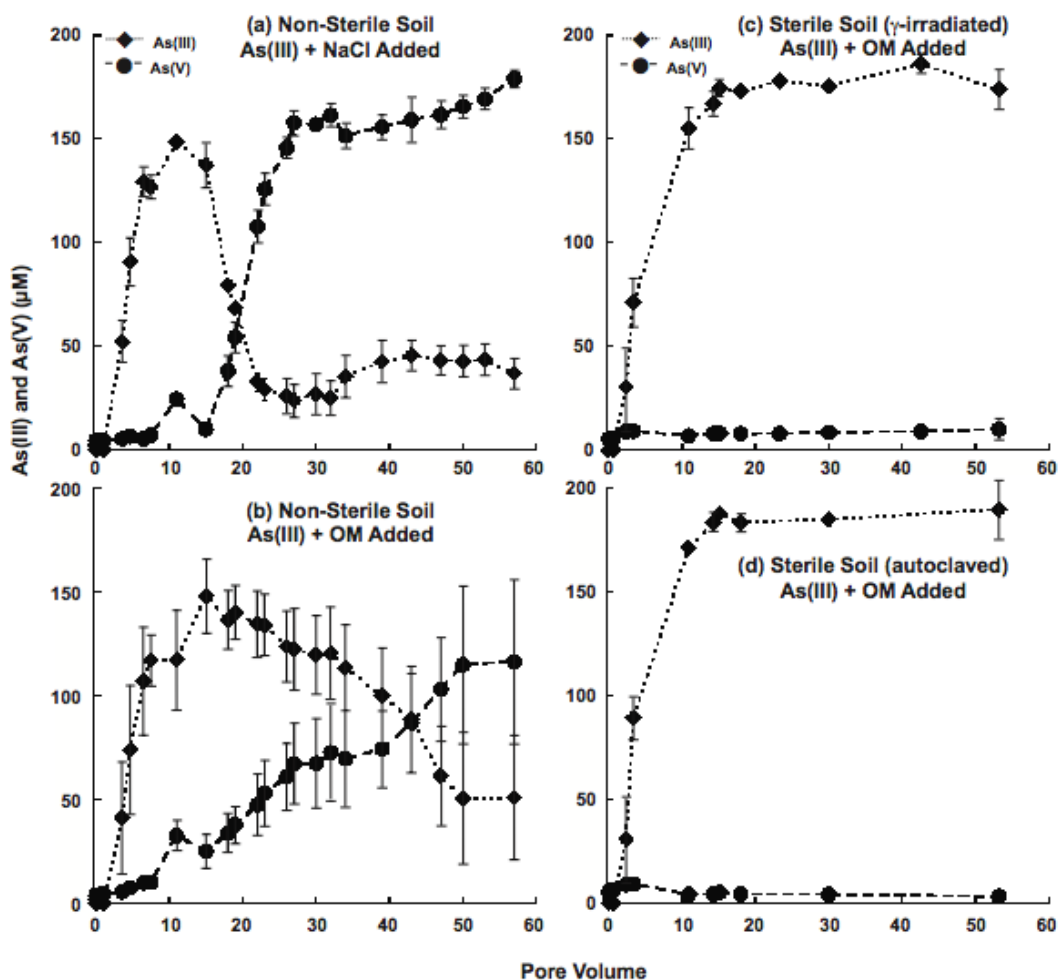


Figure 3.2 As(III) and As(V) in column effluent solution as a function of pore volume. The non-sterile soils were amended with As(III) and NaCl (a) or As(III) with organic matter (OM) (b). The γ -irradiated (c) and autoclaved (d) soil columns were amended with As(III) and OM. The non-sterile soil columns oxidized As(III) and the sterile ones did not. Error bars represent the standard deviation of triplicate columns.

Neither the γ -irradiated nor the autoclaved soils oxidized an appreciable amount of As(III) (**Fig. 3.2c, 3.2d**). Arsenic free control columns (data not shown) did not release detectable As indicating that all effluent As in As(III)-amended columns

should have originated from the influent media. Oxidizing conditions were maintained in all columns throughout the experiment as shown by the lack of dissolved Fe(II) in the effluent. The apparent oxidizing conditions indicate that the As(V) reduction was unlikely. Triplicate replicates of columns showed the NaCl-amended non-sterile columns (**Fig. 3.1a**) to be more repeatable than the OM-amended columns (**Fig. 3.1b**), suggesting that carbon source may have been a more powerful driver for microbial community change than As, assuming that variability was largely biological.

The non-sterile columns amended with a NaCl solution and an OM solution both began oxidizing As(III) at around two days (6 pore volumes). More As(V) was being eluted than As(III) by six days (20 pore volumes) in the NaCl-amended column and 11 days (45 pore volumes) in the OM-amended column (**Fig. 3.2a, 3.2b**). The observed delay in As(III) oxidation may be due to a period of microbial community modification in favor of microbes best suited to the conditions.

The concentration of total As in the soil increased by approximately 10x in all residual soil (**Table 3.1**).

Table 3.1 Total As concentration in initial soil (non-sterile and γ -irradiated) and residual soil from the column experiments.

| | As (mg kg ⁻¹) |
|-----------------------------------|---------------------------|
| Initial Soil | 9.9 ± 0.10 |
| γ -irradiated Initial Soil | 17.5 ± 0.17 |
| Non-Sterile Soil + C + As(III) | 134.4 ± 15.2 |
| Non-Sterile Soil + NaCl + As(III) | 153.6 ± 19.5 |
| γ -irradiated soil + As | 73.8 ± 5.3 |

The range of As concentration in the initial soil (9.9-17.5 mg kg⁻¹) was due to soil heterogeneity in the sampling site. The column treated with NaCl retained the most As (153.6 mg kg⁻¹) followed by the column treated with OM (134.4 mg kg⁻¹). The γ -irradiated column retained less As than either of the non-sterile columns (73.8 mg kg⁻¹). Retention of As in the treated soils shows that As(III) oxidation was accompanied by some natural sorption or sequestration of the As. This behavior is enhanced in the columns with non-sterile soil, suggesting a biological influence to As retention in the solid phase. Further research, such as with synchrotron based x-ray spectroscopy techniques, would help determine the speciation and stability of that sorbed As.

Batch experiments with sterile soil and a heterotrophic As-oxidizing bacteria isolate were performed for comparison with the soil columns (**Fig. 3.3**).

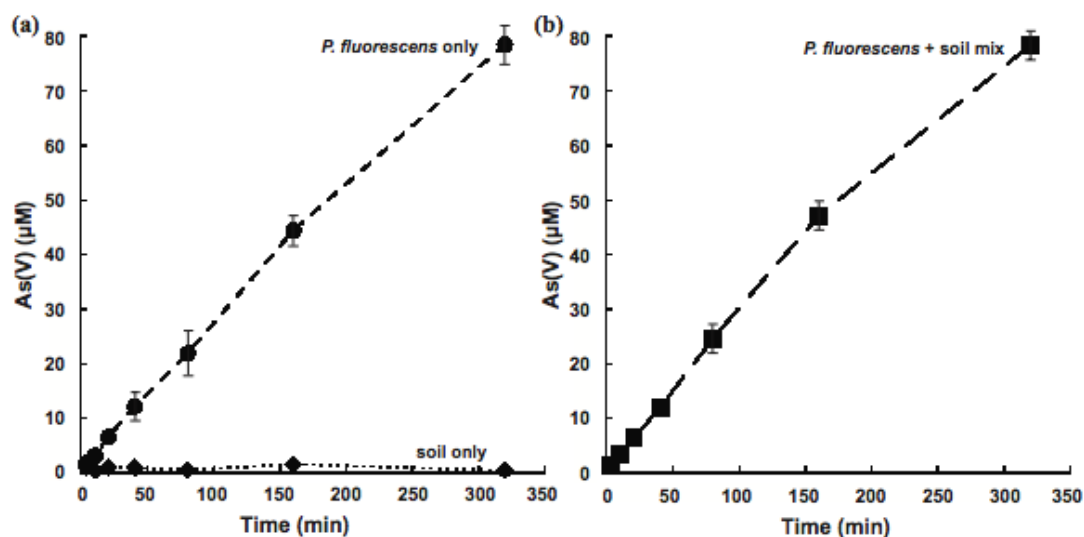


Figure 3.3 As(V) appearance in solution in batch experiments with γ -irradiated soil and *P. fluorescens* in separate batches (a) and mixed (b). The apparent rate of As(III) oxidation by *P. fluorescens* was not affected by the presence of sterile soil.

The model bacteria isolate, *Pseudomonas fluorescens*, oxidized 75 μM As(III) at a rate of $0.237 \mu\text{mol As h}^{-1} 10^{-9}$ cells in batch experiments without added soil. The rate of As(V) appearance in solution in batch experiments for *P. fluorescens* alone and *P. fluorescens* with 1 g L^{-1} sterile soil was the same. This result is in agreement with the column experiment results and it demonstrates the importance of biological As oxidation. Prior research on As(V) reduction and Cr(VI) reduction in column experiments has also shown that biological activity was essential in achieving appropriate redox conditions in the column (Macur et al., 2004; Bank et al., 2007).

3.5.3 Implications for coupled biotic-abiotic As oxidation in soils

Assuming that the only redox reaction occurring in the columns was As(III) oxidation and taking into account the flow rate and pore volume, non-sterile

columns were oxidizing As(III) at a rate of approximately $0.39 \mu\text{mol h}^{-1}$ by the end of the experiments. Based on oxidation rates obtained from batch experiments with no soil or mineral phase, 1.65×10^9 cells *P. fluorescens* per g soil would be required to support that rate in the columns. This cell concentration is higher than a reasonable soil bacteria population so it is possible that other types of As(III)-oxidizing bacteria contributed to As(III) oxidation in the columns or that As(V) reduction was occurring, which decreased the apparent rate of As(III) oxidation. In addition, the physiological and metabolic state of cells in the batch experiments, from which the oxidation rate was measured, presumably differs from the columns, which makes this calculation very rough evidence for the potential of biological As(III) oxidation. Nonetheless, the lack of As(III) oxidation in both types of sterile columns and the minimal effect of soil on the apparent rate of As(III) oxidation in batch experiments shows that the As(III) oxidation observed in column experiments could be explained by microbial activity.

Another exciting result from these experiments is that As oxidation can be induced in a soil with low As contamination. This may imply that soils with a low initial concentration of As can be made to tolerate and oxidize a large amount of As. Of course, the caveat to this result is that microbiological activity in the laboratory is not the same as in the field and the experimental method used here may have selected for organisms especially well-suited for the conditions (Madsen, 2008). A challenge of As redox and mobilization studies is making the experimental system more applicable to real-world conditions than simplistic experiments with bacteria isolates or pure minerals. By addressing a spatially heterogeneous system with a long time scale and complementary molecular methods, this study incorporates the complexity

of natural As cycling and shows the importance of biological activity in the coupled chemical and microbiological processes driving As(III) oxidation in the environment.

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3.7 References

- Bachate, S.P., Cavalca, L., Andreoni, V. 2009. Arsenic-resistant bacteria isolated from agricultural soils of Bangladesh and characterization of arsenate-reducing strains. *Journal of Applied Microbiology* 107: 145-156.
- Bank, T.L., Vishnivetskaya, T.A., Jardine, P.M., Ginder-Vogel, M.A., Fendorf, S., Baldwin, M.E. 2007. Elucidating biogeochemical reduction of chromate via carbon amendments and soil sterilization. *Geomicrobiology Journal* 24: 125-132.
- Borch, T.; Kretzschmar, R.; Kappler, A.; Van Cappellen, P.; Ginder-Vogel, M.; Voegelin, A.; Campbell, K. 2010. Biogeochemical redox processes and their impact on contaminant dynamics. *Environmental Science and Technology* 44: 15-23.
- Ellis, P.J., Conrads, T., Hille, R., Kuhn, P. 2001. Crystal structure of the 100 kDa arsenite oxidase from *Alcaligenes faecalis* in two crystal forms at 1.64 Å and 2.03 Å. *Structure* 9: 125-132.
- Gu, B., Wu, W., Ginder-Vogel, M.A., Yan, H., Fields, M.W., Zhou, J., Fendorf, S., Criddle, C.S., Jardine, P.M. 2005. Bioreduction of uranium in a contaminated soil column. *Environmental Science and Technology* 39: 4841-4847.

- Inskeep, W.P., McDermott, T., Fendorf, S. 2002. Arsenic(V)/(III) cycling in soils and natural waters: Chemical and microbiological processes. In *Environmental Chemistry of Arsenic*, Frankenberger, W.T., ed.
- Inskeep, W.P., Macur, R.E., Hamamura, N., Warelow, T.P., Ward, S.A., Santini, J.M. 2007. Detection, diversity and expression of aerobic bacterial arsenite oxidase genes. *Environmental Microbiology* 9: 934-943.
- Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.N., Lee, H., Trevors, J.T. 2004. Methods of studying soil microbial diversity. *Journal of Microbiological Methods* 58: 169-188.
- Lafferty, B.J., Ginder-Vogel, M., Sparks, D.L. 2010. Arsenite oxidation by a poorly crystalline manganese-oxide: 1. Stirred-flow experiments. *Environmental Science and Technology* 44: 8460-8466.
- Le., C.X. 2002. Arsenic speciation in the environment and humans. In *Environmental Chemistry of Arsenic*, Frankenberger, W.T., ed.
- Macur, R.E., Jackson, C.R., Botero, L.M., McDermott, T.R., Inskeep, W.P. 2004. Bacterial populations associated with the oxidation and reduction of arsenic in an unsaturated soil. *Environmental Science and Technology* 38: 104-111.
- Madsen, E.L. 2008. *Environmental Microbiology: From genomes to biogeochemistry*. Blackwell Publishing. Malden, MA. 479 p.
- McNamara, N.P., Black, H.I.J., Beresford, N.A., Parekh, N.R. 2003. Effects of acute gamma irradiation on chemical, physical, and biological properties of soils. *Applied Soil Ecology* 24: 117-132.
- Muller, D., Lievremont, D., Simeonova, D.D., Hubert, J-C., Lett., M-C. 2003. Arsenite oxidase aox genes from a metal-resistant β -proteobacterium. *Journal of Bacteriology* 185:135-141.
- Nordstrom, D.K. 2002. Worldwide occurrences of arsenic in ground water. *Science* 296: 2143-2144.
- Oremland, R.S., Stolz, J.F. 2005. Arsenic, microbes and contaminated aquifers. *Trends in Microbiology* 13: 45-49.
- Oscarson, D.W.; Huang, P.M.; Liaw, W.K.; Hammer, U.T. 1983. Kinetics of oxidation of arsenite by various manganese dioxides. *Soil Science Society of America Journal* 47, 644-648.

- Parikh, S.J.; Lafferty, B.J.; Meade, T.G.; Sparks, D.L. 2010. Evaluating environmental influences on As(III) oxidation kinetics by a poorly crystalline Mn-oxide. *Environmental Science and Technology* 44: 3772-3778.
- Polizzotto, M.L., Kocar, B.D., Benner, S.G., Sampson, M., Fendorf, S. 2008. Near-surface wetland sediments as a source of arsenic release to ground water in Asia. *Nature*. 454: 505-509.
- Quemeneur, M., Heinrich-Salmeron, A., Muller, D., Lievremont, D., Jauzerin, M., Bertin, P.N., Garrido, F., Joulain, C. 2008. Diversity surveys and evolutionary relationships of *aoxB* genes in aerobic arsenite-oxidizing bacteria. *Applied and Environmental Microbiology*. 74: 4567-4573.
- Scott, M.J.; Morgan, J.J. Reactions at oxide surfaces. 1995. 1. Oxidation of As(III) by synthetic birnessite. *Environmental Science and Technology* 29: 1898-1905.
- Stookey, L.L. 1970. Ferrozine—A new spectrophotometric reagent for iron. *Analytical Chemistry* 42: 779-781.
- Sun, W., Sierra-Alvarez, R., Milner, L., Oremland, R., Field, J.A. 2009. Arsenite and ferrous iron oxidation linked to chemolithotrophic denitrification for the immobilization of arsenic in anoxic environments. *Environmental Science and Technology* 43: 6585-6591.
- Tokunaga, T.K., Wan, J., Pena, J., Sutton, S.R., Newville, M. 2004. Hexavalent uranium diffusion into soils from concentrated acidic and alkaline solutions. *Environmental Science and Technology* 38: 3056-3062.
- Tokunaga, T.K., Wan, J., Hazen, T.C., Schwartz, E., Firestone, M.K., Sutton, S.R., Newville, M., Olson, K.R., Lanzirotti, A., Rao, W. 2003. Distribution of chromium contamination and microbial activity in soil aggregates. *Journal of Environmental Quality* 32: 541-549.
- Tufano, K.J., Fendorf, S. 2008. Confounding impacts of iron reduction on arsenic retention. *Environmental Science and Technology* 42: 4777-4783.
- Tufano, K.J., Reyes, C., Saltikov, C.W., Fendorf, S. 2008. Reductive processes controlling arsenic retention: revealing the relative importance of iron and arsenic reduction. *Environmental Science and Technology* 42: 8283-8289.
- Trevors, J.T. 1996. Sterilization and inhibition of microbial activity in soil. *Journal of Microbiological Methods* 26: 53-59.

US Environmental Protection Agency 1995. SW-846 EPA Method 3051, Microwave assisted acid digestion of sediments, sludges, soils and oils, *in*: Test Methods for Evaluating Solid Waste, 3rd Edition, 3rd Update, US Environmental Protection Agency, Washington DC.

Chapter 4

FUTURE RESEARCH

The major implications of this study are 1) the biological soil component is vital in achieving As(III) oxidation and 2) the rate of oxidation can be enhanced by mixing As(III)-oxidizing bacteria with poorly crystalline Mn oxide. Future research should confirm the kinetic findings with complementary techniques, further investigate the complex features of column studies, and characterize the stability and mobility of sorbed As. Specific tasks may include:

- Stirred-flow sorption and desorption experiments (with pH, competitive, and non-competitive ions) using the same model bacteria and Mn oxide mineral
- Synchrotron-based kinetic studies such as Q-XAFS (quick-scanning x-ray absorption fine structure) spectroscopy (as in Ginder-Vogel et al., 2009 and Landrot et al., 2010)
- Synchrotron-based analysis of As speciation retained in soil from column experiments and on the mineral phase from microbe-mineral batch experiments
- Column experiments with sterile soil inoculated with As(III)-oxidizing bacteria and soils of varying initial As contamination

These additional research thrusts would complement and advance the work presented here by further addressing the kinetics and mechanisms of As(III) oxidation in complex microbe-mineral systems. Ultimately this research may lead to better predictive modeling about As fate and transport and a kinetic model that can describe coupled biotic-abiotic redox reaction rates.

Appendix A

SUPPLEMENTARY DATA: ARSENITE OXIDATION KINETICS AT THE MICROBE-MANGANESE OXIDE INTERFACE

A.1 δ -MnO₂ Data

Preliminary experiments to determine the rate of As(III) oxidation by varying concentrations of δ -MnO₂ and sterile soil were carried out with 75 μ M As(III) and the batch method described above. The soil was the same soil as used in column experiments and it did not oxidize an appreciable amount of As(III).

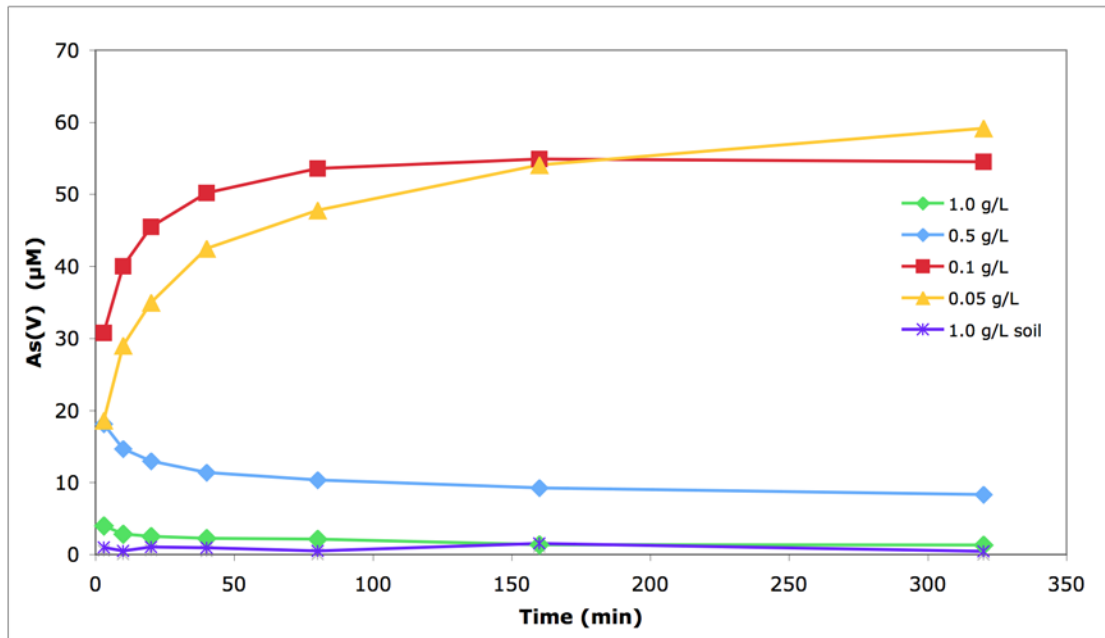


Figure A.1 As(V) appearance in solution for As(III) oxidation batch experiments with varying δ -MnO₂ concentrations (1.0, 0.5, 0.1, 0.05 g/L) and sterile soil (1.0 g/L). All experiments started with 75 μ M As(III).

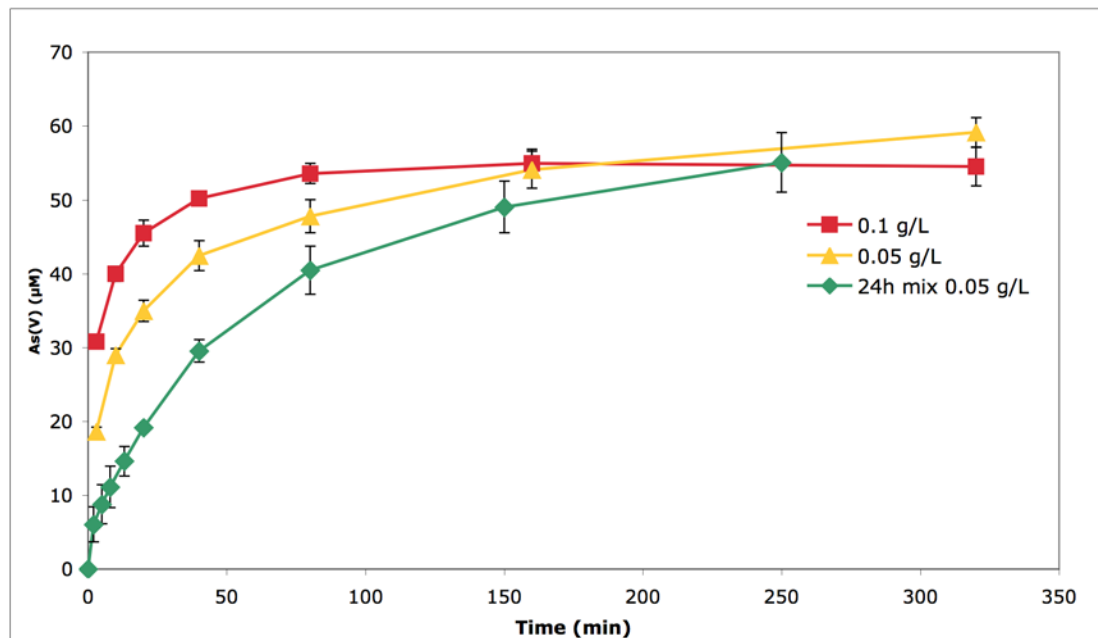


Figure A.2 As(V) appearance in solution for batch experiments with 0.1 g/L δ -MnO₂, 0.05 g/L δ -MnO₂, and 0.5 g/L δ -MnO₂ mixed for 24h before adding As(III). All experiments started with 75 μ M As(III).

A.2 Microbe Data

A.2.1 Growth Curves

The growth protocol for the As(III)-oxidizing bacteria was described above. Two liquid cultures were made, the first with inoculate from plates (“starter cultures”) and the second with inoculate from the first liquid culture (“working cultures”). The growth rate parameters for the four strains of model bacteria were described in **Table A.1**. The growth rate constants, k , were calculated as $\ln 2/DT$.

Growth curves for the second “working” culture, from which cells were harvested at late logarithmic growth phase, are presented in **Figs. A.3-A.6**.

Table A.1 Growth data for starter cultures. Cultures were harvested when OD reached “End OD” and used to inoculate working cultures at “Start OD” concentrations. All cultures were grown in sterile aerobic conditions, shaking at 100 rpm, and at ambient room temperature.

| Strain | Doubling Time (h) | k | Start OD (650 nm) | Growth Time (h) | End OD (650 nm) |
|-----------------------|--------------------------|----------|--------------------------|------------------------|------------------------|
| <i>A. tumefaciens</i> | 5.4 | 0.13 | 0.032 | 12 | 0.15 |
| <i>A. faecalis</i> | 4.9 | 0.14 | 0.018 | 12 | 0.10 |
| <i>P. fluorescens</i> | 5.9 | 0.12 | 0.037 | 12 | 0.15 |
| <i>V. paradoxus</i> | 4.9 | 0.14 | 0.029 | 12 | 0.16 |

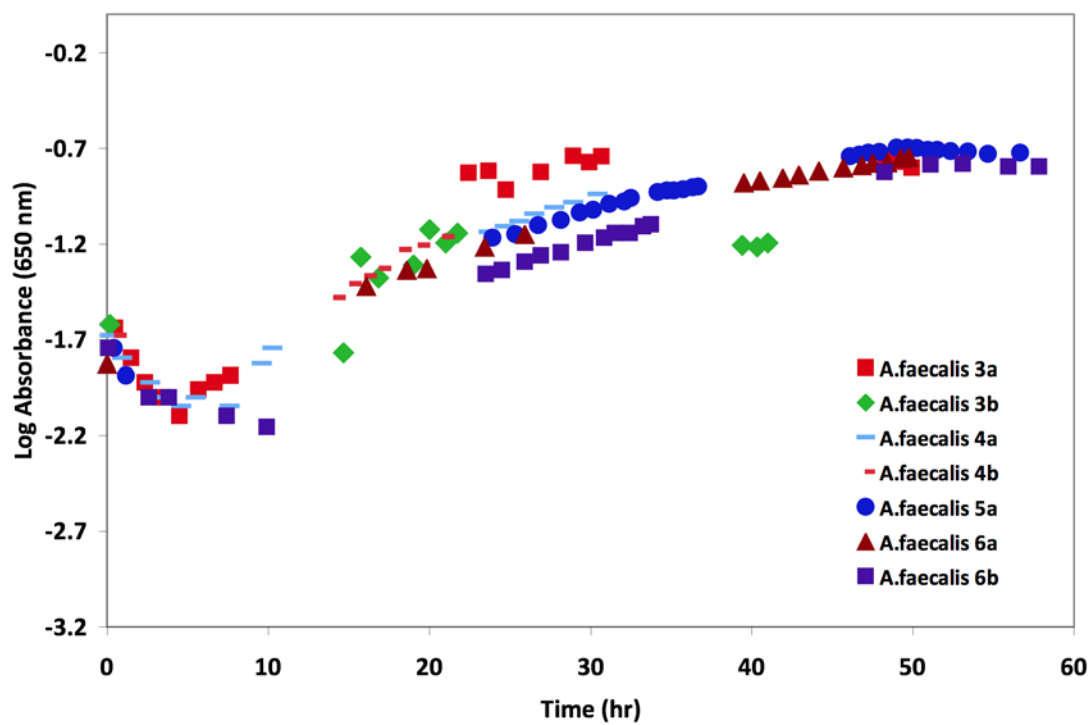


Figure A.3 *Alcaligenes faecalis* working culture growth curve with glutamic acid (5mM) carbon source.

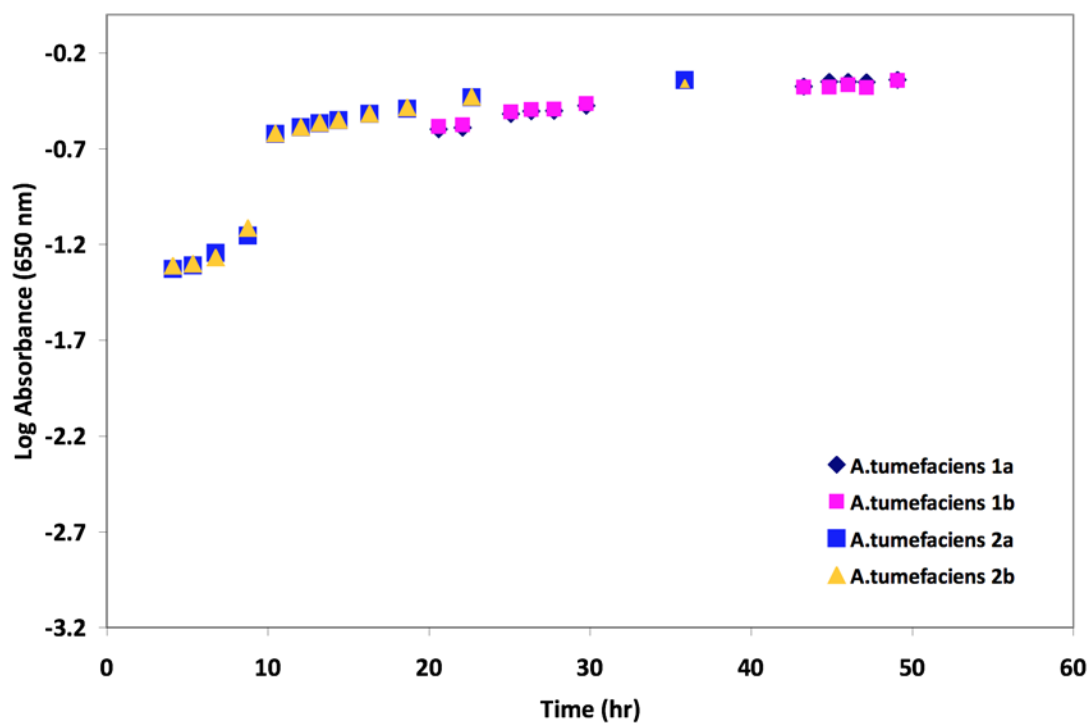


Figure A.4 *Agrobacterium tumefaciens* working culture growth curve with glucose (20 mM) carbon source.

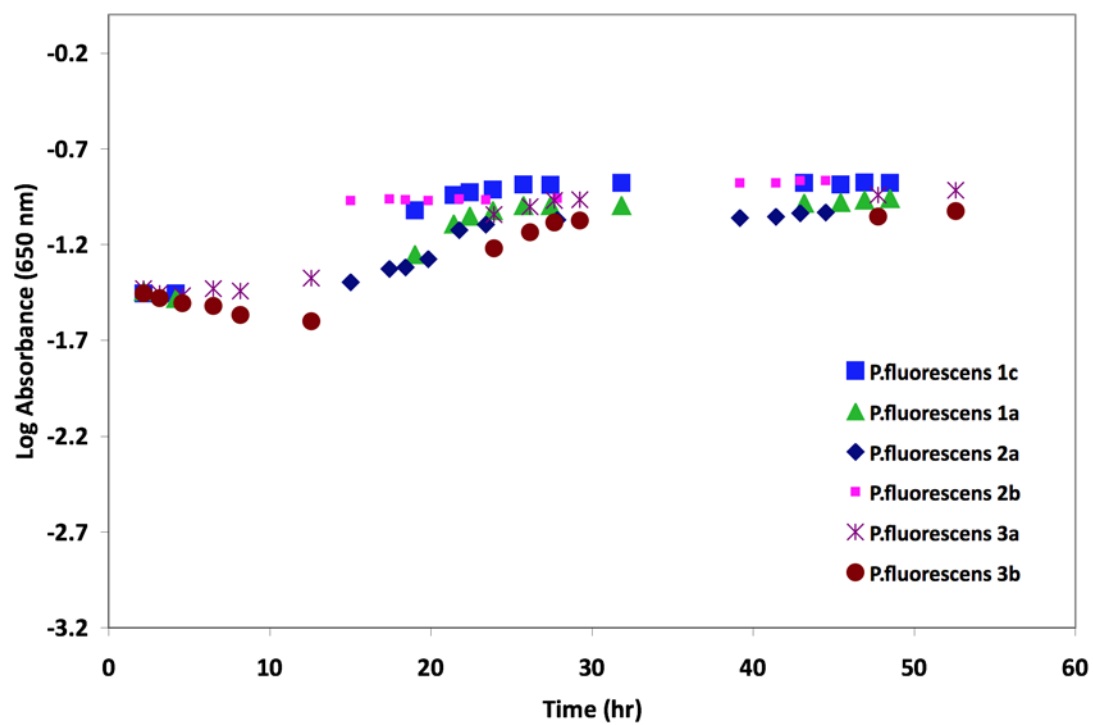


Figure A.5 *Pseudomonas fluorescens* working culture growth curve with glucose (20 mM) carbon source.

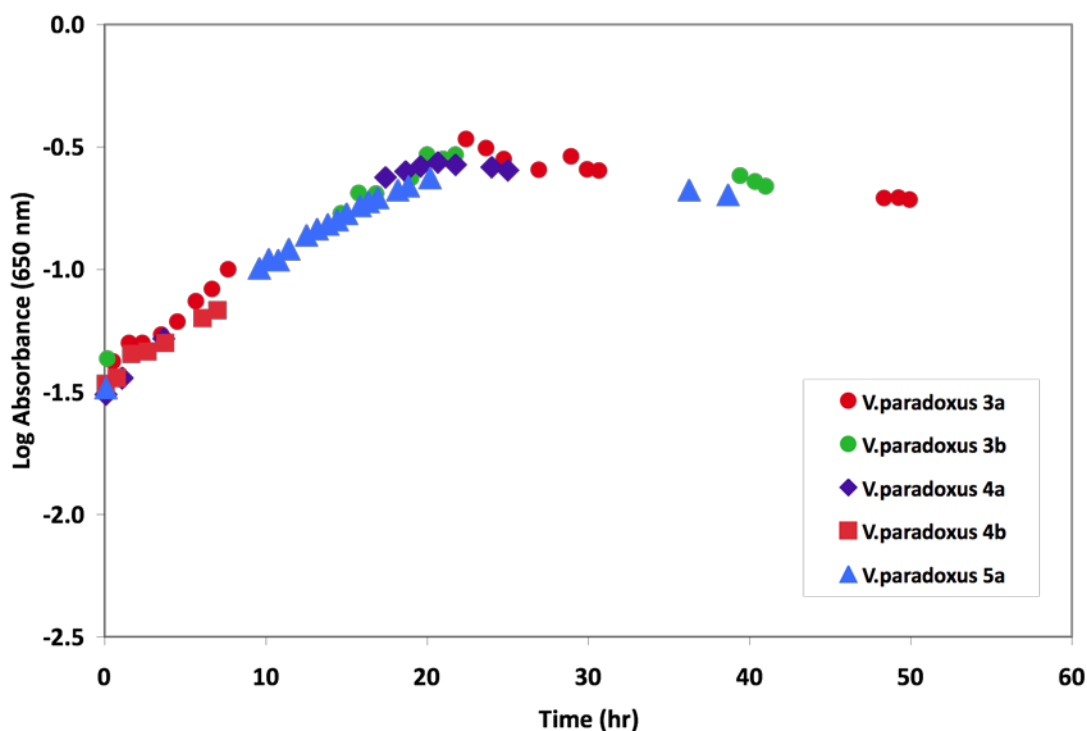


Figure A.6 *Variovorax paradoxus* working culture growth curve with glutamic acid (5 mM) carbon source.

A.2.2 *Pseudomonas fluorescens* enzyme kinetics

The initial rate of As(III) oxidation by *P. fluorescens* resting cell cultures at OD=0.036 (650 nm) in batch experiments was evaluated at various starting As(III) concentrations ranging from 0.3 μM to 140 μM . The maximum rate of As(III) oxidation occurred at 7.1 μM As(III). The rate of oxidation was lower at concentrations above and below 7.1 μM As(III), including the concentration for mixed microbe-mineral batch experiments 75 μM As(III). Initial oxidation rates and the associated error are reported in **Table A.2**. **Figure A.7** shows initial rate versus

substrate concentration with error reported as the R^2 values from linear regressions for initial oxidation rates determined in triplicate batch experiments.

Table A.2 Initial As(III) oxidation rate (appearance of As(V) in solution) for *P. fluorescens* (fixed OD) at a range of initial As(III) concentrations.

| Initial As(III) (μM) | Initial Oxidation Rate ($\mu\text{M As(III) min}^{-1}$) | R^2 |
|--------------------------------------|--|-------|
| 140 | 0.127 | 0.997 |
| 67 | 0.165 | 0.999 |
| 37 | 0.210 | 0.980 |
| 16 | 0.303 | 0.988 |
| 7.1 | 0.427 | 0.992 |
| 1.4 | 0.156 | 0.934 |
| 0.30 | 0.040 | 0.912 |

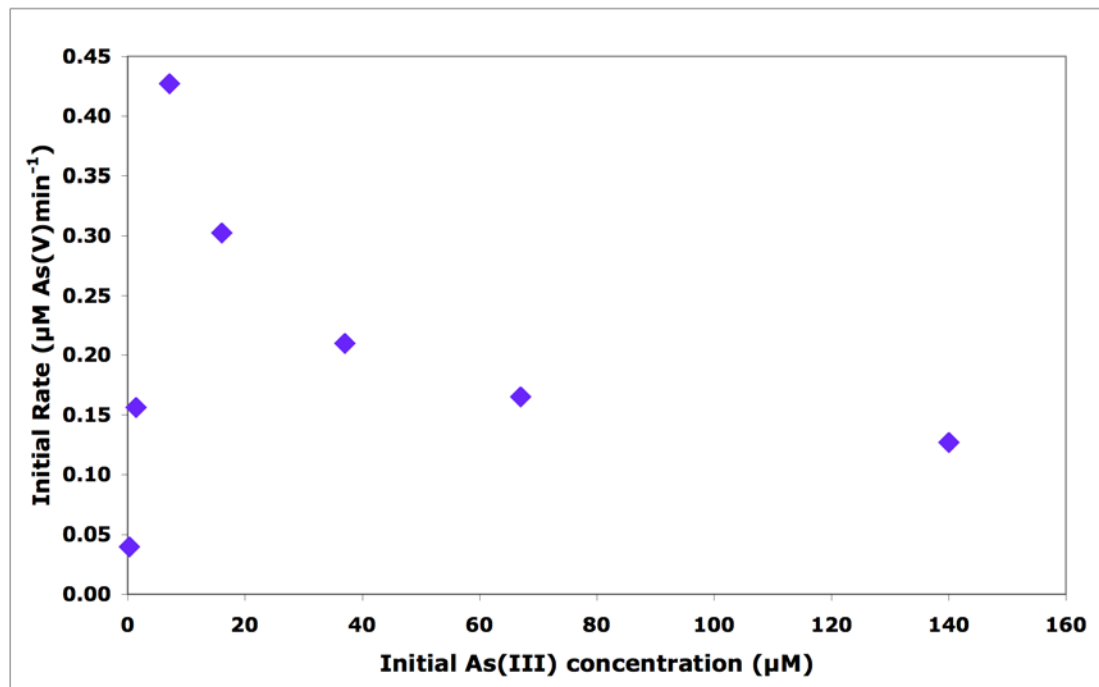


Figure A.7 *P. fluorescens* initial rate of As(III) oxidation for a fixed OD and varying initial As(III) concentrations.

A.2.3 16S rRNA Data

The identities of *A. tumefaciens* and *P. fluorescens* were confirmed using 16S rRNA sequencing analysis.

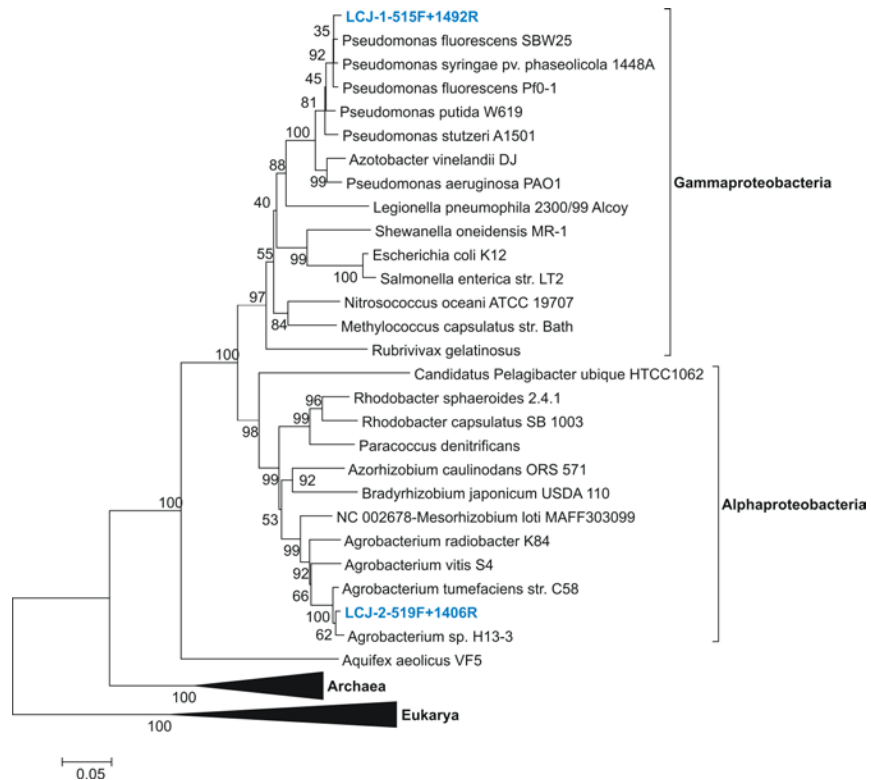


Figure A.8 A Neighbor-Joining phylogram derived from aligned 16/18S rRNA gene sequences. Strains from this study are noted in blue, bolded text. Numbers at the nodes represent the % bootstrap support observed over 500 iterations and the scale bar represents 5% sequence divergence.

Appendix B

SUPPLEMENTARY DATA: COLUMN STUDY

B.1 Preliminary Column Study

B.1.1 Materials and Methods

Preliminary column experiments were conducted with autoclaving as the sterilization method, no replicates, and a faster flow rate (10 mL h^{-1}). The four treatments were: non-sterile soil with OM media and $200 \pm 20 \mu\text{M As(III)}$; non-sterile soil with $200 \pm 20 \mu\text{M As(III)}$; autoclaved soil with OM media and $200 \pm 20 \mu\text{M As(III)}$; non-sterile soil with OM media [no As(III)].

B.1.2 Arsenic Data

Table B.1 Soil testing results from initial and final soils following the protocol described reported in Chapter 3. Total elemental analysis was by ICP mass spectrometry (ND = not detected).

| | Initial Non-Sterile Soil | Initial γ -irradiated Soil | Final Non-Sterile Soil + C + As(III) | Final Non-Sterile Soil + NaCl + As(III) | Final γ -irradiated Soil + As | Final Non-Sterile As-free control |
|----------|--------------------------|-----------------------------------|--------------------------------------|---|--------------------------------------|-----------------------------------|
| Al (ppm) | 14855.37 | 17971.51 | 4159.82 | 5258.24 | 4828.33 | 3434.27 |
| As (ppm) | 9.92 | 17.47 | 134.46 | 153.63 | 73.80 | ND |
| Ca (ppm) | 1261.99 | 1261.35 | 168.75 | 157.95 | 152.78 | 146.07 |
| Cu (ppm) | 35.15 | 40.20 | 17.60 | 17.53 | 12.56 | 14.01 |
| Fe (ppm) | 15270.62 | 16247.85 | 6280.85 | 6460.12 | 5491.59 | 5084.72 |
| Mg (ppm) | 4106.16 | 2177.75 | 690.99 | 739.98 | 636.67 | 539.37 |
| Mn (ppm) | 284.58 | 449.21 | 89.23 | 97.06 | 101.74 | 89.41 |
| Zn (ppm) | 51.58 | 53.29 | 22.75 | 227.06 | 18.06 | 19.95 |

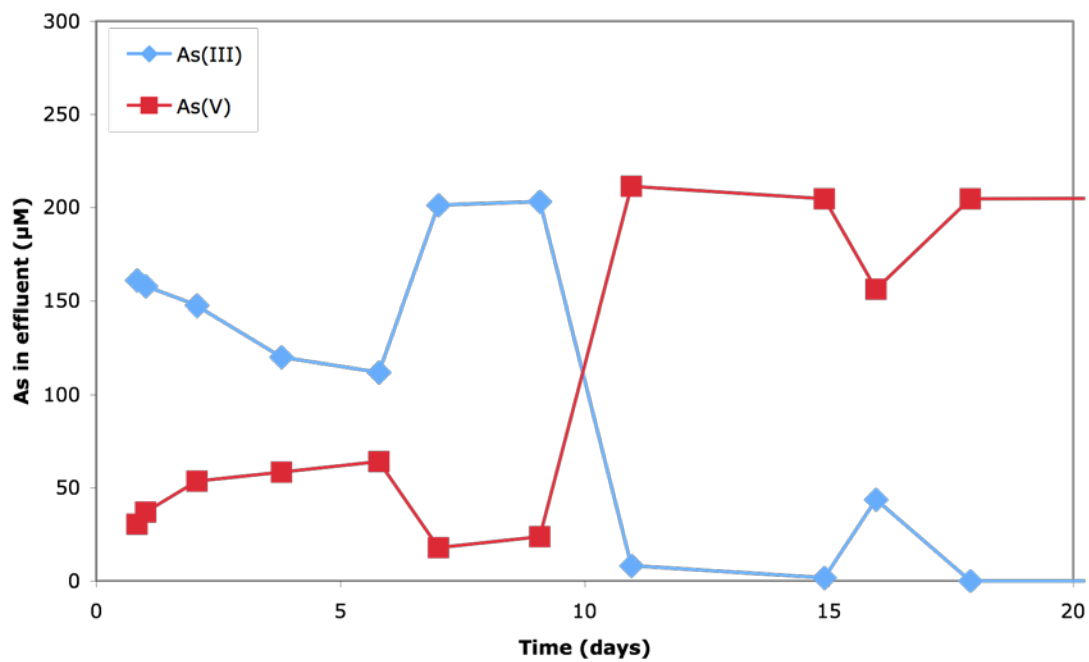


Figure B.1 As(III) and As(V) in column experiment effluent for non-sterile soil treated with OM and 200 μM As(III).

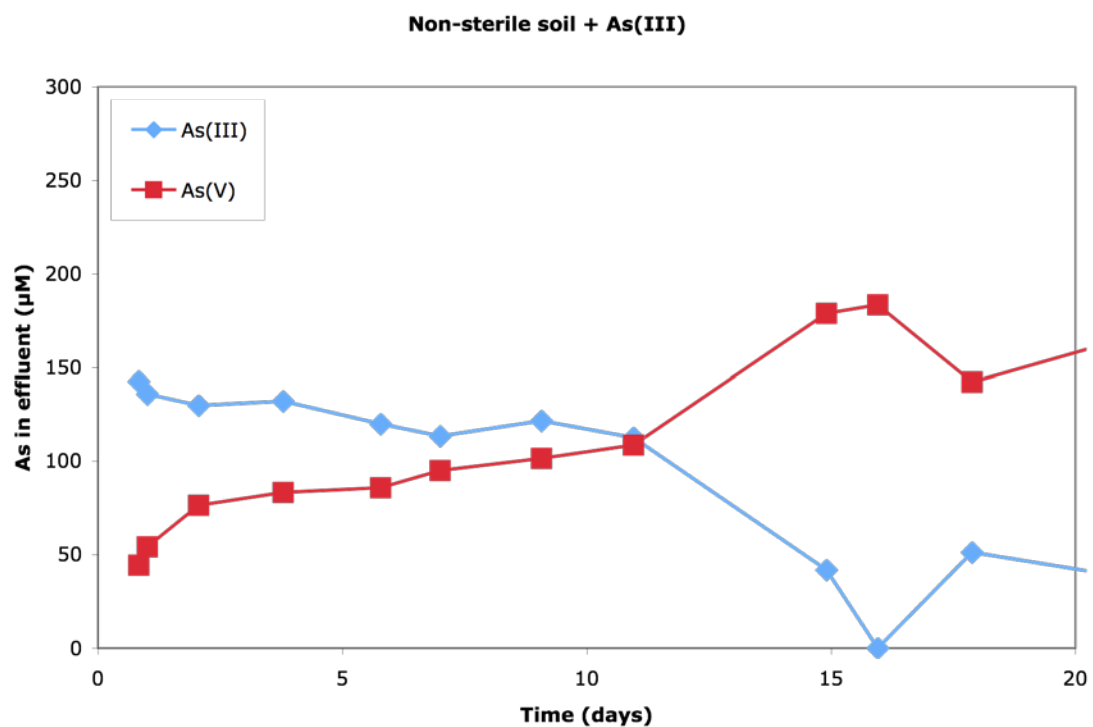


Figure B.2 As(III) and As(V) in column experiment effluent for non-sterile soil treated with no additional carbon (NaCl solution) and 200 μM As(III).

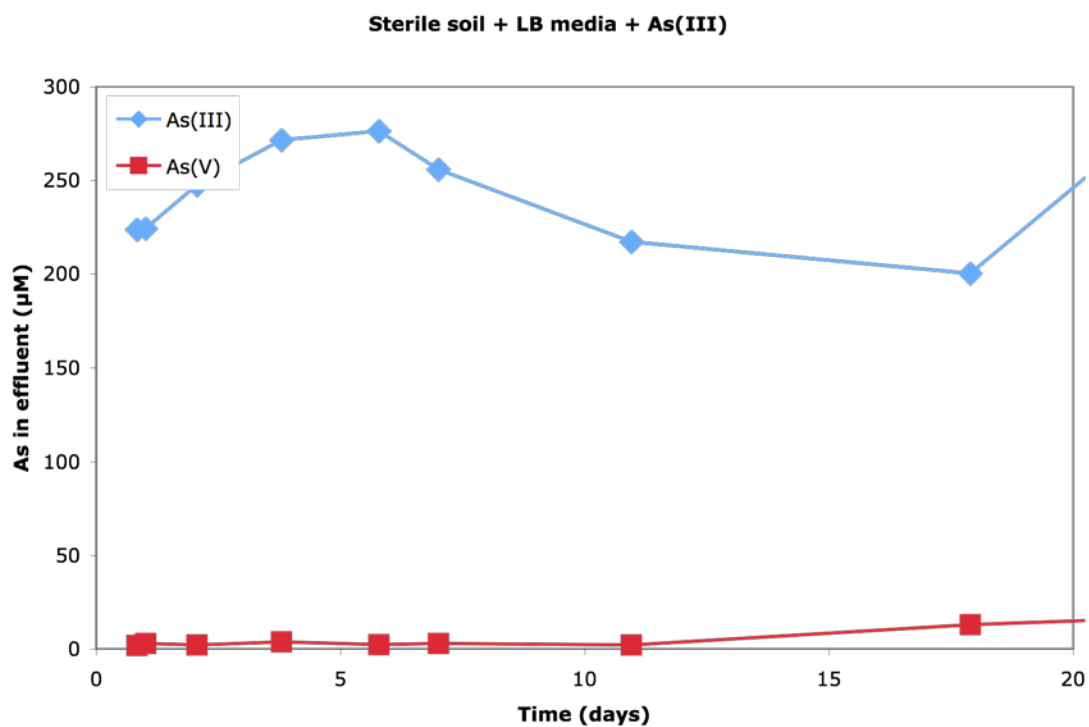


Figure B.3 As(III) and As(V) in column effluent for sterile soil treated with OM (“LB media”) and 200 μM As(III). This column did not oxidize an appreciable amount of As(III).

As-free control experiment

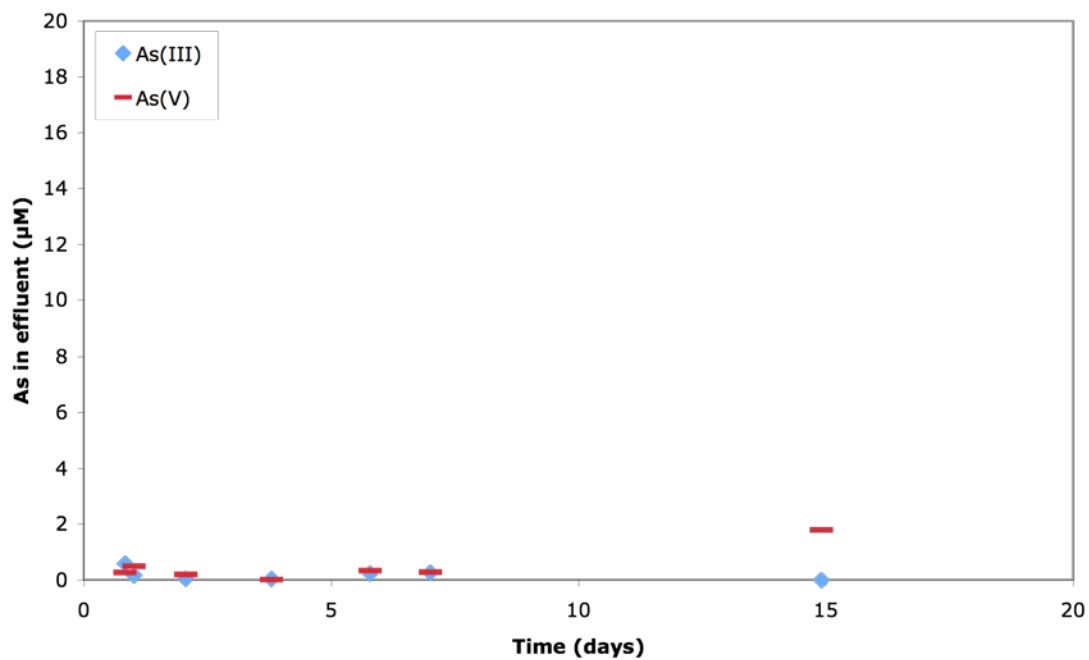


Figure B.4 As(III) and As(V) in column effluent for the As-free control experiment with non-sterile soil treated with carbon (no As added). Note the smaller y-axis scale compared with previous figures.