Biogeochemical evaluation of disposal options for arsenic-bearing wastes generated during drinking water treatment

by

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“I have spent many days stringing and unstringing my instrument,
while the song I came to sing remains unsung.”
- Rabindranath Tagore

Arsenic and iron removal plant in Jessore, Bangladesh

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Abstract

Arsenic contamination of drinking water sources threatens the health of over 50 million people around the world. Sustainable implementation of arsenic removal technologies requires disposal options for produced wastes that limit the release of arsenic. Arsenic contamination is particularly severe in Bangladesh and India, where access to landfills is limited and most wastes are disposed on the ground nearby arsenic removal units. To address concerns with arsenic release under these disposal conditions, this research characterized the stability of arsenic wastes during two alternative disposal strategies. Stabilization of wastes in concrete and mixing wastes with cow dung were tested using arsenic-bearing ferric iron wastes generated during the removal of arsenic from groundwater in West Bengal, India. Under a range of leaching tests, <1% of the total arsenic was leached from concrete stabilized waste (pH >4.2). Through the characterization of arsenic within the cement matrix by μXRF, arsenic was found to remain primarily associated with iron, consistent with the initial state of the waste. These results show that concrete stabilization can be a viable disposal option to limit arsenic release when low pH can be avoided. Cow dung mixing has been recommended based on the ability of microorganisms to create volatile arsine gases that can be subsequently diluted to safe levels in the atmosphere. However, little is known about the extent of volatilization or the microbial communities responsible for this transformation. Cow dung mesocosms with varying levels of methanogenic inhibition were used to study the impact of methanogenesis on arsenic volatilization under anaerobic conditions. Arsenic

volatilization was primarily due to the activity of methanogens, but was not a significant transformation pathway. Less than 0.02% of the total arsenic was released to the gaseous phase. Instead, more arsenic (<4%) was leached into the aqueous phase. These results highlight that cow dung disposal may increase the potential for arsenic release into the aqueous phase and is ineffective in producing volatile arsenic gases. Taken together, these studies characterized the important microbial and geochemical determinants of arsenic fate in non-landfill disposal conditions and provide additional insight on arsenic transformations over a broader range of environmental conditions.

Chapter 1
Introduction

1.1 Background

The presence of arsenic in drinking water sources is a global problem causing increased risk for cancer, cardiovascular disease, and other health problems related to development, the respiratory system, and immune response (Dhar *et al.* 1997, Das *et al.* 2009, Ravenscroft *et al.* 2009, Naujokas *et al.* 2013). Over 30 million people in South Asia are exposed to levels of arsenic above the World Health Organization's limit of 10 µg/L (Ravenscroft *et al.* 2004, Chakraborti *et al.* 2010). In some areas of Bangladesh, one in five deaths can be attributed to arsenic contamination of drinking water (Argos *et al.* 2010). The high prevalence of arsenic contaminated groundwater in Bangladesh is due in part to a massive well drilling campaign during the 1970s that switched the drinking water source from microbially contaminated surface water to shallow-tube (<200 m in depth) well water (Smith *et al.* 2000). These shallow tube wells were later discovered to be naturally contaminated with arsenic (Smith *et al.* 2000, Chakraborti *et al.* 2010). In response to this public health emergency, the scientific community has given much attention to all aspects of arsenic contamination including the causes (Kocar *et al.* 2006, Radloff *et al.* 2007, Sutton *et al.* 2009, Mladenov *et al.* 2010, Burton *et al.* 2011), arsenic field testing (Pande *et al.* 2001, Meer *et al.* 2012), and development of water treatment technologies (Ahmed 2001, Mishra and Farrell 2005, Mohan and Pittman 2007, Sarkar *et al.* 2010, Upadhyaya *et al.* 2010, Amrose *et al.* 2014).

Arsenic removal technologies typically rely on arsenic sorption to a solid phase and have been implemented at a range of scales. Removal of arsenic can be accomplished under both oxidizing (Fuller *et al.* 1993, Bissen and Frimmel 2003) and reducing conditions (Jong and Parry 2003, Upadhyaya *et al.* 2010). Many different sorbent materials have been tested under oxidizing conditions, including iron (Raven *et al.* 1998, Dixit and Hering 2003, Bang *et al.* 2005), titanium dioxide (Ferguson *et al.* 2005, Miller and Zimmerman 2010, Guan *et al.* 2012), and aluminum oxides (Lakshmanan *et al.* 2008, Sarkar *et al.* 2008). The most commonly implemented arsenic removal technologies rely on the sorption of arsenic to iron under oxidizing conditions (Mohan and Pittman 2007). Technologies have been developed for multiple scales and implementation in both developed and developing countries. A common alternative to arsenic removal around the world is switching to uncontaminated water sources, a strategy that may be the simplest and most cost effective method to provide arsenic safe drinking water (Ahmed *et al.* 2006). However, in areas without access to alternative water sources, treatment methods must be implemented to remove arsenic. Full-scale systems applied in developed countries include adsorbent media filtration, reverse osmosis, and enhanced coagulation (US EPA 2003). In developing countries, numerous point-of-use devises have been developed for water treatment at the house-hold level (Munir *et al.* 2001, Hussam and Munir 2007, Neumann *et al.* 2013), though recently more attention has been given to the development of community scale systems serving 100-500 families (Ahmad *et al.* 2003, Sarkar *et al.* 2010, Amrose *et al.* 2014). Technologies for application in developing countries include iron based sorbent media (Hussam *et al.* 2008), passive-

oxidation sand filters (Hussainuzzaman and Yokota 2006), and electro-coagulation (Amrose *et al.* 2014).

While much of the academic research on arsenic removal has focused on the scientific and engineering design of treatment technologies, the implementation of these systems has proven to present one of the largest barriers to the provision of arsenic-free drinking water. For developed and developing countries, cost is a common barrier. For example, in the US, opposition to the lowering of the maximum contaminant level for arsenic from 50 to 10 μg/L resulted from concerns over the cost of implementing arsenic removal technologies (Burnett and Hahn 2001). In developing countries, water provision in most rural areas does not take place through centralized water treatment and distribution systems and therefore solutions have been tried at a smaller scale. Barriers to the implementation of technologies at the community level include trust, convenience, operation, maintenance, and long-term performance of arsenic removal technologies (Ahmad *et al.* 2003, Hossain *et al.* 2005, Etmannski and Darton 2014). Another barrier common in developed and developing countries is the need for treatment of arsenic-bearing wastes produced during any kind of arsenic removal. In developed countries, wastes are typically treated by disposal in either municipal or hazardous waste landfills (Cornwall 2003). Even though landfills are highly engineered environments designed to keep leachate and wastes from re-entry into the environment, concerns remain about this practice of arsenic waste disposal. For example, studies have shown that arsenic-bearing wastes generated under oxidizing conditions are susceptible to reductive dissolution and release of arsenic under landfill disposal conditions (Ghosh *et al.* 2006, Cortinas *et al.* 2008, Stuckman *et al.* 2011). For developing countries, waste disposal is less tightly

controlled and access to landfills is typically limited. In many areas, discarding wastes directly on soils nearby treatment units is the most common disposal strategy (Grimshaw and Beaumont 2004, Sullivan *et al.* 2010, Etmannski and Darton 2014). Results from field sampling in the vicinity of community-scale arsenic removal plants in Bangladesh indicate that soil arsenic concentrations at and surrounding the point of waste disposal can be elevated compared to background soils (Appendix A). The potential release of arsenic from these wastes has created concern and led to several recommendations for alternative waste disposal options, including mixing wastes with cow dung, stabilization in concrete, storage in dug or concrete lined pits, or storage in an aerated coarse sand filter. The focus of this dissertation is to characterize the stability of arsenic wastes during two of these commonly recommended alternative disposal strategies, stabilizing arsenic wastes in concrete and mixing wastes with cow dung.

Concrete stabilization is a strategy used for many types of waste disposal and has been recommended for arsenic wastes (Dutre and Vandecasteele 1995, Leist *et al.* 2000, Paria and Yuet 2006). This recommendation is based on results from the commonly used Toxicity Characteristic Leaching Procedure (TCLP) (U.S. EPA 1992), the regulatory test used by the US EPA to determine if wastes are suitable for disposal in either hazardous or municipal landfills. The TCLP is a batch test in which wastes are exposed to 0.1 M acetic acid at pH values between 3 and 5 for 18 hours and the concentrations of contaminants in the aqueous phase is measured and compared against allowable standards. Several studies have used the TCLP to compare untreated arsenic-bearing wastes with cement or lime stabilized wastes and have found that cement and lime addition results in lower arsenic leachate concentrations (Akhter *et al.* 1997, Jing *et al.*

2003, Moon *et al.* 2004, Jia and Demopoulos 2008, Camacho *et al.* 2009). However, research has found the TCLP is not necessarily an accurate predictor of arsenic leaching from arsenic solid wastes in landfill environments (Ghosh *et al.* 2004). Studies have not evaluated whether the TCLP leaching test is a good predictor of the potential for arsenic to be released from cement stabilized wastes under relevant environmental disposal conditions. In developed countries, the disposal of cement stabilized arsenic-bearing wastes is sometimes practiced so that wastes can be disposed in municipal waste landfills that are less costly than hazardous waste landfills (Leist *et al.* 2000, Singh and Pant 2006). In developing countries, it would be more likely that cement stabilized wastes were stored on the ground nearby arsenic removal units. Other potential uses for such stabilized wastes could include the application of crushed concretes in the sub-base of roads or incorporation into non-load bearing concrete features, such as foot paths.

There are several reasons, which might limit the ability of the TCLP to predict the long-term stability of arsenic waste incorporated in concrete under actual disposal conditions. Firstly, the TLCP is performed by crushing the solid waste and subjecting it to the standardized leaching solution (0.1 M acetic acid). Since cementitious materials are basic and have a high capacity to increase the pH of the leaching solution, especially when crushed, they can change the pH from the targeted slightly acidic condition. Secondly, secondary solids, such as calcite, may precipitate and incorporate arsenic if the solution becomes too basic (Yokoyama *et al.* 2012) ultimately limiting the release of arsenic during the test. Previous studies have also typically used arsenic containing cements without the addition of iron (Akhter *et al.* 1997, Moon *et al.* 2004), creating conditions different from those expected when arsenic-bearing iron wastes are used.

Given these limitations, more research is needed to characterize and evaluate the effectiveness of stabilization in concrete using actual wastes relevant for drinking water treatment systems.

Mixing arsenic-bearing wastes with cow dung has been suggested with the goal of using microbial arsenic methylation and volatilization to release arsenic into the atmosphere where it can be diluted (Das 1995, Ali *et al.* 2003, Visoottiviseth and Ahmed 2008, Sullivan *et al.* 2010). This disposal strategy is intended for rural areas, where cow dung can be mixed with arsenic-bearing wastes before the mixture is dumped into a dug pit or on top of soil nearby an arsenic treatment plant. Some studies of cow dung disposal have previously been performed, but many lack a mass balance of arsenic and only a few have measured actual release of arsenic in the gaseous phase (Mohapatra *et al.* 2008, Mestrot *et al.* 2013), though wide variations exist in reported volatilization ranging from 0.3 to 32% of the total arsenic present. Studies that indirectly predict arsenic release in the gaseous phase report between 25 to 99 % of the arsenic initially present was volatilized (Ali *et al.* 2003, Banerjee 2010). In addition, the potential for oxidized arsenic solid wastes to be mobilized into the aqueous phase by reductive dissolution has not been considered (Tufano and Fendorf 2008). Further study is needed to quantify the extent to which cow dung may be an effective disposal strategy and how arsenic mobilization in the aqueous phase may be impacted.

The transformations that occur during arsenic volatilization and reductive dissolution rely on the activity of different populations of microorganisms. Many studies have used pure cultures and genetically modified bacteria to determine the genes and metabolisms that are most relevant for these transformations (Tufano *et al.* 2008, Chen *et al.* 2014),

but few have characterized the activity from complex communities likely to be present in disposal environments. Understanding the role of specific populations within complex communities is important to understand the environmental conditions that promote or inhibit volatilization and mobilization. To date, the main focus of research on microbial arsenic methylation has been on the transformation by the protein arsenite *S*-adenosylmethionine methyltransferase (ArsM) (Qin *et al.* 2006, Jia *et al.* 2013, Zhao *et al.* 2013), yet a second pathway for arsenic methylation is known and occurs during methanogenesis (Thomas *et al.* 2011). As methanogenic archaea are expected to be active in cow dung environments, arsenic methylation through methanogenesis represents an important pathway to consider. Determining the relative contribution of these two pathways as a function of environmental conditions could point to waste disposal conditions where volatilization could be maximized.

To ensure that drinking water treatment systems for the removal of arsenic are designed and implemented in ways that protect against the creation of new sources of arsenic contamination, proper disposal of arsenic wastes resulting from arsenic water treatment systems must be considered. To support this, evaluations are needed that consider the stability of arsenic waste under disposal practices and conditions relevant for countries in South Asia, where arsenic water contamination is prevalent and removal technologies are in greatest demand. This dissertation characterizes arsenic stability in non-landfill disposal scenarios, conditions of potential relevance for developing countries in South Asia, by combining techniques from microbial ecology and geochemistry. The two options considered here are concrete stabilization and cow dung mixing. These options are frequently discussed and recommended, yet few studies have characterized

the fate of arsenic during these disposal options. The data presented in this dissertation quantify the boundaries of arsenic waste stability over a range of environmental conditions, and shed light on variables that limit arsenic release under these two disposal options. By using actual arsenic-bearing waste products from an iron electrocoagulation system (Amrose *et al.* 2014), the results can serve as a guide for predicting the stability of arsenic-bearing wastes from iron media filtration or passive oxidation filters, which also rely on the sorption of arsenic to iron solids for water treatment. In addition, the results expand the fundamental knowledge of microbial and geochemical arsenic transformations relevant for arsenic mobilization and fate in contaminated environments.

1.2 Dissertation Overview

This dissertation focuses on the disposal of arsenic-bearing wastes via concrete stabilization and cow dung mixing, based on a critical review of the current state of testing and disposal practices for waste products generated during arsenic removal from drinking water (Chapter 2, Clancy *et al.* 2013). This survey of previous experimental results highlights that most research on arsenic-bearing wastes was conducted for landfill disposal and did not support decisions in areas where access to landfills is limited (Chapter 2, Clancy *et al.* 2013). To address this gap, concrete stabilization of arsenic-bearing wastes from drinking water treatment systems was evaluated through a combination of long-term leaching tests in rainwater and short-term leaching tests including the TCLP (Chapter 3, Clancy *et al.* 2015c). To explore the role of methanogenic archaea in the volatilization of arsenic in cow dung disposal, a preliminary investigation of the impact of methanogenic inhibitors on the activity of relevant microbial communities was conducted (Chapter 4, Clancy *et al.* 2015b). Building on

these results, mesocosms with arsenic wastes were created to simulate a cow dung disposal condition. Arsenic in the liquid, solid, and gaseous phases was monitored under conditions with and without methanogenic activity to elucidate the importance of methanogenic activity in arsenic transformations and volatilization and quantify the effectiveness of this strategy (Chapter 5, Clancy et al. 2015a). Drawing on these findings, recommendations for arsenic-bearing waste disposal in developing countries and areas for future research are presented (Chapter 6).

1.3 Literature Cited

Ahmad, J., B. N. Goldar, S. Misra and M. Jakariya (2003). Willingness to Pay for Arsenic-Free , Safe Drinking Water in Bangladesh.

Ahmed, M. F. (2001). An Overview of Arsenic Removal Technologies in Bangladesh and India**:** 251-269.

Ahmed, M. F., S. Ahuja, M. Alauddin, S. J. Hug, J. R. Lloyd, A. Pfaff, T. Pichler, C. Saltikov, M. Stute and A. van Geen (2006). Ensuring Safe Drinking Water in Bangladesh. *Science* 314(December): 1687-1688.

Akhter, H., F. K. Cartledge, A. Roy and M. E. Tittlebaum (1997). Solidification/stabilization of arsenic salts: Effects of long cure times. *J. Hazard. Mater.* 52(2–3): 247-264.

Ali, M. A., A. B. M. Badruzzaman, M. A. Jalil, M. F. Ahmed, M. Kamruzzaman, M. A. Rahman and A. Al Masud (2003). Fate of Arsenic in Wastes Generated From Arsenic Removal Units. Proceedings of BUET-UNU Symposium on Fate of Arsenic in the Environment, Dhaka.

Amrose, S. E., S. R. S. Bandaru, C. Delaire, C. M. van Genuchten, A. Dutta, A. DebSarkar, C. Orr, J. Roy, A. Das and A. J. Gadgil (2014). Electro-chemical arsenic remediation: Field trials in West Bengal. *Sci. Total Environ.* 488–489(0): 539-546.

Argos, M., T. Kalra, P. J. Rathouz, Y. Chen, B. Pierce, F. Parvez, T. Islam, A. Ahmed, M. Rakibuz-Zaman, R. Hasan, G. Sarwar, V. Slavkovich, A. van Geen, J. Graziano and H. Ahsan (2010). Arsenic exposure from drinking water, and all-cause and chronic-disease mortalities in Bangladesh (HEALS): a prospective cohort study. *Lancet* 376(9737): 252-258.

Banerjee, G. (2010). Treatment of Arsenic-Laden Water Plant Sludge by Anaerobic Digestion. *Pract. Period. Hazard., Toxic, Radioact. Waste Manage.* 14: 124-131.

Bang, S., G. P. Korfiatis and X. Meng (2005). Removal of arsenic from water by zero-valent iron. *J. Hazard. Mater.* 121: 61-67.

Bissen, M. and F. H. Frimmel (2003). Arsenic – a Review. Part II : Oxidation of Arsenic and its Removal in Water Treatment. *Acta Hydrochim. Hydrobiol.* 31(2): 97-107.

Burnett, J. K. and R. W. Hahn (2001). Costly Benefit: Economic Analysis Does Not Support EPA's New Arsenic Rule, A. *Regulation* 24: 44.

Burton, E. D., S. G. Johnston and R. T. Bush (2011). Microbial sulfidogenesis in ferrihydrite-rich environments: Effects on iron mineralogy and arsenic mobility. *Geochim. Cosmochim. Acta* 75(11): 3072-3087.

Camacho, J., H.-Y. Wee, T. A. Kramer and R. Autenrieth (2009). Arsenic stabilization on water treatment residuals by calcium addition. *J. Hazard. Mater.* 165(1-3): 599-603.

Chakraborti, D., M. M. Rahman, B. Das, M. Murrill, S. Dey, S. C. Mukherjee, R. K. Dhar, B. K. Biswas, U. K. Chowdhury and S. Roy (2010). Status of groundwater arsenic contamination in Bangladesh: A 14-year study report. *Water Res.* 44(19): 5789-5802.

Chen, J., G.-X. Sun, X.-X. Wang, V. d. Lorenzo, B. P. Rosen and Y.-G. Zhu (2014). Volatilization of Arsenic from Polluted Soil by Pseudomonas putida Engineered for Expression of the arsM Arsenic(III) S-Adenosine Methyltransferase Gene. *Environ. Sci. Technol.* 48(17): 10337-10344.

Clancy, T. M., K. F. Hayes and L. Raskin (2013). Arsenic Waste Management: A Critical Review of Testing and Disposal of Arsenic-Bearing Solid Wastes Generated during Arsenic Removal from Drinking Water. *Environ. Sci. Technol.* 47(19): 10799-10812.

Clancy, T. M., R. Reddy, J. Tan, K. F. Hayes and L. Raskin (2015a). Anaerobic disposal of arsenic-bearing wastes and the impact of methanogenesis on arsenic volatilization. *in prep. for Environ. Sci. Technol*.

Clancy, T. M., A. L. Smith, R. Reddy, A. J. Pinto, K. F. Hayes and L. Raskin (2015b). Anaerobic microbial community response to methanogenic inhibitors 2-bromoethanesulfonate and propynoic acid. *in prep. for Environmental Microbiology*.

Clancy, T. M., K. V. Snyder, R. Reddy, S. E. Amrose, L. Raskin and K. F. Hayes (2015c). Evaluating the cement stabilization of arsenic-bearing iron wastes from drinking water treatment. *in prep. for Journal of Hazardous Materials*.

Cornwall, D. (2003). Disposal of Waste Resulting From Arsenic Removal Processes, International Water Association.

Cortinas, I., R. Sierra-Alvarez and J. A. Field (2008). Biologically mediated mobilization of arsenic from granular ferric hydroxide in anaerobic columns fed landfill leachate. *Biotechnol. Bioeng.* 101(6): 1205-1213.

Das, B., M. M. Rahman, B. Nayak, A. Pal, U. K. Chowdhury, S. C. Mukherjee, K. C. Saha, S. Pati, Q. Quamruzzaman and D. Chakraborti (2009). Groundwater

Arsenic Contamination, Its Health Effects and Approach for Mitigation in West Bengal, India and Bangladesh. *Water Qual Expo Health* 1(1): 5-21.

Das, D. (1995). Arsenic Species Along With Other Metal/Metalloids Present and Responsible for Arsenic Episode in Groundwater of West Bengal and a Cheap Technique to Remove Arsenic, Thus Making the Groundwater Suitable for Drinking and Cooking Ph.D. Dissertation, Jadavpur University.

Dhar, R., B. Biswas, G. Samanta, B. Mandal, D. Chakraborti, S. Roy, A. Jafar, A. Islam, G. Ara, S. Kabir, A. Wadud Khan, S. Akther Ahmed and S. Abdul Hadi (1997). Groundwater arsenic calamity in Bangladesh. *Curr. Sci.* 73(1): 48-59.

Dixit, S. and J. G. Hering (2003). Comparison of arsenic(V) and arsenic(III) sorption onto iron oxide minerals: implications for arsenic mobility. *Environ. Sci. Technol.* 37(18): 4182-4189.

Dutre, V. and C. Vandecasteele (1995). Solidification/Stabilisation of Arsenic-Containing Waste: Leach Tests and Behaviour of Arsenic in the Leachate. *Waste Manage.* 15(1): 55-62.

Etmannski, T. R. and R. C. Darton (2014). A methodology for the sustainability assessment of arsenic mitigation technology for drinking water. *Sci. Total Environ.* 488–489(0): 505-511.

Ferguson, M. A., M. R. Hoffmann and J. G. Hering (2005). TiO_2-Photocatalyzed As(III) Oxidation in Aqueous Suspensions: Reaction Kinetics and Effects of Adsorption. *Environ. Sci. Technol.* 39(6): 1880-1886.

Fuller, C., J. Davis and G. Waychunas (1993). Surface chemistry of ferrihydrite: Part 2. Kinetics of arsenate adsorption and coprecipitation. *Geochim. Cosmochim. Acta* 57(10): 2271-2282.

Ghosh, A., M. Mukiibi and W. Ela (2004). TCLP underestimates leaching of arsenic from solid residuals under landfill conditions. *Environ. Sci. Technol.* 38(17): 4677-4682.

Ghosh, A., M. Mukiibi, A. E. Sáez and W. P. Ela (2006). Leaching of arsenic from granular ferric hydroxide residuals under mature landfill conditions. *Environ. Sci. Technol.* 40(19): 6070-6075.

Grimshaw, D. J. and A. E. Beaumont (2004). How can new science-led technologies contribute to mitigation of and adaptation to arsenic in the water of Bangladesh? Science for Humanity, Practical Action**:** 1-34.

Guan, X., J. Du, X. Meng, Y. Sun, B. Sun and Q. Hu (2012). Application of titanium dioxide in arsenic removal from water: A review. *J. Hazard. Mater.* 215–216(0): 1-16.

Hossain, M. A., M. K. Sengupta, S. Ahamed, M. M. Rahman, D. Mondal, D. Lodh, B. Das, B. Nayak, B. K. Roy, A. Mukherjee and D. Chakraborti (2005). Ineffectiveness and Poor Reliability of Arsenic Removal Plants in West Bengal, India. *Environ. Sci. Technol.* 39(11): 4300-4306.

Hussainuzzaman, M. M. and H. Yokota (2006). Efficiency of Arsenic Removal Unit Working in Bangladesh and Cement Stabilization of Its Sludge. *J. ASTM Int.* 3(7): 1-9.

Hussam, A., S. Ahamed and A. K. M. Munir (2008). Arsenic Filters for Groundwater in Bangladesh: Toward a Sustainable Solution. The Bridge Technologies for Clean Water, International Journal of Environmental Research and Public HealthNational Academy of Engineering.

Hussam, A. and A. K. M. Munir (2007). A simple and effective arsenic filter based on composite iron matrix: Development and deployment studies for groundwater of Bangladesh. *J. Environ. Sci. Health, Part A* 42: 1869-1878.

Jia, Y. and G. P. Demopoulos (2008). Coprecipitation of arsenate with iron(III) in aqueous sulfate media: Effect of time, lime as base and co-ions on arsenic retention. *Water Res.* 42(3): 661-668.

Jia, Y., H. Huang, M. Zhong, F.-H. Wang, L.-M. Zhang and Y.-G. Zhu (2013). Microbial Arsenic Methylation in Soil and Rice Rhizosphere. *Environ. Sci. Technol.* 47(7): 3141-3148.

Jing, C., G. P. Korfiatis and X. Meng (2003). Immobilization mechanisms of arsenate in iron hydroxide sludge stabilized with cement. *Environ. Sci. Technol.* 37(21): 5050-5056.

Jong, T. and D. L. Parry (2003). Removal of sulfate and heavy metals by sulfate reducing bacteria in short-term bench scale upflow anaerobic packed bed reactor runs. *Water Res.* 37(14): 3379-3389.

Kocar, B. D., M. J. Herbel, K. J. Tufano and S. Fendorf (2006). Contrasting Effects of Dissimilatory Iron (III) and Arsenic (V) Reduction on Arsenic Retention and Transport. *Environ. Sci. Technol.* 40(21): 6715-6721.

Lakshmanan, D., D. Clifford and G. Samanta (2008). Arsenic removal by coagulation with aluminum, iron, titanium, and zirconium. *J. - Am. Water Works Assoc.* 100(02): 76-88.

Leist, M., R. J. Casey and D. Caridi (2000). The management of arsenic wastes: problems and prospects. *J. Hazard. Mater.* 76(1): 125-138.

Meer, J. R. V. D., A. Chatzinotas and H. Harms (2012). Field Testing of Arsenic in Groundwater Samples of Bangladesh Using a Test Kit Based on Lyophilized Bioreporter Bacteria. *Environ. Sci. Technol.*

Mestrot, A., W.-Y. Xie, X. Xue and Y.-G. Zhu (2013). Arsenic volatilization in model anaerobic biogas digesters. *Appl. Geochem.* 33(0): 294-297.

Miller, S. M. and J. B. Zimmerman (2010). Novel, bio-based, photoactive arsenic sorbent: TiO_2-impregnated chitosan bead. *Water Res.* 44(19): 5722-5729.

Mishra, D. and J. Farrell (2005). Evaluation of mixed valent iron oxides as reactive adsorbents for arsenic removal. *Environ. Sci. Technol.* 39(24): 9689-9694.

Mladenov, N., Y. Zheng, M. P. Miller, D. R. Nemergut, T. Legg, B. Simone, C. Hageman, M. M. Rahman, K. M. Ahmed and D. M. McKnight (2010). Dissolved organic matter sources and consequences for iron and arsenic mobilization in Bangladesh aquifers. *Environ. Sci. Technol.* 44(1): 123-128.

Mohan, D. and C. U. Pittman (2007). Arsenic removal from water/wastewater using adsorbents--A critical review. *J. Hazard. Mater.* 142(1-2): 1-53.

Mohapatra, D., D. Mishra, G. R. Chaudhury and R. P. Das (2008). Removal of Arsenic from Arsenic Rich Sludge by Volatilization Using Anaerobic Microorganisms Treated with Cow Dung. *Soil Sediment Contam.* 17(3): 301-311.

Moon, D. H., D. Dermatas and N. Menounou (2004). Arsenic immobilization by calcium–arsenic precipitates in lime treated soils. *Sci. Total Environ.* 330(1–3): 171-185.

Munir, A. K. M., S. B. Rasul, S. D. Centre, E. Initiative, M. Habibuddowla, M. Alauddin, S. Island, N. York, A. Hussam and A. H. Khan (2001). Evaluation of Performance of Sono 3-Kolshi Filter for Arsenic Removal from Groundwater Using Zero Valent Iron Through Laboratory and Field Studies. Proceedings International Workshop on Technology for Arsenic Removal from Drinking Water, Bangladesh University of Engineering and Technology and United Nations University, Japan.

Naujokas, M. F., B. Anderson, H. Ahsan, H. V. Aposhian, J. H. Graziano, C. Thompson and W. A. Suk (2013). The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. *Environ. Health Perspect.* 121(3): 295-302.

Neumann, A., R. Kaegi, A. Voegelin, A. Hussam, A. K. M. Munir and S. J. Hug (2013). Arsenic Removal with Composite Iron Matrix Filters in Bangladesh: A Field and Laboratory Study. *Environ. Sci. Technol.*

Pande, S. P., L. S. Deshpande and S. N. Kaul (2001). Laboratory and field assessment of arsenic testing field kits in Bangladesh and West Bengal, India. *Environ. Monit. Assess.* 68(1): 1-18.

Paria, S. and P. K. Yuet (2006). Solidification-stabilization of organic and inorganic contaminants using portland cement: a literature review. *Environ. Rev.* 14(4): 217-255.

Qin, J., B. P. Rosen, Y. Zhang, G. Wang, S. Franke and C. Rensing (2006). Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase. *Proc. Natl. Acad. Sci. U. S. A.* 103(7): 2075-2080.

Radloff, K. A., Z. Cheng, M. W. Rahman, K. M. Ahmed, B. J. Mailloux, A. R. Juhl, P. Schlosser and A. van Geen (2007). Mobilization of arsenic during one-year incubations of grey aquifer sands from Araihazar, Bangladesh. *Environ. Sci. Technol.* 41(10): 3639-3645.

Raven, K. P., A. Jain and R. H. Loeppert (1998). Arsenite and Arsenate Adsorption on Ferrihydrite: Kinetics, Equilibrium, and Adsorption Envelopes. *Environ. Sci. Technol.* 32(3): 344-349.

Ravenscroft, P., H. Brammer and K. Richards (2009). Arsenic pollution: a global synthesis, Wiley-Blackwell.

Ravenscroft, P., W. G. Burgess, K. M. Ahmed, M. Burren and J. Perrin (2004). Arsenic in groundwater of the Bengal Basin, Bangladesh: Distribution, field relations, and hydrogeological setting. *Hydrogeology Journal* 13(5-6): 727-751.

Sarkar, S., L. M. Blaney, A. Gupta, D. Ghosh and A. K. Sengupta (2008). Arsenic removal from groundwater and its safe containment in a rural environment: validation of a sustainable approach. *Environ. Sci. Technol.* 42(12): 4268-4273.

Sarkar, S., J. E. Greenleaf, A. Gupta, D. Ghosh, L. M. Blaney, P. Bandyopadhyay, R. K. Biswas, A. K. Dutta and A. K. Sengupta (2010). Evolution of community-based arsenic removal systems in remote villages in West Bengal, India: Assessment of decade-long operation. *Water Res.* 44(19): 5813-5822.

Singh, T. S. and K. K. Pant (2006). Solidification/stabilization of arsenic containing solid wastes using portland cement, fly ash and polymeric materials. *J. Hazard. Mater.* 131(1–3): 29-36.

Smith, A. H., E. O. Lingas and M. Rahman (2000). Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bull. W. H. O.* 78(9): 1093-1103.

Stuckman, M. Y., J. J. Lenhart and H. W. Walker (2011). Abiotic properties of landfill leachate controlling arsenic release from drinking water adsorbents. *Water Res.* 45(16): 4782-4792.

Sullivan, C., M. Tyrer, C. R. Cheeseman and N. J. D. Graham (2010). Disposal of water treatment wastes containing arsenic - a review. *Sci. Total Environ.* 408(8): 1770-1778.

Sutton, N. B., G. M. van der Kraan, M. C. M. van Loosdrecht, G. Muyzer, J. Bruining and R. J. Schotting (2009). Characterization of geochemical constituents and bacterial populations associated with As mobilization in deep and shallow tube wells in Bangladesh. *Water Res.* 43(6): 1720-1730.

Thomas, F., R. A. Diaz-Bone, O. Wuerfel, B. Huber, K. Weidenbach, R. A. Schmitz and R. Hensel (2011). Connection between Multimetal(loid) Methylation in Methanoarchaea and Central Intermediates of Methanogenesis. *Appl. Environ. Microbiol.* 77(24): 8669-8675.

Tufano, K. J. and S. Fendorf (2008). Confounding impacts of iron reduction on arsenic retention. *Environ. Sci. Technol.* 42(13): 4777-4783.

Tufano, K. J., C. Reyes, C. W. Saltikov and S. Fendorf (2008). Reductive processes controlling arsenic retention: revealing the relative importance of iron and arsenic reduction. *Environ. Sci. Technol.* 42(22): 8283-8289.

U.S. EPA (1992). Method 1311 Toxicity Characteristic Leaching Procedure**:** 1-35.

Upadhyaya, G., J. Jackson, T. M. Clancy, S. P. Hyun, J. Brown, K. F. Hayes and L. Raskin (2010). Simultaneous removal of nitrate and arsenic from drinking water sources utilizing a fixed-bed bioreactor system. *Water Res.* 44(17): 4958-4969.

US EPA (2003). Arsenic Treatment Technology Evaluation Handbook for Small Systems. US Environmental Protection Agency. Washington, DC.

Visoottiviseth, P. and F. Ahmed (2008). Technology for Remediation and Disposal. Reviews of Environmental Contamination. D. M. Whitacre. New York, Springer. 197.

Yokoyama, Y., K. Tanaka and Y. Takahashi (2012). Differences in the immobilization of arsenite and arsenate by calcite. *Geochim. Cosmochim. Acta* 91(0): 202-219.

Zhao, F.-J., E. Harris, J. Yan, J. Ma, L. Wu, W. Liu, S. P. McGrath, J. Zhou and Y.-G. Zhu (2013). Arsenic Methylation in Soils and Its Relationship with Microbial arsM Abundance and Diversity, and As Speciation in Rice. *Environ. Sci. Technol.* 47(13): 7147-7154.

Chapter 2

Arsenic waste management: A critical review of testing and disposal of arsenic-bearing solid wastes generated during arsenic removal from drinking water

2.1 Abstract

Water treatment technologies for arsenic removal from groundwater have been extensively studied due to widespread arsenic contamination of drinking water sources. Central to the successful application of arsenic water treatment systems is the consideration of appropriate disposal methods for arsenic-bearing wastes generated during treatment. However, specific recommendations for arsenic waste disposal are often lacking or mentioned as an area for future research and the proper disposal and stabilization of arsenic-bearing waste remains a barrier to the successful implementation of arsenic removal technologies. This review summarizes current disposal options for arsenic-bearing wastes, including landfilling, stabilization, cow dung mixing, passive aeration, pond disposal, and soil disposal. The findings from studies that simulate these disposal conditions are included and compared to results from shorter, regulatory tests. In many instances, short-term leaching tests do not adequately address the range of conditions encountered in disposal environments. Future research directions are highlighted and include establishing regulatory test conditions that align with actual disposal conditions and evaluating non-landfill disposal options for developing countries.

Clancy, T. M., K. F. Hayes and L. Raskin (2013). Arsenic Waste Management: A Critical Review of Testing and Disposal of Arsenic-Bearing Solid Wastes Generated during Arsenic Removal from Drinking Water. *Environ. Sci. Technol.* 47(19): 10799-10812.

2.2 Introduction

Arsenic contamination of drinking water sources is estimated to affect over 144 million people around the world, spurring the development of numerous water treatment technologies to limit negative health impacts associated with exposure to arsenic contaminated water including skin lesions and cancers (Dhar *et al.* 1997, Smith *et al.* 2000, Ng *et al.* 2003, Caussy and Priest 2008, Ravenscroft *et al.* 2009, Chakraborti *et al.* 2010). These technologies include ion exchange, adsorptive media filtration, coagulation and flocculation, electrocoagulation, and anaerobic removal with iron sulfides. It should be noted that in areas where alternative groundwater wells with low arsenic concentrations are available, well-switching can be an effective means to provide safe drinking water (van Geen *et al.* 2002). In areas where arsenic-removal from groundwater is needed, drinking water treatment systems have been implemented in developed and developing countries, ranging in size from centralized treatment plants to smaller systems for individual households. In 1993, the World Health Organization (WHO) lowered the arsenic drinking water guideline from 50 µg/L to 10 µg/L (World Health Organization 2006). Following this change, many countries adopted a more stringent drinking water standard for arsenic, expanding the need for incorporating arsenic removal technologies in drinking water treatment processes (Patterson 2006). However, for some countries most affected by arsenic contamination, e.g., Bangladesh, India, and Nepal, the arsenic standard has remained at 50 µg/L as a result of the technical and economic challenges associated with achieving even this less stringent standard (Shrestha *et al.* 2003, Das *et al.* 2009). The higher standard in developing countries is of great concern due to the compounding effects of higher water consumption, higher rates of malnutrition, and

greater exposure to arsenic through foods irrigated with arsenic contaminated water (Smith and Smith 2004, Mukherjee *et al.* 2005). Given the increased application of arsenic removal technologies in response to the more stringent regulations, health concerns, and greater pressure to access all available water sources for drinking water production, it can be expected that greater quantities of arsenic-bearing wastes will be generated. This will require appropriate methods for treating and/or stabilizing these wastes at disposal sites.

A recent review by Sullivan et al. (2010) outlined promising options for disposal in both developed and developing countries. In developed countries, landfill disposal and stabilization are the most common disposal practices. Considering treatment options for arsenic-bearing wastes from water treatment and industrial processes in developed countries, Leist et al. (2000) indicated stabilization was the best treatment to limit arsenic release from wastes. However, many arsenic-bearing wastes from drinking water treatment are disposed directly into municipal solid waste landfills, where arsenic release due to reducing conditions has been measured in landfill leachate and gases (Pinel-Raffaitin *et al.* 2007).

Concerns over the release of arsenic from drinking water wastes are also relevant for developing countries where arsenic-bearing wastes are often directly discharged to the environment, posing a greater risk for human exposure and re-contamination of source waters. Sullivan et al. (2010) highlighted two disposal options for developing countries, i.e., mixing arsenic-bearing wastes with livestock waste and incorporating wastes into building materials. Despite these options, arsenic-bearing wastes are commonly disposed directly in nearby ponds or on open fields with little site preparation and essentially no

monitoring (Sarkar , Eriksen-Hamel and Zinia 2001, Ali *et al.* 2003, Badruzzaman 2003, Hussainuzzaman and Yokota 2006, Shafiquzzaman *et al.* 2009). Government policies for arsenic mitigation typically require some kind of disposal plan for arsenic-bearing wastes (Government of Bangladesh 2003, Planning Commission 2007), although evaluation of disposal plans and enforcement is often a challenge for stressed governments facing a multitude of environmental concerns (Metcalfe 2003, Khan 2009). Arsenic-bearing waste disposal is one of several factors that limit the implementation of arsenic removal technologies. Other factors affecting successful adoption of arsenic removal technologies include cost, maintenance, and ability to monitor effluent arsenic concentrations (Alam *et al.* 2002, Shafiquzzaman *et al.* 2009).

Although arsenic transformations, transport, and toxicity have been extensively reviewed (Bissen and Frimmel 2003a, Oremland and Stolz 2003, Caussy and Priest 2008, Khan *et al.* 2009, Lièvremont *et al.* 2009), here we highlight some of the key processes relevant to the mobility of arsenic in waste disposal environments. In aqueous environments, arsenic can be stable in a variety of oxidation states (e.g., -III, 0, II, III, and V). The most prevalent inorganic dissolved forms include arsenite, AsO_3^{3-}, in which arsenic has the oxidation state +3 (As(III)), and arsenate, AsO_4^{3-}, with arsenic in the +5 oxidation state (As(V)). Generally for mammals, As(III) is more toxic than As(V), although speciation is a secondary concern for toxicity due to biotic transformations following arsenic uptake (Thomas *et al.* 2001, Hughes 2002). While arsenic (II and III) can precipitate as reduced sulfides (e.g., realgar: AsS and orpiment: As_2S_3) or adsorb onto iron sulfides or metal oxides (Dixit and Hering 2003, Wilkin *et al.* 2003, O'Day *et al.* 2004, Kocar *et al.* 2006, Gallegos *et al.* 2007, Gallegos *et al.* 2008, Renock *et al.* 2009),

arsenate typically does not precipitate significantly and is more strongly complexed by iron and aluminum hydroxide solids (Dixit and Hering 2003). However, adsorption behavior in the natural environments can be highly variable due to differences in redox conditions, presence of complex mineral assemblages, spatial heterogeneity, and competing ions (Su and Puls 2001, O'Day *et al.* 2004, Roberts *et al.* 2004, Stachowicz *et al.* 2008, Masue-Slowey *et al.* 2013). Under sulfate reducing conditions, the presence of a variety of aqueous sulfidic arsenic species can be important in arsenic partitioning depending on the sulfur to arsenic molar concentration ratios (Wilkin *et al.* 2003, Bostick *et al.* 2005). Less common, but also important is arsine, AsH_3, in which arsenic is present in the -3 oxidation state (As(-III)). Arsine gas is highly toxic and fairly soluble in water (Klimecki and Carter 1995, Cullen and Bentley 2005). Organic arsenic compounds include mono, di-, and trimethylarsines, and a range of more complex organic arsines (Challenger 1945, Bentley and Chasteen 2002). Arsenic methylation processes are part of biological detoxification mechanisms in all domains of life (*Bacteria*, *Archaea*, and *Eukarya*) that generate organic arsenic compounds, which may be less toxic than inorganic forms (Bentley and Chasteen 2002, Hughes 2002, Cullen and Bentley 2005, Qin *et al.* 2006). Arsenic species can be converted among the various oxidation states through abiotic and biotic processes of oxidation, reduction, methylation and demethylation (Sanders 1979, Oremland and Stolz 2003, O'Day *et al.* 2004, Lièvremont *et al.* 2009, Tsai *et al.* 2009, Thomas *et al.* 2011, Yoshinaga *et al.* 2011) (Figure 2-1). Understanding the processes that drive arsenic transformation among these forms is essential for determining arsenic exposure risk and potential for leaching from arsenic-bearing solid waste disposal.

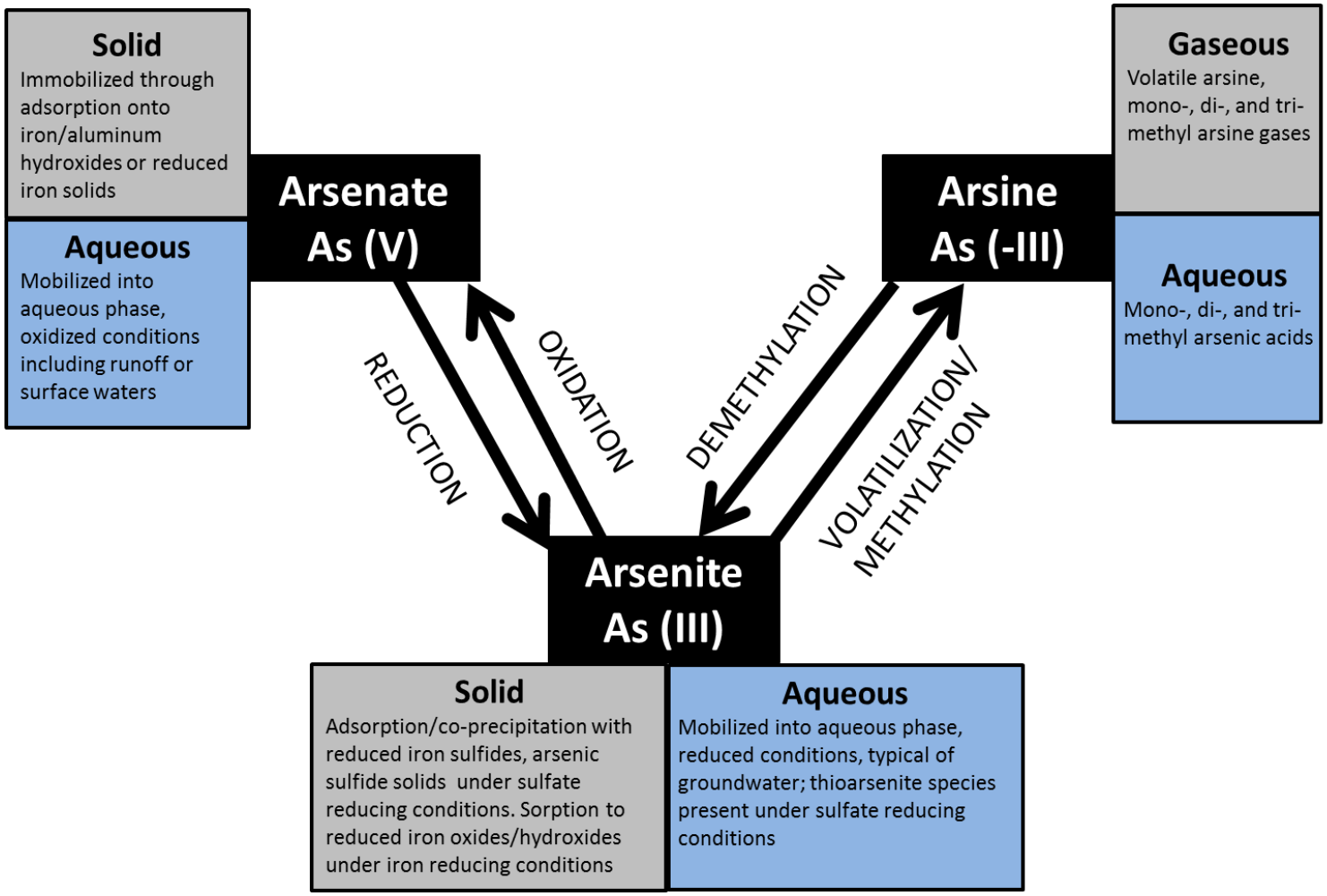


Figure 2-1. Arsenic speciation and transformation processes in the environment

In this review, we outline common arsenic-bearing wastes produced and the biogeochemistry relevant to arsenic release from these solids, discuss waste disposal options, summarize the findings of simulation experiments that have evaluated the fate of arsenic after disposal, and compare these results to the most common testing procedures used for regulating arsenic-bearing wastes. Arsenic-bearing waste management in both developing and developed countries is discussed. While previous reviews have focused on arsenic disposal options (Leist *et al.* 2000, Sullivan *et al.* 2010), this review presents the first synthesis of studies characterizing waste disposal environments and the suitability for tests used for assessing waste stability and arsenic leaching in those environments. Through this review we highlight the limitations of current knowledge and suggest new directions to enhance the applicability of research results to guide the development of best practices for arsenic-bearing waste disposal.

2.3 Arsenic removal technologies and associated arsenic-bearing wastes

All treatment methods for removing arsenic from drinking water generate arsenic-bearing wastes. Here we focus on arsenic-bearing solid wastes and do not include liquid waste (e.g., ion exchange brines, backwash waste from fixed bed bioreactors). Concentrated liquid waste disposal options have been reviewed elsewhere (Choong *et al.* 2007). Concentrations of arsenic in arsenic-bearing solid wastes vary widely, from 0.1 to 7,500 mg As/kg waste (Table S1, Appendix B). For reference, many uncontaminated soils contain arsenic concentrations on the order of 10 mg As/kg soil and the U.S. Environmental Protection Agency (EPA) upper limit of arsenic levels in biosolids for land application is 75 mg As/kg solid.(U.S. EPA 2007) However, concentration is not the only important factor as the risk posed by arsenic release also depends on the quantity of

waste generated, the chemical composition of the waste, the location of the waste generation, and the disposal environment. The most common arsenic removal technologies sequester arsenic in its oxidized form. Alternatively, arsenic removal under sulfate reducing conditions leads to the generation of reduced arsenic-bearing solid wastes. Given the importance of redox conditions on the subsequent leaching potential of the arsenic solid phase waste generated, differences between these wastes and the biogeochemistry relevant to waste disposal are discussed in more detail below.

2.3.1 Oxidized arsenic-bearing wastes

The most common arsenic removal technologies, including: ion exchange (Vagliasindi and Benjamin 1998, Ghurye *et al.* 1999, Vaaramaa and Lehto 2003), adsorbent media filtration (Selvin *et al.* 2002, Mohan and Pittman 2007), coagulation and flocculation (Meng *et al.* 2001a, Laky and Licskó 2011), and electrocoagulation (Kumar *et al.* 2004, Martínez-Villafañe *et al.* 2009, van Genuchten *et al.* 2012), remove arsenic as arsenate under oxic conditions. When treating waters in which arsenic is initially present as arsenite, abiotic(Pettine *et al.* 1999, Bissen and Frimmel 2003b, Ferguson *et al.* 2005, Leupin and Hug 2005, Miller and Zimmerman 2010) or biotic (Sun *et al.* 2009) oxidation steps are commonly included before arsenic removal due to typically higher adsorption capacities on many adsorbents for arsenate (Bissen and Frimmel 2003b, Bissen and Frimmel 2003a, Dixit and Hering 2003, Hug and Leupin 2003, Wolthers *et al.* 2005). The effectiveness of arsenate adsorption is dependent on pH (Raven *et al.* 1998, Dixit and Hering 2003) and concentrations of competing ions including phosphate, silicate, natural organic matter and carbonate/bicarbonate (Peryea and Kammereck 1997, Ghosh *et al.* 2006b, Laky and Licskó 2011, Stuckman *et al.* 2011, Wan *et al.* 2011).

For most of these, arsenate is typically adsorbed to iron or other metal based oxides. The redox state of arsenic, iron, and other metals in these wastes has implications for arsenic leaching under different disposal conditions. Particularly problematic is the release of arsenic from oxidized wastes in reduced environments due to reductive dissolution of iron oxides (Ghosh *et al.* 2004, Islam *et al.* 2004, van Geen *et al.* 2004, Anawar *et al.* 2006, Jing *et al.* 2008, Corsini *et al.* 2011) and the production of sulfide when microbial sulfate reduction occurs (Saalfield and Bostick 2009, Burton *et al.* 2011). It should be noted that sulfide formation does not always result in arsenic release. As described above and below, sulfide can cause arsenic removal from the aqueous phase, depending on environmental conditions like pH (Burton *et al.* 2011) and concentrations of sulfide and iron (Lee *et al.* 2005).

2.3.2 Reduced arsenic-bearing waste

Under reducing conditions, arsenic can be sequestered through co-precipitation or adsorption with iron sulfides, produced either abiotically or through the production of sulfide by sulfate-reducing bacteria or archaea (Rochette *et al.* 2000). Arsenic removal under reduced conditions by iron sulfides has been shown to be unaffected by the presence of silica, providing a potential advantage over arsenic removal under oxic conditions in the presence of high silica concentrations (Han *et al.* 2013). The stimulation of biotic sulfate reduction and the subsequent formation of iron sulfides has been used to remove arsenic and other heavy metals from acid mine drainage contaminated waters (Jong and Parry 2003, Kaksonen and Puhakka 2007, Luo *et al.* 2008), landfills (Keimowitz *et al.* 2007), and aquifers (Kirk *et al.* 2010). Arsenic removal with iron sulfide coated sand also has been demonstrated (Han *et al.* 2011). Kirk et al. (2010) found

arsenic removal under sulfate-reducing conditions was limited by pyrite formation and the solubility of arsenic sulfides. In simulated groundwater, cultures of sulfate-reducing bacteria have been shown to remove both arsenite and arsenate (Teclu *et al.* 2008). An anaerobic biologically-active carbon fixed-bed bioreactor has recently been used to remove arsenic from drinking water through the production of iron and arsenic sulfides (Upadhyaya *et al.* 2010).

The wastes generated during anaerobic arsenic removal under sulfate-reducing conditions differ from those generated during solid phase removal under oxidizing conditions, and typically contain reduced arsenic, sulfur and iron phases. The stability and potential for arsenic leaching from reduced arsenic-bearing wastes have not been widely tested, but these wastes are expected to be more stable in reduced environments, such as those found in landfills (Jong and Parry 2005).

Abiotic oxidation of As(III) or As(II) sorbed to reduced iron solids and iron sulfides depends on the type of solids and the pH (Saulnier and Mucci 2000, Jeong *et al.* 2010a, Jeong *et al.* 2010b). Acid volatile sulfides (AVS), defined as sulfides that generate hydrogen sulfide gas upon addition of hydrochloric acid, typically oxidize rapidly in the presence of oxygen (Holmes 1999, Caetano *et al.* 2003). Arsenic has been shown to be associated with AVS in reduced sediments, and released to the aqueous phase upon exposure to oxidized waters (Saulnier and Mucci 2000). The pH is also important in controlling arsenic mobilization during oxidation as arsenic mobilization depends on the relative rates of iron dissolution and precipitation of oxidized iron solids (Jeong *et al.* 2010b). At low pH, dissolution of iron sulfides is fast and precipitation of oxidized iron is slow, resulting in the release of arsenic in the aqueous phase (Jeong *et al.* 2010b). Release

to the aqueous phase is minimized under alkaline conditions because of direct conversion of iron sulfides to oxidized iron phases (Jeong *et al.* 2010b).

2.4 Arsenic waste disposal environments

The stability of arsenic-bearing wastes depends on the characteristics of the waste and the ultimate disposal environment. As discussed above, the risks posed by arsenic-bearing wastes also depend on the arsenic concentration, overall quantity of waste generated, and the location of the waste generation. Given their relevance to either developing or developed countries, we review characteristics of the environmental conditions for the following disposal options for arsenic-bearing wastes: landfills, stabilization, cow dung, passive aeration systems, ponds, and soil. For each option we summarize the findings of studies that have been carried out to mimic these disposal environments. These types of experiments are critical to understanding the fate of arsenic under disposal conditions and for evaluating the suitability of regulatory tests to assess actual disposal conditions.

2.4.1 Landfill environments

Arsenic-bearing solid wastes from drinking water treatment in the U.S. and other developed countries are typically disposed in municipal solid waste landfills (Cornwall 2003). Waste degradation in landfills is usually divided into four stages based on the chemical environment and microbial activity characteristic of each stage. These stages are initial aerobic, acidogenic, initial methanogenic, and stable methanogenic (Christensen and Kjeldsen 1995). In landfills, both biotic and abiotic processes are extant and determine the stability of arsenic wastes.

Considering abiotic reactions in landfills, arsenic fate depends mostly on the presence of oxygen and hydrogen sulfides and their associated redox conditions (Hounslow 1980). In a simplified model, based solely on the presence and absence of oxygen and hydrogen sulfides, iron is expected to be present as iron(III) hydroxides in the initial oxic phase, whereas soluble iron(II) sulfides predominate during the later anoxic stages. In the presence of oxygen without hydrogen sulfides, arsenic is likely to be bound to oxidized iron or aluminum oxyhydroxide solids. When neither oxygen nor hydrogen sulfides are present, arsenic is found in the reduced form, i.e., arsenite, in the aqueous phase. When oxygen is absent and hydrogen sulfides are present, the precipitation of arsenic sulfides and iron sulfides can remove arsenic from the aqueous phase, reducing arsenic mobility (Hounslow 1980, O'Day *et al.* 2004).

While abiotic reactions provide the thermodynamic basis for predicting arsenic speciation and by inference arsenic mobility, microbial activity, resulting from the availability of organic matter and various electron acceptors, typically controls the overall redox conditions of a landfill. Consequently, microbial activity affects the principal oxidation states and concentrations of complexing ligands or metals that influence the release of arsenic from solids. During the initial aerobic phase, aerobic or facultative microbes rapidly remove oxygen and degrade organics resulting in anaerobic conditions. Then denitrifying and sulfate-reducing bacteria and archaea convert nitrate and sulfate to dinitrogen gas and hydrogen sulfide, respectively (Palmisano and Barlaz 1996). Microbial arsenic and iron reduction under reducing conditions can cause the release of arsenic and have been shown to be a major factor in arsenic release from natural sediment contaminated with landfill leachate plumes (DeLemos *et al.* 2006). Under anaerobic

conditions, fermenting bacteria create acidic conditions (pH between 4.5 and 7.5). In the last two anaerobic stages, methane is produced by methanogenic archaea (Demirel and Scherer 2008).

The importance of microbial arsenic methylation in landfills has been recognized only recently (Pinel-Raffaitin *et al.* 2007). However, the potential for arsenic methylation under reducing conditions in general has been studied for some time (Bright *et al.* 1994, Michalke *et al.* 2000). Techniques have been developed to measure the methylated arsenic species and the genes responsible for arsenic methylation (Qin *et al.* 2006, Mestrot *et al.* 2009) and should be applied to more studies to quantify how widespread and important arsenic methylation is in the disposal of arsenic treatment wastes in reducing conditions.

Landfill conditions have been simulated in long-term experiments using leachate from municipal solid waste landfills and synthetic leachate. Arsenic has been shown to be released from ferric hydroxide solids used in drinking water treatment under reducing conditions in simulated landfill experiments (Ghosh *et al.* 2006a). Jing et al. (2008) used spent media from five different types of arsenic adsorbent media filtration treatment plants to investigate the leaching potential under reducing conditions over 70 days. They found that neither the Toxicity Characteristic Leaching Procedure (TCLP) nor the California Waste Extraction Test (Cal WET), regulatory tests described in more detail below, were appropriate tests for predicting leaching under reducing conditions (Jing *et al.* 2008). In a column simulating mature landfill disposal for arsenic-bearing wastes from SONO filters, the TCLP under predicted arsenic leaching over the 102 day operation of the column (Islam *et al.* 2011). While regulatory tests depend on abiotic predictions of

leaching, it has been shown that microbial activity can increase arsenic leaching as described above. Abiotic and biotic column studies in which ferric hydroxide and activated alumina waste were exposed to synthetic landfill leachate showed that leaching of arsenic occurred more rapidly and to a greater extent in the biotic columns (Sierra-Alvarez *et al.* 2005, Cortinas *et al.* 2008).

2.4.2 Stabilization

Stabilization of hazardous waste to reduce the toxicity and mobility of contaminants is a common treatment strategy. This is often accomplished through the addition of lime, concrete, or iron containing amendments (Raj *et al.* 2005). Novel materials, like polymeric matrices, are also being developed specifically for arsenic-bearing wastes (Shaw *et al.* 2008). Concrete stabilization has been used for mine tailings (Choi *et al.* 2009) and solid wastes from a constructed wetland for arsenic removal (Nakwanit *et al.* 2011). Following stabilization, arsenic wastes have been disposed on soils (Kumpiene *et al.* 2008), in landfills (Kameswari *et al.* 2001, Sullivan *et al.* 2010), and used in bricks (Mahzuz *et al.* 2009). Stabilization has been used primarily in developed countries, but is receiving increased attention in developing countries (Sullivan *et al.* 2010).

Environmental conditions including pH, relative humidity, and wetting and drying cycles impact the leaching of arsenic from stabilized wastes. Arsenic in these solids is typically stabilized by the formation of calcium arsenic precipitates (Jing *et al.* 2003, Moon *et al.* 2004). Paria and Yuet (2006) provide an overview of the use of Portland cement for stabilization and highlight that leaching behavior is pH dependent. Leaching

behavior also depends on the relative humidity and cycles of wetting and drying due to the carbonation of calcium hydroxides (Sanchez *et al.* 2002).

Following mixing with amendments, such as cement or clay, arsenic-bearing wastes can be incorporated into bricks or in recycled concrete mixtures used for road construction. Incorporating arsenic-bearing waste from drinking water treatment into useable bricks has not been widely adopted, but is of considerable interest in Bangladesh and other developing countries (Mahzuz *et al.* 2009). Although the use of fly ash and incinerator waste in recycled aggregates for road construction has been evaluated and is commonly used around the world (Jurič *et al.* 2006, Kumar and Patil 2006, Moon and Dermatas 2007), the use of recycled concrete containing arsenic-bearing wastes from drinking water treatment systems has not been directly studied for such applications. Future research should evaluate the feasibility of this disposal option, taking into account the location and amount of wastes generated.

Using arsenic-bearing waste materials in construction assumes that amended and traditional materials have comparable compressive strengths. Variables that can contribute to the strength of bricks include curing time, temperature, and chemical characteristics of the waste. Iron containing wastes have been found to hinder cement hydration (Sullivan *et al.* 2010) and decrease compressive strength (Olmo *et al.* 2001). However, there is large variation in reported fractions of iron containing wastes that can be incorporated without affecting the compressive strength (Banerjee and Chakraborty 2005, Mahzuz *et al.* 2009). Interactions between wastes and concrete that can affect arsenic stability and concrete strength are specific for each type of waste and should be considered when determining waste disposal options. For this strategy to be adopted on a

larger scale, the costs of waste transport should also be taken into account. Additionally, laboratory and field studies are needed to evaluate long-term arsenic leaching potential.

Cement stabilized drinking water treatment waste has been frequently characterized using regulatory leaching tests (described below). Cement stabilization has been shown to decrease the concentration of arsenic in the leachate relative to un-stabilized controls (Leist *et al.* 2003, Jing *et al.* 2005, Camacho *et al.* 2009). Concerns about testing cement stabilized solids have been raised due to the ability of these solids to increase the solution pH, which can subsequently affect the solubility of solid phases that stabilize arsenic. Since there may be large differences in the buffering capacity of leaching test solutions, attempts to control the pH of the leaching solution or to simulate actual disposal conditions may be compromised when performing tests with cements (Halim *et al.* 2003).

Experiments have also been used to develop ways to reduce leaching from arsenic wastes stabilized with amendments. In one study, higher firing temperatures correlated with slight decreases in leaching from bricks using different extraction liquids, including distilled water and rainwater (Rouf and Hossain 2003). In one of the few studies evaluating environmental conditions, less arsenic was leached from concrete stored under conditions of higher relative humidity (Sanchez *et al.* 2002). Future research should focus on establishing the environmental conditions, during both production and use phases that limit arsenic leaching from these solids.

Only a few studies have evaluated the long-term stability of stabilized arsenic-bearing wastes under disposal conditions. For example, mining waste treated with iron and lime was studied after 20 years of pit disposal (Pantuzzo and Ciminelli 2010).

Leaching from soluble calcium-arsenic phases was observed and it was concluded that long-term stability requires high iron to arsenic ratios (Pantuzzo and Ciminelli 2010). An alternative waste treatment approach using biological arsenite oxidation during or after lime treatment has also been evaluated. Enrichments of autotrophic arsenite-oxidizing bacteria were used to treat mining waste and were shown to reduce arsenic leaching, compared to controls without added bacteria (Battaglia-Brunet *et al.* 2011). Further research should build on these studies to expand our understanding of stabilized waste in different disposal environments. These additional studies should also include simulations of stabilized wastes integrated into buildings and roads, for which typical, long-term conditions have yet to be studied.

2.4.3 Disposal with cow dung

Mixing arsenic wastes with cow dung is intended to promote microbial arsenic methylation to produce gaseous methylarsines that are less toxic to mammals than inorganic forms. This approach was initially suggested based on observations of decreasing solid phase arsenic concentrations following the addition of cow dung to arsenic wastes (Das 1995, Das *et al.* 2001). Although limited additional research has been performed to indicate that substantial arsenic removal could be achieved through volatilization, mixing arsenic-bearing wastes with cow dung has since been recommended as an optimal disposal strategy, especially for rural areas in developing countries (Mudgal 2001, Ali *et al.* 2003, Visoottiviseth and Ahmed 2008, Sullivan *et al.* 2010). However, studies of arsenic contaminated soils have reported only a small fraction of arsenic volatilized with the addition of cow dung. For example, by measuring gaseous arsenic emissions over 10 days, Turpeinen et al. (2002) found that natural soil microbes

volatilized less than 0.5% of the arsenic contained in a contaminated soil (arsenic concentrations varied between 2,125 and 3,632 mg As/kg soil). Genetically engineered bacteria, containing the *arsM* gene for arsenic methylation, were shown to volatilize up to 4.5% of arsenic from a contaminated soil containing 42 mg As/kg soil over 30 days (Liu *et al.* 2011). The addition of fungi capable of arsenic volatilization to contaminated soil mixed with cow dung increased the amount of volatilized arsenic by 3.7 times as compared to the naturally present microbes, which volatilized only about 0.03% of the arsenic in five months (1,387 mg As/kg soil) (Edvantoro *et al.* 2004). In a three-week study of paddy soils, Mestrot et al. (2009) reported arsenic volatilization took place only in rice paddies to which manure was applied, but was less than 0.014% of the arsenic soil content (24.2 mg As/kg soil).

Mixing arsenic-bearing wastes with cow dung has also been studied, with large variations in the amount of arsenic volatilization reported. In one of the few studies to measure gaseous arsenic emissions, Mohapatra et al. (2008) mixed cow dung with arsenic-bearing ferrihydrite (18.5 mg As/g) and determined that 10% of the total arsenic was volatilized in 40 days. In addition, 32% of the arsenic originally present in the waste was released into the aqueous phase (Mohapatra *et al.* 2008). In another anaerobic incubation, arsenic wastes from a drinking water treatment plant were mixed with organic food waste and sewage sludge and incubated in an anaerobic digester for 50 days (Banerjee 2010). This study reported arsenic removal up to 99% based on determining arsenic content in the waste through solid digestions and measuring arsenic concentrations in the effluent. They cited volatilization as the removal pathway, but no direct measurements of gaseous arsenic emissions were performed (Banerjee 2010). In

another study, cow dung was added to arsenic-bearing wastes from household coagulation units and incubated for 36 days (Ali *et al.* 2003). Arsenic volatilization up to 50% was reported based on differences in the arsenic measured in acid digested solid samples (Ali *et al.* 2003). In a more recent study of anaerobic digestion with added arsenic (1 mg As/kg digestion solids), actual gaseous emissions were measured, finding up to 2% arsenic volatilization after 42 days (Mestrot *et al.* 2013).

These studies show that conditions in anaerobic digesters can promote volatilization of arsenic. However, the potential for arsenic release in the aqueous phase should also be evaluated. This is particularly important for cow dung pit disposal of wastes or on soils exposed to rainwater. Future studies should also include measurements of all arsenic phases to accurately report gaseous and aqueous emissions.

2.4.4 Passive aeration disposal

To avoid reductive dissolution and the subsequent release of arsenic from oxidized arsenic-bearing wastes, a passive aeration system was developed to keep wastes in their oxidized forms (Sarkar *et al.* 2010). This system is designed using a container to hold arsenic-bearing wastes, with slotted pipes running through the container, allowing air to pass through the wastes. The passive aeration provided by this air is intended to maintain oxic conditions, so that arsenate will remain bound to the resins/iron hydroxides/aluminum hydroxide media. This disposal option has been implemented in West Bengal, India, where over 200 community based arsenic removal filters have been installed (Sarkar *et al.* 2010). Spent iron/aluminum hydroxide based adsorbent media from the filters are collected and disposed in one central location (Sarkar *et al.* 2008).

While this type of disposal system may be an excellent option for oxidized arsenic-bearing wastes in developed and developing countries, long-term studies have not been conducted to monitor its effectiveness. The location of pipes and vents for optimal aeration have not been determined or experimentally verified. Additionally, studies have not monitored wastes over time to show long-term stability. Further research is needed to determine the effects of changing environmental conditions, including redox and pH, caused by flooding and cycles of wetting and drying.

2.4.5 Pond disposal

In rural areas of developing countries, including Bangladesh, ponds are sometimes used as the ultimate disposal location for arsenic-bearing solid wastes from arsenic-removing sand filters and household filters (Yokota *et al.* 2001, Hussainuzzaman and Yokota 2006, Shafiquzzaman *et al.* 2009). During filter maintenance, slurries of water containing arsenic-bearing iron hydroxides particles are disposed in ponds. However, pond disposal is not widely studied, nor included as an option in many discussions of waste management. Beyond measuring arsenic concentrations, only a few studies have characterized the chemical composition of pond water in arsenic affected areas (Yokota *et al.* 2001, Neumann *et al.* 2009, Knappett *et al.* 2011). These studies have reported arsenic concentrations (0-40 µg/L), dissolved oxygen concentrations (0.006 – 0.047 mM, 0.2-1.5 mg/L), pH (7.5-9), and organic carbon concentrations (~1 mM, 12 mg/L) in pond waters in Bangladesh (Yokota *et al.* 2001, Neumann *et al.* 2009, Knappett *et al.* 2011). These concentrations vary with the types of activities in and nearby the ponds, including the location of latrines and the use of ponds for bathing or growing fish

(Knappett *et al.* 2011). Future research should focus on improving our understanding of this environment and monitor ponds in which arsenic-bearing waste is disposed.

Only two studies have simulated the fate of arsenic-bearing wastes disposed in ponds. In one study, arsenic wastes were mixed with sewage and pond sediments and incubated anaerobically for one year (Badruzzaman 2003). Arsenic was detected in the aqueous phase only during the first twelve weeks (Badruzzaman 2003). Methylation and volatilization of arsenic was proposed as a removal mechanism, although gaseous samples were not taken (Badruzzaman 2003). During long-term disposal simulations, pond water, distilled water, rainwater, and groundwater were compared as leachant solutions for columns of adsorbent media waste (Ali *et al.* 2003). Pond water resulted in the lowest release of arsenic, but the composition of each leachant was not reported, making it difficult to understand the cause of the variation (Ali *et al.* 2003). Future research should combine improved chemical characterization of pond waters with lab and field studies of arsenic-bearing waste disposal in ponds.

2.4.6 Direct soil disposal

In many areas of developing countries without access to engineered landfills, one of the most common waste disposal strategies is direct disposal on soil (Ali *et al.* 2003, Grimshaw and Beaumont 2004, Sullivan *et al.* 2010). While scant research exists on the fate of arsenic from drinking water treatment wastes disposed on soil, it is the recommended disposal strategy for waste generated by the SONO filter (Hussam *et al.* 2008). However, critics have stressed the need for further analyses of these solids and the fate of arsenic under field disposal conditions (Grimshaw and Beaumont 2004, Adel and Husain 2008). Arsenic release and contamination of surface water sources has been

mentioned as a primary concern with the direct disposal of arsenic-bearing wastes on soils. Furthermore, direct soil disposal may lead to arsenic uptake by crops and fish, increasing the potential arsenic exposure for people in arsenic affected areas (Grimshaw and Beaumont 2004, Watanabe *et al.* 2004, Khan *et al.* 2009). Studies of rice grown in areas with high arsenic soil concentrations and irrigated with arsenic contaminated water highlight this threat to public health (Meharg and Rahman 2003, Williams *et al.* 2006). Using arsenic-free water sources along with aerobic irrigation has been suggested to reduce this risk (Williams *et al.* 2006), and has been found to decrease arsenic availability for rice plant uptake (Xu *et al.* 2008).

To date no studies have been conducted to simulate direct soil disposal. Researchers have only performed regulatory testing procedures, primarily the TCLP, on arsenic-bearing solids, which have not been shown to accurately predict long-term leaching in these environments. While in some cases arsenic-bearing wastes from drinking water treatment have concentrations similar to or below the concentration of arsenic in surrounding soil, the potential for release from these solids may not be the same and should be evaluated. One study of actual disposal environments used the TCLP to test soils and cow dung in areas where arsenic wastes had been disposed (Eriksen-Hamel and Zinia 2001). However, this approach did not include a mass balance or monitor arsenic from the waste over time making it difficult to draw conclusions about the ultimate fate of arsenic from drinking water wastes. Because regulatory testing procedures were developed and intended to provide information for landfill disposal scenarios, further research is needed to understand if they are suitable to predicting the potential for arsenic release from arsenic solid wastes disposed directly onto soils.

2.5 Arsenic waste testing procedures

While disposal practices vary among countries, testing procedures for determining arsenic leaching potential of solid wastes are more often shared. These testing procedures aim to quantify the potential for arsenic leaching in disposal environments. The most common test is the U.S. Environmental Protection Agency (EPA) toxicity characteristic leaching procedure (TCLP) (U.S. EPA 1992). Leaching tests, such as the TCLP, quantify the extent of extraction of arsenic from a solid waste using a single solution. In most cases, the solution is chosen to cause leaching of contaminants at a similar lever or to a greater extent than the leaching expected in the disposal environment. However, this is difficult given the high number of contaminants of interest and their varied leaching behavior (Hooper *et al.* 1998). Many of these tests, including the TCLP, are used to categorize wastes as either hazardous, requiring disposal in a hazardous waste landfill, or non-hazardous, suitable for discarding in municipal solid waste landfills, depending on the resulting concentration of contaminant in the leachate. In developing countries, wastes classified as suitable for non-hazardous landfill disposal are often claimed to pose no concern for disposal conditions that differ greatly from landfills (Eriksen-Hamel and Zinia 2001, Ali *et al.* 2003, Hussam and Munir 2007). In non-landfill disposal environments, such leaching tests may not adequately cover the range of conditions that can result in arsenic leaching. As such, more research is needed to verify the suitability for assessing arsenic release over the range of conditions that may occur upon direct land disposal.

An overview of the most commonly applied testing procedures is given in Table 2-1. Most of these leaching tests have been developed for the purpose of regulating waste

disposal in landfills for a variety of contaminants. Each test has slightly different conditions that affect predicted leaching and are used in different countries for regulations and recommendations for waste disposal.

Table 2-1. Testing Procedures for Determining Disposal of Arsenic Wastes

Testing Procedure	Application	Extraction Liquid	pH	Duration (hours)	Leachant to solid mass ratio	Reference
Toxicity characteristic leaching procedure (TCLP)	U.S. EPA, hazardous material classification and suitability for landfill disposal	Acetic acid (0.1 M)	5	18	20:1	(U.S. EPA 1992)
California waste extraction test (Cal WET)	California, hazardous material classification and suitability for landfill disposal	Citric acid (0.2 M)	5	48	10:1	(U.S. EPA 2003)
Synthetic precipitation leaching procedure (SPLP)	U.S., Non-regulatory, simulation of leaching in rainwater	Distilled water with nitric acid and sulfuric acid [#]	4.2 or 5	18	20:1	(U.S. EPA 1994)
Total available leaching procedure (TALP) or Maximum availability leaching test	European Union, hazardous material characterization	Distilled water with nitric acid[†]	4 and 7	3	50:1	(Environment Agency 2005)
Korean waste standard test	Korea, soils and solid waste	Heated 1:1 (w/w) nitric acid and hydrochloric acid	not adjusted	2	unspecified	(Lim *et al.* 2009)
pH-stat	European Union, waste characterization	Distilled water with nitric acid[†]	4	24	10:1	(Oberman n and Cremer 1992)
German leaching test (DIN 38414)	Germany, stabilized waste	Distilled water	not adjusted	24	10:1	(Dutre and Vandecasteele 1995)
United Kingdom leaching test	U.K., soils and solid waste	Distilled water	not adjusted	1	5:1	(Hartley *et al.* 2004)
America Society of	U.S., soils and solid waste	Distilled water	not adjusted	48	4:1	(Eisenberg

Testing and Materials (ASTM) test						*et al.* 1986)
BS EN 12457	European Union, hazardous material classification and suitability for landfill disposal	Distilled water	not adjusted	24	10:1	(BS EN 2002)
Japanese standard procedure for leaching test	Japan, municipal solid waste	Distilled water	not adjusted	6	10:1	(Environment Agency of Japan 1973)
modified Dutch column test	European Union, soils	Distilled water with nitric acid (0.1 mM)	4	504	continuous flow	(Hartley *et al.* 2004)
ANS 16.1 Short-term leaching test	U.S., low level nuclear waste and stabilized waste	Acetic acid (0.014 M)	3.25	2160	semi-batch	(ANS 1986, Moon and Dermatas 2007)

[†]Acid concentration not reported because it varies with wastes, for these tests the pH reported is controlled after the addition of the waste.

[#]Nitric and sulfuric acid concentrations depend on the location relative to the Mississippi River, for locations east of the Mississippi River the extraction fluid has pH 4.2 (0.038 mM nitric acid and 0.013 mM sulfuric acid). Tests for locations west of the Mississippi River use pH 5 (0.006 mM nitric acid and 0.002 mM sulfuric acid).

2.5.1 TCLP Toxicity Characteristic Leaching Procedure (TCLP)

The TCLP is the most widely used test for arsenic-bearing wastes around the world. In the U.S., the EPA regulates hazardous waste based on the TCLP. Specifically, TCLP leachate concentrations of arsenic greater than 5 mg/L require disposal in a hazardous waste landfill (U.S. EPA 1992). When the leachate arsenic concentration is below 5 mg/L, the waste is classified as non-hazardous and suitable for disposal in a municipal solid waste landfill.

As a test for landfill disposal of arsenic-bearing wastes, the TCLP may not be ideal. Several studies have shown the TCLP to under-predict the leaching of arsenic under landfill conditions (Hooper *et al.* 1998, Ghosh *et al.* 2004, Jing *et al.* 2008). Additionally, reported results from the TCLP show large variation for different types of arsenic-bearing wastes, including drinking water treatment wastes (Table S1, Appendix B). None of the reported studies include TCLP leachate concentrations greater than 5 mg/L, the U.S. EPA regulatory limit for non-hazardous waste classification. The leachate limit in a test similar to the TCLP used in Australia is 0.7 mg/L, and this value is also rarely exceeded (Environment Australia 2002).

Results from TCLP tests on drinking water treatment waste from Table S1 (Appendix B) are plotted in Figure 2-2 as the arsenic concentration in the TCLP leachate versus the corresponding arsenic solids concentrations. No obvious relationship exists between arsenic solids concentration and the TCLP leachate concentration. This could be expected due to different waste generation conditions, variations in the headspace volume to leachant volume ratios used in the TCLP tests, and total iron concentration of the media. However, even when comparing the same waste types within a single study, orders of magnitude differences in arsenic waste concentrations result in only small changes in TCLP leachate concentration. This analysis suggests that TCLP test results may not accurately reflect the increased risk for leaching from

wastes with higher arsenic solids concentrations since actual disposal environments may not be well accounted for by conditions in the TCLP as noted below.

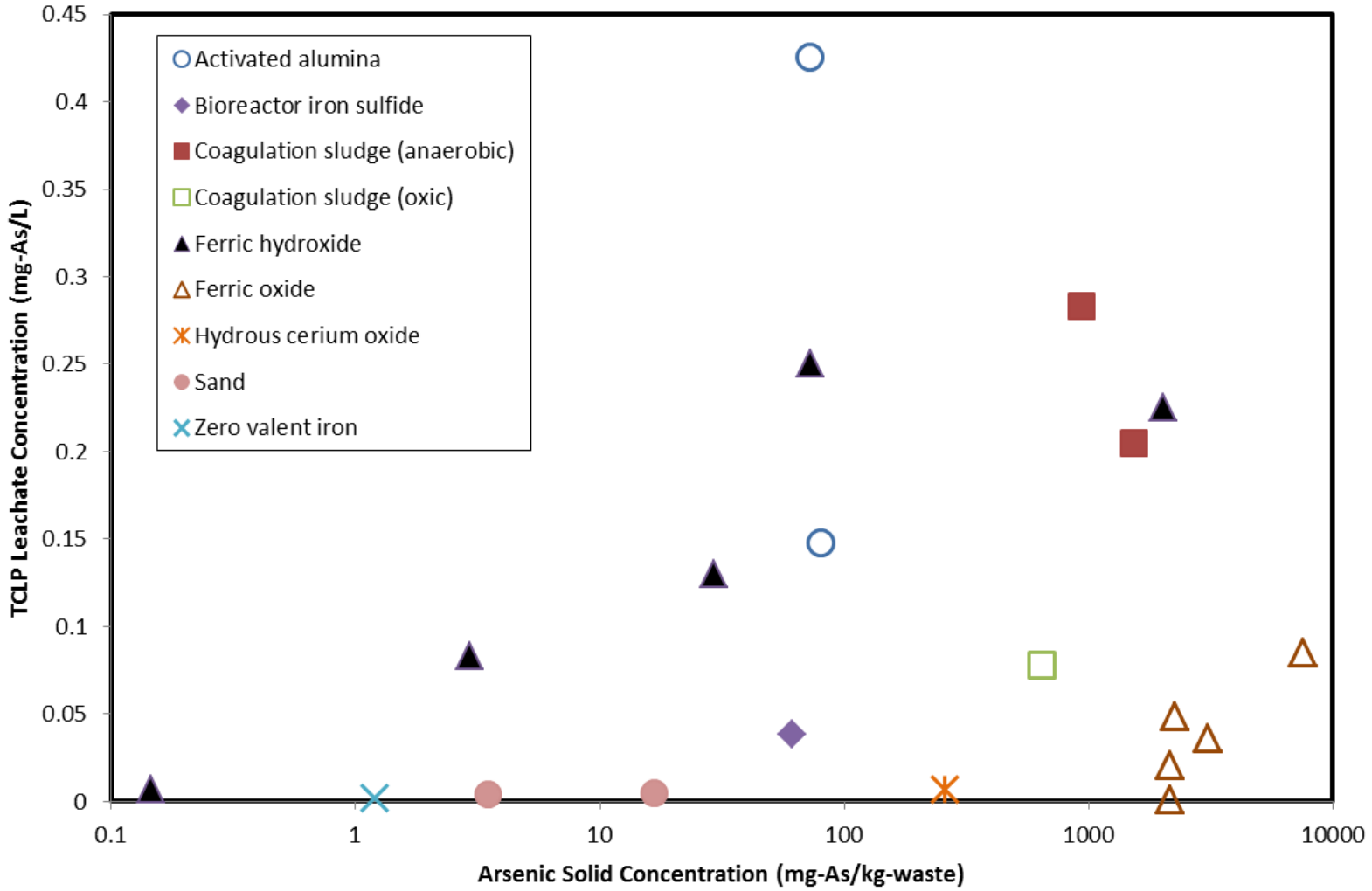


Figure 2-2. TCLP leachate concentrations versus arsenic concentration in waste solids from drinking water treatment systems. (Hooper *et al.* 1998, Meng *et al.* 2001b, Hossain 2003, Banerjee and Chakraborty 2005, Jong and Parry 2005, Choi *et al.* 2009, Stuckman *et al.* 2011)

2.5.2 Other tests

Several alternative tests have been developed and used to test wastes for regulations and research. In the European Union, the total available leaching procedure (TALP) or maximum availability test is used for waste characterization (Environment Agency 2005) and the BS EN 12457 is used for classification of wastes for hazardous and non-hazardous waste landfill disposal (BS EN 2002, Zandi *et al.* 2007). In California, the TCLP and the California waste extraction test (Cal WET) have been implemented to classify wastes as hazardous or non-hazardous (U.S. EPA 2003). Another, less commonly applied test is the U.S. EPA synthetic precipitation leaching procedure (SPLP) used to mimic leaching in rainwater (U.S. EPA 1994). The German test (DIN 38414) is used primarily for the evaluation of leaching from stabilization disposal of wastes (Perera *et al.* 2005). The pH-stat test has also been used in Europe and measures arsenic concentration over time while maintaining a constant pH (Obermann and Cremer 1992, Förstner and Haase 1998). Other tests that have been used to evaluate arsenic leaching, but have not been widely used for arsenic-bearing wastes, and include a test for comparing soil leaching (Hartley *et al.* 2004), the Japanese standard procedure for leaching test (Environment Agency of Japan 1973, Hussainuzzaman and Yokota 2006), and the Korean waste standard test (Lim *et al.* 2009).

While all previously mentioned leaching tests are performed in batch mode, with the same leachant for a set duration of time, leachant replacement tests have also been used. The modified Dutch column method uses a flow-through column and has been used to compare arsenic leaching from soils (Hartley *et al.* 2004). Another kind of leachant replacement test is modeled after tests for low-level nuclear wastes for stabilized solids

and is operated in a semi-batch mode where the leachant is replaced after intervals of batch leaching (ANS 1986, Moon and Dermatas 2007). While these tests have not been widely applied to arsenic-bearing drinking water treatment wastes, they offer alternatives to current tests.

2.5.3 Extraction liquids

Acid-based extraction liquids are most commonly used for leaching tests. Their use has been justified based on the presence of acids in landfills (Halim *et al.* 2003, Environment Agency 2005). Acetic acid is used in the TCLP, while citric acid is used in the Cal WET (U.S. EPA 2003). The Cal WET measured higher arsenic leaching than the TCLP and a leaching test using municipal solid waste leachate (Hooper *et al.* 1998, Jing *et al.* 2005). This is likely due to citrate chelation of iron following the dissolution of arsenopyrites and subsequent mobilization of arsenic (Hooper *et al.* 1998) and increased sorbent dissolution (Jing *et al.* 2005). The TALP uses distilled water with nitric acid at pH 4 and 7 and is intended to predict leaching potential under landfill conditions (Environment Agency 2005).

In the SPLP, nitric and sulfuric acids are used in appropriate concentrations to mimic acid rain (U.S. EPA 1994, New Jersey Department of Environmental Protection 2008). In comparison to the TCLP and Cal WET, the arsenic leaching potential measured by the SPLP is typically lower (Hooper *et al.* 1998, Choi *et al.* 2009). This test may provide a more realistic assessment of arsenic release under environmental disposal conditions, such as soil disposal, for which arsenic-bearing wastes come into direct contact with rainwater. The extraction liquid in the TALP has also been modified to mimic local rainwater and used in an evaluation of SONO filter waste (Hussam and

Munir 2007). Arsenic leaching results using this modified TALP were reported to be similar or lower than the corresponding TCLP measurements (Hussam and Munir 2007). While these tests may be accurate predictors of the potential for arsenic release in rainwater, environmental conditions in soil or pond water disposals are likely to be more complex and include microbially mediated release of arsenic from wastes.

The pH-stat test uses distilled water and titration with nitric acid and sodium hydroxide to keep the solution at a set pH, typically pH 4, with samples taken at different times during the test (Obermann and Cremer 1992). The test has been applied to soils and building wastes (Herreweghe and Swennen 2002, Cappuyns and Swennen 2008) and drinking water treatment wastes (Förstner and Haase 1998). In the only comparison with other leaching tests, the pH-stat test resulted in lower arsenic release than the TCLP (Cappuyns and Swennen 2008). Distilled water with nitric acid at pH 4 was used in the modified Dutch column test and resulted in much higher arsenic leaching compared to the UK Environmental Agency test and the ASTM test, both of which use distilled water as the extraction liquid (Hartley *et al.* 2004). In the Korean standard waste test, concentrated nitric and hydrochloric acids are heated, resulting in a measurement close to the total amount of arsenic in a waste (Lim *et al.* 2009). If the aim is to provide a conservative measure of the potential for release under unknown or uncharacterized disposal conditions, the Korean standard waste test or other total digestions may be more appropriate.

Alternatively, distilled water without pH adjustment is used in several leaching tests. The DIN 38414 test uses distilled water and has primarily been used to test stabilization treatments of arsenic waste and results in arsenic concentrations similar to

the TCLP, but lower than the Cal WET (Dutre and Vandecasteele 1995, Banerjee and Chakraborty 2005, Perera *et al.* 2005). The UK Environmental Agency leaching test and ASTM tests also use distilled water, but have primarily been used for testing soils (Hartley *et al.* 2004). Without pH adjustment, the pH of these tests depends on the properties of each waste and is likely to greatly affect the measured arsenic leaching potential. This is not ideal for predicting leaching in disposal environments, as in most disposal conditions the overall pH will not be dependent on the waste, but will be dictated by the landfill leachate, pond water, or soils.

2.5.4 Test duration

By design, leaching tests attempt to estimate the leaching potential of a waste in a time period much shorter than that of disposal. The tests described here range in duration from 1 hour to 13 weeks. The 18 hour TCLP extraction has been tested for longer periods (up to 84 hours) and greater arsenic leaching has been found for longer test durations (Hooper *et al.* 1998, Stuckman *et al.* 2011). The duration of the pH-stat test has been recommended to be increased from 24 hours to 96 hours to better estimate the maximum leaching potential (Paschke *et al.* 1999, Herreweghe and Swennen 2002). Increasing the duration of these tests may be helpful in generating more conservative predictions of long-term leaching. However, future work should link these leaching test measurements with actual leaching measurements under field conditions.

2.5.5 Criticisms of testing procedures

The most common criticism of the currently used leaching tests is that air is used as the headspace gas to predict long-term leaching behavior under anaerobic disposal conditions found in landfills (Hooper *et al.* 1998, Leist *et al.* 2000, Meng *et al.* 2001b,

Halim *et al.* 2003, Hartley *et al.* 2004, Jong and Parry 2005, Sullivan *et al.* 2010). The use of air as a headspace gas has been shown to result in lower arsenic leachate concentrations as compared to a nitrogen headspace for oxidized arsenic-bearing wastes (Ghosh *et al.* 2004). The volume of the headspace gas is not specified in leaching tests and is of concern for reduced arsenic-bearing wastes. Comparisons of the TCLP test for reduced arsenic-bearing wastes found that higher air headspace volumes resulted in lower arsenic concentrations in the leachate (Meng *et al.* 2001b, Jong and Parry 2005). This is consistent with the oxidation of iron and increased arsenic stability in solid phases. To avoid the variability of different headspace volumes and more accurately account for anaerobic landfill conditions, nitrogen purged bottles or bottles without headspace have been recommended for performing TCLP extractions (Meng *et al.* 2001b, Jong and Parry 2005).

In general, leaching tests based on extractions with a single liquid at a static pH and redox condition may be poor predictors of a waste's long-term leaching potential, since they ignore the changing conditions encountered in disposal environments (Kjeldsen *et al.* 2002). Leachant replacement tests are one option for evaluating changing conditions and may more accurately simulate the introduction of fresh leaching liquids in landfills or other disposal environments. A series of tests with different leachants and different headspace gases may also be helpful to better assess leaching potential under changing pH and redox conditions in disposal environments. Additionally, tests should include cycles of wetting and drying for arsenic-bearing solid wastes that are exposed to environmental conditions through direct dumping on soils or through the use of concrete containing arsenic-bearing wastes.

Presently, there are no short term tests to evaluate the potential for microbial activity to enhance the release of arsenic from arsenic-bearing wastes. However, as described earlier, in the presence of organic carbon, microbial activity in landfills, cow dung, soils, and ponds can be a driving factor affecting the release of arsenic (Ali *et al.* 2003, Pinel-Raffaitin *et al.* 2007, Cortinas *et al.* 2008). Future research could be used to develop tests to evaluate the stability of wastes under conditions with stimulated microbial activity that are representative of these environments. Tests should use realistic organic carbon concentrations and microbial seeds from relevant environments to accurately predict the potential for leaching in these conditions.

All the tests described here aim to assess the long-term leaching potential of a waste. However, for some disposal options, a mass-based loading standard (mass of arsenic applied to an area over a given time) may be more appropriate. Mass-based loading limits for a variety of pollutants are used in the U.S. to regulate land application of biosolids generated during wastewater treatment (U.S. EPA 2007). These regulations may be relevant for direct environmental disposal of arsenic waste including soil and pond disposal. They state that the limit for the total mass of arsenic applied to fields through biosolids is 41 kg/hectare and the annual maximum application limit is 2 kg/hectare (U.S. EPA 2007). Since mass-based loading standards do not take into account waste-specific characteristics, like speciation and leaching potential, these standards may overestimate the risks posed by some wastes. However, given that leaching tests have not been shown to accurately measure long-term leaching potential for direct environmental disposal conditions, this more conservative approach may be warranted.

2.6 Recommendations and Research Needs

Arsenic-bearing wastes should be disposed in environments that limit the potential risks for release of arsenic to the environment and contamination of water and food sources. Ideal environments are those that are similar to the conditions under which arsenic was removed, for example containment in a passive aeration system for oxidized arsenic-bearing wastes. Additionally, given the ability of microbially mediated transformations of arsenic and iron, wastes should be stored in environments with limited microbial activity. Concrete stabilization might provide such a condition. However, concrete that incorporates arsenic should be used only in applications with minimal exposure to microbial activity. Future research should explore the changing conditions that occur in landfills and other disposal environments over both time and space. Particularly lacking are simulations of non-landfill disposal conditions relevant for developing countries. Studies should include characterization of chemical and microbial changes during disposal. Most importantly, the results from such simulation experiments should be incorporated into recommendations for appropriate disposal and for developing improved regulatory testing procedures.

Short-term testing procedures used for regulations and disposal recommendations can be useful in predicting the relative potential for leaching, but should be modified to represent the conditions of disposal environments to more accurately assess the long-term leaching potential of wastes. To evaluate the long-term leaching potential of arsenic-bearing wastes in anaerobic landfill environments, regulatory tests should include the use of zero-headspace and nitrogen purged vessels, to better simulate the reducing conditions. Sequential leachant cycle tests mimicking the different stages in a landfill may also be

useful for better predicting long-term arsenic release. For non-landfill disposal conditions, more information about the fate of arsenic for different disposal scenarios is needed to develop tests that accurately measure the potential for arsenic leaching. Until more is known about arsenic leaching under different and variable environmental conditions representative of various disposal options, regulations based on the total mass loading of arsenic, such as those used for land application of biosolids, may be a more conservative approach.

2.7 Literature Cited

Adel, M. M. and M. Husain (2008). Sono Filter Waste Disposals Contradict Safe Environmental Regulations. UNESCO-Sponsored Conference on Water Scarcity, Global Changes and Groundwater Management Responses. Irvine, California, University of California at Irvine: 1-21.

Alam, M. G., G. Allinson, F. Stagnitti, A. Tanaka and M. Westbrooke (2002). Arsenic contamination in Bangladesh groundwater: a major environmental and social disaster. Int. J. Environ. Health Res. 12(3): 235-253.

Ali, M. A., A. B. M. Badruzzaman, M. A. Jalil, M. F. Ahmed, M. Kamruzzaman, M. A. Rahman and A. Al Masud (2003). Fate of Arsenic in Wastes Generated From Arsenic Removal Units. Proceedings of BUET-UNU Symposium on Fate of Arsenic in the Environment, Dhaka.

Anawar, H. M., J. Akai, T. Yoshioka, E. Konohira, J. Y. Lee, H. Fukuhara, M. Tari Kul Alam and A. Garcia-Sanchez (2006). Mobilization of arsenic in groundwater of Bangladesh: evidence from an incubation study. Environ. Geochem. Health 28(6): 553-565.

ANS (1986). Measurements of the leachability of solidified low-level radioactive wastes by a short-term test procedure. Standard ANSI/ANS 16.1. American Nuclear Society. La Grange Park, Illinois.

Badruzzaman, A. B. M. (2003). Leaching of Arsenic from Wastes of Arsenic Removal Systems. Fate of Arsenic in the Environment. M. F. Ahmed, Bangladesh University of Engineering and Technology, The United Nations University.

Banerjee, G. (2010). Treatment of Arsenic-Laden Water Plant Sludge by Anaerobic Digestion. Pract. Period. Hazard., Toxic, Radioact. Waste Manage. 14: 124-131.

Banerjee, G. and R. Chakraborty (2005). Management of arsenic-laden water plant sludge by stabilization. Clean Technol. Environ. Policy 7(4): 270-278.

Battaglia-Brunet, F., C. Crouzet, D. Breeze, H. Tris and D. Morin (2011). Decreased leachability of arsenic linked to biological oxidation of As(III) in solid wastes from bioleaching liquors. Hydrometallurgy 107(1-2): 34-39.

Bentley, R. and T. G. Chasteen (2002). Microbial methylation of metalloids: arsenic, antimony, and bismuth. Microbiol. Mol. Biol. Rev. 66(2): 250-250.

Bissen, M. and F. H. Frimmel (2003a). Arsenic – a Review. Part I : Occurrence , Toxicity, Speciation, Mobility. Acta Hydrochim. Hydrobiol. 31(1): 9-18.

Bissen, M. and F. H. Frimmel (2003b). Arsenic – a Review. Part II : Oxidation of Arsenic and its Removal in Water Treatment. Acta Hydrochim. Hydrobiol. 31(2): 97-107.

Bostick, B. C., S. Fendorf and G. E. Brown Jr (2005). In situ analysis of thioarsenite complexes in neutral to alkaline arsenic sulphide solutions. Mineral. Mag. 69(5): 781-781.

Bright, D. A., S. Brock, K. J. Reimer, W. R. Cullen, G. M. Hewitt and J. Jafaar (1994). Methylation of arsenic by anaerobic microbial consortia isolated from lake sediment. Appl. Organomet. Chem. 8(4): 415-422.

BS EN (2002). 12457, Characterisation of waste leaching compliance test for leaching of granular waste materials and sludges. British Standard, UK.

Burton, E. D., S. G. Johnston and R. T. Bush (2011). Microbial sulfidogenesis in ferrihydrite-rich environments: Effects on iron mineralogy and arsenic mobility. Geochim. Cosmochim. Acta 75(11): 3072-3087.

Caetano, M., M.-J. Madureira and C. Vale (2003). Metal remobilisation during resuspension of anoxic contaminated sediment: Short-term laboratory study. Water, Air, Soil Pollut. 143: 23-40.

Camacho, J., H.-Y. Wee, T. A. Kramer and R. Autenrieth (2009). Arsenic stabilization on water treatment residuals by calcium addition. J. Hazard. Mater. 165(1-3): 599-603.

Cappuyns, V. and R. Swennen (2008). "Acid extractable" metal concentrations in solid matrices: a comparison and evaluation of operationally defined extraction procedures and leaching tests. Talanta 75(5): 1338-1347.

Caussy, D. and N. D. Priest (2008). Introduction to Arsenic Contamination and Health Risk Assessment with Special Reference to Bangladesh. Reviews of Environmental Contamination. H. Garelick and H. Jones. New York, Springer. 197: 1-15.

Chakraborti, D., M. M. Rahman, B. Das, M. Murrill, S. Dey, S. C. Mukherjee, R. K. Dhar, B. K. Biswas, U. K. Chowdhury and S. Roy (2010). Status of groundwater arsenic contamination in Bangladesh: A 14-year study report. Water Res. 44(19): 5789-5802.

Challenger, F. (1945). Biological methylation. Chem. Rev.

Choi, W. H., S. R. Lee and J. Y. Park (2009). Cement based solidification/stabilization of arsenic-contaminated mine tailings. Waste Manage. 29(5): 1766-1771.

Choong, T., T. Chuah, Y. Robiah, F. Gregorykoay and I. Azni (2007). Arsenic toxicity, health hazards and removal techniques from water: an overview. Desalination 217(1-3): 139-166.

Christensen, T. H. and P. Kjeldsen (1995). Landfill emissions and environmental impact: An introduction. SARDINIA '95 Fifth International Landfill Symposium, Proceedings. T. H. Christensen, R. Cossu and R. Stegmann. Cagliari, Italy, CISA. 3.

Cornwall, D. (2003). Disposal of Waste Resulting From Arsenic Removal Processes, International Water Association.

Corsini, A., L. Cavalca, P. Zaccheo, L. Crippa and V. Andreoni (2011). Influence of microorganisms on arsenic mobilization and speciation in a submerged contaminated soil: Effects of citrate. Appl. Soil Ecol. 49: 99-106.

Cortinas, I., R. Sierra-Alvarez and J. A. Field (2008). Biologically mediated mobilization of arsenic from granular ferric hydroxide in anaerobic columns fed landfill leachate. Biotechnol. Bioeng. 101(6): 1205-1213.

Cullen, W. R. and R. Bentley (2005). The toxicity of trimethylarsine: an urban myth. J. Environ. Monit.: 11-15.

Das, B., M. M. Rahman, B. Nayak, A. Pal, U. K. Chowdhury, S. C. Mukherjee, K. C. Saha, S. Pati, Q. Quamruzzaman and D. Chakraborti (2009). Groundwater Arsenic Contamination, Its Health Effects and Approach for Mitigation in West Bengal, India and Bangladesh. Water Qual Expo Health 1(1): 5-21.

Das, D. (1995). Arsenic Species Along With Other Metal/Metalloids Present and Responsible for Arsenic Episode in Groundwater of West Bengal and a Cheap Technique to Remove Arsenic, Thus Making the Groundwater Suitable for Drinking and Cooking Ph.D. Dissertation, Jadavpur University.

Das, D., A. Chatterjee, G. Samanta, T. R. Chowdhury, B. K. Mandal, R. Dhar, C. R. Chanda, D. Lodh, P. P. Chowdhury, G. K. Basu, B. K. Biswas, U. K. Chowdhury, M. M. Rahman, K. Paul and D. Chakraborti (2001). A Simple Household Device to Remove Arsenic from Groundwater and Two Years Performance Report of Arsenic Removal Plant for Treating Groundwater with Community Participation. Technologies for Arsenic Removal from Drinking Water. M. F. Ahamed, M. A. Ali and Z. Adeel. BUET, Dhaka, The United Nations University, Tokyo.

DeLemos, J. L., B. C. Bostick, C. E. Renshaw, S. Stürup and X. Feng (2006). Landfill-stimulated iron reduction and arsenic release at the Coakley Superfund Site (NH). Environ. Sci. Technol. 40(1): 67-73.

Demirel, B. and P. Scherer (2008). The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. Environ. Sci. Bio/Technol. 7: 173-190.

Dhar, R., B. Biswas, G. Samanta, B. Mandal, D. Chakraborti, S. Roy, A. Jafar, A. Islam, G. Ara, S. Kabir, A. Wadud Khan, S. Akther Ahmed and S. Abdul Hadi (1997). Groundwater arsenic calamity in Bangladesh. Curr. Sci. 73(1): 48-59.

Dixit, S. and J. G. Hering (2003). Comparison of arsenic(V) and arsenic(III) sorption onto iron oxide minerals: implications for arsenic mobility. Environ. Sci. Technol. 37(18): 4182-4189.

Dutre, V. and C. Vandecasteele (1995). Solidification/Stabilisation of Arsenic-Containing Waste: Leach Tests and Behaviour of Arsenic in the Leachate. Waste Manage. 15(1): 55-62.

Edvantoro, B. B., R. Naidu, M. Megharaj, G. Merrington and I. Singleton (2004). Microbial formation of volatile arsenic in cattle dip site soils contaminated with arsenic and DDT. Appl. Soil Ecol. 25(3): 207-217.

Eisenberg, S. H., M. E. Tittlebaum, H. C. Eaton and M. M. Soroczack (1986). Chemical characteristics of selected fly ash leachates. J. Environ. Sci. Health, Part A 21: 383-402.

Environment Agency (2005). Leaching Characteristics of Granular Building and Waste Materials. EA NEN 7371:2004: 1-12.

Environment Agency of Japan (1973). Japanese Environment Agency Notification No.13.

Environment Australia (2002). Guidance on whether wastes containing metals or metal compounds are regulated under the Hazardous Waste Act, Department of the Environment and Heritage.

Eriksen-Hamel, N. and B. K. N. Zinia (2001). A Study of Arsenic Treatment Technologies and Leaching Characteristics of Arsenic Contaminated Sludge. Technologies for Arsenic Removal from Drinking Water. Bangladesh University of Engineering and Technology, Dhaka, Bangladesh, United Nation Univeristy, Tokyo: 207-213.

Ferguson, M. A., M. R. Hoffmann and J. G. Hering (2005). TiO2-Photocatalyzed As(III) Oxidation in Aqueous Suspensions: Reaction Kinetics and Effects of Adsorption. Environ. Sci. Technol. 39(6): 1880-1886.

Förstner, U. and I. Haase (1998). Geochemical demobilization of metallic pollutants in solid waste—implications for arsenic in waterworks sludges. J. Geochem. Explor. 62(1-3): 29-36.

Gallegos, T. J., Y.-S. Han and K. F. Hayes (2008). Model predictions of realgar precipitation by reaction of As(III) with synthetic Mackinawite under anoxic conditions. Environ. Sci. Technol. 42(24): 9338-9343.

Gallegos, T. J., S. P. Hyun and K. F. Hayes (2007). Spectroscopic investigation of the uptake of arsenite from solution by synthetic mackinawite. Environ. Sci. Technol. 41(22): 7781-7786.

Ghosh, A., M. Mukiibi and W. Ela (2004). TCLP underestimates leaching of arsenic from solid residuals under landfill conditions. Environ. Sci. Technol. 38(17): 4677-4682.

Ghosh, A., M. Mukiibi, A. E. Sáez and W. P. Ela (2006a). Leaching of arsenic from granular ferric hydroxide residuals under mature landfill conditions. Environ. Sci. Technol. 40(19): 6070-6075.

Ghosh, A., A. E. Sáez and W. Ela (2006b). Effect of pH, competitive anions and NOM on the leaching of arsenic from solid residuals. Sci. Total Environ. 363(1–3): 46-59.

Ghurye, G. L., D. A. Clifford and A. R. Tripp (1999). Combined arsenic and nitrate removal by ion exchange. J. - Am. Water Works Assoc. 91(10): 85-96.

Government of Bangladesh (2003). Draft Implementation Plan for Arsenic Mitigation in Bangladesh. Dhaka, Bangladesh.

Grimshaw, D. J. and A. E. Beaumont (2004). How can new science-led technologies contribute to mitigation of and adaptation to arsenic in the water of Bangladesh? Science for Humanity, Practical Action: 1-34.

Halim, C. E., R. Amal, D. Beydoun, J. A. Scott and G. Low (2003). Evaluating the applicability of a modified toxicity characteristic leaching procedure (TCLP) for the classification of cementitious wastes containing lead and cadmium. J. Hazard. Mater. 103(1-2): 125-140.

Han, Y.-S., A. H. Demond and K. F. Hayes (2013). Impact of dissolved silica on arsenite removal by nano-particulate FeS and FeS-coated sand. Chemosphere in press.

Han, Y.-S., T. J. Gallegos, A. H. Demond and K. F. Hayes (2011). FeS-coated sand for removal of arsenic(III) under anaerobic conditions in permeable reactive barriers. Water Res. 45(2): 593-604.

Hartley, W., R. Edwards and N. W. Lepp (2004). Arsenic and heavy metal mobility in iron oxide-amended contaminated soils as evaluated by short- and long-term leaching tests. Environ. Pollut. 131(3): 495-504.

Herreweghe, S. V. and R. Swennen (2002). Chemical associations of heavy metals and metalloids in contaminated soils near former ore treatment plants : a differentiated approach with emphasis on pH stat-leaching. J. Geochem. Explor. 76: 113-138.

Holmes, J. (1999). Fate of incorporated metals during mackinawite oxidation in sea water. Appl. Geochem. 14(3): 277-281.

Hooper, K., M. Iskander, G. Sivia, F. Hussein, J. Hsu, M. DeGuzman, Z. Odion, Z. Ilejay, F. Sy, M. Petreas and B. Simmons (1998). Toxicity Characteristic Leaching Procedure Fails To Extract Oxoanion-Forming Elements That Are Extracted by Municipal Solid Waste Leachates. Environ. Sci. Technol. 32(23): 3825-3830.

Hossain, M. A. (2003). Performance Evaluation and Verification of Five Arsenic Removal Technologies. Dhaka, Bangladesh, Bangladesh Council of Scientific and Industrial Research.

Hounslow, A. W. (1980). Ground-Water Geochemistry: Arsenic in Landfills. Ground Water 18(4): 331-333.

Hug, S. J. and O. Leupin (2003). Iron-Catalyzed Oxidation of Arsenic (III) by Oxygen and by Hydrogen Peroxide: pH-Dependent Formation of Oxidants in the Fenton Reaction. Environ. Sci. Technol. 37(12): 2734-2742.

Hughes, M. F. (2002). Arsenic toxicity and potential mechanisms of action. Toxicol. Lett. 133(1): 1-16.

Hussainuzzaman, M. M. and H. Yokota (2006). Efficiency of Arsenic Removal Unit Working in Bangladesh and Cement Stabilization of Its Sludge. J. ASTM Int. 3(7): 1-9.

Hussam, A., S. Ahamed and A. K. M. Munir (2008). Arsenic Filters for Groundwater in Bangladesh: Toward a Sustainable Solution. The Bridge Technologies for Clean Water, International Journal of Environmental Research and Public HealthNational Academy of Engineering.

Hussam, A. and A. K. M. Munir (2007). A simple and effective arsenic filter based on composite iron matrix: Development and deployment studies for groundwater of Bangladesh. J. Environ. Sci. Health, Part A 42: 1869-1878.

Islam, F. S., A. G. Gault, C. Boothman, D. A. Polya, J. M. Charnock, D. Chatterjee and J. R. Lloyd (2004). Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. Nature 430(6995): 68-71.

Islam, M. S., M. A. A. Mamun and M. N. Islam (2011). Leachability of arsenic from wastes of arsenic removal units using modified toxicity characteristic leaching procedure. J. Life Earth Sci. 6: 19-26.

Jeong, H. Y., Y.-S. Han and K. F. Hayes (2010a). X-ray absorption and X-ray photoelectron spectroscopic study of arsenic mobilization during mackinawite (FeS) oxidation. Environ. Sci. Technol. 44(3): 955-961.

Jeong, H. Y., Y.-S. Han, S. W. Park and K. F. Hayes (2010b). Aerobic oxidation of mackinawite (FeS) and its environmental implication for arsenic mobilization. Geochim. Cosmochim. Acta 74(11): 3182-3198.

Jing, C., G. P. Korfiatis and X. Meng (2003). Immobilization mechanisms of arsenate in iron hydroxide sludge stabilized with cement. Environ. Sci. Technol. 37(21): 5050-5056.

Jing, C., J. S. Liu, M. Patel and X. Meng (2005). Arsenic leachability in water treatment adsorbents. Environ. Sci. Technol. 39(14): 5481-5487.

Jing, C., S. Liu and X. Meng (2008). Arsenic remobilization in water treatment adsorbents under reducing conditions: Part I. Incubation study. Sci. Total Environ. 389(1): 188-194.

Jong, T. and D. L. Parry (2003). Removal of sulfate and heavy metals by sulfate reducing bacteria in short-term bench scale upflow anaerobic packed bed reactor runs. Water Res. 37(14): 3379-3389.

Jong, T. and D. L. Parry (2005). Evaluation of the stability of arsenic immobilized by microbial sulfate reduction using TCLP extractions and long-term leaching techniques. Chemosphere 60: 254-265.

Jurič, B., L. Hanžič, R. Ilić and N. Samec (2006). Utilization of municipal solid waste bottom ash and recycled aggregate in concrete. Waste Manage. 26(12): 1436-1442.

Kaksonen, A. H. and J. A. Puhakka (2007). Sulfate Reduction Based Bioprocesses for the Treatment of Acid Mine Drainage and the Recovery of Metals. Eng. Life Sci. 7(6): 541-564.

Kameswari, K. S. B., A. G. Bhole and R. Paramasivam (2001). Evaluation of Solidification/Stabilization (S/S) Process for the Disposal of Arsenic-Bearing Sludges in Landfill Sites. Environ. Eng. Sci. 18(3): 167-176.

Keimowitz, A. R., B. J. Mailloux, P. Cole, M. Stute, H. J. Simpson and S. N. Chillrud (2007). Laboratory Investigations of Enhanced Sulfate Reduction as a Groundwater Arsenic Remediation Strategy. Environ. Sci. Technol. 41(19): 6718-6724.

Khan, M. M. (2009). Governance and Management of Environmental Policies in Bangladesh. Environmental Policy: A Multi-National Conference on Policy Analysis and Teaching Methods. Seol, Korea.

Khan, N. I., G. Owens, D. Bruce and R. Naidu (2009). Human arsenic exposure and risk assessment at the landscape level: a review. Environ. Geochem. Health 31: 143-166.

Kirk, M. F., E. E. Roden, L. J. Crossey, A. J. Brealey and M. N. Spilde (2010). Experimental analysis of arsenic precipitation during microbial sulfate and iron reduction in model aquifer sediment reactors. Geochim. Cosmochim. Acta 74(9): 2538-2555.

Kjeldsen, P., M. Barlaz, A. Rooker, A. Baun, A. Ledin and T. Christensen (2002). Present and Long-Term Composition of MSW Landfill Leachate: A Review. Crit. Rev. Environ. Sci. Technol. 32(4): 297-336.

Klimecki, W. T. and D. E. Carter (1995). Arsine Toxicity: Chemical and Mechanistic Implications. J. Toxicol. Environ. Health 46: 399-409.

Knappett, P. S. K., V. Escamilla, A. Layton, L. D. McKay, M. Emch, D. E. Williams, R. Huq, J. Alam, L. Farhana, B. J. Mailloux, A. Ferguson, G. S. Sayler, K. M. Ahmed and A. van Geen (2011). Impact of population and latrines on fecal contamination of ponds in rural Bangladesh. Sci. Total Environ. 409(17): 3174-3182.

Kocar, B. D., M. J. Herbel, K. J. Tufano and S. Fendorf (2006). Contrasting Effects of Dissimilatory Iron (III) and Arsenic (V) Reduction on Arsenic Retention and Transport. Environ. Sci. Technol. 40(21): 6715-6721.

Kumar, P. R., S. Chaudhari, K. C. Khilar and S. P. Mahajan (2004). Removal of arsenic from water by electrocoagulation. Chemosphere 55(9): 1245-1252.

Kumar, S. and C. B. Patil (2006). Estimation of resource savings due to fly ash utilization in road construction. Resour., Conserv. Recycl. 48(2): 125-140.

Kumpiene, J., A. Lagerkvist and C. Maurice (2008). Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments--a review. Waste Manage. 28(1): 215-225.

Laky, D. and I. Licskó (2011). Arsenic removal by ferric-chloride coagulation--effect of phosphate, bicarbonate and silicate. Water Sci. Technol. 64(5): 1046-1055.

Lee, M.-K., J. A. Saunders, R. T. Wilkin and S. Mohammad (2005). Geochemical Modeling of Arsenic Speciation and Mobilization: Implications for Bioremediation. Advances in Arsenic Research, American Chemical Society. 915: 398-413.

Leist, M., R. J. Casey and D. Caridi (2000). The management of arsenic wastes: problems and prospects. J. Hazard. Mater. 76(1): 125-138.

Leist, M., R. J. Casey and D. Caridi (2003). The fixation and leaching of cement stabilized arsenic. Waste Manage. 23(4): 353-359.

Leupin, O. X. and S. J. Hug (2005). Oxidation and removal of arsenic (III) from aerated groundwater by filtration through sand and zero-valent iron. Water Res. 39(9): 1729-1740.

Lièvremont, D., P. N. Bertin and M.-C. Lett (2009). Arsenic in contaminated waters: biogeochemical cycle, microbial metabolism and biotreatment processes. Biochimie 91(10): 1229-1237.

Lim, M., G.-C. Han, J.-W. Ahn, K.-S. You and H.-S. Kim (2009). Leachability of arsenic and heavy metals from mine tailings of abandoned metal mines. Int. J. Environ. Res. Public Health 6(11): 2865-2879.

Liu, S., F. Zhang, J. Chen and G. Sun (2011). Arsenic removal from contaminated soil via biovolatilization by genetically engineered bacteria under laboratory conditions. J. Environ. Sci. 23(9): 1544-1550.

Luo, Q., T. K. Tsukamoto, K. L. Zamzow and G. C. Miller (2008). Arsenic, Selenium, and Sulfate Removal using an Ethanol-Enhanced Sulfate-Reducing Bioreactor. Mine Water Environ. 27(2): 100-108.

Mahzuz, H. M. A., R. Alam, M. N. Alam, R. Basak and M. S. Islam (2009). Use of arsenic contaminated sludge in making ornamental bricks. Int. J. Environ. Sci. Tech. 6(2): 291-298.

Martínez-Villafañe, J. F., C. Montero-Ocampo and A. M. García-Lara (2009). Energy and electrode consumption analysis of electrocoagulation for the removal of arsenic from underground water. J. Hazard. Mater. 172(2-3): 1617-1622.

Masue-Slowey, Y., S. C. Ying, B. D. Kocar, C. E. Pallud and S. Fendorf (2013). Dependence of Arsenic Fate and Transport on Biogeochemical Heterogeneity Arising from the Physical Structure of Soils and Sediments. J. Environ. Qual. 42(4): 1119-1129.

Meharg, A. A. and M. M. Rahman (2003). Arsenic contamination of Bangladesh paddy field soils: implications for rice contribution to arsenic consumption. Environ. Sci. Technol. 37(2): 229-234.

Meng, X., G. P. Korfiatis, C. Christodoulatos and S. Bang (2001a). Treatment of arsenic in Bangladesh well water using a household co-precipitation and filtration system. Water Res. 35(12): 2805-2810.

Meng, X., G. P. Korfiatis, C. Jing and C. Christodoulatos (2001b). Redox transformations of arsenic and iron in water treatment sludge during aging and TCLP extraction. Environ. Sci. Technol. 35(17): 3476-3481.

Mestrot, A., M. K. Uroic, T. Plantevin, M. R. Islam, E. M. Krupp, J. Feldmann and A. A. Meharg (2009). Quantitative and qualitative trapping of arsines deployed to assess loss of volatile arsenic from paddy soil. Environ. Sci. Technol. 43(21): 8270-8275.

Mestrot, A., W.-Y. Xie, X. Xue and Y.-G. Zhu (2013). Arsenic volatilization in model anaerobic biogas digesters. Appl. Geochem. 33(0): 294-297.

Metcalfe, I. (2003). Environmental concerns for Bangladesh*. S Asia 26(3): 423-438.

Michalke, K., E. B. Wickenheiser, M. Mehring, A. V. Hirner and R. Hensel (2000). Production of volatile derivatives of metal(loid)s by microflora involved in anaerobic digestion of sewage sludge. Appl. Environ. Microbiol. 66(7): 2791-2796.

Miller, S. M. and J. B. Zimmerman (2010). Novel, bio-based, photoactive arsenic sorbent: TiO2-impregnated chitosan bead. Water Res. 44(19): 5722-5729.

Mohan, D. and C. U. Pittman (2007). Arsenic removal from water/wastewater using adsorbents--A critical review. J. Hazard. Mater. 142(1-2): 1-53.

Mohapatra, D., D. Mishra, G. R. Chaudhury and R. P. Das (2008). Removal of Arsenic from Arsenic Rich Sludge by Volatilization Using Anaerobic Microorganisms Treated with Cow Dung. Soil Sediment Contam. 17(3): 301-311.

Moon, D. H. and D. Dermatas (2007). Arsenic and lead release from fly ash stabilized/solidified soils under modified semi-dynamic leaching conditions. J. Hazard. Mater. 141(2): 388-394.

Moon, D. H., D. Dermatas and N. Menounou (2004). Arsenic immobilization by calcium–arsenic precipitates in lime treated soils. Sci. Total Environ. 330(1–3): 171-185.

Mudgal, A. K. (2001). Draft Review of the Household Arsenic Removal Technology Options: 1-18.

Mukherjee, A., M. K. Sengupta, M. A. Hossain, S. Ahamed, D. Lodh, B. Das, B. Nayak, K. C. Saha, S. C. Mukherjee, S. Pati, R. N. Dutta, G. Chatterjee and D. Chakraborti (2005). Are some animals more equal than others? Toxicology 208(1): 165-169.

Nakwanit, S., P. Visoottiviseth, S. Khokiattiwong and W. Sangchoom (2011). Management of arsenic-accumulated waste from constructed wetland treatment of mountain tap-water. J. Hazard. Mater. 185(2-3): 1081-1085.

Neumann, R. B., K. N. Ashfaque, A. B. M. Badruzzaman, M. Ashraf Ali, J. K. Shoemaker and C. F. Harvey (2009). Anthropogenic influences on groundwater arsenic concentrations in Bangladesh. Nat. Geosci. 3(1): 46-52.

New Jersey Department of Environmental Protection (2008). Guidance for the use of the Synthetic Precipitation Leaching Procedure to Develop Site-Specific Impact to Ground Water Remediation Standards.

Ng, J. C., J. Wang and A. Shraim (2003). A global health problem caused by arsenic from natural sources. Chemosphere 52(9): 1353-1359.

O'Day, P. A., D. Vlassopoulos, R. Root and N. Rivera (2004). The influence of sulfur and iron on dissolved arsenic concentrations in the shallow subsurface under changing redox conditions. Proc. Natl. Acad. Sci. U. S. A. 101(38): 13703-13708.

Obermann, P. and S. Cremer (1992). Mobilisierung von Schwermetallen in Porenwassern von belasteten Boden und Deponien. Mater. Ermittlung Sanierung Altlasten 6(Landesamt fur Wasser und Abfall Nordrhein-Westfalen, Germany).

Olmo, I. F., E. Chacon and A. Irabien (2001). Influence of lead, zinc, iron (III) and chromium (III) oxides on the setting time and strength development of Portland cement. Cem. Concr. Res. 31(8): 1213-1219.

Oremland, R. S. and J. F. Stolz (2003). The Ecology of Arsenic. Science 300: 939-944.

Palmisano, A. C. and M. A. Barlaz (1996). Microbiology of Solid Waste, CRC.

Pantuzzo, F. L. and V. S. T. Ciminelli (2010). Arsenic association and stability in long-term disposed arsenic residues. Water Res. 44(19): 5631-5640.

Paria, S. and P. K. Yuet (2006). Solidification-stabilization of organic and inorganic contaminants using portland cement: a literature review. Environ. Rev. 14(4): 217-255.

Paschke, A., R. Wennrich and P. Morgenstern (1999). Comparison of 24 h and Long-term pH stat Leaching Tests for Heavy Metal Mobilization from Solid Matrices. Acta Hydrochim. Hydrobiol. 27(4): 223-229.

Patterson, G. (2006). Current Issues--High Efficiency Ion Exchange Technology Brings Arsenic Compliance to Baldy Mesa Water District. J. - Am. Water Works Assoc. 98(4): 26-28.

Perera, A. S. R., A. Al-Tabbaa, J. M. Reid and J. A. Stegemann (2005). State of practice reports UK Stabilisation/Solidification Treatment and Remediation Part IV: Testing and Performance criteria. Proceedings of the International Conference on Stabilization/Solidification Treatment and Remediation. London, Balkema: 415-435.

Peryea, F. J. and R. Kammereck (1997). Phosphate-enhanced movement of arsenic out of lead arsenate-contaminated topsoil and through uncontaminated subsoil. Water, Air, Soil Pollut. 93: 243-254.

Pettine, M., L. Campanella and F. J. Millero (1999). Arsenite oxidation by H2O2 in aqueous solutions. Geochim. Cosmochim. Acta 63(18): 2727-2735.

Pinel-Raffaitin, P., I. Le Hecho, D. Amouroux and M. Potin-Gautier (2007). Distribution and fate of inorganic and organic arsenic species in landfill leachates and biogases. Environ. Sci. Technol. 41(13): 4536-4541.

Planning Commission (2007). Report of the Task Force on Formulating Action Plan for Removal of Arsenic Contamination in West Bengal. New Delhi, Government of India.

Qin, J., B. P. Rosen, Y. Zhang, G. Wang, S. Franke and C. Rensing (2006). Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase. Proc. Natl. Acad. Sci. U. S. A. 103(7): 2075-2080.

Raj, D. S. S., C. Aparna, P. Rekha, V. H. Bindhu and Y. Anjaneyulu (2005). Stabilisation and solidification technologies for the remediation of contaminated soils and sediments: an overview. Land Contam. Reclam. 13(1): 23-48.

Raven, K. P., A. Jain and R. H. Loeppert (1998). Arsenite and Arsenate Adsorption on Ferrihydrite: Kinetics, Equilibrium, and Adsorption Envelopes. Environ. Sci. Technol. 32(3): 344-349.

Ravenscroft, P., H. Brammer and K. Richards (2009). Arsenic pollution: a global synthesis, Wiley-Blackwell.

Renock, D., T. Gallegos, S. Utsunomiya, K. Hayes, R. C. Ewing and U. Becker (2009). Chemical and structural characterization of As immobilization by nanoparticles of mackinawite (FeSm). Chem. Geol. 268(1-2): 116-125.

Roberts, L. C., S. J. Hug, T. Ruettimann, M. D. M. Billah, A. W. Khan and M. T. Rahman (2004). Arsenic removal with iron(II) and iron(III) in waters with high silicate and phosphate concentrations. Environ. Sci. Technol. 38(1): 307-315.

Rochette, E. A., B. C. Bostick, G. Li and S. Fendorf (2000). Kinetics of Arsenate Reduction by Dissolved Sulfide. Environ. Sci. Technol. 34(22): 4714-4720.

Rouf, M. A. and M. D. Hossain (2003). Effects of Using Arsenic-Iron Sludge in Brick Making. Fate of Arsenic in the Environment. M. F. Ahmed, Bangladesh University of Engineering and Technology, The United Nations University: 193-208.

Saalfield, S. L. and B. C. Bostick (2009). Changes in iron, sulfur, and arsenic speciation associated with bacterial sulfate reduction in ferrihydrite-rich systems. Environ. Sci. Technol. 43(23): 8787-8793.

Sanchez, F., C. Gervais, A. C. Garrabrantsa, R. C. Barna and D. S. Kosson (2002). Leaching of inorganic contaminants from cement-based waste materials as a result of carbonation during intermittent wetting. Waste Manage. 22(2): 249-260.

Sanders, J. G. (1979). Microbial role in the demethylation and oxidation of methylated arsenicals in seawater. Chemosphere 8(3): 135-137.

Sarkar, A. Participatory Governance and Inter-Sector Coordination for Sustainable Solutions of Arsenic Contamination of Groundwater in India: An Explorative Study. Amsterdam Conference on the Human Dimensions of Global Environmental Change-Earth System Governance: Theories and Strategies for Sustainability.

Sarkar, S., L. M. Blaney, A. Gupta, D. Ghosh and A. K. Sengupta (2008). Arsenic removal from groundwater and its safe containment in a rural environment: validation of a sustainable approach. Environ. Sci. Technol. 42(12): 4268-4273.

Sarkar, S., J. E. Greenleaf, A. Gupta, D. Ghosh, L. M. Blaney, P. Bandyopadhyay, R. K. Biswas, A. K. Dutta and A. K. Sengupta (2010). Evolution of community-based arsenic removal systems in remote villages in West Bengal, India: Assessment of decade-long operation. Water Res. 44(19): 5813-5822.

Saulnier, I. and A. Mucci (2000). Trace metal remobilization following the resuspension of estuarine sediments: Saguenay Fjord, Canada. Appl. Geochem. 15(2): 191-210.

Selvin, N., J. Upton, J. Simms and J. Barnes (2002). Arsenic treatment technology for groundwaters. Water Sci. Technol. 2: 11-16.

Shafiquzzaman, M., M. S. Azam, I. Mishima and J. Nakajima (2009). Technical and social evaluation of arsenic mitigation. J Health Popul Nutr 27(5): 674-683.

Shaw, J. K., S. Fathordoobadi, B. J. Zelinski, W. P. Ela and A. E. Sáez (2008). Stabilization of arsenic-bearing solid residuals in polymeric matrices. J. Hazard. Mater. 152(3): 1115-1121.

Shrestha, R., M. Shrestha, N. Upadhyay, R. Pradhan, R. Khadka, A. Maskey, M. Maharjan, S. Tuladhar, B. Dahal and K. Shrestha (2003). Groundwater Arsenic Contamination, Its Health Impact and Mitigation Program in Nepal. J. Environ. Sci. Health, Part A 38(1): 185-200.

Sierra-Alvarez, R., J. A. Field, I. Cortinas, G. Feijoo, M. T. Moreira, M. Kopplin and A. J. Gandolfi (2005). Anaerobic microbial mobilization and biotransformation of arsenate adsorbed onto activated alumina. Water Res. 39(1): 199-209.

Smith, A. H., E. O. Lingas and M. Rahman (2000). Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. Bull. W. H. O. 78(9): 1093-1103.

Smith, A. H. and M. M. H. Smith (2004). Arsenic drinking water regulations in developing countries with extensive exposure. Toxicology 198(1–3): 39-44.

Stachowicz, M., T. Hiemstra and W. H. van Riemsdijk (2008). Multi-competitive interaction of As(III) and As(V) oxyanions with Ca2+, Mg2+, PO3- 4, and CO2- 3 ions on goethite. J. Colloid Interface Sci. 320(2): 400-414.

Stuckman, M. Y., J. J. Lenhart and H. W. Walker (2011). Abiotic properties of landfill leachate controlling arsenic release from drinking water adsorbents. Water Res. 45(16): 4782-4792.

Su, C. and R. W. Puls (2001). Arsenate and arsenite removal by zero valent iron: effects of phosphate, silicate, carbonate, borate, sulfate, chromate, molybdate, and nitrate, relative to chloride. Environ. Sci. Technol. 35(22): 4562-4568.

Sullivan, C., M. Tyrer, C. R. Cheeseman and N. J. D. Graham (2010). Disposal of water treatment wastes containing arsenic - a review. Sci. Total Environ. 408(8): 1770-1778.

Sun, W., R. Sierra-Alvarez, L. Milner, R. Oremland and J. A. Field (2009). Arsenite and ferrous iron oxidation linked to chemolithotrophic denitrification for the immobilization of arsenic in anoxic environments. Environ. Sci. Technol. 43(17): 6585-6591.

Teclu, D., G. Tivchev, M. Laing and M. Wallis (2008). Bioremoval of arsenic species from contaminated waters by sulphate-reducing bacteria. Water Res. 42(19): 4885-4893.

Thomas, D. J., M. Styblo and S. Lin (2001). The Cellular Metabolism and Systemic Toxicity of Arsenic. Toxicol. Appl. Pharmacol. 176(2): 127-144.

Thomas, F., R. A. Diaz-Bone, O. Wuerfel, B. Huber, K. Weidenbach, R. A. Schmitz and R. Hensel (2011). Connection between Multimetal(loid) Methylation in Methanoarchaea and Central Intermediates of Methanogenesis. Appl. Environ. Microbiol. 77(24): 8669-8675.

Tsai, S.-L., S. Singh and W. Chen (2009). Arsenic metabolism by microbes in nature and the impact on arsenic remediation. Curr. Opin. Biotechnol. 20(6): 659-667.

Turpeinen, R., M. Pantsar-Kallio and T. Kairesalo (2002). Role of microbes in controlling the speciation of arsenic and production of arsines in contaminated soils. Sci. Total Environ. 285(1-3): 133-145.

U.S. EPA (1992). Method 1311 Toxicity Characteristic Leaching Procedure: 1-35.

U.S. EPA (1994). Method 1312 Synthetic Precipitation Leaching Procedure: 1-30.

U.S. EPA (2003). U.S. EPA Workshop on Managing Arsenic Risks to the Environment: Characterization of Waste , Chemistry , and Treatment and Disposal Proceedings and Summary Report, U.S. EPA.

U.S. EPA (2007). Part 503 Standards for the Use of Disposal of Sewage Sludge. Title 40: Protection of the Environment.

Upadhyaya, G., J. Jackson, T. M. Clancy, S. P. Hyun, J. Brown, K. F. Hayes and L. Raskin (2010). Simultaneous removal of nitrate and arsenic from drinking water sources utilizing a fixed-bed bioreactor system. Water Res. 44(17): 4958-4969.

Vaaramaa, K. and J. Lehto (2003). Removal of metals and anions from drinking water by ion exchange. Desalination 155: 157-170.

Vagliasindi, F. G. A. and M. M. Benjamin (1998). Arsenic Removal in Fresh and NOM-Preloaded Ion Exchange Packed Bed Adsorption Reactors. Water Sci. Technol. 38(6): 337-343.

van Geen, A., H. Ahsan, A. H. Horneman, R. K. Dhar, Y. Zheng, I. Hussain, K. M. Ahmed, A. Gelman, M. Stute, H. J. Simpson, S. Wallace, C. Small, F. Parvez, V. Slavkovich, N. J. Loiacono, M. Becker, Z. Cheng, H. Momotaj, M. Shahnewaz,

A. A. Seddique and J. H. Graziano (2002). Promotion of well-switching to mitigate the current arsenic crisis in Bangladesh. Bull. W. H. O. 80(9): 732-737.

van Geen, A., J. Rose, S. Thoral, J. M. Garnier, Y. Zheng and J. Y. Bottero (2004). Decoupling of As and Fe release to Bangladesh groundwater under reducing conditions. Part II: Evidence from sediment incubations. Geochim. Cosmochim. Acta 68(17): 3475-3486.

van Genuchten, C. M., S. E. A. Addy, J. Peña and A. J. Gadgil (2012). Removing Arsenic from Synthetic Groundwater with Iron Electrocoagulation: An Fe and As K-Edge EXAFS Study. Environ. Sci. Technol. 46(2): 986-994.

Visoottiviseth, P. and F. Ahmed (2008). Technology for Remediation and Disposal. Reviews of Environmental Contamination. D. M. Whitacre. New York, Springer. 197.

Wan, W., T. J. Pepping, T. Banerji, S. Chaudhari and D. E. Giammar (2011). Effects of water chemistry on arsenic removal from drinking water by electrocoagulation. Water Res. 45(1): 384-392.

Watanabe, C., A. Kawata, N. Sudo, M. Sekiyama, T. Inaoka, M. Bae and R. Ohtsuka (2004). Water intake in an Asian population living in arsenic-contaminated area. Toxicol. Appl. Pharmacol. 198(3): 272-282.

Wilkin, R. T., D. Wallschlager and R. G. Ford (2003). Speciation of arsenic in sulfidic waters. Geochem. Trans. 4(1): 1-7.

Williams, P. N., M. R. Islam, E. E. Adomako, A. Raab, S. A. Hossain, Y. G. Zhu, J. Feldmann and A. A. Meharg (2006). Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. Environ. Sci. Technol. 40(16): 4903-4908.

Wolthers, M., L. Charlet, C. H. van Der Weijden, P. R. van der Linde and D. Rickard (2005). Arsenic mobility in the ambient sulfidic environment: Sorption of arsenic(V) and arsenic(III) onto disordered mackinawite. Geochim. Cosmochim. Acta 69(14): 3483-3492.

World Health Organization (2006). Guidelines for Drinking-water Quality.

Xu, X. Y., S. P. McGrath, A. A. Meharg and F. J. Zhao (2008). Growing Rice Aerobically Markedly Decreases Arsenic Accumulation. Environ. Sci. Technol. 42(15): 5574-5579.

Yokota, H., K. Tanabe, M. Sezaki, Y. Akiyoshi, T. Miyata, K. Kawahara, S. Tsushima, H. Hironaka, H. Takafuji, M. Rahman, S. A. Ahmad, M. H. S. U. Sayed and M.

H. Faruquee (2001). Arsenic contamination of ground and pond water and water purification system using pond water in Bangladesh. Eng. Geol. 60: 323-331.

Yoshinaga, M., Y. Cai and B. P. Rosen (2011). Demethylation of methylarsonic acid by a microbial community. Environ. Microbiol. 13(5): 1205-1215.

Zandi, M., N. V. Russell, R. G. Edyvean, R. J. Hand and P. Ward (2007). Interpretation of standard leaching test BS EN 12457-2: is your sample hazardous or inert? J. Environ. Monit. 9(12): 1426-1429.

Chapter 3

Evaluating the cement stabilization of arsenic-bearing iron wastes from drinking water treatment

3.1 Abstract

Cement stabilization of arsenic-bearing wastes is recommended to limit arsenic release from wastes following disposal. Such stabilization has been demonstrated to reduce the arsenic concentration in the Toxicity Characteristic Leaching Procedure (TCLP), the test regulating landfill disposal of arsenic waste. However, few studies have evaluated leaching from actual wastes under conditions similar to those in ultimate disposal environments. In this study, direct land disposal in areas where flooding would be likely was simulated to test arsenic release from cement stabilized arsenic-bearing iron oxide wastes. When submersed for 406 days in chemically simulated rainwater, <0.4% of total arsenic was leached, an amount comparable to the leaching measured during the TCLP (<0.3%). Short-term (18 h) modified TCLP tests (pH 3-12) found that cement stabilization lowered arsenic leaching at high pH, but increased leaching at pH <4.2 compared to non-stabilized wastes. The application of μXRF revealed the majority of arsenic in cement stabilized waste remained associated with iron, and by μXRD, the presence of calcite and other cementitious minerals was confirmed. This distribution of arsenic differed from previous reports in which arsenic salts were stabilized with cement and arsenic-calcium solid phases were observed. This illustrates that the initial form of arsenic-bearing wastes influences the final stabilized form. Overall, cement stabilization is effective for arsenic-bearing wastes when acidic conditions can be avoided.

Clancy, T. M., K. V. Snyder, R. Reddy, S. E. Amrose, L. Raskin and K. F. Hayes (2015).*in prep.for J. Hazard.Mater*.

3.2 Introduction

Worldwide contamination of drinking water by arsenic has spurred the development and implementation of numerous arsenic-removal technologies, many of which rely on the sorption of arsenic to oxidized iron solids (Jain and Ali 2000, Kumar *et al.* 2004, Choong *et al.* 2007, Mohan and Pittman 2007, Ravenscroft *et al.* 2009, van Genuchten *et al.* 2012, Neumann *et al.* 2013). The resulting arsenic-bearing iron solid waste can become a new source of arsenic contamination depending on the disposal environment. This is of concern in developed or developing countries where waste solids from filters used to remove arsenic from drinking water are disposed in either landfills or soil environments near filtration facilities, respectively. Landfill conditions may cause the release of arsenic from ferric iron solids through reductive dissolution and changes in pH (Ghosh *et al.* 2006, Stuckman *et al.* 2011). However, for conditions in which arsenic solid wastes are disposed directly on land, such as in Bangladesh, the potential for arsenic release has not been well characterized.

To minimize the potential for release, arsenic-bearing wastes have been mixed with cement for disposal (Dutre and Vandecasteele 1995, Leist *et al.* 2000, Paria and Yuet 2006). Cement stabilization includes mixtures of cement, water, and sand that are used to create mortars and if aggregates, such as crushed gravel or stone, are included the resulting material is a concrete. While the effectiveness of cement stabilization has been shown to vary widely based on both waste and concrete characteristics (Leist *et al.* 2003, Sullivan *et al.* 2010), studies have found that cement or lime addition typically reduces arsenic measured in the leachate of the Toxicity Characteristic Leaching Procedure (TCLP) when compared to non-stabilized wastes (Akhter *et al.* 1997, Jing *et al.* 2003,

Moon *et al.* 2004, Jia and Demopoulos 2008, Camacho *et al.* 2009). The TCLP is frequently used to measure leaching potential and is the test applied by the US E.P.A. to determine if wastes are safe for municipal solid waste landfill disposal or must be treated as a hazardous waste (U.S. EPA 1992a).

The leachant solution for the TCLP is comprised of acetic acid (pK_a of 4.75, 25 °C), and is typically designed to result in a pH < 5, though the final pH of the test is not always reported, and can vary depending on the alkalinity of the waste tested. For a typical TCLP formulation, the testing of cement- or lime-stabilized waste can result in pH > 5, which may confound the interpretation of the leaching results. In studies measuring the leaching of arsenic from cement stabilized arsenic wastes by the TLCP over a range of pH values, higher pH conditions have been shown to result in lower leaching (Jing *et al.* 2003, Yoon *et al.* 2010).

Few studies have evaluated arsenic leaching from cement stabilized wastes over long time periods (>24 hours) (Sanchez *et al.* 2002, Singh and Pant 2006), yet such studies are warranted to assess their true stability in disposal environments. Sanchez *et al.* (2002) evaluated the leaching of arsenic, added as a salt, from a mortar under environments with varied relative humidity and CO_2 levels and found arsenic release continued over 94 days regardless of conditions. Singh and Pant (2006) similarly observed continuous leaching from cement stabilized arsenite adsorbed to activated alumina over a 90 period. More information linking the effectiveness of short-term leaching tests for predicting longer term leaching under actual disposal conditions is needed to assess whether short-term leaching tests can be used to predict the ultimate arsenic release over the much longer exposure periods expected in common disposal environments.

When cement stabilized arsenic wastes are stored near treatment plants or used in non-load bearing structural elements, rainwater is expected to be the natural leachant. In South Asia, the potential for flooding may be especially high during the monsoon seasons (Shahid 2010). While the TLCP (with acetic acid) is expected to provide a more aggressive leaching condition than rainwater, it remains to be demonstrated whether the standard short-term TLCP test is a good predictor of the efficacy of cement stabilized waste for keeping arsenic from leaching over long periods of rainwater exposure.

The stability of arsenic stabilized wastes depends on the type of solid phases that bind arsenic within the cement. Characterization of cement and lime stabilized arsenic by bulk X-ray diffraction (XRD) has identified crystalline arsenic calcium phases, including: $NaCaAsO_4 \cdot 7.5H_2O$, $Ca_4(OH)_2(AsO_4)_2 \cdot 4H_2O$, $Ca_3(AsO_4)_2 \cdot 3^{2/3} H_2O$, $Ca_5(AsO_4)_3(OH)$ (Akhter *et al.* 1997, Bothe Jr and Brown 1999, Moon *et al.* 2004). However, in most cases, these calcium-arsenic solids originated from arsenic salts and therefore may not be representative of those that form from arsenic drinking water treatment wastes in which arsenic is typically sorbed to iron solids. In the only studies characterizing cement or lime stabilization of arsenic sorbed to iron solids, calcium arsenic solids were observed by FTIR (Jing *et al.* 2003) and the calcium iron arsenic solid, yukonite ($Ca_2Fe_3(AsO_4)_4(OH) \cdot 12H_2O$) was found by XRD (Jia and Demopoulos 2008). Since bulk XRD detection is limited to crystalline solids, if amorphous phases are present, XRD would not identify arsenic associated with these phases. Similarly, other techniques such as bulk X-ray absorption spectroscopy can be difficult to interpret when samples contain multiple phases. These challenges require the application of new techniques to

determine the distribution of arsenic from iron-based drinking water treatment in cement stabilized solids.

In this study, wastes from a pilot-scale iron electrocoagulation system operated for arsenic removal from a contaminated groundwater source in West Bengal, India (Amrose *et al.* 2014) were used to: (i) evaluate leaching from cement stabilized wastes over long-term exposure to simulated rainwater, (ii) compare long-term simulated leaching with short-term leaching tests, and (iii) characterize the distribution of arsenic in cement stabilized wastes using μXRF and crystalline phases using μXRD. These results are used to assess the potential for cement stabilization arsenic waste to provide long term stability against leaching under representative disposal conditions, to determine if short term leaching test are effective predictors of this stability, and to identify the arsenic solid phase associations responsible for its stability against leaching.

3.3 Experimental

3.3.1 Mortar preparation and waste characteristics

The cement stabilization of arsenic-bearing wastes was performed by creating a mortar through the hand mixing a combination of ordinary Portland cement, sand, and water at a ratio of 1:3:0.5 by mass (Jing *et al.* 2003, Sugiyama *et al.* 2007). An iron oxide arsenic-bearing waste from a pilot scale Electro-Chemical Arsenic Remediation (ECAR) unit operating in West Bengal, India (Amrose *et al.* 2014) was dried at 105 °C and incorporated into the mortar at either 1% or 3% of the total mass, replacing some of the sand. The arsenic and iron coordination environments in wastes from similar systems have been extensively characterized (van Genuchten *et al.* 2012, van Genuchten *et al.* 2014). Wet mortar was poured into plastic molds to create 0.83 cm^3 cubes. Molds were

sealed with plastic wrap and cured for 48 hours before cubes were removed from the molds. Cubes were then stored in plastic bags and inside an anaerobic glove box to reduce exposure to carbon dioxide before use in leaching tests. To compare the impact of curing conditions, another set of 2.05 cm^3 cubes was prepared using three different curing conditions. One set, as described above, was cured for 48 hours wrapped in plastic. Two other sets were prepared and cured in water for a total curing time of either 6 days or 28 days. Cubes from all conditions were removed from molds after 48 hours.

3.3.2 Long-term rainwater leaching tests

Leaching tests were performed by exposing uncrushed mortar cubes to rainwater for 406 days to simulate long-term leaching. Synthetic rainwater was made based on an average major ion concentrations measured in rainwater samples collected in areas surrounding Dhaka, Bangladesh (Hadi *et al.* 1999) (Table S2, Appendix C). 100 mortar cubes containing 1% ECAR waste were placed into each of three glass containers with 300 mL of the synthetic rainwater. Liquid samples were collected and filtered through 0.2 μm nylon filters. The liquid volume removed at various sampling times was replaced with an equivalent volume of synthetic rainwater. Dionized water, typically less than 10 mL per week, was added to replace water loss due to evaporation to maintain a constant volume. Triplicate experiments were also set-up with containers holding 100 mortar cubes and filled with 300 mL of synthetic rainwater for 1 hour daily after which the rainwater was drained and a sample collected. For the remaining 23 hours the cubes would be left to air dry. This process was continued for 70 days. After the pH was measured (Mettler Toledo, Columbus, OH), the samples were acidified with nitric acid for the measurement of total arsenic, iron, and calcium using inductively-couple plasma

mass spectroscopy (ICP-MS) (PerkinElmer ALEN DRC-e, Waltham, MA). The detection limit for arsenic was 1.1 µg/L. The instrument error was 5%, as determined by the variation from known standards. The minimum measurement error was 0.8 µg/L. Errors are reported as the highest of the standard deviation between replicate samples, the instrumental error, or the minimum measurement error.

3.3.3 Short-term leaching: TCLP and modified TCLP

For short-term leaching tests, the TCLP was performed as outlined by the U.S. EPA (1992a). Briefly, 0.1 M acetic acid was used as the leachant with an initial pH of either 2.88 or pH 4.93, adjusted with 1 M NaOH. In modified TCLP tests, the acetic acid concentration was varied (0.1 M, 1.5 M, 3 M, 4.5 M, and 6 M) to change the final leachate pH. In TCLP tests, the leachant to solid ratio was 20:1 and was typically performed with 1 g of dried and crushed mortar or raw waste in a 50 mL centrifuge tube. Replicate samples, between three and five, were mixed by using an end-over-end sample tube rotator for 18 hours. Samples were filtered through 0.7 µm pre-washed glass microfiber filters (EMD Millipore, Darmstadt, Germany) and acidified with nitric acid for total arsenic measurement via ICP-MS.

Short-term leaching tests to compare leaching from differentially cured mortars were performed using uncrushed 2.05 cm^3 mortar cubes. Three curing conditions tested included: 2 day curing wrapped in plastic, 6 day curing in water, and 28 day curing in water. For each of these curing conditions five replicate leaching tests were conducted at three different acetic acid concentrations, 0.1 M, 1.5 M, and 3 M. Each replicate test was performed with one cube and 40 mL of leachant. The percentage of arsenic leached from each cube was calculated based on total digestions of mortar cubes from the same batch

of mortar and the mass of the dry cubes before the leaching test. Cubes were placed into the leaching solution inside of 50 mL centrifuge tubes and were mixed on a shaker plate for 18 hours before samples were filtered and acidified for total arsenic measurement via ICP-MS.

3.3.4 Short-term leaching: NaOH Leaching

To assess the leaching of raw ECAR waste at high pH, a short-term leaching test in 0.1 M NaOH was performed, where 0.01 g of dried ECAR waste was added to 40 mL of leachant and rotated in an end-over-end rotator for either 1 or 3 days. This was also repeated in a12 mM CaO solution to evaluate whether the presence of calcium impacted the results. Samples were filtered and acidified as described above.

3.3.5 Total digestions

Total digestions were performed on the waste and mortar solids according to the US EPA Method 3050B to determine total solid arsenic concentrations (U.S. EPA 1992b). Between 0.5 and 1.0 g of dried and crushed solids from a subset of at least five cubes or a sample of ECAR waste were treated with 10 mL of 7.8 M nitric acid and incubated at 100 °C for 2 hours followed by the addition of hydrogen peroxide, typically 1 mL, or until vigorous bubbling stopped, and then incubated for another 2 hours at 100 °C. Cooled samples were filtered through Whatman no. 41(GE Healthcare Life Sciences, Pittsburg, PA) filter paper, diluted, and acidified with nitric acid for total arsenic measurement by ICP-MS.

3.3.6 Solids characterization

Samples of 1% ECAR waste mortar cubes taken prior to rainwater incubation and on day 386 of rainwater leaching were prepared for X-ray microprobe analysis at beamline

13-ID-E, Sector 13 GeoSoilEnviroCARS, Advanced Photon Source at Argonne National Laboratory (Sutton *et al.* 2002). Polished thin sections (30 µm) of the mortar cubes were prepared on quartz glass slides by Spectrum Petrographics (Vancouver, WA). Microfocused X-ray fluorescence (µXRF) compositional mapping was performed using a monochromatic X-ray beam focused to a spot size of 1 x 2 µm (V x H) using a pair of 200 mm long, elliptically-bent, Rh-coated silicon mirrors in a Kirkpatrick–Baez geometry. X-ray fluorescence spectra were collected using a 4-element Vortex-ME4 silicon-drift-diode detector (Hitachi, Tokyo, Japan) coupled to XMap digital spectrometers (XIA, Hayward, CA). Maps were collected in a continuous scan mode using incident X-ray energy of 13.0 keV, a pixel size of 2 µm, and a scan rate of 30 msec/pixel. The energy dispersive detector was filtered using 230 µm of Kapton film to minimize calcium K fluorescence. This reduced detector dead time and pile up. XRF energy dispersive maps were processed using beamline specific software (http://gsecars.uchicago.edu). Elemental mass ratios were calculated using the NRLXRF program (Criss *et al.* 1978) using a standardless fundamental parameters approach (Kanngießer 2003), referenced to measured Ca Kα emission intensities and assuming a calcite bulk chemical composition. At points of interest, µXRD patterns were collected in transmission mode using a Perkin Elmer 16 inch amorphous silicon digital X-ray detector (XRD1621) with incident beam energy of 17.0 keV and a 5 second collection time per frame. Background subtraction and image processing was performed with FIT2D (Hammersley 1997). Alumina was used as a detector calibration standard. XRD pattern recognition was performed using MDI Jade 2010 (Livermore, CA).

3.4 Results & Discussion

3.4.1 ECAR Waste and Mortar Characterization

The total arsenic concentration in the mortar cubes was determined by the total digestion method and found to be 14.6 ± 2.1 and 32.7 ± 1.6 µg As/g mortar for 1% and 3% ECAR wastes, respectively. The arsenic concentration of the raw ECAR waste was 995 ± 50 µg As/g waste. In order to compare leaching across different solids, the arsenic leached is presented as a percentage of the total arsenic based on these digestions.

3.4.2 Long-term rainwater leaching compared to TCLP estimate

The pH in the long-term rainwater leaching experiment (406 days) started out at a relatively high pH of 12.7, but then gradually decreased to a final pH of ~8.9 (Figure 3-3). The high pH was caused by the dissolution of portlandite ($Ca(OH)_2$) from the mortar, which resulted in Ca leaching. Over time calcite precipitation likely occurred, given the exposure of the rainwater to carbon dioxide from the atmosphere. Consistent with this, dissolved calcium concentrations were above 100 mg/L before day 16, but decreased to below 1 mg/L after day 29 (data not shown). Iron concentrations were around 1 mg/L, but decreased to below the detection limit (0.1 mg/L) after day 20 (data not shown). Before day 18, arsenic concentrations in the aqueous phase were below the detection limit (0.1 ug/L), but began to increase over time, reaching an average concentration of 21 ± 4.8 µg/L by day 406 among the three replicates. The lag in arsenic release has been observed previously (Singh and Pant 2006) and was likely caused by arsenic sorption/co-precipitation with calcite in the leaching solution (Alexandratos *et al.* 2007, Lee *et al.* 2007, Sø *et al.* 2008, Yokoyama *et al.* 2012). After 406 days, the total arsenic leached from the cubes represented about 0.34% of the total amount of arsenic present in the

mortar. In the 70 day experiment, where cubes were exposed to rainwater for 1 hour daily, followed by 23 hours of drying, concentrations of arsenic above the detection limit were not measured. In this test, due to the high volume of rainwater used (300 mL per day), up to 1.45 % of the total arsenic present could have leached without being detected. Taken together, the results from both long-term leaching studies indicate that cement stabilization is very effective in keeping arsenic from leaching by rainwater.

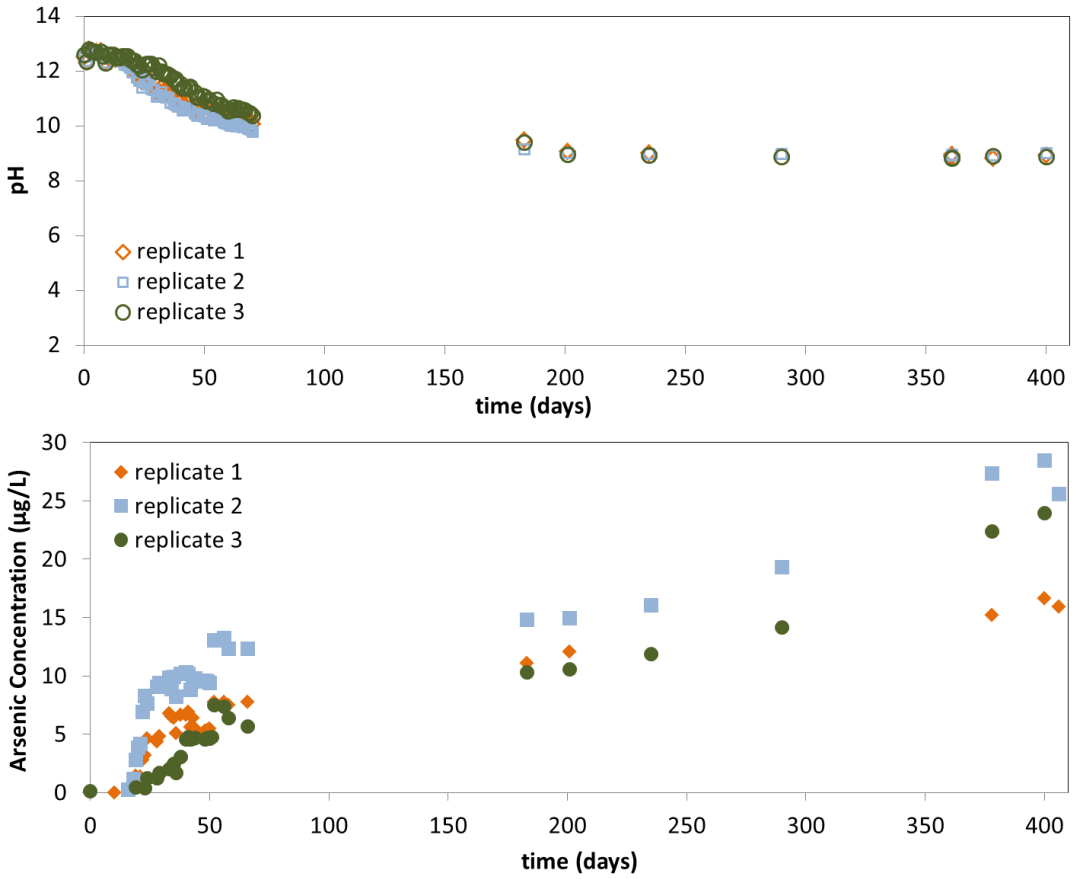


Figure 3-3. pH (a) and total arsenic (b) in filtered rainwater leachate over time for triplicate incubations of 100 mortar cubes containing 1% ECAR.

The standard TCLP test, using 0.1 M acetic acid and initial pH of 2.9 as the leachant, resulted in an average leachate arsenic concentration of 2.0 ± 0.8 µg/L from triplicate tests. This is far below the limit for disposal in municipal solid waste landfills (<5,000 µg/L) (U.S. EPA 1992a). The final pH of the test leachate varied between 6.3 and 11.5.

This pH range was the largest among all replicate tests. Possible causes include the physical and chemical heterogeneity of the cement stabilized waste cubes and the small sample size (1 g) taken for each test. However, as discussed below, this pH range is not expected to result in large changes in the arsenic leaching. The amount of arsenic leached from the ECAR mortar under this test, represented 0.27% of the total arsenic present in the mortar. No statistically significant difference was found between the arsenic leached during the long-term rainwater experiment with uncrushed cubes compared with the short-term TCLP on crushed cubes (p-value > 0.1;Table 31).

Table 3-1. Comparison of short-term (TCLP) and long-term (rainwater) leaching

Leaching Test	% of total arsenic leached average (std. deviation in percentage points)	time (days)	initial leachant pH	final leachate pH
Long-term Rainwater Leaching	0.34 (±0.1)	406	6.2	8.9
Toxicity Characteristic Leaching Procedure (TCLP)	0.27 (±0.1)	0.75	2.9	6.3-11.5

3.4.3 Short-term leaching tests with varying pH

While the design of the TCLP is to determine leaching from solids at a pH between 3 and 5, solids such as cements result in a much higher final pH. To determine arsenic leaching over a wider pH range, acetic acid concentrations were varied. Results from the leaching test for cement stabilized ECAR waste and raw ECAR waste are shown in Figure 3-2, where the mass of arsenic leached is plotted as the percentage of the total arsenic in each solid versus the final leachate pH.

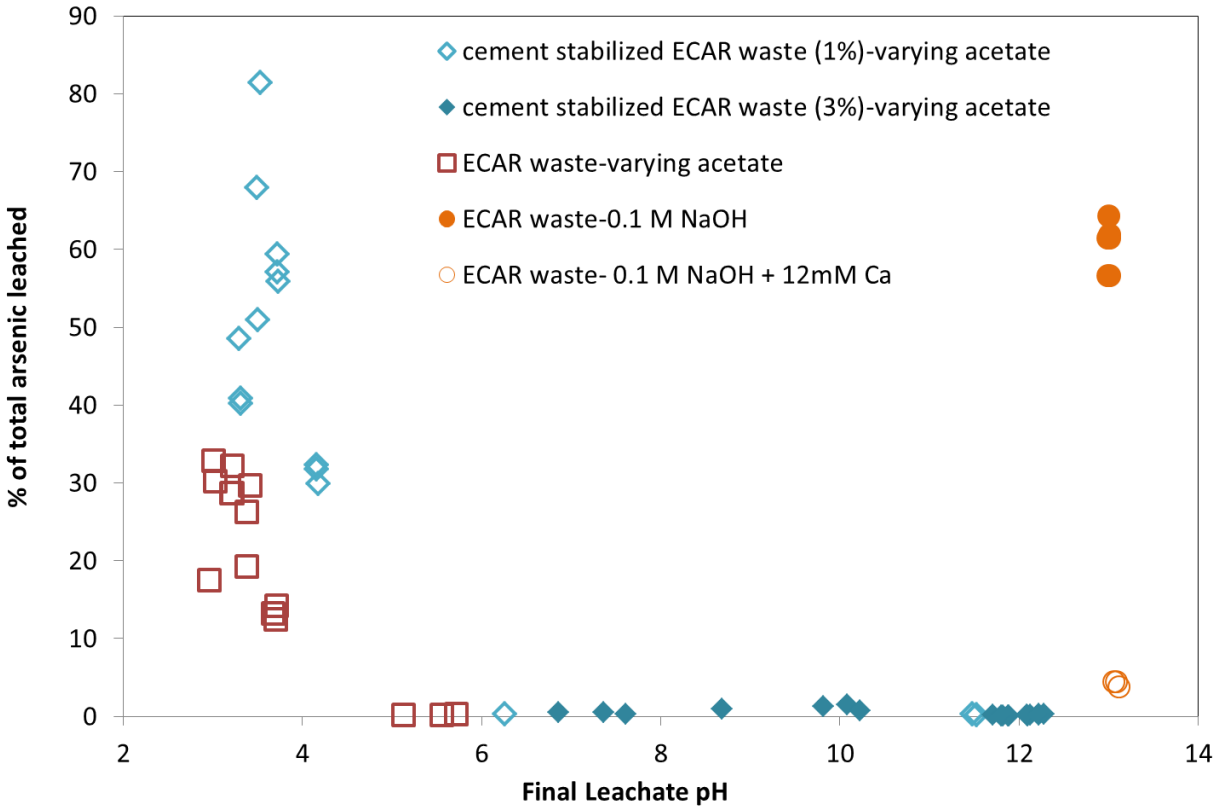


Figure 3-4. Arsenic leaching versus final leachate pH from short-term leaching tests of ECAR waste with and without cement stabilization.

When raw ECAR waste was subjected to a high pH environment, i.e. 0.1 M NaOH, up to 64% of the total arsenic was mobilized. However, in the presence of calcium, the mobilization of arsenic from ECAR waste at high pH was greatly decreased (<4%). This had been previously observed and could be caused by arsenate co-precipitation or sorption to calcite (Parks *et al.* 2003, Jia and Demopoulos 2008). A reduction in leaching was also observed when the ECAR is cement stabilized, which resulted in less than 1.5% mobilization in acetic acid where the final leachate pH is between 6.2 and 12.3. Given the potential for $Ca(OH)_2$ dissolution from the crushed mortar and the subsequent precipitation of $CaCO_3$ over this pH range, it is not possible to distinguish between arsenic that remains in its original phase and arsenic that is mobilized and subsequently

adsorbed or co-precipitated with calcite. The stability of calcium solids at high pH is also important in preventing the release of arsenic from the cement stabilized cubes under the long-term rainwater leaching. Leaching from raw ECAR waste was also found to be low as a percentage of the total arsenic present at pH 5.1-5.7. Leachate arsenic concentrations were between 13.4 and 66.2 µg/L. This is likely due to the strong sorption of arsenate to iron oxides in the pH range 4-8 (Fuller *et al.* 1993, Dixit and Hering 2003, Wee and Kramer 2005).

At pH less than 4.2, arsenic mobilization increased with decreasing pH for both raw ECAR waste and cement stabilized waste. Below this pH, the dissolution of iron oxides would be expected and would result in the release of arsenic. Under these low pH conditions, slightly more arsenic was leached from the cement stabilized waste than from the raw ECAR waste. Compared to the raw ECAR waste, where lower pH would increase arsenate sorption, until iron dissolution, cement stabilized wastes may also have some arsenic associated with calcium solids, which could cause more As to leach from the cement stabilized wastes as pH is lowered and both iron oxides and calcium solids begin to dissolve. These results are consistent with previous reports (Jing *et al.* 2003, Moon *et al.* 2004), though the increased release from stabilized solids compared to non-stabilized solids at low pH is often overlooked. This is an important consideration for disposal conditions where wastes may be exposed to low pH solutions, such as acid rain (pH ~4.3), animal wastes (pH as low as 4) (Bertron *et al.* 2005), or landfill leachate during early stages of landfill degradation (pH 4.5) (Kjeldsen *et al.* 2002). Even though cement stabilization is used as a pre-treatment for landfill disposal (Singh and Pant 2006), the long-term effects of landfill leachate exposure are not well characterized. There is

some indication that cement stabilized materials will not dissolve in landfill leachates which are typically oversaturated with respect to calcite (Bennett *et al.* 2000, Manning 2001). However, if dissolution of the cement stabilized solids were to occur, the reducing conditions would likely limit re-sorption/co-precipitation of arsenite with calcite (Sø *et al.* 2008, Wang *et al.* 2012, Yokoyama *et al.* 2012). For storage conditions above ground, low pH and reducing conditions would be less likely, and the pH inside the cement stabilized solids would remain high, producing conditions where the mortar would remain intact and low arsenic leaching would be expected. Generally, the advantage of cement stabilized waste versus raw ECAR iron waste is due to the encapsulation and the maintenance of a high pH condition that would protect against reductive dissolution likely for a long time, until the mortar was dissolved.

In addition to pH, other variables may affect the leaching from cement stabilized wastes. One factor is curing conditions, which can affect the properties of mortar or concrete. Increased curing times have been associated with increases in compressive strength and decreases in water permeability, carbonation depth, and porosity (Ramezanianpour and Malhotra 1995, Al-Khaiat and Haque 1998, Bai *et al.* 2002). However, there has been limited research relating these properties with leaching behavior. Previous studies have compared leaching using the TCLP, with a final pH above 5, and have found slightly decreased leaching with increased curing time, from 14 to 28 days, and very little change between leaching from samples cured 28 days and samples cured longer than 1 year (Akhter *et al.* 1997, Kameswari *et al.* 2001, Jing *et al.* 2005, Nakwanit *et al.* 2011). In this study, we found little difference in curing conditions on arsenic leaching at a final pH 3.8-4.0 (Figure 3-5). At the lowest final pH, the

percentage of the total arsenic leached was greatest for the 6 day water cured samples (27.6% ± 3.1 pp), followed by 28 day water cured samples (15.7% ± 1.2 pp), and lowest for 2 day plastic wrapped samples (10.6% ± 1.0 pp) (Figure 3-5). This indicates that the leaching potential for the cubes used in the long-term leaching (which were also cured for 2 days in plastic) may be lower than that from mortars cured in water. Standard lab tests for mortar and concrete are typically performed after water curing, but actual conditions may vary in the field application of cement stabilization for wastes. The differences in leaching observed here warrant further study to determine the optimal curing conditions for field applications.

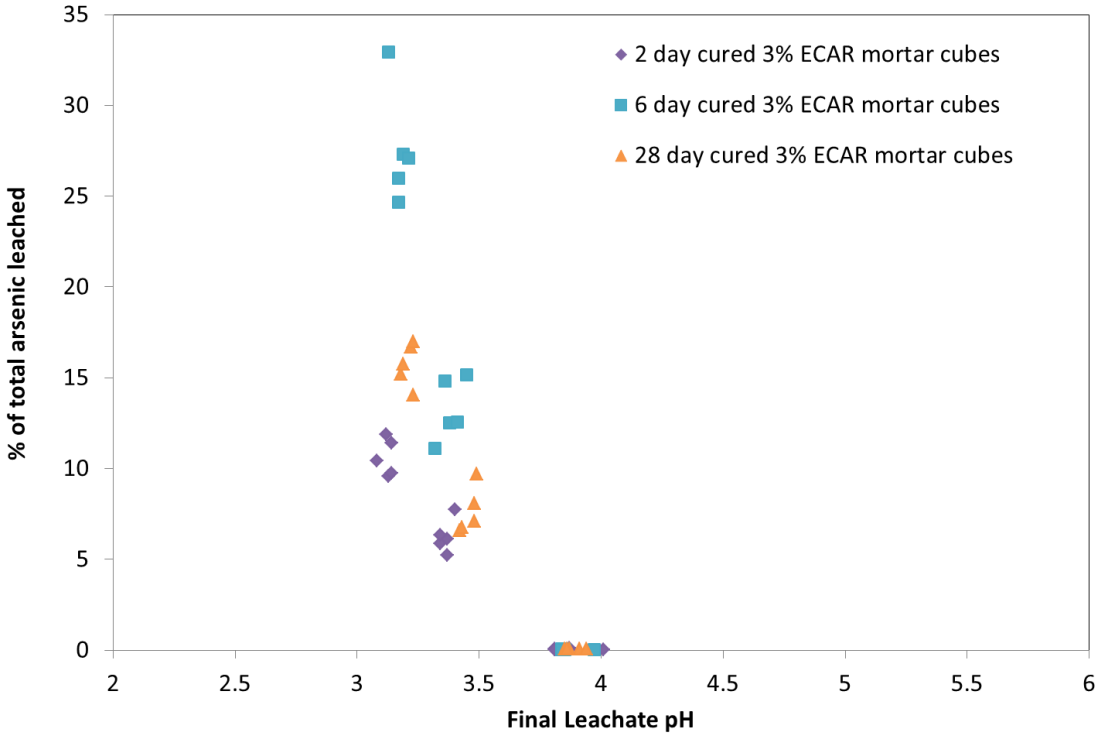


Figure 3-5. The percent of total arsenic leached from uncrushed mortar cubes containing 3% ECAR waste in leaching tests with varying concentrations of acetic acid.

3.4.4 Solids characterization

μXRF elemental mapping shows the distribution of calcium, arsenic, and iron in areas near ECAR waste particles within the mortar cube (Figure 3-6). In maps from both the initial time point and after 386 days in rainwater, arsenic and iron were found together. Calcium was more abundant near the edges of the ECAR waste particle and away from the waste. A linear relationship exists between As and Fe intensities, but not between As and Ca intensities (Figure S1, Appendix C). Elemental mass ratios for three areas with a range of arsenic intensities (areas shown Figure S2, Appendix C) show that the ratio of Fe to As is between 152 and 187 to 1. These results highlight that the majority of arsenic present in the cement stabilized waste remains associated with iron, similar to the initial state of the ECAR waste. Of importance is also the areas surrounding the ECAR waste particle with lower arsenic. This may be caused by some desorption of arsenic from the iron under the high pH conditions of the cement matrix and subsequent association of with calcium phases. This is consistent with the finding that at low pH (<4) more arsenic leaches under short-term leaching tests from cement stabilized samples compared with raw ECAR waste as a result of the additional arsenic released from the ECAR waste at high pH while in the cement matrix. These findings are also supported by evidence that arsenic is preferentially associated with iron in natural environments and agricultural lime samples, but associated with calcium when iron sorption is limited (Schmidt *et al.* 2009, Bardelli *et al.* 2011, Costagliola *et al.* 2013, Winkel *et al.* 2013).

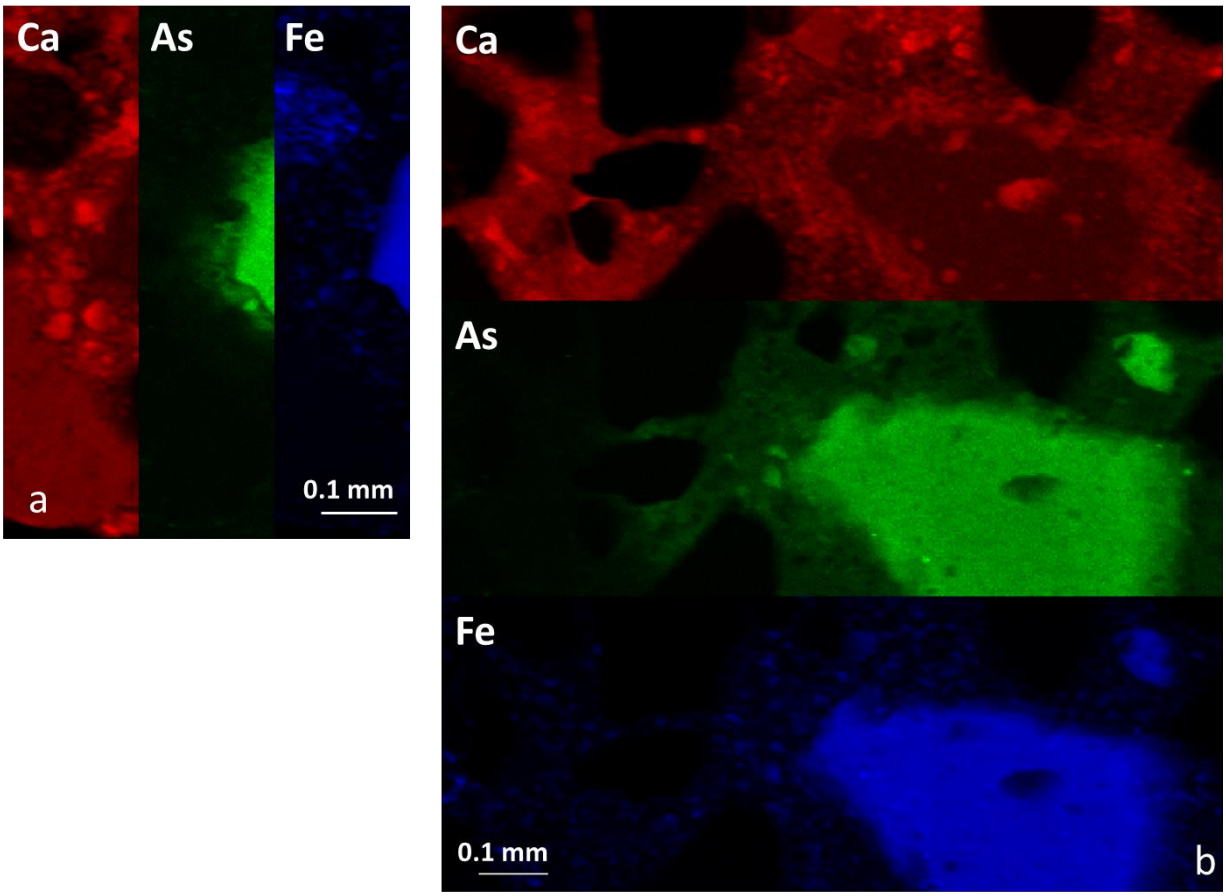
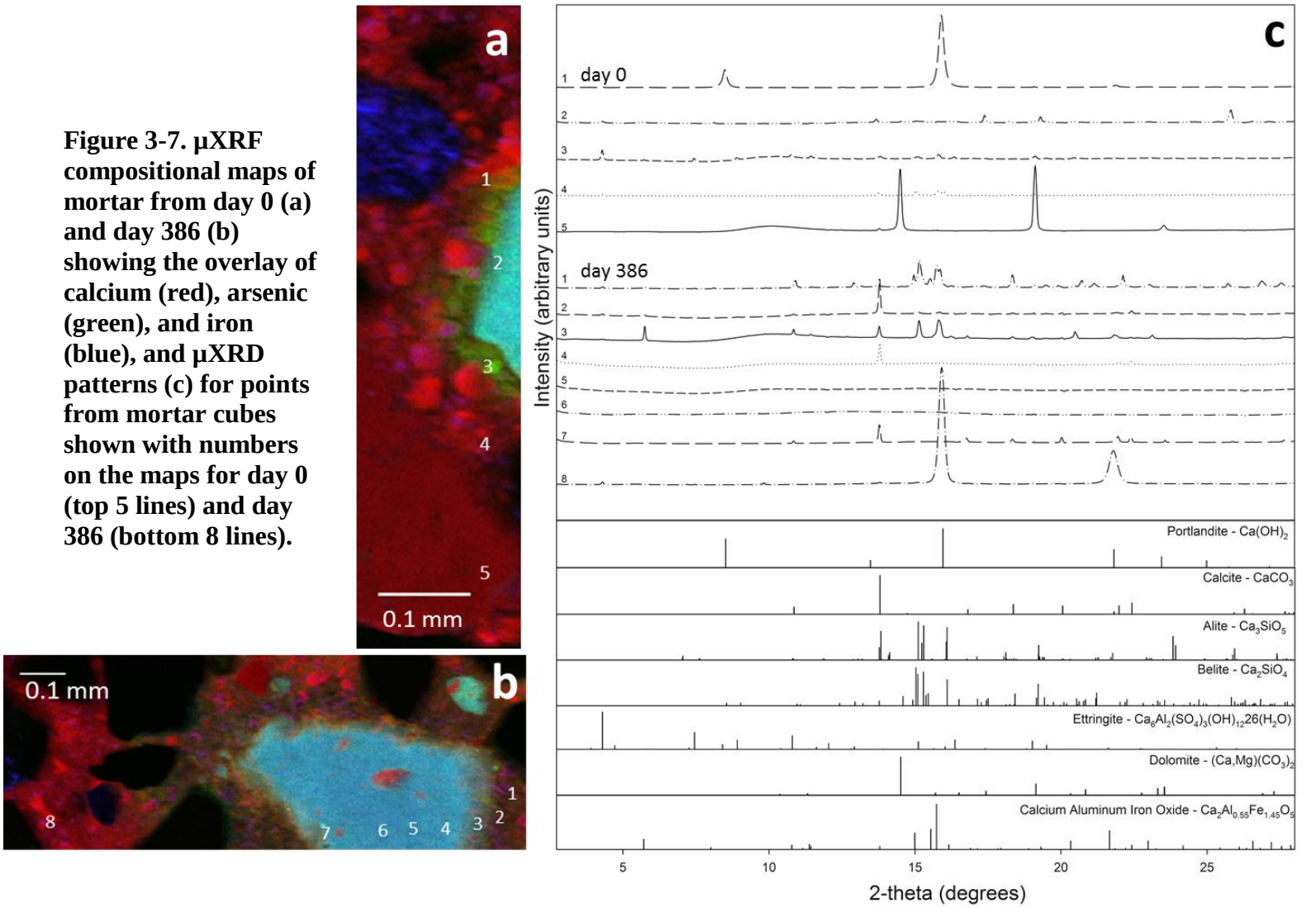


Figure 3-6. µXRF elemental maps of calcium (red), arsenic (green), and iron (blue) in thin sections of cement stabilized ECAR waste from day 0 before flooding (a) and day 386 of rainwater flooding (b).

µXRD patterns from across the cubes show evidence for calcite, ettringite, portlandite, calcium aluminum iron oxide, dolomite, and calcium silica solids (Figure 3-7c), all phases commonly found in cementitious materials (Scheidegger *et al.* 2006, Schlegel *et al.* 2011, Dilnesa *et al.* 2014). In these samples no evidence for crystalline calcium arsenic phases was found. This is in contrast to studies of cement stabilization with arsenic salts (Akhter *et al.* 1997, Bothe Jr and Brown 1999, Moon *et al.* 2004) and is likely due to the use of an iron-based arsenic waste in this study. In one other study of arsenic-bearing iron wastes treated with lime, yukonite was identified using XRD, but was observed only after an accelerated aging performed at 70 °C for 7 weeks (Jia and

Demopoulos 2008). In a second study of iron wastes, evidence for calcium arsenic phases was found using Fourier transform infrared spectroscopy (FTIR) and X-ray absorption fine structure (EXAFS) (Jing *et al.* 2003) though it can be difficult to distinguish between arsenic co-precipitated with calcite and calcium arsenic solids, such as johnbaumite, using these techniques (Alexandratos *et al.* 2007, Yokoyama *et al.* 2012). Calcite, although present at day 0, became more abundant in the mortar after long-term incubation. The presence of calcite as the primary crystalline phase in points across and around the ECAR particle suggests that arsenate sorption to or co-precipitation with calcite plays only a minor role in arsenic stability over time as only small amounts of arsenic dissolves from the ECAR waste at high pH into the surrounding cement matrix.

Figure 3-7. µXRF compositional maps of mortar from day 0 (a) and day 386 (b) showing the overlay of calcium (red), arsenic (green), and iron (blue), and µXRD patterns (c) for points from mortar cubes shown with numbers on the maps for day 0 (top 5 lines) and day 386 (bottom 8 lines).



3.5 Conclusions

The results presented here highlight the importance of pH on the predicted mobility of arsenic from cement stabilized wastes. For long-term exposure of cement stabilized arsenic-bearing waste to a simulated rainwater, pH remained high (>8) and arsenic leaching was low (about 0.34% of the total arsenic present). This was similar to the amount predicted in the standard TCLP, where the pH was also high (pH 6.3-11.5). Under short-term leaching conditions with acetic acid, little leaching from cement stabilized arsenic-bearing iron wastes occurred between pH 6.2-12.3. At pH <4.2, increased leaching was observed from cement stabilized and raw wastes. Characterization of the cement stabilized arsenic-bearing wastes revealed that arsenic was primarily associated with iron, similar to the starting waste material. The primary crystalline phase identified was calcite and no crystalline calcium arsenic phases were identified, a result that differs from previous studies of cement stabilization with arsenic salts. Taken together, these results indicate that cement stabilization is a promising technique to limit arsenic release into the environment under conditions where exposure to low pH can be avoided. Future studies should further evaluate optimal curing conditions for field applications and the stability of mortar and concrete in other potential disposal environments such as landfills or ponds.

3.6 Literature Cited

Akhter, H., F. K. Cartledge, A. Roy and M. E. Tittlebaum (1997). Solidification/stabilization of arsenic salts: Effects of long cure times. *J. Hazard. Mater.* 52(2–3): 247-264.

Al-Khaiat, H. and M. N. Haque (1998). Effect of initial curing on early strength and physical properties of a lightweight concrete. *Cem. Concr. Res.* 28(6): 859-866.

Alexandratos, V. G., E. J. Elzinga and R. J. Reeder (2007). Arsenate uptake by calcite: Macroscopic and spectroscopic characterization of adsorption and incorporation mechanisms. *Geochim. Cosmochim. Acta* 71(17): 4172-4187.

Amrose, S. E., S. R. S. Bandaru, C. Delaire, C. M. van Genuchten, A. Dutta, A. DebSarkar, C. Orr, J. Roy, A. Das and A. J. Gadgil (2014). Electro-chemical arsenic remediation: Field trials in West Bengal. *Sci. Total Environ.* 488–489(0): 539-546.

Bai, J., S. Wild and B. B. Sabir (2002). Sorptivity and strength of air-cured and water-cured PC–PFA–MK concrete and the influence of binder composition on carbonation depth. *Cem. Concr. Res.* 32(11): 1813-1821.

Bardelli, F., M. Benvenuti, P. Costagliola, F. Di Benedetto, P. Lattanzi, C. Meneghini, M. Romanelli and L. Valenzano (2011). Arsenic uptake by natural calcite: An XAS study. *Geochim. Cosmochim. Acta* 75(11): 3011-3023.

Bennett, P. J., F. J. Longstaffe and R. K. Rowe (2000). The stability of dolomite in landfill leachate-collection systems. *Canadian Geotechnical Journal* 37(2): 371-378.

Bertron, A., J. Duchesne and G. Escadeillas (2005). Attack of cement pastes exposed to organic acids in manure. *Cement and Concrete Composites* 27(9–10): 898-909.

Bothe Jr, J. V. and P. W. Brown (1999). Arsenic immobilization by calcium arsenate formation. *Environ. Sci. Technol.* 33(21): 3806-3811.

Camacho, J., H.-Y. Wee, T. A. Kramer and R. Autenrieth (2009). Arsenic stabilization on water treatment residuals by calcium addition. *J. Hazard. Mater.* 165(1-3): 599-603.

Choong, T., T. Chuah, Y. Robiah, F. Gregorykoay and I. Azni (2007). Arsenic toxicity, health hazards and removal techniques from water: an overview. *Desalination* 217(1-3): 139-166.

Costagliola, P., F. Bardelli, M. Benvenuti, F. Di Benedetto, P. Lattanzi, M. Romanelli, M. Paolieri, V. Rimondi and G. Vaggelli (2013). Arsenic-Bearing Calcite in

Natural Travertines: Evidence from Sequential Extraction, μXAS, and μXRF. *Environ. Sci. Technol.* 47(12): 6231-6238.

Criss, J. W., L. S. Birks and J. V. Gilfrich (1978). Versatile x-ray analysis program combining fundamental parameters and empirical coefficients. *Analytical Chemistry* 50(1): 33-37.

Dilnesa, B. Z., E. Wieland, B. Lothenbach, R. Dähn and K. L. Scrivener (2014). Fe-containing phases in hydrated cements. *Cem. Concr. Res.* 58(0): 45-55.

Dixit, S. and J. G. Hering (2003). Comparison of arsenic(V) and arsenic(III) sorption onto iron oxide minerals: implications for arsenic mobility. *Environ. Sci. Technol.* 37(18): 4182-4189.

Dutre, V. and C. Vandecasteele (1995). Solidification/Stabilisation of Arsenic-Containing Waste: Leach Tests and Behaviour of Arsenic in the Leachate. *Waste Manage.* 15(1): 55-62.

Fuller, C., J. Davis and G. Waychunas (1993). Surface chemistry of ferrihydrite: Part 2. Kinetics of arsenate adsorption and coprecipitation. *Geochim. Cosmochim. Acta* 57(10): 2271-2282.

Ghosh, A., M. Mukiibi, A. E. Sáez and W. P. Ela (2006). Leaching of arsenic from granular ferric hydroxide residuals under mature landfill conditions. *Environ. Sci. Technol.* 40(19): 6070-6075.

Hadi, D. A., M. Billah, K. F. Biswas and I. Rafiqul (1999). A study on chemical composition of atmospheric precipitation at Savar, Joydevpur, Tongi and Narayanganj, Bangladesh. *Water, Air, Soil Pollut.* 111: 377-384.

Hammersley, A. P. (1997). FIT2D: an Introduction and Overview; Internal Report ESRF97HA02. Grenoble, France, ESRF.

Jain, C. K. and I. Ali (2000). Arsenic: occurrence, toxicity and speciation techniques. *Water Res.* 34(17): 4304-4312.

Jia, Y. and G. P. Demopoulos (2008). Coprecipitation of arsenate with iron(III) in aqueous sulfate media: Effect of time, lime as base and co-ions on arsenic retention. *Water Res.* 42(3): 661-668.

Jing, C., G. P. Korfiatis and X. Meng (2003). Immobilization mechanisms of arsenate in iron hydroxide sludge stabilized with cement. *Environ. Sci. Technol.* 37(21): 5050-5056.

Jing, C., S. Liu and X. Meng (2005). Arsenic leachability and speciation in cement immobilized water treatment sludge. *Chemosphere* 59(9): 1241-1247.

Kameswari, K. S. B., A. G. Bhole and R. Paramasivam (2001). Evaluation of Solidification/Stabilization (S/S) Process for the Disposal of Arsenic-Bearing Sludges in Landfill Sites. *Environ. Eng. Sci.* 18(3): 167-176.

Kanngießer, B. (2003). Quantification procedures in micro X-ray fluorescence analysis. *Spectrochimica Acta Part B: Atomic Spectroscopy* 58(4): 609-614.

Kjeldsen, P., M. Barlaz, A. Rooker, A. Baun, A. Ledin and T. Christensen (2002). Present and Long-Term Composition of MSW Landfill Leachate: A Review. *Crit. Rev. Environ. Sci. Technol.* 32(4): 297-336.

Kumar, P. R., S. Chaudhari, K. C. Khilar and S. P. Mahajan (2004). Removal of arsenic from water by electrocoagulation. *Chemosphere* 55(9): 1245-1252.

Lee, M., I. S. Paik, I. Kim, H. Kang and S. Lee (2007). Remediation of heavy metal contaminated groundwater originated from abandoned mine using lime and calcium carbonate. *J. Hazard. Mater.* 144(1–2): 208-214.

Leist, M., R. J. Casey and D. Caridi (2000). The management of arsenic wastes: problems and prospects. *J. Hazard. Mater.* 76(1): 125-138.

Leist, M., R. J. Casey and D. Caridi (2003). The fixation and leaching of cement stabilized arsenic. *Waste Manage.* 23(4): 353-359.

Manning, D. A. C. (2001). Calcite precipitation in landfills: an essential product of waste stabilization. *Mineral. Mag.* 65(5): 603-610.

Mohan, D. and C. U. Pittman (2007). Arsenic removal from water/wastewater using adsorbents--A critical review. *J. Hazard. Mater.* 142(1-2): 1-53.

Moon, D. H., D. Dermatas and N. Menounou (2004). Arsenic immobilization by calcium–arsenic precipitates in lime treated soils. *Sci. Total Environ.* 330(1–3): 171-185.

Nakwanit, S., P. Visoottiviseth, S. Khokiattiwong and W. Sangchoom (2011). Management of arsenic-accumulated waste from constructed wetland treatment of mountain tap-water. *J. Hazard. Mater.* 185(2-3): 1081-1085.

Neumann, A., R. Kaegi, A. Voegelin, A. Hussam, A. K. M. Munir and S. J. Hug (2013). Arsenic Removal with Composite Iron Matrix Filters in Bangladesh: A Field and Laboratory Study. *Environ. Sci. Technol.*

Paria, S. and P. K. Yuet (2006). Solidification-stabilization of organic and inorganic contaminants using portland cement: a literature review. *Environ. Rev.* 14(4): 217-255.

Parks, J. L., J. Novak, M. MacPhee, C. Itle and M. Edwards (2003). Effect of Ca on As release from ferric and alum residuals. *American Water Works Association. Journal* 95(6): 108.

Ramezanianpour, A. A. and V. M. Malhotra (1995). Effect of curing on the compressive strength, resistance to chloride-ion penetration and porosity of concretes incorporating slag, fly ash or silica fume. *Cement and Concrete Composites* 17(2): 125-133.

Ravenscroft, P., H. Brammer and K. Richards (2009). Arsenic pollution: a global synthesis, Wiley-Blackwell.

Sanchez, F., C. Gervais, A. C. Garrabrantsa, R. C. Barna and D. S. Kosson (2002). Leaching of inorganic contaminants from cement-based waste materials as a result of carbonation during intermittent wetting. *Waste Manage.* 22(2): 249-260.

Scheidegger, A. M., M. Vespa, D. Grolimund, E. Wieland, M. Harfouche, I. Bonhoure and R. Dähn (2006). The use of (micro)-X-ray absorption spectroscopy in cement research. *Waste Manage.* 26(7): 699-705.

Schlegel, M. C., U. Mueller, U. Panne and F. Emmerling (2011). Deciphering the Sulfate Attack of Cementitious Materials by High-Resolution Micro-X-ray Diffraction. *Analytical Chemistry* 83(10): 3744-3749.

Schmidt, G. T., K. H. Lui and M. Kersten (2009). Speciation and Mobility of Arsenic in Agricultural Lime. *J. Environ. Qual.* 38(5): 2058-2069.

Shahid, S. (2010). Rainfall variability and the trends of wet and dry periods in Bangladesh. *International Journal of Climatology* 30(15): 2299-2313.

Singh, T. S. and K. K. Pant (2006). Solidification/stabilization of arsenic containing solid wastes using portland cement, fly ash and polymeric materials. *J. Hazard. Mater.* 131(1–3): 29-36.

Sø, H. U., D. Postma, R. Jakobsen and F. Larsen (2008). Sorption and desorption of arsenate and arsenite on calcite. *Geochim. Cosmochim. Acta* 72(24): 5871-5884.

Stuckman, M. Y., J. J. Lenhart and H. W. Walker (2011). Abiotic properties of landfill leachate controlling arsenic release from drinking water adsorbents. *Water Res.* 45(16): 4782-4792.

Sugiyama, T., S. Takahashi, S. Honda and E. Sakai (2007). Current State of the JSCE Standard on Test Method for Leaching of Trace Elements from Hardened Concrete.

Sullivan, C., M. Tyrer, C. R. Cheeseman and N. J. D. Graham (2010). Disposal of water treatment wastes containing arsenic - a review. *Sci. Total Environ.* 408(8): 1770-1778.

Sutton, S. R., P. M. Bertsch, M. Newville, M. Rivers, A. Lanzirotti and P. Eng (2002). Microfluorescence and microtomography analyses of heterogeneous earth and environmental materials. Reviews in Mineralogy & Geochemistry: Applications of Synchrotron Radiation in Low-Temperature Geochemistry & Environmental Science. P. A. Fenter, M. L. Rivers, N. C. Sturchio and S. R. Sutton. Chantilly, VA, Mineral Soc Am. 49**:** 429-483.

U.S. EPA (1992a). Method 1311 Toxicity Characteristic Leaching Procedure**:** 1-35.

U.S. EPA (1992b). Method 3050B Acid Digestion of Sediments, Sludges, and Soils. Test Methods for Evaluating Solid Waste, Physical/Chemical Methods SW-846.

van Genuchten, C. M., S. E. A. Addy, J. Peña and A. J. Gadgil (2012). Removing Arsenic from Synthetic Groundwater with Iron Electrocoagulation: An Fe and As K-Edge EXAFS Study. *Environ. Sci. Technol.* 46(2): 986-994.

van Genuchten, C. M., J. Peña, S. E. Amrose and A. J. Gadgil (2014). Structure of Fe(III) precipitates generated by the electrolytic dissolution of Fe(0) in the presence of groundwater ions. *Geochim. Cosmochim. Acta* 127(0): 285-304.

Wang, Y., S. Sikora, H. Kim, B. Dubey and T. Townsend (2012). Mobilization of iron and arsenic from soil by construction and demolition debris landfill leachate. *Waste Manage.* 32(5): 925-932.

Wee, H.-Y. and T. Kramer, A. (2005). Characterization of Arsenic-Containing Water Treatment Residuals. Advances in Arsenic Research, American Chemical Society. 915**:** 321-332.

Winkel, L. H. E., B. Casentini, F. Bardelli, A. Voegelin, N. P. Nikolaidis and L. Charlet (2013). Speciation of arsenic in Greek travertines: Co-precipitation of arsenate with calcite. *Geochim. Cosmochim. Acta* 106(0): 99-110.

Yokoyama, Y., K. Tanaka and Y. Takahashi (2012). Differences in the immobilization of arsenite and arsenate by calcite. *Geochim. Cosmochim. Acta* 91(0): 202-219.

Yoon, I.-H., D. H. Moon, K.-W. Kim, K.-Y. Lee, J.-H. Lee and M. G. Kim (2010). Mechanism for the stabilization/solidification of arsenic-contaminated soils with Portland cement and cement kiln dust. *Journal of Environmental Management* 91(11): 2322-2328.

Chapter 4

Anaerobic microbial community response to methanogenic inhibitors 2-bromoethanoesulfonate and propynoic acid

4.1 Summary

Studies of methanogenesis often include the use of chemical inhibitors to limit the activity of methanogenic archaea. When used in studies of complex microbial communities, it is important to characterize inhibitor induced structural and functional changes of archaeal and bacterial communities. We characterized microbial community structure and activity in cow dung and municipal wastewater treatment plant anaerobic digester sludge seeded mesocosms after exposure to two methanogenesis inhibitors, 2-bromoethanesulfonate (BES) and propynoic acid (PA). Methane production was reduced by 89 % (0.5 mM BES), 100 % (10 mM BES), 24 % (0.1 mM PA), and 95 % (10 mM PA). Using modified primers targeting the methyl-coenzyme M reductase (*mcrA*) gene, involved in the final step of methanogenesis, changes in *mcrA* gene expression were found to correlate with changes in methane production and the relative activity of methanogens. Methanogenic activity was determined by the relative abundance of methanogen 16S rRNA cDNA as a percentage of the total community 16S rRNA cDNA and decreased with increasing concentrations of inhibitors. Aceticlastic methanogens were inhibited to a greater extent than hydrogenotrophic methanogens. The relative abundance of the 16S rRNA cDNA of some syntrophic bacteria was reduced following

Clancy, T. M., A. L. Smith, R. Reddy, A. J. Pinto, K. F. Hayes and L. Raskin (2015). *in prep. for Environmental Microbiology*.

exposure to both inhibitors, but the overall structure of the active bacterial community was not significantly different.

4.2 Introduction

Methane can be viewed as a potent greenhouse gas, an energy source, a dangerous and explosive byproduct of anaerobic biodegradation, a waste product diverting energy from animal feed, or a driver of microbial carbon cycling (Hallam *et al.* 2003, Dupont and Accorsi 2006, Knittel and Boetius 2009, Appels *et al.* 2011, Chowdhury and Dick 2013, IPCC 2013, Patra and Yu 2013). Due to methane's importance in fields ranging from climate science to animal husbandry, much research has focused on understanding the activity of methanogenic archaea under anaerobic conditions (Reeve *et al.* 1997, Conrad 2007). Aerobic methane generation has also been identified and may be an important source of methane from oceans (Karl *et al.* 2008), however this study will focus on methane production under anaerobic conditions. All known methanogenic archaea contain genes that encode for the methyl-coenzyme M reductase (MCR) the final step of methanogenesis. There are two isoenzymes, MCRI and MCRII, and the *mcrA* and *mrtA* genes encode for α-subunit of each, respectively (Reeve *et al.* 1997). The mcrA/mrtA genes have been a common target for measuring methanogen abundance, activity, and diversity. The agreement between phylogenetic trees based on 16S rRNA genes and *mcrA* genes (Luton *et al.* 2002) has been used to support the use of the *mcrA* gene as a methanogen specific phylogenetic target.

Compounds that inhibit methanogenesis have been important in research to study pure cultures of methanogens (Ungerfeld *et al.* 2004, Watkins *et al.* 2012), and mixed communities to understand carbon cycling in soils (Sugimoto and Wada 1993, Wu *et al.*

2001), the inhibition of ruminal methanogens (Ungerfeld *et al.* 2006, Zhou *et al.* 2011b), and microbial populations involved in dechlorination (Perkins *et al.* 1994, Chiu and Lee 2001), mercury methylation (Han *et al.* 2010, Avramescu *et al.* 2011), and the degradation of nitrosamines (Tezel *et al.* 2011). Further, inhibitors have been useful in elucidating the activity of methanogens related to metal and metalloid methylation (Meyer *et al.* 2008, Thomas *et al.* 2011). A variety of chemicals have been applied to inhibit methanogenesis in livestock to either reduce methane emissions or to direct more of the feed energy to animals for increased agricultural output (i.e., milk and meat) (Machmüller and Kreuzer 1999, Boadi *et al.* 2004, Beauchemin *et al.* 2009). Regardless of the intended use, when methanogenic inhibitors are used in mixed communities, detailed characterization of inhibitor-induced changes to both archaeal and bacterial populations is needed to ensure that the observed effects can be accurately ascribed to the inhibition of methanogenic activity or to elucidate indirect effects. This is especially important given that a wide diversity of methanogenic inhibitors with varying properties and mechanisms of action have been used. Methanogen inhibitors can be divided into several categories (as reviewed by (Liu *et al.* 2011)), including analogs of coenzyme M (Gunsalus *et al.* 1978, Zinder *et al.* 1984), inhibitors of methanopterin biosynthesis (Dumitru *et al.* 2003), medium and long chain fatty acids (Prins *et al.* 1972, Soliva *et al.* 2003), nitrocompounds (Zhou *et al.* 2011b), halogenated hydrocarbons (Denman *et al.* 2007), ethylene (Oremland and Taylor 1975), acetylene (Oremland and Taylor 1975, Sprott *et al.* 1982), and unsaturated analogs of propionate and butyrate (Ungerfeld *et al.* 2003, Ungerfeld *et al.* 2004, Ungerfeld *et al.* 2006, Zhou *et al.* 2011b).

While many inhibitors are considered methanogen-specific, various studies have found that other microorganisms can be affected. The most commonly used methanogenesis inhibitor, 2-bromoethanesulfonate (BES), a coenzyme M analog, has been found to also inhibit dechlorinating bacteria (Loffler *et al.* 1997, Chiu and Lee 2001) and affect bacterial growth on aliphatic alkenes (Boyd *et al.* 2006). Propynoic acid (PA), an unsaturated propionate analog with one triple carbon bond, is an effective inhibitor of methanogenesis (Ungerfeld *et al.* 2004, Zhou *et al.* 2011b). However, limited studies have been performed on the effects of PA on the structure of microbial communities (Patra and Yu 2013). To date, studies of the impacts of methanogenesis inhibitors on bacterial and archaeal communities have relied on clone libraries, denaturing gel electrophoresis (DGGE), or terminal restriction fragment length polymorphism (TRFLP) targeting the 16S rRNA gene (Chiu and Lee 2001, Xu *et al.* 2010a, Xu *et al.* 2010b, Patra and Yu 2013) and the *mcrA* gene (Denman *et al.* 2007). Results from DGGE based evaluations of the impact of inhibitors have shown changes in the overall community structure and did not yield insights into how specific populations were impacted (Chiu and Lee 2001, Patra and Yu 2013). Studies using TRFLP and clone libraries of the 16S rRNA gene have reported decreases in the relative abundance of aceticlastic methanogens and syntrophic bacteria and increases in the relative abundance of homoacetogens after exposure of mesophilic anaerobic digester sludge to BES and chloroform (Xu *et al.* 2010a, Xu *et al.* 2010b). In a study of cow rumen communities, *mcrA* gene clone libraries and quantitative PCR revealed a decrease in the most abundant methanogen genus, *Methanobrevibacter*, under BES inhibited conditions (Denman *et al.* 2007). Since these studies relied on DNA-based techniques (Chiu and Lee 2001,

Denman *et al.* 2007, Xu *et al.* 2010a, Xu *et al.* 2010b, Patra and Yu 2013) they may not have revealed short-term changes in microbial activity in batch mesocosms or in systems with low yield, because of low growth rates and retention of dead or inactive biomass and extracellular DNA (Chiao *et al.* 2014, Smith *et al.* 2014).

In this study, we evaluated a modification to commonly used PCR primer sets targeting the *mcrA* gene. We then applied this primer set to track the expression of *mcrA* genes by using reverse transcriptase quantitative PCR (RT-qPCR) in mixed communities seeded with anaerobic digester sludge and cow dung at different levels of inhibition by either BES or PA. The effects of BES and PA on methanogenic and bacterial populations were characterized through a combination of DNA- and RNA- based Illumina sequencing targeting theV4 region of the 16S rRNA genes and 16S rRNA cDNA and *mcrA* genes and *mcrA* transcript cDNA.

4.3 Results & Discussion

To target the *mcrA* gene in methanogens, the mlas forward primer described by Steinberg and Regan (2009) was modified with additional degeneracies and used with the previously reported mcra-rev reverse primer (Steinberg and Regan 2008). These modifications improved the predicted amplification for 10 of the 32 methanogens with complete genomes available (Table S3, Appendix D). Amplification was confirmed using ten DNA extracts from pure cultures of methanogens (Table S3 and S4, Appendix D). These extracts were pooled to create two mock communities A and B, to represent either a relatively even community (A) or an uneven community (B) with relative methanogen abundances similar to what would be found in an anaerobic digester (Smith *et al.* 2013). For these mock communities both the 16S rRNA genes and *mcrA* genes were sequenced.

Similar trends were observed when comparing mock community A with mock community B for both genes, though there were differences in the percent relative abundances predicted by the two genes (Figure 4-8). In a previous comparison of methanogen mock communities with TRFLP greater differences between the expected and observed communities were found based on the *mcrA* gene as compared to the 16S rRNA genes and attributed to the higher number of degeneracies in the primers used for the *mcrA* gene (Lueders and Friedrich 2003). These differences highlight several challenges in quantification using amplicon sequencing due to biases dependent on gene target, PCR conditions, quantification method, and primer biases (Suzuki and Giovannoni 1996, Zhou *et al.* 2011a, Pinto and Raskin 2012).

In this study, the relative abundance of *Methanobacterium* was much greater than expected for both the 16S rRNA and *mcrA* genes, while *Methanosaeta* was much lower in relative abundance than expected (Figure 4-8). Although, as expected, both genera were in greater abundance in mock community B compared to mock community A for both genes. For *Methanobrevibacter, Methanococcus,* and *Methanosphaera*, the relative abundance as measured by the *mcrA* gene was much lower in both mock communities as compared to the expected values and those measured by the 16S rRNA gene. There are no mismatches between the primers and the gene sequences for these organisms (Table S5, Appendix D) and a similar under-representation was observed for a mock community generated by pooling individually amplified PCR products of the *mcrA* gene from each strain (Figure S3, Appendix D).

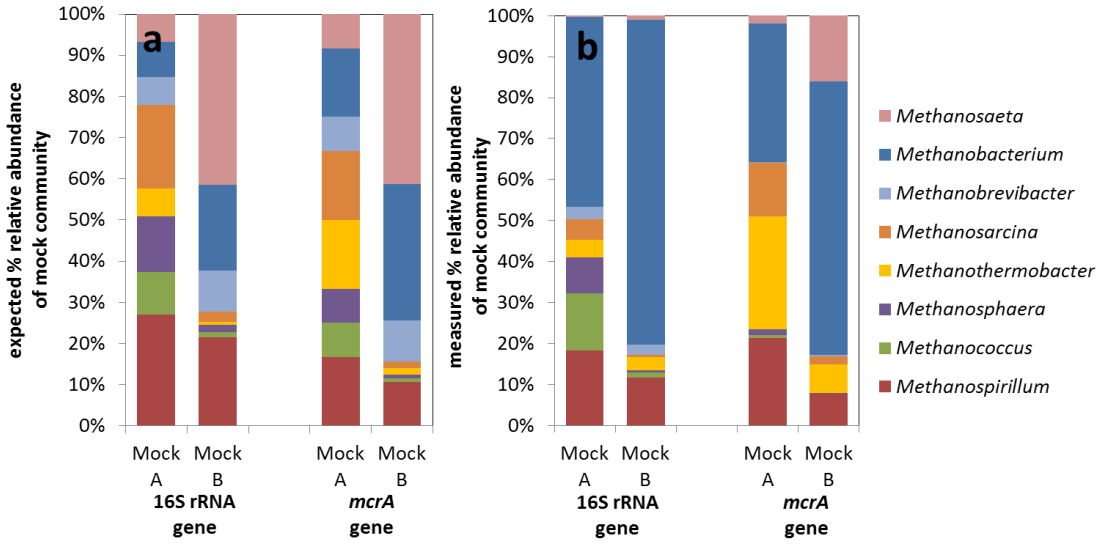


Figure 4-8. Comparison of expected (a) and measured (b) methanogen relative abundance from two mock communities A and B with 16S rRNA gene and *mcrA* gene sequencing.

To characterize changes in mixed communities induced by methanogenic inhibitors, biomass samples were collected from cow dung and anaerobic digester sludge mesocosms with varying levels of methanogenic activity controlled by the addition of BES and PA. Methanogenic activity was monitored through the measurement of methane production and *mcrA* gene expression. The microbial communities were characterized using sequencing of the 16S rRNA gene, 16S rRNA cDNA, *mcrA* genes, and *mcrA* transcript cDNA. As expected, with increasing concentrations of the methanogen inhibitors BES and PA, the rate of methane production and cumulative methane produced decreased (Figure 4-9 and Figure S4, Appendix D). Expression of the *mcrA* gene positively correlated with methane production (Figure 4-9). A similar correlation between methane production and the 16S rRNA cDNA sequences (referred to here as "relative activity") of methanogens over the total community (including *Bacteria* and *Archaea*) was also found (Figure 4-9). While there are biases associated with using

16S rRNA cDNA as a marker of activity (Blazewicz *et al.* 2013), the correlation between methanogen 16S rRNA cDNA and a functional gene specific to methanogens indicates that 16S rRNA activity can be a reliable metric for methanogen activity in these conditions.

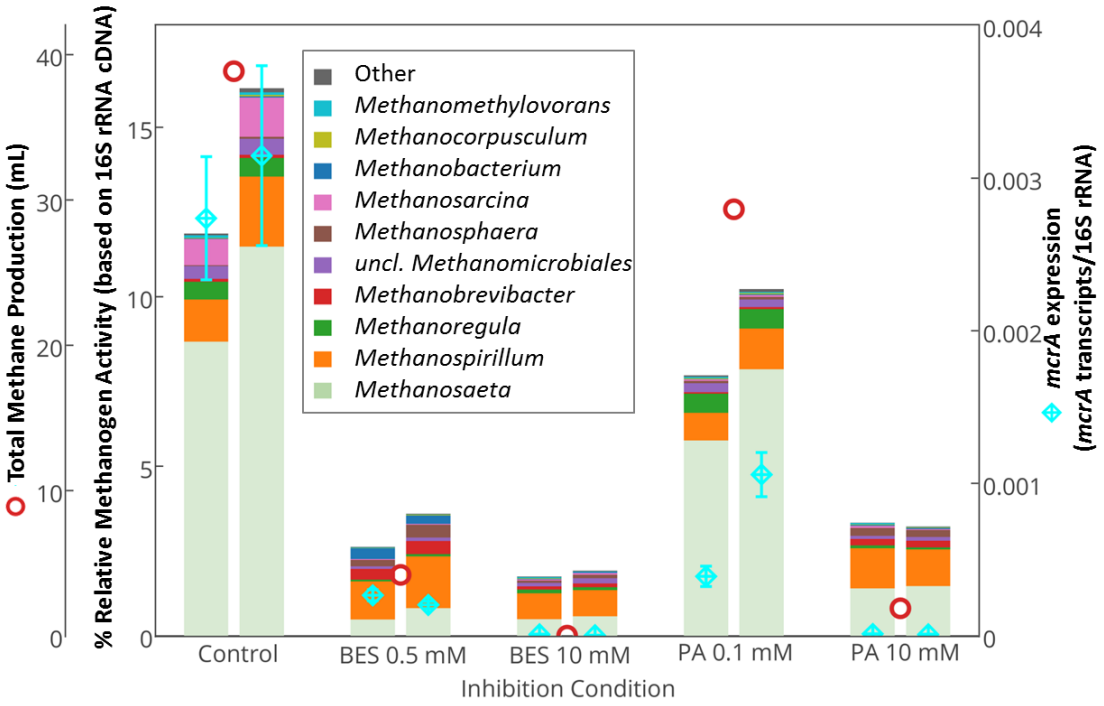


Figure 4-9. Relative methanogen activity (based on methanogen 16S rRNA cDNA as a % of the total community (including *Bacteria* and *Archaea*) 16S rRNA cDNA (bars), *mcrA* expression normalized by 16S rRNA cDNA (diamonds) determined with RT-qPCR, and cumulative methane production (circles). The *mcrA* expression is displayed as the averages and standard deviations of triplicate qPCR reactions. Duplicates shown represent duplicate biomass samples from the same reactors. No inhibitor was added in control conditions.

Sequencing of 16S rRNA gene, 16S rRNA cDNA, *mcrA* genes, and *mcrA* transcript cDNA was performed to characterize the changes in the community structure of methanogenic populations under different inhibition conditions. As expected, given the short duration of this experiment (9 days), the differences in the archaeal DNA-based

sequencing results for the different conditions were modest (Figure 4-10a and c) compared to RNA-based sequencing results (Figure 4-10b and d), which revealed significant differences in the methanogenic communities for the different conditions. As with the mock communities, when comparing the results between the two different genes sequenced (16S rRNA gene/16S rRNA cDNA and *mcrA* genes/*mcrA* transcript cDNA) (Figure 4-10 a and b compared to c and d) for any given methanogenic genus, the trends in relative abundance and activity are similar across inhibition conditions. However there are differences in the percent relative abundance and activity. Similar to the results from the mock communities, *Methanosaeta* spp. made up a larger percent relative abundance and activity in *mcrA*-based sequencing, while *Methanospirillum* spp. made up a larger percent relative abundance and activity according to 16S rRNA-based sequencing.

In results from both 16S rRNA gene and 16S rRNA cDNA sequencing, *Methanosaeta* spp. was the most abundant and active methanogen, representing 38 % of the archaeal 16S rRNA gene and 71 % of the archaeal 16S rRNA cDNA sequences (Figure 4-10). Results from *mcrA* gene and transcript cDNA sequencing also show *Methanosaeta* spp. was the most abundant and active methanogen, representing 86 % and 93 % of the methanogen community and active methanogen community, respectively. Further, the activity of *Methanosaeta* spp. was reduced in both BES and PA 10 mM inhibition conditions, shown by both 16S rRNA cDNA and *mcrA* transcript cDNA results (Figure 4-10b and d). Very little difference was observed between *Methanosaeta* spp. activity in PA 0.1 mM compared to the control condition. This is consistent with the methane generation results where among inhibited conditions PA 0.1 mM generated the most methane (Figure 4-8). Results from both the 16S rRNA gene and 16S rRNA cDNA

sequencing found *Methanosphaera* spp. and *Methanobrevibacter* spp. represented a greater fraction of the archaeal community and active archaeal community under all inhibited conditions compared to the control (Figure 4-10a and b). These genera made up a smaller fraction of the *mcrA*-based communities, though *Methanobrevibacter* spp. was found to be more active in most inhibited conditions as compared to the control based on *mcrA* transcript cDNA (Figure 4-10c). *Methanoregula* spp. was between 15-33 % of the archaeal community according to 16S rRNA gene sequencing, and its activity was a smaller fraction, between 2-6 %, based on 16S rRNA cDNA sequencing in all conditions. Using *mcrA*-based sequencing *Methanoregula* was less than 2 % of the abundance and activity of methanogens under all conditions.

The mock community results showed that *Methanobacterium* was less abundant in the *mcrA*-based communities compared to the 16S rRNA gene-based communities (Figure 4-8) and this was similarly observed in the 16S rRNA gene-based mesocosm results compared to the *mcrA* gene sequencing (Figure 4-10a compared to c). However, the RNA-based sequencing of the *mcrA* transcript cDNA revealed much higher activity of hydrogenotrophic methanogens *Methanobacterium* spp. and *Methanomicrobium* spp. at high PA and both BES conditions compared to the control (Figure 4-10d). The 16S rRNA cDNA based activity difference for *Methanobacterium* spp. was less substantial, but shows a similar trend (Figure 4-10b).

One explanation for this drastic difference in *mcrA*-based activity may be the presence of a second gene that encodes for an isoenzyme of methyl-coenzyme M, the *mrtA* gene. This gene has been found in members of both *Methanobacterium* and *Methanomicrobium* genera (Bonacker *et al.* 1992, Luton *et al.* 2002), but to date has not

been reported in aceticlastic methanogens. Other genera with identified *mrtA* genes include *Methanothermobacter* spp. and *Methanosphaera* spp., though the gene is not well annotated or differentiated from reported *mcrA* gene sequences. A comparison between representative *mcrA* sequences from the different operational taxonomic units (OTUs) that were identified as *Methanobacterium* and *Methanomicrobium* shows that of the seven OTUs, one is highly similar (95.9%) to a *Methanobacterium mrtA* gene (OTU 6, Figure S5, Appendix D) and is highest in relative activity in the BES and PA 10 mM conditions (Figure S6, Appendix D). Interestingly, pure culture studies with *Methanobacterium thermoautotrophicum* have found differential expression of the *mcrA* and *mrtA* genes, with the *mrtA* being more highly expressed during the exponential growth phase of methanogens with the gene and under conditions of high substrate availability (Bonacker *et al.* 1992, Pihl *et al.* 1994, Pennings *et al.* 1997).

The other OTUs observed here were more closely related to known *mcrA* sequences. OTU 2 is also highest in relative activity during these conditions and is more closely related to the *mcrA* gene from *Methanobacterium* sp. T01 which is only 71.8% similar to the *Methanobacterium mrtA* gene. We suspect that there are reasons beyond the increase in *mrtA* expression that allow *Methanobacterium* and *Methanomicrobium* to continue expressing the *mcrA* gene during inhibitor exposure. This is also consistent with other studies that found hydrogenotrophic methanogens to be less sensitive to inhibition than aceticlastic methanogens (Zinder *et al.* 1984, Perkins *et al.* 1994, Xu *et al.* 2010a). Multiple explanations for these results have been hypothesized, including differences in cell envelopes that might result in higher exposure to inhibitors or differences in coenzyme M transport rates (Xu *et al.* 2010a).

It is important to note that the shifts in Figure 4-10 represent relative changes in total methanogen abundance and activity. Given the challenges with quantitative nucleic acid extractions from heterogeneous biomass samples, these relative abundance and activity data were not converted to an absolute quantification of abundance or activity per biomass. However, by comparing the abundance and activity of methanogens as a fraction of the total community abundance and activity (*Bacteria* and *Archaea*) (Figure 4-9) it is clear that the methanogenic activity is lower for higher inhibitor concentrations.

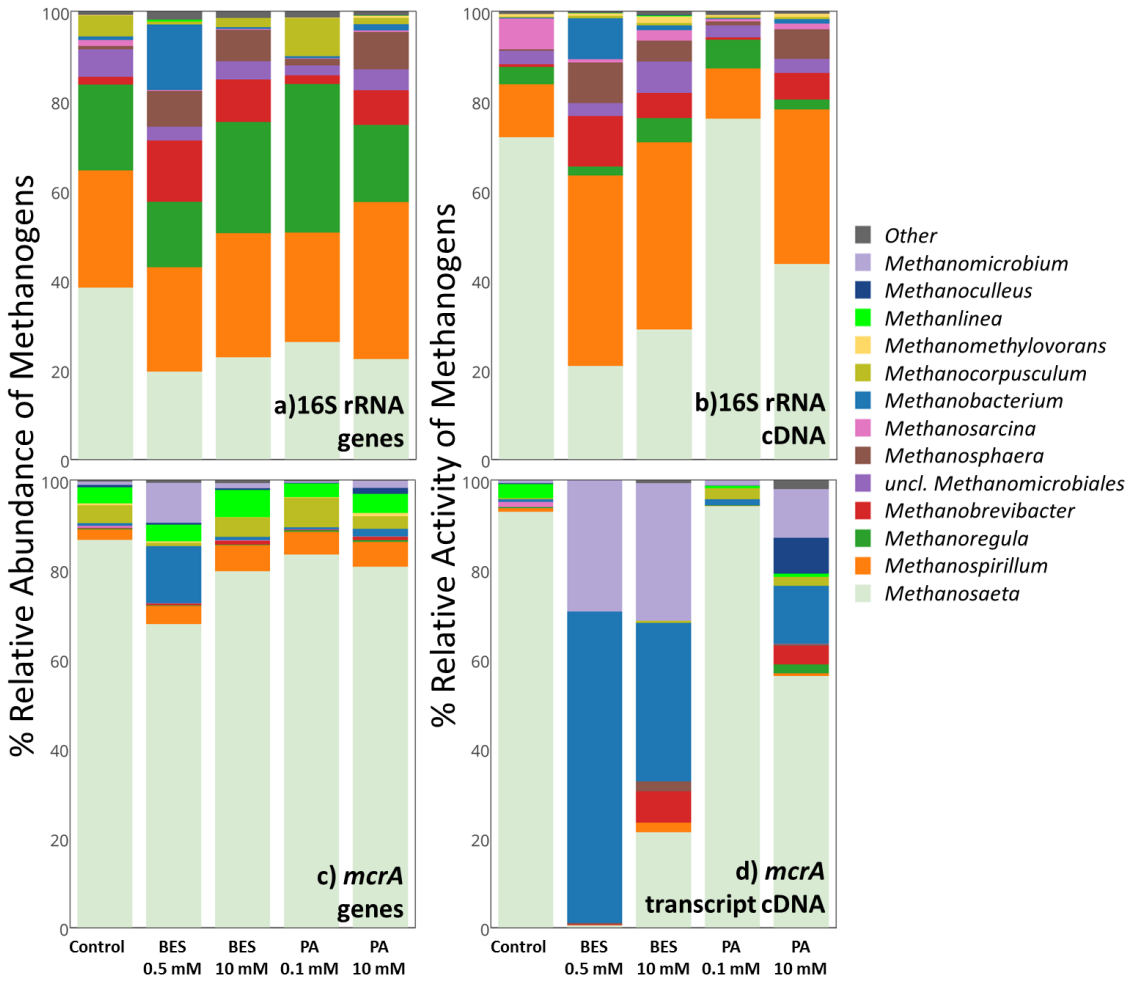


Figure 4-10. Relative abundance (DNA) and activity (RNA) of methanogens based on 16S rRNA (top) and *mcrA* (bottom) sequencing. Sequences from duplicate samples for each condition are combined.

Seven populations of previously described syntrophic fatty-acid oxidizing bacteria were identified in these mesocosm samples. The communities were predominantly comprised of *Syntrophomonas*, a butyrate and higher VFA oxidizer (Sousa *et al.* 2007), and *Smithella*, a propionate oxidizer (Liu *et al.* 1999) (Figure 4-11). These populations have a coupled metabolism with hydrogenotrophic methanogens that keep the partial pressure of H_2 low such that their metabolism is energetically favorable. Due to this important relationship between syntrophic bacteria and methanogens, the inhibition of hydrogenotrophic methanogens (Figure S7, Appendix D) is likely to cause an increase in the partial pressure of hydrogen and therefore changes in syntrophic bacteria activity. Using fluorescence in situ hybridization (FISH) in sewage sludge digesters exposed to BES, Xu *et al.* (2010b) observed a lower abundance of syntrophic bacteria under methanogenesis inhibited conditions compared to a control. In the current study, greater changes were observed in relative activity (RNA-based) as compared to relative abundance (DNA-based) as expected, given the short duration of the experiment (Figure 4-11). *Syntrophomonas* abundance and activity were lower during inhibited conditions compared to the control (Figure 4-11). The relative abundance and activity of *Smithella* did not decrease with the presence of either inhibitor. The energetics of butyrate and propionate oxidation is dependent on the partial pressure of hydrogen, which was not measured in this study, but may have contributed to the differential response (Figure S8, Appendix D). Other factors that may contribute to these observed differences include the production and degradation rates of 16S rRNA levels. While these rates are not known, there are differences in 16S rRNA gene copy number between *Syntrophomonas* and *Smithella* that have three and one copies, respectively. This difference can be indicative

of differential growth strategies where higher copy numbers are associated with faster growth rates following environmental changes (Klappenbach *et al.* 2000). This would be consistent with our finding that *Syntrophomonas* responded more quickly to environmental changes due to inhibition of methanogenesis.

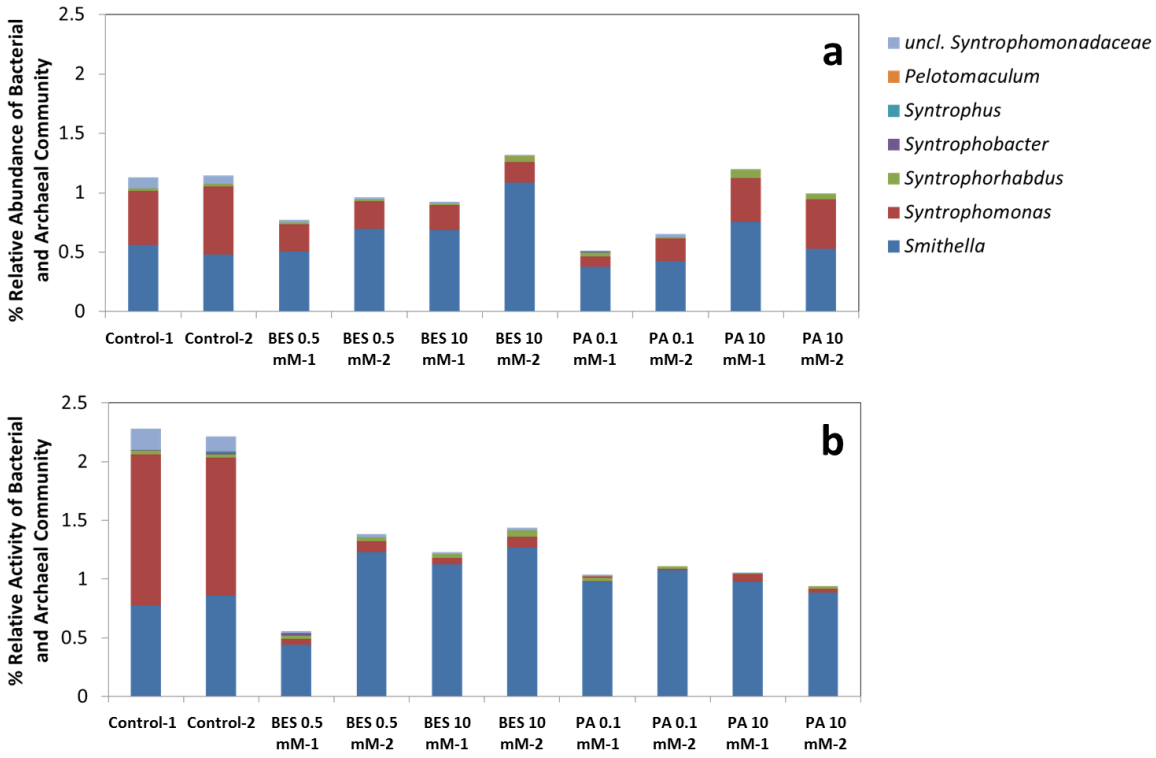


Figure 4-11. Relative abundance (a) and activity (b) of syntrophic bacteria as a percentage of the total bacterial and archaeal communities based on 16S rRNA gene and 16S rRNA cDNA sequencing.

Overall, the bacterial community present in these mesocosms is quite diverse, containing greater than 9000 OTUs, grouped at a 0.03 cut-off, and 600 phylotypes, grouped taxonomically at the genus level. The overall shifts in the structure of the active bacterial community are not significant between the different conditions (θ_{yc} AMOVA, p-value > 0.05). There are few changes in the relative activity of the 20 most abundant phylotypes (Figure 4-12a). Other studies have found general evidence for a community

shift using less resolved techniques for community fingerprinting. Chiu and Lee (2001) used DGGE and measurements of dechlorination products and found a shift in the bacterial community over long-term exposure to BES. DGGE also revealed shifts in rumen fluid mesocosms exposed to PA when used in combination with other inhibitors (Patra and Yu 2013). It is difficult to compare these previous findings with the current study as DGGE the specific bacterial groups responsible for these shifts were not identified.

In this study, an indicator analysis (Dufrêne and Legendre 1997) was applied to determine the bacterial groups whose presence, or in this case activity (based on 16S rRNA cDNA) is indicative of each condition. The statistically significant groups (p-value < 0.05) are shown in Figure 4-12b. Of the bacterial groups identified as indicators of the control samples, two are syntrophic bacterial groups (*Syntrophomonas* and an unclassified member of *Syntrophomonadaceae*). As previously described this result is expected due to the inhibition of these groups in both BES and PA conditions. An unclassified member of the order *Fusobacteriales* is also more active in control samples compared to all other conditions. Groups identified as indicators of inhibited conditions include cellulose degraders and groups commonly found in rumen and plant matter digesters, including the groups *Cellulosilyticum* (Li *et al.* 2014), *Clostridium III* and *IV* (Collins *et al.* 1994), *Prevotella* (Williams *et al.* 2013), and *Succinivibrio* (Yue *et al.* 2013). Future studies employing chemical inhibitors of methanogenesis should recognize the potential for these genera to increase in activity and for the activity of some syntrophic bacteria to decrease.

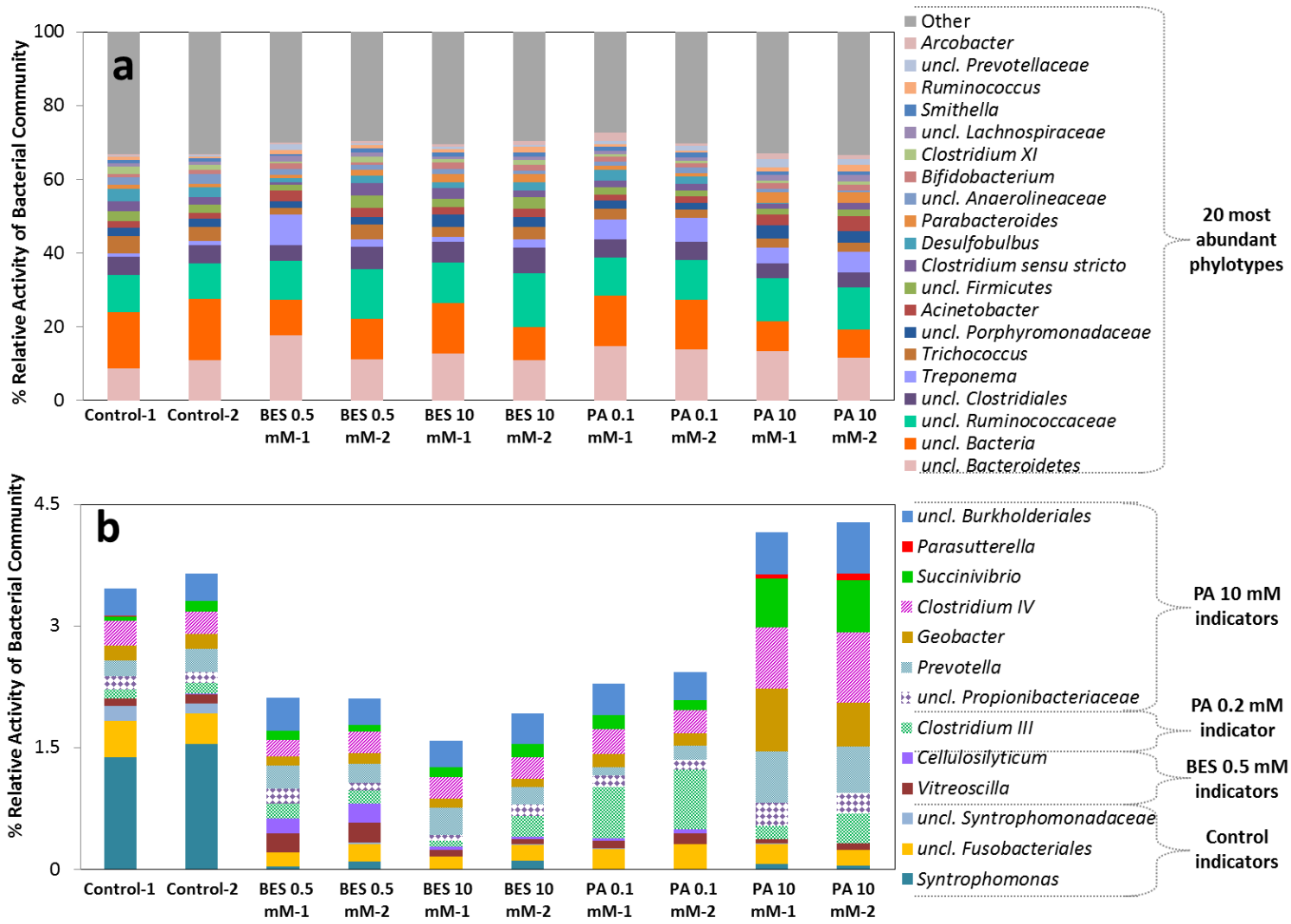


Figure 4-12. Relative activity of the 20 most abundant bacterial phylotypes (a) and the phylotypes identified as indicator organisms ($p < 0.05$) (b) for each inhibition condition.

4.4 Experimental Procedures

4.4.1 Mesocosm set-up

Mesocosms were seeded with 6 g wet cow dung, collected from a field where grass and corn fed cattle were grazing using sterile plastic scoops, in 100 mL of concentrated (approximately 5000 mg/L total suspended solids) anaerobic digester sludge collected from a mesophilic (32 °C) wastewater treatment plant anaerobic sludge digester (Northfield Wastewater Treatment Plant, Whitmore Lake, MI). Control mesocosms contained no added inhibitor. The effect of 2-bromoethane sulfonate (BES) addition was evaluated at concentrations of 0.5 and 10 mM, whereas propynoic acid (PA) was tested at concentrations of 0.1 and 10 mM. Some conditions were evaluated in replicate bottles and additional inhibitor concentrations (50 mM BES and 1 mM PA) were also tested (Figure S4, Appendix D). The pH was measured before capping the mesocosms and brought to pH 7.0 using sodium hydroxide. The 150 mL serum bottles were capped with a butyl rubber stopper, crimp sealed, and purged with N_2 gas before the start of the experiment. Incubations were carried out in a 31 °C water bath and the mesocosm contents were mixed on magnetic stir plates.

4.4.2 Mesocosm sampling

A glass syringe (Chemglass Life Sciences, Vineland, New Jersey) was used to measure gas production and collect gas for composition measurements using a needle about every other day. The CH_4, CO_2, and N_2 composition in the headspace gas was measured using a gas chromatograph (Gow-Mac, Bethlehem, PA) coupled with a thermal conductivity detector (TCD). On day 9, after a final collection of the headspace gas, the bottles were opened and the biomass centrifuged at 4 °C. The supernatant was decanted

and biomass samples were collected for DNA and RNA extraction, the latter being preserved with RNAlater (Qiagen, Valencia, California). Following collection, biomass samples were frozen at -80 °C until extraction.

4.4.3 Nucleic acid extractions and cDNA synthesis

DNA and RNA was extracted from biomass samples using the automated extraction Maxwell 16 Blood LEV kit or Maxwell 16 simplyRNA tissue kit, for DNA or RNA, respectively according to the manufacturer's instructions. Briefly, zirconium beads (0.1 mm) and lysis buffer were added to each sample and three 2 minute bead beating steps were performed, replacing the lysis buffer after each bead beating. Proteinase K was added to each sample for DNA extraction prior to the automatic extraction. For RNA extraction, the method was the same, except bead beating was performed in 1-thiolyglycerol homogenization buffer and 10 µL of DNase 1 (instead of 5 µL) was added to the extraction kit. Nucleic acid quality and quantity were determined using spectrophotometry (Nanodrop 1000, Thermo Fischer Scientific, Wilmington, DE), fluorospectrometry (Quantifluor dsDNA and RNA systems (Promega, Madison, WI) and Nanodrop 3000 (Thermo Fischer Scientific, Wilmington, DE)), and for RNA samples using electrophoresis with the Experion RNA analysis kit (Bio-Rad, Hercules, CA). cDNA was synthesized using SuperScript® VILO cDNA synthesis kit according to the manufacturer's instructions (Invitrogen, Carlsbad, CA).

4.4.4 Primer design

Primers targeting the *mcrA* gene were designed through an *in silico* analysis followed by testing with pure cultures and mock communities. First, existing primer sets (Juottonen *et al.* 2006, Steinberg and Regan 2008, Steinberg and Regan 2009, Zeleke *et*

al. 2013) were compared to partial *mcrA* sequences downloaded from GenBank (NCBI, Bethesda, MD) and back translated full length McrA protein sequences using EMBOSS Backtranseq with the *Methanothermobacter thermoautotrophicus* strain Delta H codon usage table (EMBL EBI, Hinxton, UK) using MEGA 6.0 (Tamura *et al.* 2013). The forward primer mlas was modified with additional degeneracies (5'GGYGGTGTMGGNTTCACHCARTA-3') (Steinberg and Regan 2009). The reverse primer mcra-rev was used as reported previously (5'-CGTTCATBGCGTAGTTVGGRTAGT-3') (Steinberg and Regan 2008). Primer specificity and coverage was assessed *in silico* using MFE primer 2.0 (Qu *et al.* 2012). The v4 region of 16S rRNA gene was targeted using universal primers F515 (5'-GTGCCAGCMGCCGCGGTAA-3') and R806 (5'- GGACTACHVGGGTWTCTAAT-3') (Caporaso *et al.* 2011). The coverage of these primers was verified with TestPrime 1.0 (Klindworth *et al.* 2012). Both primer sets were checked for complementarity with sequences from the complete genomes of the methanogens used in the mock communities (Table S5, Appendix D).

PCR and RT-qPCR

To verify the amplification of the *mcrA* gene from a range of methanogens, DNA extracts from pure cultures of methanogens were used as a template for PCR over a range of annealing temperatures. PCR was performed using 20 µL reactions with primers at 500 nM, 0.5 ng of template, 0.3 mg/mL bovine serum albumin (BSA), 10 µL Phusion High Fidelity Master Mix (NEB, Ipswich, MA), and nuclease-free water. An initial 2 minute denaturation at 95 °C was followed by 30 cycles of denaturing at 95 °C for 20 s, annealing at 55 °C for 15 s, and extension at 72 °C for 30 s, with a final extension at 72

°C for 5 min. PCR products were visualized on a 1.5 % agarose gel. PCR products for use as qPCR standards were generated in the same fashion for both *mcrA* and 16S rRNA gene amplicons, using DNA extracts from mesocosm samples pooled by equal mass as the template (He and McMahon 2011, Sonthiphand *et al.* 2013). PCR products were visualized on a 1.5 % agarose gel and the band was excised and purified with the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). Amplified and purified pools were quantified using the Quantifluor dsDNA system and fluorospectrometry. Serial dilutions of the pools were prepared for qPCR standards and ranged from 10^7-10^2 copies/µL for *mcrA* and 10^8-10^4 copies/µL for 16S rRNA genes.

The Mastercycler Realplex Ep (Eppendorf, Hamburg, Germany) was used to perform RT-qPCR with triplicate wells for each sample and reaction volumes of 19 µL using Fast Plus EvaGreen Master Mix (Biotium, Hayward, CA). Forward and reverse primer concentrations were 500 nM, except the reverse *mcrA* primer was 250 nM. The conditions used for thermocycling were as described above with slight modification. Instead of 30 cycles, 50 cycles were performed and a melting curve analysis was performed as the final step. To improve annealing conditions for the *mcrA* transcript cDNA quantification, there was an initial 2 min denaturation of the cDNA at 95 °C, then 5 cycles of 95 °C for 20 s, 55 °C for 15 s, followed by a temperature ramp of 0.1 °C/s to 72 °C (Luton *et al.* 2002, Morris *et al.* 2014), and extension for 72 °C for 30 s. Then 45 cycles were performed without the temperature ramp with a final extension at 72 °C for 5 min. The standard curves R^2 were 0.995 and 0.998 and efficiencies were 74% and 89%, for *mcrA* and 16S rRNA genes, respectively.

Mock communities

Three different mock communities (even, even-PCR, and digester) were created by mixing varying amounts of either DNA extracts or amplified PCR products. The even mock community was made by mixing DNA extracts based on concentration and genome length to achieve an even community. The even-PCR mock community was made by mixing mcrA-amplified PCR products from each methanogen mixed evenly based on PCR product concentration. The digester mock community was mixed from DNA extracts from each methanogen based on genome length to achieve a community representative of an anaerobic digester, expected communities based on these calculations are shown in Figure S3 (Appendix D).

Sequencing and analysis

Samples from the mock community, mesocosm DNA, and mesocosm cDNA were submitted for sequencing of the v4 region of the 16S rRNA gene at the Host Microbiome Initiative (University of Michigan, Ann Arbor, MI). Primers F515 and R806 (Caporaso *et al.* 2011) were modified for sequencing as described by Kozich *et al.* (2013). PCR was performed using Accuprime TAQ (Invitrogen) and thermocylcing conditions were 2 min 95 °C denaturation, followed by 30 cycles of denaturation at 95 °C for 20 s, annealing at 55 ° C for 15 s, and extension at 72 °C for 5 min, the final extension was performed at 72 °C for 5 min. Samples were also submitted for sequencing of the *mcrA* gene, amplification was performed as described above. Following amplification of either gene, the SequalPrep Normalization Plate Kit (Life Technologies, Grand Island, NY) was used to pool samples by equal mass. Amplicons were multiplexed and sequenced using the Illumina MiSeq, Reagent Kit V2 was used for *mcrA* amplicons resulting in a total of 20,842 paired-end reads after quality filtering, there were between 193 and 2240

sequences per sample. For 16S rRNA gene amplicons Reagent Kit V3 was used and resulted in a total of 15,152 sequences per sample after quality filtering and subsampling. The resulting sequences were processed with mothur (Schloss *et al.* 2009) following the Schloss MiSeq SOP and classified using the 16S taxonomy from the Ribosomal Database Project (Cole *et al.* 2013) and the *mcrA* taxonomic database from (Yang *et al.* 2014). For *mcrA* sequences 4 ambiguous base pairs were allowed and a similarity cutoff of 85.8% was used for the genus level corresponding to a 97% cutoff for the 16S rRNA (Yang *et al.* 2014).

4.5 Literature Cited

Appels, L., J. Lauwers, J. Degrève, L. Helsen, B. Lievens, K. Willems, J. Van Impe and R. Dewil (2011). Anaerobic digestion in global bio-energy production: Potential and research challenges. *Renewable and Sustainable Energy Reviews* 15(9): 4295-4301.

Avramescu, M.-L., E. Yumvihoze, H. Hintelmann, J. Ridal, D. Fortin and D. R.S. Lean (2011). Biogeochemical factors influencing net mercury methylation in contaminated freshwater sediments from the St. Lawrence River in Cornwall, Ontario, Canada. *Sci. Total Environ.* 409(5): 968-978.

Beauchemin, K. A., S. M. McGinn, C. Benchaar and L. Holtshausen (2009). Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: Effects on methane production, rumen fermentation, and milk production. *Journal of Dairy Science* 92(5): 2118-2127.

Blazewicz, S. J., R. L. Barnard, R. A. Daly and M. K. Firestone (2013). Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses. *ISME J* 7(11): 2061-2068.

Boadi, D., C. Benchaar, J. Chiquette and D. Massé (2004). Mitigation strategies to reduce enteric methane emissions from dairy cows: Update review. *Canadian Journal of Animal Science* 84(3): 319-335.

Bonacker, L. G., S. Baudner and R. K. Thauer (1992). Differential expression of the two methyl-coenzyme M reductases in Methanobacterium thermoautotrophicum as determined immunochemically via isoenzyme-specific antisera. *European Journal of Biochemistry* 206(1): 87-92.

Boyd, J. M., A. Ellsworth and S. A. Ensign (2006). Characterization of 2-Bromoethanesulfonate as a Selective Inhibitor of the Coenzyme M-Dependent Pathway and Enzymes of Bacterial Aliphatic Epoxide Metabolism. *Journal of Bacteriology* 188(23): 8062-8069.

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer and R. Knight (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. U. S. A.* 108(Supplement 1): 4516-4522.

Chiao, T.-H., T. M. Clancy, A. J. Pinto, C. Xi and L. Raskin (2014). Differential resistance of drinking water bacterial populations to monochloramine disinfection. *Environmental Science and Technology* 48(7): 4038-4047.

Chiu, P. C. and M. Lee (2001). 2-Bromoethanesulfonate Affects Bacteria in a Trichloroethene-Dechlorinating Culture. *Appl. Environ. Microbiol.* 67(5): 2371-2374.

Chowdhury, T. R. and R. P. Dick (2013). Ecology of aerobic methanotrophs in controlling methane fluxes from wetlands. *Appl. Soil Ecol.* 65(0): 8-22.

Cole, J. R., Q. Wang, J. A. Fish, B. Chai, D. M. McGarrell, Y. Sun, C. T. Brown, A. Porras-Alfaro, C. R. Kuske and J. M. Tiedje (2013). Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res.*

Collins, M. D., P. A. Lawson, A. Willems, J. J. Cordoba, J. Fernandez-Garayzabal, P. Garcia, J. Cai, H. Hippe and J. A. E. Farrow (1994). The Phylogeny of the Genus Clostridium: Proposal of Five New Genera and Eleven New Species Combinations. *International Journal of Systematic Bacteriology* 44(4): 812-826.

Conrad, R. (2007). Microbial Ecology of Methanogens and Methanotrophs. Adv. Agron. L. S. Donald, Academic Press. Volume 96**:** 1-63.

Denman, S. E., N. W. Tomkins and C. S. McSweeney (2007). Quantitation and diversity analysis of ruminal methanogenic populations in response to the antimethanogenic compound bromochloromethane. *FEMS Microbiology Ecology* 62(3): 313-322.

Dufrêne, M. and P. Legendre (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs* 67(3): 345-366.

Dumitru, R., H. Palencia, S. D. Schroeder, B. A. DeMontigny, J. M. Takacs, M. E. Rasche, J. L. Miner and S. W. Ragsdale (2003). Targeting Methanopterin Biosynthesis To Inhibit Methanogenesis. *Appl. Environ. Microbiol.* 69(12): 7236-7241.

Dupont, L. and A. Accorsi (2006). Explosion characteristics of synthesised biogas at various temperatures. *J. Hazard. Mater.* 136(3): 520-525.

Gunsalus, R. P., J. A. Romesser and R. S. Wolfe (1978). Preparation of coenzyme M analogs and their activity in the methyl coenzyme M reductase system of Methanobacterium thermoautotrophicum. *Biochemistry* 17(12): 2374-2377.

Hallam, S. J., P. R. Girguis, C. M. Preston, P. M. Richardson and E. F. DeLong (2003). Identification of Methyl Coenzyme M Reductase A (mcrA) Genes Associated with Methane-Oxidizing Archaea. *Appl. Environ. Microbiol.* 69(9): 5483-5491.

Han, S., P. Narasingarao, A. Obraztsova, J. Gieskes, A. C. Hartmann, B. M. Tebo, E. E. Allen and D. D. Deheyn (2010). Mercury Speciation in Marine Sediments under Sulfate-Limited Conditions. *Environ. Sci. Technol.* 44(10): 3752-3757.

He, S. and K. D. McMahon (2011). `Candidatus Accumulibacter' gene expression in response to dynamic EBPR conditions. *ISME J* 5(2): 329-340.

IPCC (2013). Climate Change 2013 The Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. T. F. Stocker, D. Qin, G.-K. Plattner et al. NY, USA.

Juottonen, H., P. E. Galand and K. Yrjälä (2006). Detection of methanogenic Archaea in peat: comparison of PCR primers targeting the mcrA gene. *Research in Microbiology* 157(10): 914-921.

Karl, D. M., L. Beversdorf, K. M. Bjorkman, M. J. Church, A. Martinez and E. F. Delong (2008). Aerobic production of methane in the sea. *Nature Geosci* 1(7): 473-478.

Klappenbach, J. A., J. M. Dunbar and T. M. Schmidt (2000). rRNA Operon Copy Number Reflects Ecological Strategies of Bacteria. *Appl. Environ. Microbiol.* 66(4): 1328-1333.

Klindworth, A., E. Pruesse, T. Schweer, J. Peplies, C. Quast, M. Horn and F. O. Glöckner (2012). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.*

Knittel, K. and A. Boetius (2009). Anaerobic Oxidation of Methane: Progress with an Unknown Process. *Annu. Rev. Microbiol.* 63(1): 311-334.

Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander and P. D. Schloss (2013). Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. *Appl. Environ. Microbiol.* 79(17): 5112-5120.

Li, J., J. Rui, Z. Pei, X. Sun, S. Zhang, Z. Yan, Y. Wang, X. Liu, T. Zheng and X. Li (2014). Straw- and slurry-associated prokaryotic communities differ during co-fermentation of straw and swine manure. *Appl. Microbiol. Biotechnol.* 98(10): 4771-4780.

Liu, H., J. Wang, A. Wang and J. Chen (2011). Chemical inhibitors of methanogenesis and putative applications. *Appl. Microbiol. Biotechnol.* 89(5): 1333-1340.

Liu, Y., D. L. Balkwill, H. C. Aldrich, G. R. Drake and D. R. Boone (1999). Characterization of the anaerobic propionate-degrading syntrophs Smithella propionica gen. nov., sp. nov. and Syntrophobacter wolinii. *International Journal of Systematic Bacteriology* 49(2): 545-556.

Loffler, F. E., K. M. Ritalahti and J. M. Tiedje (1997). Dechlorination of chloroethenes is inhibited by 2-bromoethanesulfonate in the absence of methanogens. *Appl. Environ. Microbiol.* 63(12): 4982-4985.

Lueders, T. and M. W. Friedrich (2003). Evaluation of PCR Amplification Bias by Terminal Restriction Fragment Length Polymorphism Analysis of Small-Subunit rRNA and mcrA Genes by Using Defined Template Mixtures of Methanogenic Pure Cultures and Soil DNA Extracts. *Appl. Environ. Microbiol.* 69(1): 320-326.

Luton, P. E., J. M. Wayne, R. J. Sharp and P. W. Riley (2002). The mcrA gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. *Microbiology* 148(11): 3521-3530.

Machmüller, A. and M. Kreuzer (1999). Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep. *Canadian Journal of Animal Science* 79(1): 65-72.

Meyer, J., K. Michalke, T. Kouril and R. Hensel (2008). Volatilisation of metals and metalloids: an inherent feature of methanoarchaea? *Systematic and applied microbiology* 31(2): 81-87.

Morris, R., A. Schauer-Gimenez, U. Bhattad, C. Kearney, C. A. Struble, D. Zitomer and J. S. Maki (2014). Methyl coenzyme M reductase (mcrA) gene abundance correlates with activity measurements of methanogenic H2/CO2-enriched anaerobic biomass. *Microbial Biotechnology* 7(1): 77-84.

Oremland, R. S. and B. F. Taylor (1975). Inhibition of Methanogenesis in Marine Sediments by Acetylene and Ethylene: Validity of the Acetylene Reduction Assay for Anaerobic Microcosms. *Applied Microbiology* 30(4): 707-709.

Patra, A. K. and Z. Yu (2013). Effective reduction of enteric methane production by a combination of nitrate and saponin without adverse effect on feed degradability, fermentation, or bacterial and archaeal communities of the rumen. *Bioresour. Technol.* 148(0): 352-360.

Pennings, J. L. A., J. L. J. de Wijs, J. T. Keltjens and C. van der Drift (1997). Medium-reductant directed expression of methyl coenzyme M reductase isoenzymes in Methanobacterium thermoautotrophicum (strain ΔH). *FEBS Letters* 410(2–3): 235-237.

Perkins, P. S., S. J. Komisar, J. A. Puhakka and J. F. Ferguson (1994). Effects of electron donors and inhibitors on reductive dechlorination of 2,4,6-trichlorophenol. *Water Res.* 28(10): 2101-2107.

Pihl, T. D., S. Sharma and J. N. Reeve (1994). Growth phase-dependent transcription of the genes that encode the two methyl coenzyme M reductase isoenzymes and N5-

methyltetrahydromethanopterin:coenzyme M methyltransferase in Methanobacterium thermoautotrophicum delta H. *Journal of Bacteriology* 176(20): 6384-6391.

Pinto, A. J. and L. Raskin (2012). PCR biases strongly distort bacterial and archaeal community structure in pyrosequencing. *PLoS One* 7(8).

Prins, R. A., C. J. Van Nevel and D. I. Demeyer (1972). Pure culture studies of inhibitors for methanogenic bacteria. *Antonie Van Leeuwenhoek* 38: 281-287.

Qu, W., Y. Zhou, Y. Zhang, Y. Lu, X. Wang, D. Zhao, Y. Yang and C. Zhang (2012). MFEprimer-2.0: a fast thermodynamics-based program for checking PCR primer specificity. *Nucleic Acids Res.* 40(W1): W205-W208.

Reeve, J. N., J. Nölling, R. M. Morgan and D. R. Smith (1997). Methanogenesis: genes, genomes, and who's on first? *Journal of Bacteriology* 179(19): 5975-5986.

Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. a. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G. Thallinger, D. J. Van Horn and C. F. Weber (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75(23): 7537-7541.

Smith, A. L., S. J. Skerlos and L. Raskin (2013). Psychrophilic anaerobic membrane bioreactor treatment of domestic wastewater. *Water Res.* 47: 1655-1665.

Smith, A. L., S. J. Skerlos and L. Raskin (2014). Improving anaerobic membrane bioreactor treatment of wastewater through membrane biofilm development. *ISME Journal* submitted.

Soliva, C. R., I. K. Hindrichsen, L. Meile, M. Kreuzer and A. Machmüller (2003). Effects of mixtures of lauric and myristic acid on rumen methanogens and methanogenesis in vitro. *Lett. Appl. Microbiol.* 37(1): 35-39.

Sonthiphand, P., E. Cejudo, S. L. Schiff and J. D. Neufeld (2013). Wastewater Effluent Impacts Ammonia-Oxidizing Prokaryotes of the Grand River, Canada. *Appl. Environ. Microbiol.* 79(23): 7454-7465.

Sousa, D. Z., H. Smidt, M. M. Alves and A. J. M. Stams (2007). Syntrophomonas zehnderi sp. nov., an anaerobe that degrades long-chain fatty acids in co-culture with Methanobacterium formicicum. *International Journal of Systematic and Evolutionary Microbiology* 57(3): 609-615.

Sprott, G. D., K. F. Jarrell, K. M. Shaw and R. Knowles (1982). Acetylene as an Inhibitor of Methanogenic Bacteria. *Journal of General Microbiology* 128(10): 2453-2462.

Steinberg, L. M. and J. M. Regan (2008). Phylogenetic Comparison of the Methanogenic Communities from an Acidic, Oligotrophic Fen and an Anaerobic Digester Treating Municipal Wastewater Sludge. *Appl. Environ. Microbiol.* 74(21): 6663-6671.

Steinberg, L. M. and J. M. Regan (2009). mcrA-Targeted Real-Time Quantitative PCR Method To Examine Methanogen Communities. *Appl. Environ. Microbiol.* 75(13): 4435-4442.

Sugimoto, A. and E. Wada (1993). Carbon isotopic composition of bacterial methane in a soil incubation experiment: Contributions of acetate and CO2H2. *Geochim. Cosmochim. Acta* 57(16): 4015-4027.

Suzuki, M. T. and S. J. Giovannoni (1996). Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl. Environ. Microbiol.* 62(2): 625-630.

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30(12): 2725-2729.

Tezel, U., L. P. Padhye, C.-H. Huang and S. G. Pavlostathis (2011). Biotransformation of Nitrosamines and Precursor Secondary Amines under Methanogenic Conditions. *Environ. Sci. Technol.* 45(19): 8290-8297.

Thomas, F., R. A. Diaz-Bone, O. Wuerfel, B. Huber, K. Weidenbach, R. A. Schmitz and R. Hensel (2011). Connection between Multimetal(loid) Methylation in Methanoarchaea and Central Intermediates of Methanogenesis. *Appl. Environ. Microbiol.* 77(24): 8669-8675.

Ungerfeld, E. M., S. R. Rust, D. R. Boone and Y. Liu (2004). Effects of several inhibitors on pure cultures of ruminal methanogens*. *J. Appl. Microbiol.* 97(3): 520-526.

Ungerfeld, E. M., S. R. Rust and R. Burnett (2003). Use of some novel alternative electron sinks to inhibit ruminal methanogenesis. *Reprod. Nutr. Dev* 43: 189-202.

Ungerfeld, E. M., S. R. Rust and R. Burnett (2006). Effects of butyrate precursors on electron relocation when methanogenesis is inhibited in ruminal mixed cultures. *Lett. Appl. Microbiol.* 42(6): 567-572.

Watkins, A. J., E. G. Roussel, G. Webster, R. J. Parkes and H. Sass (2012). Choline and N,N-Dimethylethanolamine as Direct Substrates for Methanogens. *Appl. Environ. Microbiol.* 78(23): 8298-8303.

Williams, A. G., S. Withers and A. D. Sutherland (2013). The potential of bacteria isolated from ruminal contents of seaweed-eating North Ronaldsay sheep to

hydrolyse seaweed components and produce methane by anaerobic digestion in vitro. *Microbial Biotechnology* 6(1): 45-52.

Wu, X. L., K. J. Chin, S. Stubner and R. Conrad (2001). Functional patterns and temperature response of cellulose-fermenting microbial cultures containing different methanogenic communities. *Appl. Microbiol. Biotechnol.* 56(1-2): 212-219.

Xu, K., H. Liu and J. Chen (2010a). Effect of classic methanogenic inhibitors on the quantity and diversity of archaeal community and the reductive homoacetogenic activity during the process of anaerobic sludge digestion. *Bioresour. Technol.* 101(8): 2600-2607.

Xu, K., H. Liu, X. Li, J. Chen and A. Wang (2010b). Typical methanogenic inhibitors can considerably alter bacterial populations and affect the interaction between fatty acid degraders and homoacetogens. *Appl. Microbiol. Biotechnol.* 87(6): 2267-2279.

Yang, S., S. Liebner, M. Alawi, O. Ebenhöh and D. Wagner (2014). Taxonomic database and cut-off value for processing mcrA gene 454 pyrosequencing data by MOTHUR. *J. Microbiol. Methods* 103(0): 3-5.

Yue, Z.-B., W.-W. Li and H.-Q. Yu (2013). Application of rumen microorganisms for anaerobic bioconversion of lignocellulosic biomass. *Bioresour. Technol.* 128(0): 738-744.

Zeleke, J., S.-L. Lu, J.-G. Wang, J.-X. Huang, B. Li, A. Ogram and Z.-X. Quan (2013). Methyl Coenzyme M Reductase A (mcrA) Gene-Based Investigation of Methanogens in the Mudflat Sediments of Yangtze River Estuary, China. *Microbial Ecology* 66(2): 257-267.

Zhou, J., L. Wu, Y. Deng, X. Zhi, Y.-H. Jiang, Q. Tu, J. Xie, J. D. Van Nostrand, Z. He and Y. Yang (2011a). Reproducibility and quantitation of amplicon sequencing-based detection. *ISME J* 5(8): 1303-1313.

Zhou, Z., Q. Meng and Z. Yu (2011b). Effects of methanogenic inhibitors on methane production and abundances of methanogens and cellulolytic bacteria in in vitro ruminal cultures. *Appl. Environ. Microbiol.* 77(8): 2634-2639.

Zinder, S. H., T. Anguish and S. C. Cardwell (1984). Selective Inhibition by 2-Bromoethanesulfonate of Methanogenesis from Acetate in a Thermophilic Anaerobic Digestor. *Appl. Environ. Microbiol.* 47(6): 1343-1345.

Chapter 5

Anaerobic disposal of arsenic-bearing wastes and the impact of methanogenesis on arsenic volatilization

5.1 Abstract

The prevalence of arsenic contaminated drinking water sources has led to the development and implementation of a variety of water treatment systems to remove arsenic, all of which generate arsenic-bearing wastes. For rural areas, one proposed disposal option for arsenic waste is mixing the waste with cow dung to promote microbially mediated arsenic volatilization under anaerobic conditions. Arsenic volatilization is expected to facilitate the dilution of arsenic in the atmosphere. However, little is known about the degree of these transformations or the microbial populations responsible for the volatilization. Volatilization of arsenic can be mediated by the protein arsenite *S*-adenosylmethionine methyltransferase (ArsM), which is present in a wide diversity of microorganisms, or through the activity of enzymes involved in methanogenesis. In this study, mesocosms containing an arsenic-bearing ferric iron waste from an electrocoagulation drinking water treatment system were seeded with cow dung and anaerobic digester sludge to promote methanogenesis. To evaluate the role of methanogenesis in arsenic volatilization, methanogenesis was inhibited in some of the mesocosms using 2-bromoethanesulfonate and propynoic acid. Arsenic was measured in the gaseous, aqueous, and solid phases and compared to the initial amount of arsenic added. Arsenic volatilization was highest in the control mesocosms in which

methanogenesis was not inhibited, but represented less than 0.02 % of the total amount of arsenic added. Molecular analyses, including 16S rRNA cDNA sequencing, qPCR of *mcrA* transcripts, and microarray-based analysis of *arsM* expression, revealed that arsenic volatilization was correlated with methanogenic activity. Mobilization into the aqueous phase was substantial although less than 4 % of the total arsenic, indicating that environmental contamination may be of concern using this disposal strategy. Based on these findings, anaerobic disposal strategies using cow dung and anaerobic digestion sludge are not recommended for arsenic-bearing oxidized iron waste disposal.

5.2 Introduction

Human exposure to arsenic increases the risk for cancers, skin legions, cardiovascular problems, and other negative health outcomes (Argos et al. 2010, Naujokas et al. 2013). Naturally occurring arsenic contaminates groundwater used as a source for drinking water and crop irrigation (Ravenscroft et al. 2009, Zhao et al. 2010). Anthropogenic arsenic contamination originates from several industries, including wood processing and meat production (Yao et al. 2012, Nachman et al. 2013, Mercer and Frostick 2014). As a result, a variety of processes generate arsenic-bearing waste and appropriate waste disposal options are needed. Several existing practices include waste disposal under anaerobic conditions. Anaerobic digestion is commonly used for the treatment of agricultural biomass or livestock wastes (Kelleher et al. 2002, Mussoline et al. 2014), which often contain arsenic due to plant uptake of arsenic in irrigation water (Zhao et al. 2010, Bhattacharya et al. 2012) or as a result of arsenic containing feed additives consumed by livestock (Chapman and Johnson 2002, Nachman et al. 2013). Drinking water treatment wastes in developed countries are often disposed in landfills

(Cornwall 2003). As an alternative to landfill disposal in developing countries, mixing arsenic containing drinking water treatment wastes with cow dung has been proposed to remove arsenic by volatilization (Das 1995, Ali et al. 2003, Visoottiviseth and Ahmed 2008, Sullivan et al. 2010).

Microbial activity largely determines the fate of arsenic under anaerobic conditions. For oxidized arsenic-bearing iron wastes, reductive dissolution through the activity of arsenate- and iron- reducing microorganisms can lead to the release of arsenic from solid wastes into the aqueous phase (DeLemos et al. 2006, Ghosh et al. 2006, Jing et al. 2008, Tufano et al. 2008). More recently, the ability of microorganisms to transform arsenic into volatile arsine and methylarsine gases has been of interest as a potentially desirable treatment outcome (Qin et al. 2006, Zhao et al. 2013, Chen et al. 2014). Indeed, arsenic disposal with cow dung is suggested based on the expectation that through microbial activity, arsenic is volatilized and transferred to the atmosphere where dilution reduces exposure risk (Visoottiviseth and Ahmed 2008). However, the extent to which this pathway is important in arsenic transformations under anaerobic conditions remains unclear. Measurements of volatilization from soils and in rice paddies find only a small fraction, less than 0.1% of the total arsenic, is released from solids via volatilization (Turpeinen et al. 2002, Edvantoro et al. 2004, Mestrot et al. 2009). In studies that have measured gaseous arsenic release from anaerobic digesters, between 0.3 and 32% of the total arsenic was reported to have been volatilized (Mohapatra et al. 2008, Mestrot et al. 2013). Other studies using the differences between the initial arsenic added and the measurement of arsenic in the liquid and solid phases after incubation have reported higher amounts of volatilized arsenic (Ali et al. 2003, Banerjee 2010). However, these

studies have not provided a complete mass balance of arsenic, the absence of which calls into question whether cow dung disposal leads to significant volatilization or is caused by errors in different measurement techniques.

The potential for microbial arsenic methylation and volatilization has been known for some time (Challenger et al. 1933, Bentley and Chasteen 2002). Two pathways have been identified. The first occurs during methanogensis through the demethylation of methylcobalamin and results in the production of arsine and mono-, di-, and tri-methylarsine gases (Thomas et al. 2011). The second pathway occurs through the activity of the arsenite *S*-adenosylmethionine methyltransferase (ArsM) and is thought to be a detoxification mechanism found in all domains of life (Bentley and Chasteen 2002, Qin et al. 2006). Recently, studies have focused on quantifying the abundance of the second pathway in natural environments (Qin et al. 2009, Jia et al. 2013, Zhao et al. 2013) and the potential for the *arsM* gene to be used in genetically modified microorganisms for the remediation of arsenic contaminated sites (Liu et al. 2011, Chen et al. 2014). Much less research has evaluated the impact of methanogenesis on arsenic volatilization in natural or engineered systems, even though volatilization has been observed in environments with high methanogenic activity, including rice paddies (Mestrot et al. 2009), landfills (Pinel-Raffaitin et al. 2007), and anaerobic digesters (Michalke et al. 2000, Mestrot et al. 2013). To date, studies have not investigated the relative importance of these two pathways or the activity of specific populations within mixed microbial communities.

The objectives of this study were to 1) evaluate the potential for arsenic volatilization to be used as a remediation strategy for arsenic-bearing ferric iron wastes from drinking

water treatment disposed under anaerobic conditions, 2) compare the relative contribution of methanogenic activity to arsenic volatilization, and 3) characterize changes in the expression of *arsM* genes as it relates to the fate of arsenic.

5.3 Methods

5.3.1 Mesocosm set-up and incubation

Mesocosms were set up in 500 mL glass serum bottles seeded with 24 g cow dung (wet weight) and 400 mL of anaerobic digester sludge solids, resulting in approximately 5000 mg/L total suspended solids. Triplicate bottles were seeded similarly for each of four conditions: 1) a control with no added arsenic, 2) arsenic waste (As waste), 3) arsenic waste and methanogenesis inhibited by 2-bromoethanesulfonate (As-BES), and 4) arsenic waste and methanogenesis inhibited by propynoic acid (As-PA). The liquid and solids from the control bottles without added arsenic were later used as the background matrix to create arsenic standards. For the three conditions with arsenic, 0.667 g of dried arsenic waste was added to achieve a final arsenic concentration of 1.66 mg As/L with a total working volume of about 410 mL. This concentration was selected to represent a realistic ratio of cow dung to arsenic waste and be low enough to avoid the potential impacts of arsenic toxicity. The arsenic-bearing iron waste was produced at a pilot Electro-Chemical Arsenic Remediation (ECAR) system for the removal of arsenic from groundwater in West Bengal, India (Amrose et al. 2014). Preliminary experiments were also performed (as described in the supporting information (SI)) in which arsenic was added as a liquid (AsO_3). To inhibit methanogenesis, two different inhibitors were selected for comparison: 2-bromoethanesulfonate (BES), a commonly used coenzyme-M analog (Gunsalus et al. 1978, Zinder et al. 1984, Ungerfeld et al. 2004) and propynoic

acid, an unsaturated analog to propionate (Ungerfeld et al. 2003, Zhou et al. 2011). Two inhibitors were selected to identify potential artifacts arising from non-specific inhibition. Concentrations of 10 mM for each inhibitor were selected based on previous batch studies with the same mesocosm community targeting complete inhibition of methanogenesis (Chapter 4). Bottles were capped, crimp sealed, and purged with N_2 gas before incubation for 17 days on a shaker table at 31-33 °C. Two mesocosms were also prepared with anaerobic digester sludge, cow dung, and arsenic-bearing waste, for immediate sampling to determine the initial distribution of arsenic in the liquid and solid phases.

5.3.2 Mesocosm sampling

Over a 17 day incubation period, gas samples were collected to quantify gas production and characterize its composition. Sampling was done by connecting a glass syringe (via a needle through the bottle septum) fitted with an arsenic trap, made from a glass tube containing silver nitrate impregnated silica gel (1% w/v) (SKC Inc., Eighty Four, PA) (Mestrot et al. 2009). All gas generated during the incubation went through the trap, which was later digested to measure the amount of arsenic volatilization. Tests of gas trap efficiency were performed as described in the SI. At the final day of sampling before uncapping the bottles, the headspace was again purged with N_2 gas through the gas trap. For each of the four conditions (control without added arsenic, As waste, As-BES, and As-PA), one of each of the triplicate bottles was selected for biomass collection and preservation, centrifuged at 4 °C to collect solids for molecular analyses. Biomass samples were immediately frozen at -80 °C. The remaining liquid was separated by filtration with Whatman no. 41 filters (GE Healthcare Life Sciences, Pittsburg, PA) and

acidified with nitric acid. The other two mesocosms for each condition were centrifuged at 20 °C, followed by filtration through Whatman no. 41 filters to separate liquid and solids. These solid and liquid samples were used for further chemical analysis.

5.3.3 Analytical methods

Samples of gas produced were analyzed to quantify CH_4, CO_2, and N_2 using a gas chromatograph (Gow-Mac, Bethlehem, PA) coupled with a thermal conductivity detector (TCD) (Smith et al. 2013). Total volatilized arsenic was measured by digesting the silica beads from the gas trap first with 2 mL of 5% nitric acid followed by 2 mL of 1% nitric acid, and incubation at 100 °C, a procedure modified slightly from that described in Mestrot et al. (2009). Total arsenic in the concentrated acid digestion fluid was measured with inductively coupled plasma mass spectroscopy (ICP-MS) (Upadhyaya et al. 2010). ICP-MS instrumental error was determined to be 5% based on the variation from known standards. Errors reported include the larger of either 5% or the standard deviation between triplicate samples. For concentrations less than 2 µg/L the minimum error was 0.8 µg/L with a detection limit of 1.1 µg/L. Liquid phase arsenic in filtered and acidified samples was measured by ICP-MS. The pH of the liquid samples was also measured (Mettler Toledo, Columbus, OH). Arsenic in the solid fraction, including the filters from liquid separation, was measured following a total digestion. Total digestions were performed at 100 °C for 2 hours with 1:1 nitric acid (10 mL/g solid), followed by the addition of 2 mL of 15% hydrogen peroxide and a second incubation at 100 °C for 2 hours (U.S. EPA 1992). The liquid and solid fractions from control bottles, without arsenic added, were treated identically to the samples and then used as the matrix for

ICP-MS standards through the addition of known quantities of arsenic ICP standard (Ricca Chemical, Arlington, TX).

5.3.4 Molecular methods

RNA extractions were performed with the Power Soil RNA kit (MoBio Laboratories, Carlsbad, CA) and treated with DNase using the TURBO DNA-free kit (Ambion, Grand Island, NY) according to the manufacturer's instructions. RNA quantity and quality was determined using fluorospectrometry with the Quantifluor RNA system (Promega, Madison, WI) and electrophoresis with the Experion RNA analysis kit (Bio-Rad, Hercules, CA). Single-stranded cDNA for reverse transcriptase quantitative PCR (RT-qPCR) and sequencing was synthesized using SuperScript VILO kit (Life Technologies, Grand Island, NY) according the manufacturer's instructions, including an extended incubation time of 120 min to maximize yield.

RT-qPCR was performed as described previously (Chapter 4). Briefly, standards were created from a template of pooled mesocosm DNA extracts (McMahon and Daugulis 2008, Sonthiphand et al. 2013). The *mcrA* and 16S rRNA genes amplified with 20 µL reactions containing 0.5 ng template, 500 nM primers, 0.3 mg/mL bovine serum albumin, 10 µL Phusion High Fidelity Master Mix (NEB, Ipswich, MA), and nuclease-free water. Thermocycling conditions included an initial denaturation at 95 °C, followed by 30 cycles of 20 s at 95 °C, 15 s at 55 °C, and 30 s at 72 °C, with a final 5 min extension at 72 °C. Products were run on a 1.5% agarose gel, excised, and purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). Amplified and purified pools were quantified with fluorospectrometry using the Quantifluor dsDNA system (Promega) and diluted for standards (10^7-10^2 copies/µL for *mcrA* and 10^8-10^4 copies/µL for 16S

rRNA amplicons). Real time quantitative PCR (RT-qPCR) was performed using the Mastercycler Realplex Ep (Eppendorf, Hamburg, Germany) with triplicate wells for each sample and reaction volumes of 20 µL using Fast Plus EvaGreen Master Mix (Biotium, Hayward, CA). Primer concentrations were 500 nM for the forward primers and the reverse 16S rRNA gene primer and the reverse *mcrA* primer was 250 nM. The first cycle included an initial 2 min denaturation at 95 °C, then 5 cycles of 95 °C for 20 s, 55 °C for 15s, followed by a temperature ramp of 0.1 °C/s to 72 °C (Luton et al. 2002, Morris et al. 2014), and extension for 72 °C for 30 s. Then 45 cycles were performed without the temperature ramp with a final extension at 72 °C for 5 min. The final step was a melting curve analysis. The standard curve R^2 values were 0.99 and 0.99 and efficiencies were 68% and 76%, for *mcrA* and 16S rRNA, respectively.

Sequencing was performed at the Host Microbiome Initiative (University of Michigan, Ann Arbor, MI) using universal primers F515 and R806 targeting the v4 region of 16S rRNA (Caporaso et al. 2011) as modified by Kozich et al. (2013). Amplification from cDNA samples was performed with Accuprime TAQ (Invitrogen). Thermocycling conditions were as follows: initial 2 min denaturation at 95 °C, followed by 30 cycles of 20 s at 95 °C, 15 s at 55 °C, and 5 min at 72 °C. A final extension was performed for 5 min at 72 °C. Amplicons were pooled by equal mass using the SequalPrepNormalization Plate Kit (Life Technologies), multiplexed and sequenced using the Illumina MiSeq, Reagent Kit V2. Sequences were processed with mothur (Schloss et al. 2009) and classified using the 16S rRNA gene taxonomy from the Ribosomal Database Project (Cole et al. 2013). There were 222,482 paired-end reads

after quality filtering. Bacterial sequences were separated and sub-sampled for subsequent analyses of community structure to a depth of 17,424 sequences per sample.

Double-stranded cDNA for microarray analysis was prepared by concentrating equal mass of RNA, about 5 µg, from each sample by overnight precipitation at -20 °C with 0.1 volume sodium acetate and 2.5 volume ethanol followed by an ethanol wash and re-suspension in 10 µL nuclease-free water. Random primers (Invitrogen, Carlsbad, CA) were added to the RNA and incubated at 65 °C for 5 min followed by incubation on ice. First strand synthesis was then performed by adding 2 µL 5x first strand buffer (Invitrogen), 1 µL of 10 mM dNTP mix (Invitrogen), 2 µL of 0.1 M DTT (Invitrogen), 1 µL of RNase Inhibitor (Promega), and 1 µL of linear acryl amide (Ambion, Grand Island, NY). This mixture was incubated at room temperature for 10 min followed by the addition of 1 µL reverse transcriptase SuperScript III (Invitrogen). A 10 min incubation at 25 °C was followed by 3 hours at 50 °C and cooling at 4 °C. With the samples on ice, reagents for the second strand synthesis were added which included: 91 µL nuclease free water, 30 µL 5x Second Strand reaction buffer (Invitrogen), 3 µL 10 mM dNTP mix, 10 U *E. coli* DNA ligase (Invitrogen), 40 U *E. coli* DNA polymerase (Invitrogen), 1 U *E. coli* RNase H (Promega, Madison, WI) per reaction. The tubes were incubated at 16 °C for 2 hours. The reaction was stopped by adding 10 µL 0.5 M EDTA (pH 8.0) (Lonza, Basel, Switzerland) and 10 µL 1 M NaOH (Sigma Aldrich, St. Louis, MO) and incubating at 65 °C for 10 min. Phenol chloroform isoamyl was used to purify the samples which were then precipitated overnight at -20 °C with 1 µL linear acryl amid, 0.5 volumes 7.5 M ammonium acetate, and 2.5 volumes of ethanol. cDNA was re-suspended and shipped to Glomics Inc. (Norman, OK) for hybridization onto the microarray

GeoChip version 5.0. Methods for hybridization and scanning have been previously described (He et al. 2010, Xu et al. 2010). The normalized signal intensity was log transformed and scaled by dividing each probe signal by the mean of all positive probes on that array. To identify other Bacteria and Archaea that may have the arsM gene, 25 annotated ArsM protein sequences were downloaded from NCBI and used as the database for blastx searches from all complete genomes available from NCBI. Initial parameters limited results to matches with 50% identity and an alignment length of at least 150 amino acids. Resulting sequences were aligned and compared to the initial 25 protein sequences and only sequences that maintained conservation in the same areas were selected as potential ArsM proteins. These conserved regions included the cysteine residues that were determined to be necessary for arsenic binding (Qin et al. 2006).

5.4 Results

Through measurements of arsenic in the gas, liquid, and solid phases, a mass balance of arsenic was achieved (Figure 5-13). These results include the measurements from duplicate mesocosms from the uninhibited As waste condition (sampled at day 0 and day 17) and inhibited conditions (As-BES and As-PA sampled at day 17). The recovery of arsenic was 97-101% of the total arsenic expected based on the total digestion of the arsenic-bearing waste (995 ± 49.8 μg As/g ECAR waste) and the mass added to each bottle (0.667 g). These results show that the majority of arsenic remained in the solid phase over the 17 day experiment. This is consistent with the observations of arsenic distribution in mesocosms where arsenic was added as a liquid arsenite and the majority of the arsenic was found in the solid phase following 11 days of incubation (Figure S10, Appendix E).

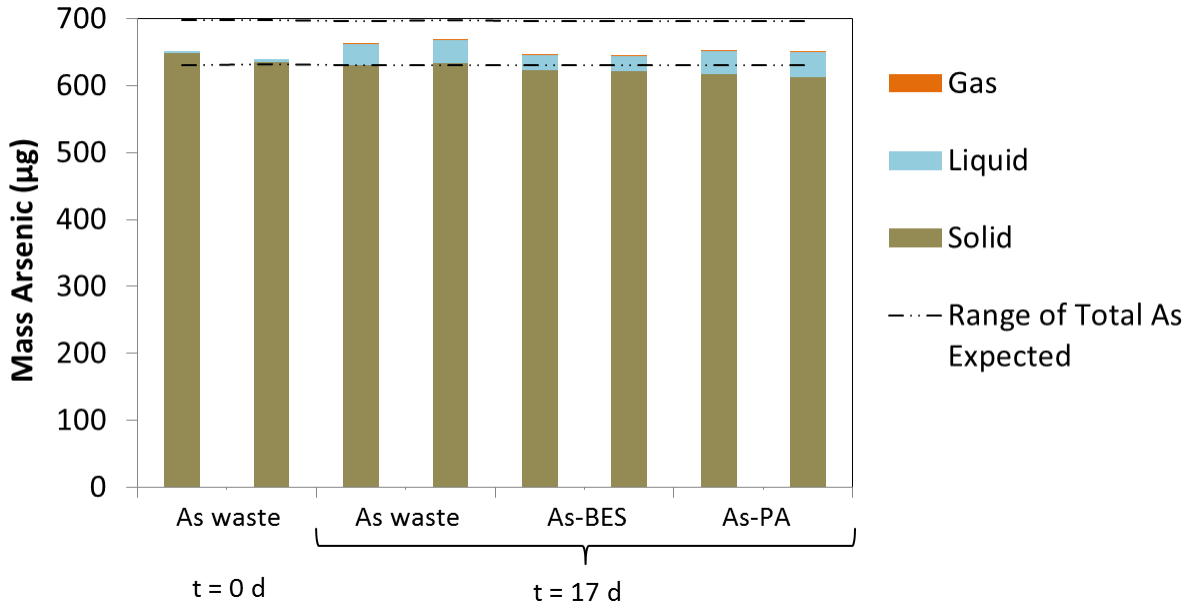


Figure 5-13. Arsenic mass balance in duplicate mesocosms from each condition and the expected range of total arsenic, based on the average from total digestions of arsenic-bearing waste (range determined by 5% instrument error).

Measurements of arsenic in the aqueous phase (Figure 5-14) show that, immediately after mixing the arsenic-bearing wastes, cow dung, and anaerobic digester sludge, about 5 µg of arsenic was in the aqueous phase. Following 17 days of incubation, the amount of aqueous phase arsenic was higher for all conditions, with and without methanogenesis inhibition. Aqueous arsenic increased by a factor 7.2, 5.0, and 7.9 for As waste, As-BES, and As-PA, respectively. This represents <4% of the total mass of arsenic added to the mesocosms. The average final pH was 7.1, 6.4, and 6.6 for the As, As-BES, and As-PA mesocosms, respectively.

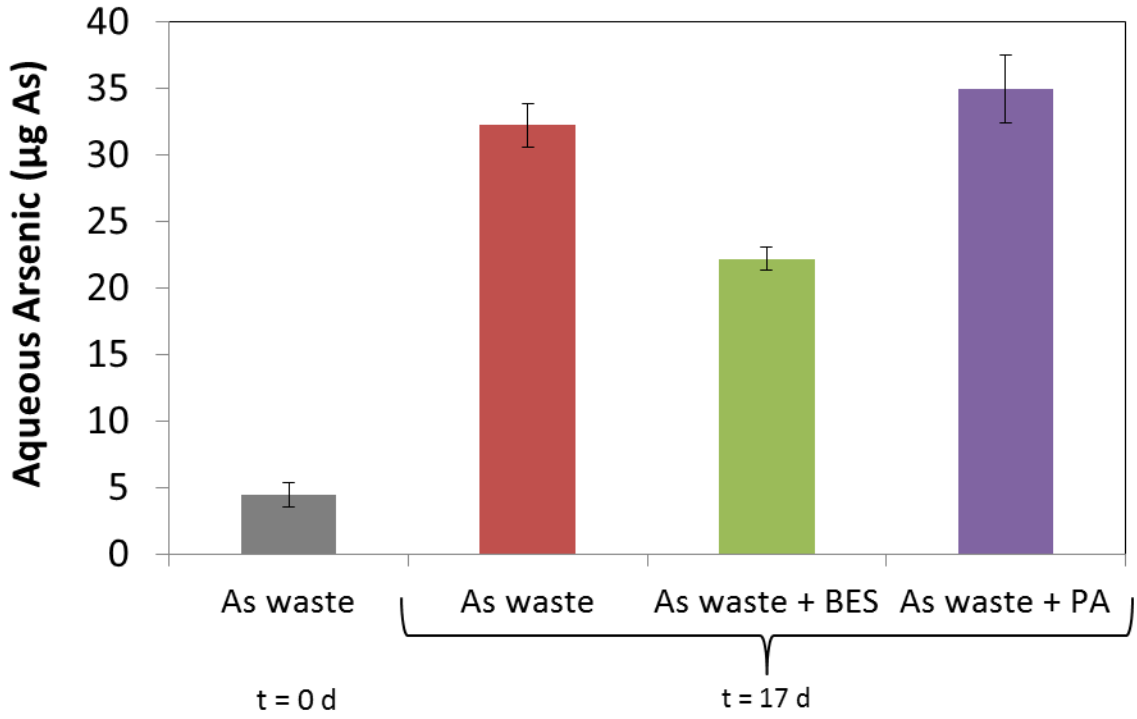


Figure 5-14. Mass of arsenic in the liquid measured at the start of the mesocosm experiments and after 17 days of incubation with either the ECAR waste or ECAR waste and inhibitors (BES or PA). Bars represent the average and standard deviation from triplicate mesocosms, except for the measurement from t = 0 which represents the average and range of duplicate mesocosms. The average and standard deviation of the liquid volumes for all mesocosms was 408 ± 32 mL.

As expected, the methane generation from mesocosms with arsenic waste and without inhibitors was greatest, resulting in the production of 740 mL methane in 17 days (Figure 5-15). Methane generation in both mesocosms with methanogenesis inhibitors was low; no methane was measured in the presence of BES and only 4.5 mL of methane was measured when PA was present. Arsenic volatilization was much higher in mesocosms with active methanogenesis as compared to inhibited conditions (Figure 5-15). Arsenic measured in the traps of the control mesocosms without arsenic waste added was near the detection limit of 4 ng with an average and standard deviation of 4.3 ± 3.2 ng. Compared to the volatilization observed in the As waste condition,

volatilization from inhibited As waste conditions was 88% and 96% lower, for BES and PA respectively.

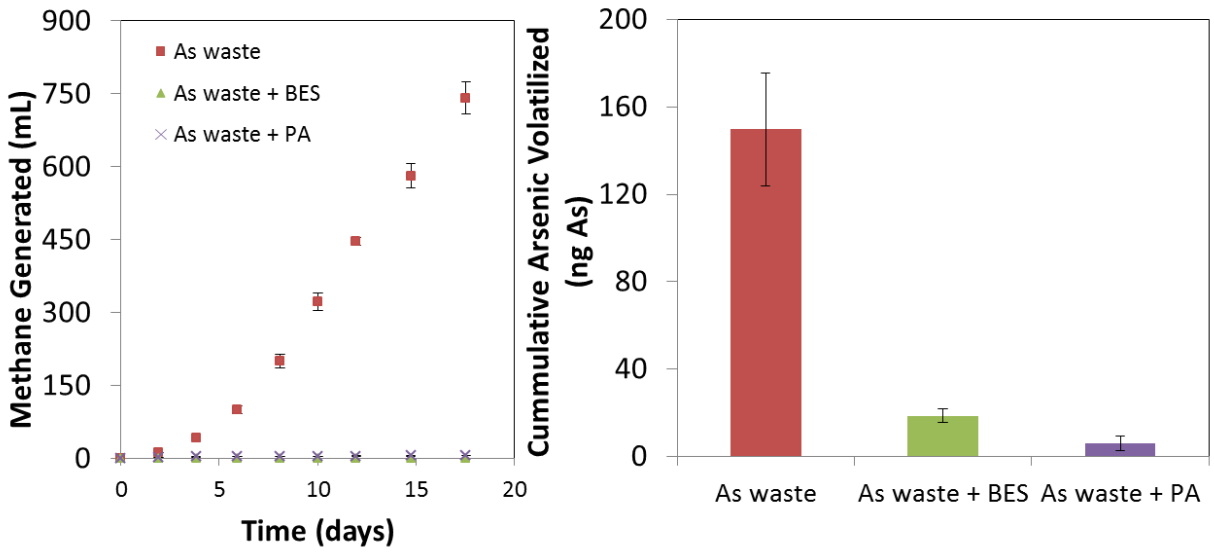


Figure 5-15. Methane generation over time (left) and cumulative arsenic volatilized after 17 days (right). Measurements represent the average from triplicate bottles.

Results from 16S rRNA cDNA sequencing and RT-qPCR of *mcrA* expression confirm the reduced activity of methanogens in inhibited conditions (Figure 5-16). Methanogens were identified as making up about 18% of the active community in non-inhibited conditions, based on 16S rRNA cDNA sequences, which was similar between control and arsenic waste containing mesocosms. Methanogenic activity as measured by *mcrA* expression was highest in the control without arsenic added, followed by the arsenic waste mesocosm, and very low for the inhibited conditions (Figure 5-16). As observed in Chapter 4, *Methanosaeta* activity was greatly reduced under inhibited conditions and there were some differences in inhibition levels by genera.

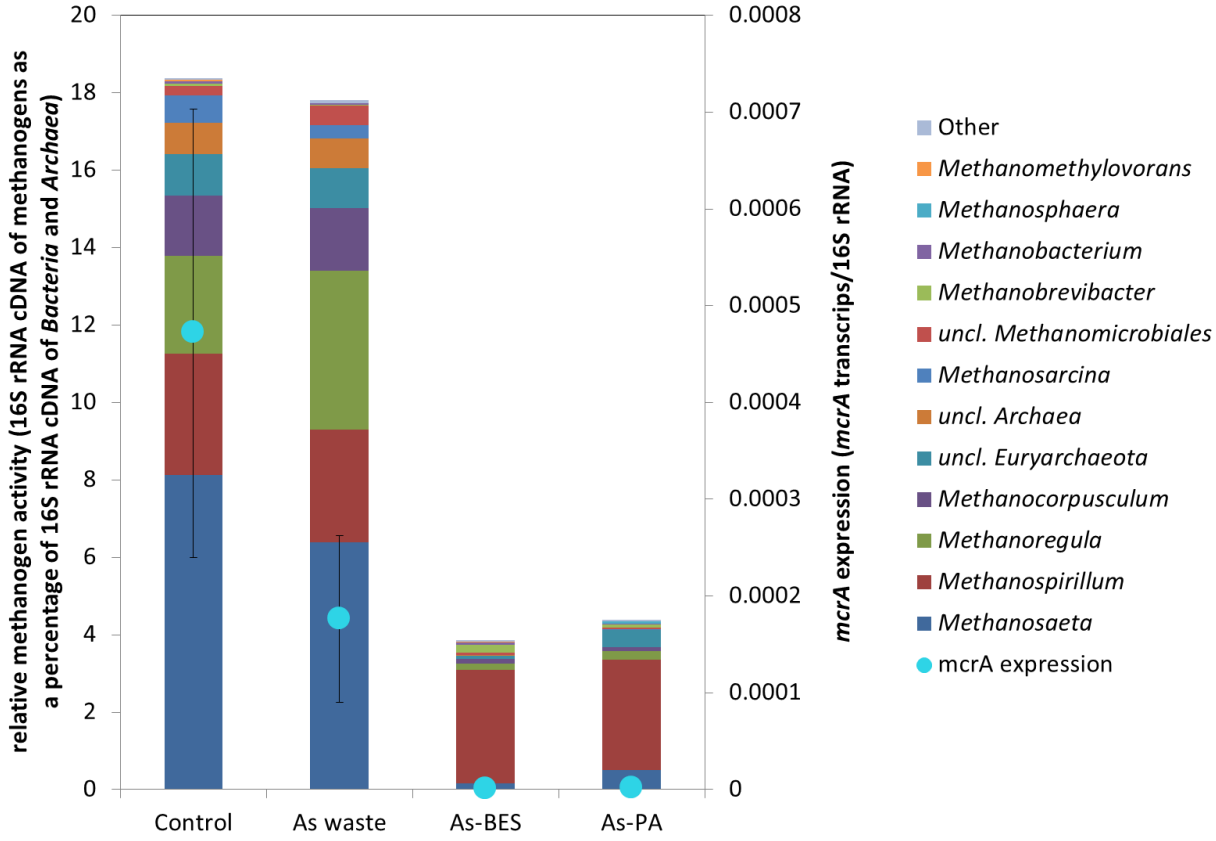


Figure 5-16. Methanogenic activity in mesocosms based on the percent relative activity of methanogens over all bacterial and archaeal 16S rRNA cDNA sequences (bars) and *mcrA* expression (circles).

The overall bacterial community structure (θ_{yc}) of the active bacterial populations was significantly different (AMOVA, $p < 0.01$) among the various conditions evaluated based on 16S rRNA cDNA sequencing. Compared to the control samples, the inhibited community structures were more different than those from the arsenic waste mesocosm samples (Figure S11, Appendix E). Consistent with earlier findings, syntrophic bacterial activity determined by 16S rRNA cDNA sequencing was also inhibited with BES and PA (Figure S12, Appendix E). Genera that were more abundant in uninhibited conditions (control and arsenic waste mesocosm) included *Syntrophomonas*, *Syntrophorhabdus*, and uncl. *Syntrophomonadaceae* (Table S8, Appendix E). Unlike the previous inhibitor study

where arsenic waste was not present (Chapter 4), in this study, *Pelotomaculum* spp., another syntrophic bacterial group, was found to be very active in the arsenic waste mesocosm, but not in either the control or inhibited conditions (Figure S12, Appendix E). Interestingly, using the GeoChip 5.0, there was positive detection of *arsM* gene expression from *Pelotomaculum thermopropionicum* strain SI under both the control and As waste conditions, but no detection of this gene under inhibited conditions (Figure 5-17). Of the 167,044 probes contained on the GeoChip 5.0 microarray, probes exist for the *arsM* genes of 73 species of *Bacteria* and *Archaea* (Table S10, Appendix E). By searching complete genomes available through NCBI, an additional 28 potential *arsM* genes were also identified (Table S10, Appendix E). While most of the genera with newly identified potential *arsM* genes were not active in these mesocosms (based on 16S rRNA cDNA sequencing), the presence of a potential *arsM* gene in *Methanosaeta* spp. is relevant to this study, as this was the most active methanogenic genus in the uninhibited conditions.

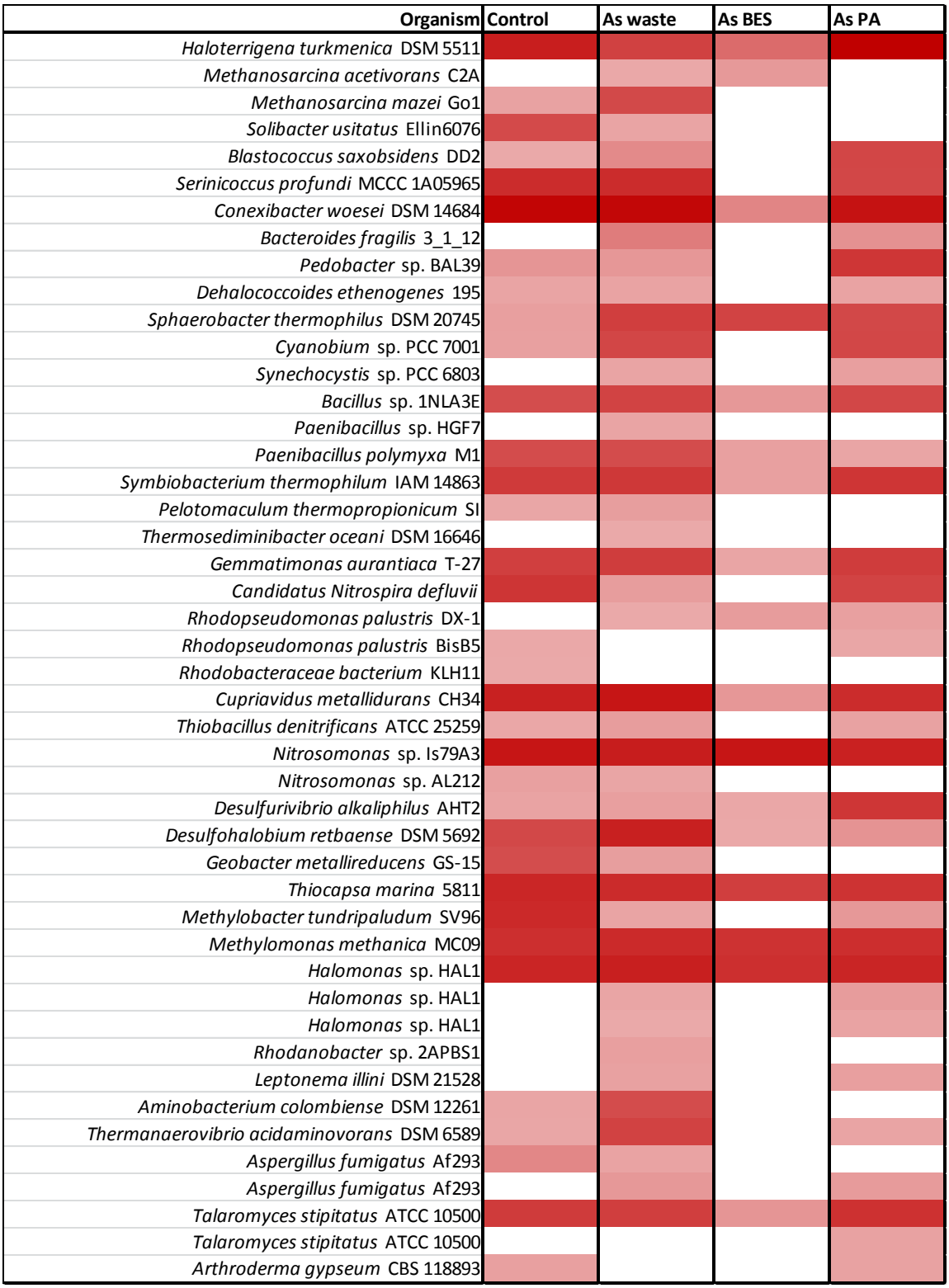


Figure 5-17. Average intensity of *arsM* transcripts assayed by the GeoChip 5.0 from duplicate biomass samples for each condition. Intensity ranges from 0 represented by white and 1.5 represented by the darkest red.

5.5 Discussion

5.5.1 Arsenic volatilization was low, with higher mobilization in the aqueous phase

The distribution of arsenic in the gaseous, aqueous, and solid phases after incubation under anaerobic conditions shows that cow dung mixing is not an effective disposal strategy for arsenic-bearing wastes. After 17 days, most of the arsenic (>90%) remained in the solid phase (Figure 5-13). This was also consistent with results from preliminary tests in which arsenic was added as aqueous-phase AsO_3 (Figure S10, Appendix E). The pathway of arsenic release to the atmosphere through volatilization was not realized, as even under conditions with the greatest volatilization, less than 0.02% of the total arsenic present was volatilized (Figure 5-15 and Figure S10, Appendix E). The amount of arsenic volatilized in this study is similar to some studies of arsenic volatilization in natural environments and anaerobic digesters (Turpeinen et al. 2002, Edvantoro et al. 2004, Mestrot et al. 2009, Mestrot et al. 2013). Other studies that reported greater volatilization did not measure arsenic in the gaseous phase, but relied on differences in the liquid and solid arsenic measurements to estimate gaseous arsenic production (Ali et al. 2003, Banerjee 2010) and likely had large errors associated with these measurements, although the errors were not estimated or given. In the present study, the errors associated with the determination of arsenic concentrations through total digestion of solids were higher than the total amount of measured arsenic volatilization.

Compared to the amount volatilized, the arsenic released into the aqueous phase was much higher and accounted for up to 4% of the total arsenic (Figure 5-14). This is likely due to the activity of iron- and arsenate- reducing microorganisms. Under field conditions, where arsenic wastes would be mixed with cow dung and disposed on the

ground or in pits, this mobilization of arsenic into the aqueous phase could result in the transport of arsenic to surrounding areas during rain or flooding events. The time scales for actual disposal would be considerably longer than 17 days. Given increased time, both arsenic volatilization and mobilization in the aqueous phase would be expected to increase although their relative magnitudes would likely remain similar. This is supported by two other studies of arsenic mobilization over longer time periods. In experiments tracking the arsenic volatilization from anaerobic digesters in which arsenic was added in the aqueous phase, the arsenic volatilization rate remained linear and resulted in low amounts (<2 %) of the total arsenic to be volatilized over 42 days (Mestrot et al. 2013). In a different study where arsenic-bearing ferric iron wastes were incubated under anaerobic landfill conditions for over 800 days, about 49 % of the arsenic initially present was mobilized into the aqueous phase and arsenic loss due to volatilization was not measured (Ghosh et al. 2006).

5.5.2 Methanogenic activity correlates with arsenic volatilization

The highest amount of arsenic volatilization occurred under conditions with the highest methanogenic activity, indicating that methanogenic activity is likely responsible for the majority of the volatilization. It is possible that the volatilization under these methanogenic conditions is due to either the enzymes involved in methanogenesis, the ArsM proteins found in methanogens, or some combination of the two pathways. Recently, the ArsM from the methanogen *Methanosarcina acetivorans* C2A has been identified and characterized (Wang et al. 2014) and through our search of complete genomes a potential ArsM protein was identified in two species of *Methanosaeta*. As *Methanosaeta* spp. was the most active methanogen in the uninhibited mesocosms and no

probe for the *arsM* is present on the GeoChip, the data presented here cannot distinguish between these two pathways. Interestingly, the ArsM pathway in bacteria associated with methanogens may also contribute to arsenic volatilization. In this case, the *arsM* expression of *Pelotomaculum* was identified under uninhibited conditions, but not when methanogenesis was inhibited (Figure 5-17). The potential for this syntroph to be involved in arsenic volatilization is linked to methanogenic activity, as syntrophic bacteria rely on the activity of methanogens to keep H_2 partial pressures low enough to create energetically favorable conditions for their metabolisms.

The 16S rRNA cDNA sequences reveal that methanogens comprise a large fraction (~18%) of the active microbial community in these mesocosms (Figure 5-16). In field application of cow dung mixing, anaerobic conditions would prevail and methanogenesis would be supported (Gattinger et al. 2007, Radl et al. 2007), although methanogenic activity would likely not reach levels greater than those studied here in well-mixed conditions that foster the coupled interactions between methanogens and their syntrophic partners (Ghanimeh et al. 2012). Since these stirred mesocosms represent ideal conditions for methanogenic activity, these findings indicate that while methanogenic activity correlated with increased arsenic volatilization, the potential to further increase arsenic volatilization rate by increasing the activity of methanogens is unlikely. This is further indication that volatilization through cow dung mixing will not be an effective strategy.

5.5.3 Alternate ArsM volatilization pathways

The potential for the ArsM pathway to contribute to arsenic volatilization was measured in part by the GeoChip 5.0 microarray (Figure 5-17). These results showed

that *arsM* gene expression was greatest in the uninhibited As waste condition and lowest in the As-BES condition. PA inhibition caused some decrease in the number of microorganisms expressing *arsM*, but was similar to the expression levels observed in the control mesocosms, based on number of species from which *arsM* transcripts were detected. The lower total expression of *arsM* observed in the BES inhibited conditions compared to the PA condition does not correspond to the observed arsenic volatilization, which was higher in the BES condition as compared to the PA condition (Figure 5-15). Because there was no methane detected in the BES condition, it is likely that the observed arsenic volatilization occurred due to the activity of either the groups identified with the GeoChip or other groups of bacteria that have yet to be identified as possessing the ability to methylate and volatilize arsenic. While the genes included on this microarray contain most of the currently known *arsM* genes, the potential for organisms with unidentified *arsM* genes cannot be ruled out. Furthermore, the difference in arsenic volatilization observed between the uninhibited As waste and the PA inhibited mesocosms cannot be explained by the differences in *arsM* expression based on the GeoChip, indicating that methanogenic activity is likely the most important factor in determining arsenic volatilization under these conditions.

While the ArsM pathway has been the focus of recent studies demonstrating the potential of genetically modified bacteria with the *arsM* gene to remediate arsenic contaminated sites, the maximum volatilization reported has been only 4.5% of the total arsenic present (Liu et al. 2011, Chen et al. 2014). Rather than relying on genetically modified organisms that have a limited ability to compete with natural microbial communities (Ji et al. 2013), future research should focus on increasing the understanding

of natural conditions that promote arsenic volatilization to determine the potential for volatilization as a remediation tool. Previous studies have found differences in the speciation and volatilization of arsenic under aerobic vs. anaerobic conditions, indicating that redox conditions are likely important differentiators of the volatilization pathway (Woolson 1977). Studies should evaluate aerobic conditions or changing redox conditions and elucidate the key populations for arsenic volatilization under these conditions, which may also be of interest for global arsenic cycling (Mestrot et al. 2013, Wang et al. 2014). For remediation strategies, the importance of fungal activity should also be further explored, given that much higher volatilization, up to 30% of the total arsenic, have been previously reported (Liu et al. 2013, Wang et al. 2014). Future studies of fungal volatilization should also include mass balances of arsenic and evaluate actual arsenic-bearing wastes as most previous studies have been limited to measurements of aqueous arsenic in culture media.

5.6 Environmental Significance

The combination of very low arsenic volatilization and increased release of arsenic to the aqueous phase shows that cow dung mixing, when anaerobic conditions prevail, is not a beneficial disposal strategy for arsenic-bearing drinking water wastes. The small amount of volatilization that did occur was attributed to methanogenic activity, a finding with relevance for the use of anaerobic digesters for arsenic contaminated wastes where some arsenic volatilization should be expected. Future studies should evaluate other redox environments and microbial activities, including fungal activity, to assess whether arsenic volatilization under other conditions may provide a more promising result. Other

disposal strategies should also be explored as volatilization may not always be an ideal pathway especially in conditions where adequate gas-phase dilution cannot be achieved.

5.7 Literature Cited

Ali, M. A., A. B. M. Badruzzaman, M. A. Jalil, M. F. Ahmed, M. Kamruzzaman, M. A. Rahman and A. Al Masud (2003). *Fate of Arsenic in Wastes Generated From Arsenic Removal Units*. Proceedings of BUET-UNU Symposium on Fate of Arsenic in the Environment, Dhaka.

Amrose, S. E., S. R. S. Bandaru, C. Delaire, C. M. van Genuchten, A. Dutta, A. DebSarkar, C. Orr, J. Roy, A. Das and A. J. Gadgil (2014). "Electro-chemical arsenic remediation: Field trials in West Bengal." *Science of The Total Environment* **488–489**(0): 539-546.

Argos, M., T. Kalra, P. J. Rathouz, Y. Chen, B. Pierce, F. Parvez, T. Islam, A. Ahmed, M. Rakibuz-Zaman, R. Hasan, G. Sarwar, V. Slavkovich, A. van Geen, J. Graziano and H. Ahsan (2010). "Arsenic exposure from drinking water, and all-cause and chronic-disease mortalities in Bangladesh (HEALS): a prospective cohort study." *The Lancet* **376**(9737): 252-258.

Banerjee, G. (2010). "Treatment of Arsenic-Laden Water Plant Sludge by Anaerobic Digestion." *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management* **14**: 124-131.

Bentley, R. and T. G. Chasteen (2002). "Microbial methylation of metalloids: arsenic, antimony, and bismuth." *Microbiology and Molecular Biology Reviews* **66**(2): 250-250.

Bhattacharya, S., K. Gupta, S. Debnath, U. C. Ghosh, D. Chattopadhyay and A. Mukhopadhyay (2012). "Arsenic bioaccumulation in rice and edible plants and subsequent transmission through food chain in Bengal basin: a review of the perspectives for environmental health." *Toxicological & Environmental Chemistry* **94**(3): 429-441.

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer and R. Knight (2011). "Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample." *Proceedings of the National Academy of Sciences* **108**(Supplement 1): 4516-4522.

Challenger, F., C. Higginbottom and L. Ellis (1933). "The formation of organo-metalloid compounds by microorganisms. Part I. Trimethylarsine and dimethylarsine." *Journal of the Chemical Society*: 95-101.

Chapman, H. D. and Z. B. Johnson (2002). "Use of Antibiotics and Roxarsone in Broiler Chickens in the USA: Analysis for The Years 1995 to 2000." *Poultry Science* **81**(3): 356-364.

Chen, J., G.-X. Sun, X.-X. Wang, V. d. Lorenzo, B. P. Rosen and Y.-G. Zhu (2014). "Volatilization of Arsenic from Polluted Soil by Pseudomonas putida Engineered for Expression of the arsM Arsenic(III) S-Adenosine Methyltransferase Gene." *Environmental Science & Technology* **48**(17): 10337-10344.

Cole, J. R., Q. Wang, J. A. Fish, B. Chai, D. M. McGarrell, Y. Sun, C. T. Brown, A. Porras-Alfaro, C. R. Kuske and J. M. Tiedje (2013). "Ribosomal Database Project: data and tools for high throughput rRNA analysis." *Nucleic Acids Research*.

Cornwall, D. (2003). *Disposal of Waste Resulting From Arsenic Removal Processes*, International Water Association.

Das, D. (1995). *Arsenic Species Along With Other Metal/Metalloids Present and Responsible for Arsenic Episode in Groundwater of West Bengal and a Cheap Technique to Remove Arsenic, Thus Making the Groundwater Suitable for Drinking and Cooking* Ph.D. Dissertation, Jadavpur University.

DeLemos, J. L., B. C. Bostick, C. E. Renshaw, S. Stürup and X. Feng (2006). "Landfill-stimulated iron reduction and arsenic release at the Coakley Superfund Site (NH)." *Environmental Science & Technology* **40**(1): 67-73.

Edvantoro, B. B., R. Naidu, M. Megharaj, G. Merrington and I. Singleton (2004). "Microbial formation of volatile arsenic in cattle dip site soils contaminated with arsenic and DDT." *Applied Soil Ecology* **25**(3): 207-217.

Gattinger, A., M. G. Höfle, M. Schloter, A. Embacher, F. Böhme, J. C. Munch and M. Labrenz (2007). "Traditional cattle manure application determines abundance, diversity and activity of methanogenic Archaea in arable European soil." *Environmental Microbiology* **9**(3): 612-624.

Ghanimeh, S., M. El Fadel and P. Saikaly (2012). "Mixing effect on thermophilic anaerobic digestion of source-sorted organic fraction of municipal solid waste." *Bioresource Technology* **117**(0): 63-71.

Ghosh, A., M. Mukiibi, A. E. Sáez and W. P. Ela (2006). "Leaching of arsenic from granular ferric hydroxide residuals under mature landfill conditions." *Environmental Science & Technology* **40**(19): 6070-6075.

Gunsalus, R. P., J. A. Romesser and R. S. Wolfe (1978). "Preparation of coenzyme M analogs and their activity in the methyl coenzyme M reductase system of Methanobacterium thermoautotrophicum." *Biochemistry* **17**(12): 2374-2377.

He, Z., M. Xu, Y. Deng, S. Kang, L. Kellogg, L. Wu, J. D. Van Nostrand, S. E. Hobbie, P. B. Reich and J. Zhou (2010). "Metagenomic analysis reveals a marked

divergence in the structure of belowground microbial communities at elevated CO2." *Ecology Letters* **13**(5): 564-575.

Ji, X., S. A. Ripp, A. C. Layton, G. S. Sayler and J. M. DeBruyn (2013). "Assessing Long Term Effects of Bioremediation: Soil Bacterial Communities 14 Years after Polycyclic Aromatic Hydrocarbon Contamination and Introduction of a Genetically Engineered Microorganism." *Journal of Bioremediation & Biodegradation* **4**(8).

Jia, Y., H. Huang, M. Zhong, F.-H. Wang, L.-M. Zhang and Y.-G. Zhu (2013). "Microbial Arsenic Methylation in Soil and Rice Rhizosphere." *Environmental Science & Technology* **47**(7): 3141-3148.

Jing, C., S. Liu and X. Meng (2008). "Arsenic remobilization in water treatment adsorbents under reducing conditions: Part I. Incubation study." *Science of the Total Environment* **389**(1): 188-194.

Kelleher, B. P., J. J. Leahy, A. M. Henihan, T. F. O'Dwyer, D. Sutton and M. J. Leahy (2002). "Advances in poultry litter disposal technology – a review." *Bioresource Technology* **83**(1): 27-36.

Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander and P. D. Schloss (2013). "Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform." *Applied and Environmental Microbiology* **79**(17): 5112-5120.

Liu, S., Y. Hou and G. Sun (2013). "Synergistic degradation of pyrene and volatilization of arsenic by cocultures of bacteria and a fungus." *Frontiers of Environmental Science & Engineering* **7**(2): 191-199.

Liu, S., F. Zhang, J. Chen and G. Sun (2011). "Arsenic removal from contaminated soil via biovolatilization by genetically engineered bacteria under laboratory conditions." *Journal of Environmental Sciences* **23**(9): 1544-1550.

Luton, P. E., J. M. Wayne, R. J. Sharp and P. W. Riley (2002). "The mcrA gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill." *Microbiology* **148**(11): 3521-3530.

McMahon, M. J. L. and A. J. Daugulis (2008). "Enhancement of biogenic sulfide production in a packed-bed bioreactor via critical inoculum design and carrier material selection." *Biotechnology and Bioengineering* **100**(5): 855-863.

Mercer, T. G. and L. E. Frostick (2014). "Evaluating the potential for environmental pollution from chromated copper arsenate (CCA)-treated wood waste: A new mass balance approach." *Journal of Hazardous Materials* **276**(0): 10-18.

Mestrot, A., B. Planer-Friedrich and J. Feldmann (2013). "Biovolatilisation: a poorly studied pathway of the arsenic biogeochemical cycle." *Environmental Science: Processes & Impacts*.

Mestrot, A., M. K. Uroic, T. Plantevin, M. R. Islam, E. M. Krupp, J. Feldmann and A. A. Meharg (2009). "Quantitative and qualitative trapping of arsines deployed to assess loss of volatile arsenic from paddy soil." *Environmental Science & Technology* **43**(21): 8270-8275.

Mestrot, A., W.-Y. Xie, X. Xue and Y.-G. Zhu (2013). "Arsenic volatilization in model anaerobic biogas digesters." *Applied Geochemistry* **33**(0): 294-297.

Michalke, K., E. B. Wickenheiser, M. Mehring, A. V. Hirner and R. Hensel (2000). "Production of volatile derivatives of metal(loid)s by microflora involved in anaerobic digestion of sewage sludge." *Applied and Environmental Microbiology* **66**(7): 2791-2796.

Mohapatra, D., D. Mishra, G. R. Chaudhury and R. P. Das (2008). "Removal of Arsenic from Arsenic Rich Sludge by Volatilization Using Anaerobic Microorganisms Treated with Cow Dung." *Soil and Sediment Contamination* **17**(3): 301-311.

Morris, R., A. Schauer-Gimenez, U. Bhattad, C. Kearney, C. A. Struble, D. Zitomer and J. S. Maki (2014). "Methyl coenzyme M reductase (mcrA) gene abundance correlates with activity measurements of methanogenic H2/CO2-enriched anaerobic biomass." *Microbial Biotechnology* **7**(1): 77-84.

Mussoline, W., G. Esposito, P. Lens, G. Garuti and A. Giordano (2014). "Electrical energy production and operational strategies from a farm-scale anaerobic batch reactor loaded with rice straw and piggery wastewater." *Renewable Energy* **62**(0): 399-406.

Nachman, K. E., P. A. Baron, G. Raber, K. A. Francesconi, A. Navas-Acien and D. C. Love (2013). "Roxarsone, Inorganic Arsenic, and Other Arsenic Species in Chicken: A U.S.-Based Market Basket Sample." *Environmental Health Perspectives* **121**(7): 818-824.

Naujokas, M. F., B. Anderson, H. Ahsan, H. V. Aposhian, J. H. Graziano, C. Thompson and W. A. Suk (2013). "The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem." *Environmental health perspectives* **121**(3): 295-302.

Pinel-Raffaitin, P., I. Le Hecho, D. Amouroux and M. Potin-Gautier (2007). "Distribution and fate of inorganic and organic arsenic species in landfill leachates and biogases." *Environmental Science & Technology* **41**(13): 4536-4541.

Qin, J., C. R. Lehr, C. Yuan, X. C. Le, T. R. McDermott and B. P. Rosen (2009). "Biotransformation of arsenic by a Yellowstone thermoacidophilic eukaryotic alga." *Proceedings of the National Academy of Sciences of the United States of America* **106**(13): 5213-5217.

Qin, J., B. P. Rosen, Y. Zhang, G. Wang, S. Franke and C. Rensing (2006). "Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase." *Proceedings of the National Academy of Sciences of the United States of America* **103**(7): 2075-2080.

Radl, V., A. Gattinger, A. Chronakova, A. Nemcova, J. Cuhel, M. Simek, J. C. Munch, M. Schloter and D. Elhottova (2007). "Effects of cattle husbandry on abundance and activity of methanogenic archaea in upland soils." *ISME J* **1**(5): 443-452.

Ravenscroft, P., H. Brammer and K. Richards (2009). *Arsenic pollution: a global synthesis*, Wiley-Blackwell.

Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. a. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G. Thallinger, D. J. Van Horn and C. F. Weber (2009). "Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities." *Applied and Environmental Microbiology* **75**(23): 7537-7541.

Smith, A. L., S. J. Skerlos and L. Raskin (2013). "Psychrophilic anaerobic membrane bioreactor treatment of domestic wastewater." *Water Research* **47**: 1655-1665.

Sonthiphand, P., E. Cejudo, S. L. Schiff and J. D. Neufeld (2013). "Wastewater Effluent Impacts Ammonia-Oxidizing Prokaryotes of the Grand River, Canada." *Applied and Environmental Microbiology* **79**(23): 7454-7465.

Sullivan, C., M. Tyrer, C. R. Cheeseman and N. J. D. Graham (2010). "Disposal of water treatment wastes containing arsenic - a review." *Science of the Total Environment* **408**(8): 1770-1778.

Thomas, F., R. A. Diaz-Bone, O. Wuerfel, B. Huber, K. Weidenbach, R. A. Schmitz and R. Hensel (2011). "Connection between Multimetal(loid) Methylation in Methanoarchaea and Central Intermediates of Methanogenesis." *Applied and Environmental Microbiology* **77**(24): 8669-8675.

Tufano, K. J., C. Reyes, C. W. Saltikov and S. Fendorf (2008). "Reductive processes controlling arsenic retention: revealing the relative importance of iron and arsenic reduction." *Environmental Science & Technology* **42**(22): 8283-8289.

Turpeinen, R., M. Pantsar-Kallio and T. Kairesalo (2002). "Role of microbes in controlling the speciation of arsenic and production of arsines in contaminated soils." *Science of the Total Environment* **285**(1-3): 133-145.

U.S. EPA (1992). Method 3050B Acid Digestion of Sediments, Sludges, and Soils. *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods SW-846*.

Ungerfeld, E. M., S. R. Rust, D. R. Boone and Y. Liu (2004). "Effects of several inhibitors on pure cultures of ruminal methanogens*." *Journal of Applied Microbiology* **97**(3): 520-526.

Ungerfeld, E. M., S. R. Rust and R. Burnett (2003). "Use of some novel alternative electron sinks to inhibit ruminal methanogenesis." *Reprod. Nutr. Dev* **43**: 189-202.

Upadhyaya, G., J. Jackson, T. M. Clancy, S. P. Hyun, J. Brown, K. F. Hayes and L. Raskin (2010). "Simultaneous removal of nitrate and arsenic from drinking water sources utilizing a fixed-bed bioreactor system." *Water Research* **44**(17): 4958-4969.

Visoottiviseth, P. and F. Ahmed (2008). Technology for Remediation and Disposal. *Reviews of Environmental Contamination*. D. M. Whitacre. New York, Springer. **197**.

Wang, P.-P., G.-X. Sun and Y.-G. Zhu (2014). "Identification and Characterization of Arsenite Methyltransferase from an Archaeon, Methanosarcina acetivorans C2A." *Environmental Science & Technology*.

Wang, P., G. Sun, Y. Jia, A. A. Meharg and Y. Zhu (2014). "A review on completing arsenic biogeochemical cycle: Microbial volatilization of arsines in environment." *Journal of Environmental Sciences* **26**(2): 371-381.

Woolson, E. A. (1977). "Generation of Alkylarsines from Soil." *Weed Science* **25**(5): 412-416.

Xu, M., W.-M. Wu, L. Wu, Z. He, J. D. Van Nostrand, Y. Deng, J. Luo, J. Carley, M. Ginder-Vogel, T. J. Gentry, B. Gu, D. Watson, P. M. Jardine, T. L. Marsh, J. M. Tiedje, T. Hazen, C. S. Criddle and J. Zhou (2010). "Responses of microbial community functional structures to pilot-scale uranium in situ bioremediation." *ISME J* **4**(8): 1060-1070.

Yao, L., L. Huang, Z. He, C. Zhou and G. Li (2012). "Occurrence of Arsenic Impurities in Organoarsenics and Animal Feeds." *Journal of Agricultural and Food Chemistry* **61**(2): 320-324.

Zhao, F.-J., E. Harris, J. Yan, J. Ma, L. Wu, W. Liu, S. P. McGrath, J. Zhou and Y.-G. Zhu (2013). "Arsenic Methylation in Soils and Its Relationship with Microbial arsM Abundance and Diversity, and As Speciation in Rice." *Environmental Science & Technology* **47**(13): 7147-7154.

Zhao, F.-J., S. P. McGrath and A. A. Meharg (2010). "Arsenic as a Food Chain Contaminant: Mechanisms of Plant Uptake and Metabolism and Mitigation Strategies." *Annual Review of Plant Biology* **61**(1): 535-559.

Zhou, Z., Q. Meng and Z. Yu (2011). "Effects of methanogenic inhibitors on methane production and abundances of methanogens and cellulolytic bacteria in in vitro ruminal cultures." *Applied and environmental microbiology* **77**(8): 2634-2639.

Zinder, S. H., T. Anguish and S. C. Cardwell (1984). "Selective Inhibition by 2-Bromoethanesulfonate of Methanogenesis from Acetate in a Thermophilic Anaerobic Digestor." *Applied and Environmental Microbiology* **47**(6): 1343-1345.

Chapter 6
Conclusions, Environmental Significance, and Future Research Needs

6.1 Overview

This dissertation evaluated the potential for disposal options to limit arsenic release from the wastes generated during drinking water treatment for the removal of arsenic from groundwater. Non-landfill options were selected because of their applicability for developing countries, including Bangladesh and India, where over 30 million people are exposed to arsenic through their drinking water (Ravenscroft *et al.* 2009). The disposal options tested were concrete stabilization and cow dung mixing. These options have both been recommended for application in developing countries, where access to landfills can be limited (Leist *et al.* 2000, Ali *et al.* 2003, Visoottiviseth and Ahmed 2008, Sullivan *et al.* 2010). The studies presented in this dissertation tested an arsenic-bearing waste produced during iron electrocoagulation treatment of contaminated groundwater in West Bengal, India (Amrose *et al.* 2014). Concrete stabilization was shown to be effective at limiting arsenic release to less than 1% of the total arsenic present under non-acidic conditions (pH >4.2) (Chapter 3, Clancy *et al.* 2015c). Through the characterization of arsenic waste within the cement matrix, arsenic was shown to remain primarily associated with iron, similar to the initial state of raw arsenic-bearing waste (Chapter 3, Clancy *et al.* 2015c). These results highlight the ability of cements to limit arsenic release even without changing the phase of arsenic to calcium arsenic solids, as had been previously suggested (Chapter 3, Clancy *et al.* 2015c). Cow dung mixing has been

suggested based on the ability of microorganisms to transform arsenic to volatile arsine and methylarsine gases that can be diluted in the atmosphere. However, very little research has characterized the extent of volatilization or the relevant microbial communities. Anaerobic mesocosms were set-up to simulate cow dung mixing conditions and create an arsenic mass balance by measuring arsenic in the gaseous, liquid, and solid phases (Chapter 5, Clancy *et al.* 2015a). Results showed that very little (<0.02%) arsenic was released through volatilization, but much higher amounts (4%) were released into the aqueous phase (Chapter 5, Clancy *et al.* 2015a). To determine the relative contribution of methanogenesis to arsenic volatilization, molecular techniques were applied to verify that two inhibitors were specific to methanogenic archaea (Chapter 4, , Clancy *et al.* 2015b). Through the application of these inhibitors, it was found that over 87% of the volatilized arsenic could be attributed to the activity of methanogens (Chapter 5, Clancy *et al.* 2015a). These results can also be useful in predicting the volatilization of arsenic under other anaerobic disposal conditions, such as landfills and anaerobic digesters. The findings from these studies show that cow dung mixing should not be recommended due to the potential for increased arsenic release in the aqueous phase. Alternatively, concrete stabilization is a more promising technique and can be recommended for disposal as long as low pH conditions can be avoided.

6.2 Main findings and significance

6.2.1 Concrete stabilization of waste can limit the release of arsenic in non-acidic environments (pH > 4.2)

Stabilizing arsenic-bearing wastes with cement may be a practical method of disposal at community scale arsenic removal plants, where wastes could be mixed with

cements and stored on the ground near removal plants. To simulate potential arsenic release during this disposal scenario, arsenic-bearing wastes were mixed with cement and sand to create mortars and subjected to numerous leaching tests (Chapter 4, Clancy *et al.* 2015b). A long-term leaching simulation in a static simulated rainwater solution was performed and arsenic release was monitored over 406 days. Less than 0.4% of the total arsenic present in the mortar was released to the aqueous phase under these conditions. Through short-term (18 hour) tests, leaching over a range of final pH values was studied using leachant solutions with varying concentrations of acetic acid. Acetic acid was selected because of its use as the leachant in the Toxicity Characteristic Leaching Procedure, the US EPA regulatory test for waste stability. Comparisons between leaching from the raw waste and cement stabilized waste showed that at high pH, very little arsenic was released to the aqueous phase in cement stabilized wastes. At low pH (<4.2) increased arsenic release for wastes was observed, although higher amounts of arsenic were leached from cement stabilized wastes compared to raw wastes. This result is particularly important for disposal environments where low pH conditions dominate, such as landfills where some cement stabilized arsenic-bearing wastes are disposed (Kjeldsen *et al.* 2002, Singh and Pant 2006).

Results from the characterization of the cement stabilized wastes with μXRF showed that, within the cement matrix, most of the arsenic remains associated with iron, which is the same as the starting waste. There is some evidence for arsenic diffusion away from the waste particles, as can be expected from iron wastes in high pH conditions and consistent with observations from the short-term leaching tests with raw waste. This result supports the finding that arsenic was more susceptible to leaching from cement

stabilized wastes under acidic conditions compared to raw waste. In the cement stabilized matrix, any arsenic that was separated from the iron in the waste could have been bound by calcite, but would ultimately dissolve under low pH conditions. The presence of calcite was identified as the primary crystalline phase in areas with the highest arsenic concentration.

Unlike previous studies that reported evidence for crystalline calcium-arsenic solid phases, none of these phases were identified using μXRD. This indicates that cement stabilization can limit arsenic release from iron wastes without changing the phase of arsenic and that cements can create a physical barrier that can limit the exposure of wastes to leaching solutions. For treating wastes before disposal on soil near arsenic removal units, these results show that most of the arsenic would be expected to remain in the solid phase and risk of environmental recontamination is low.

6.2.2 Methanogen inhibitors 2-bromoethanesulfonate and propynoic acid primarily affect methanogens and syntrophic bacteria

The overall effect of inhibitors on the active populations in anaerobic digester sludge and cow dung microbial communities was characterized (Chapter 3, Clancy *et al.* 2015c), before the application of methanogenic inhibitors to study the effects of methanogenesis on arsenic volatilization (Chapter 5, Clancy *et al.* 2015a). Especially in complex microbial communities, chemical inhibitors can have unintended effects on the activity of non-targeted populations. Through the application of RNA- and DNA-based techniques, the changes in activity of methanogens and the overall bacterial and archaeal community were characterized. Using new primers for the methyl-coenzyme M reductase (*mcrA*) gene, the activity of methanogens based on this functional gene was compared to the

activity of methanogens as measured by targeting the 16S rRNA (determined based on amplicon sequencing of cDNA from RNA extracts). The expression of the *mcrA* gene, the relative activity of methanogens (based on 16S rRNA), and the generation of methane were shown to correlate with varying levels of methanogenic inhibition. Analysis of the bacterial community highlighted that the overall community structure of active populations was not significantly changed by the addition of inhibitors. Specific bacterial groups, primarily syntrophic bacteria, were somewhat inhibited. This is expected as syntrophic bacteria rely on methanogens to keep the partial pressures of hydrogen low for their metabolisms to be energetically favorable. These results help to interpret the findings of studies reported in Chapter 5, in which inhibitors were applied to arsenic waste containing mesocosms.

6.2.3 Cow dung disposal results in low volatilization of arsenic and higher release of arsenic in the aqueous phase

The recommendation to mix arsenic-bearing wastes with cow dung has been especially prevalent around discussions of arsenic removal in developing countries (Ali *et al.* 2003). This has continued even though there are few studies that evaluate this method's effectiveness and the potential for arsenic mobilization. Previous simulations of arsenic-bearing wastes mixed with cow dung report large variations in the amount of volatilized arsenic and have not studied the potential for arsenic to be reductively dissolved and mobilized in the aqueous phase. In the study described in this dissertation, arsenic-bearing wastes were incubated under anaerobic conditions in mesocosms seeded with anaerobic digester sludge and cow dung to create conditions mimicking environmental disposal and create optimum potential for methanogenic activity. By

creating a mass balance of arsenic through measurements of arsenic in the gaseous, liquid, and solid phases, these results showed that very little arsenic was volatilized (<0.02% of the total arsenic added) (Chapter 5, Clancy *et al.* 2015a). While the majority of the arsenic remained in the solid phase, arsenic release to the aqueous phase was much higher than the amount volatilized (4% of the total arsenic added). This finding highlights the potential for mobilization to surrounding environments if arsenic wastes were mixed with cow dung and disposed on soils or in pits where rainwater could carry arsenic to the surrounding environment.

6.2.4 Methanogenic activity is correlated with arsenic volatilization under anaerobic conditions

There are two potential pathways for the microbially mediated volatilization of arsenic, through direct arsenic methylation by arsenite *S*-adenosylmethionine methyltransferase (ArsM protein) or through the enzymes involved in methanogenesis. Most research has focused on the volatilization due to ArsM, though little is known about how these pathways are affected by environmental conditions or the activity of different naturally occurring microbial populations. The results presented here represent the first quantification of the relative importance of these two pathways and the specific microbial populations involved in volatilization. Using the inhibitors studied previously (Chapter 4, Clancy *et al.* 2015b), methanogenic activity was controlled in mesocosms with arsenic-bearing wastes. The highest arsenic volatilization occurred in conditions with the highest methanogenic activity, indicating that methanogenesis was the primary pathway for the volatilization of arsenic under these conditions. This is important as it indicates that even under relatively ideal conditions for methanogenesis, limited arsenic volatilization was

observed (<0.2% of the total arsenic). Based on this study, it can be concluded that arsenic volatilization through the activity of methanogens in cow dung is not an effective disposal strategy.

These results are also important to understand the fate of arsenic in anaerobic digesters. Due to the use of arsenic as a feed additive for livestock (Chapman and Johnson 2002, Arai *et al.* 2003, Nachman *et al.* 2013) and the growth of crops in arsenic contaminated areas (Zhao *et al.* 2010, Bhattacharya *et al.* 2012), these animal waste and crops contain arsenic concentrations between 0.007-50 mg/kg (Arai *et al.* 2003, Bhattacharya *et al.* 2012) and depending on the composition of waste mixtures may contribute a significant arsenic load to anaerobic digesters. Previous studies have been motivated by the concern for arsenic volatilization in anaerobic digesters and the potential to harm the health of nearby residents or workers when large amounts of arsenic contaminated waste is treated (Mestrot *et al.* 2013). Because the primary function of anaerobic digesters is the conversion of organics to methane through methanogenesis, the findings from this study reveal that arsenic volatilization cannot be avoided during active methanogenesis in anaerobic digesters, which may treat higher amounts of total waste products compared to those produced at drinking water arsenic removal units. Therefore, designs to mitigate this problem should focus on limiting the arsenic load to anaerobic digesters or treating gases before environmental release.

6.3 Future Research Directions

To support the implementation of proper disposal strategies for arsenic-bearing wastes from drinking water treatment, several areas of research should build on the findings from this dissertation. Studies should include both lab-scale experiments and

field-scale studies of environmental release from actual disposal sites. Further monitoring in areas with a range of disposal practices would be helpful to focus efforts on areas with the highest risk of contamination from disposal sites.

The leaching studies presented here indicate the potential for concrete stabilization of arsenic-bearing wastes to limit the release of arsenic into a rainwater solution. Further research should evaluate specific parameters for optimal application in the field. Curing conditions were shown to affect the leaching of concrete in low pH conditions (Chapter 3, Clancy *et al.* 2015c) and further studies should evaluate the optimal curing environments at a scale appropriate for waste disposal near an arsenic removal unit. This research should also explore the links between changes in the concrete matrix, including arsenic leaching, water permeability, carbonation depth, and strength, as a function of curing condition.

Further research on the stability of concretes in landfill environments and pond water should be performed before cement stabilized wastes are disposed in either environment. There has been very little research on the long-term stability of concrete in landfills, even though cement stabilization can be used as a pre-treatment before landfilling (Singh and Pant 2006) and is typically performed to meet the TCLP limits for disposal in municipal solid waste landfills. In both ponds and landfills, anaerobic conditions promote fermentation of organic compounds resulting in lowering of the pH. Under very low pH (<4.2) conditions, concretes are not expected to be stable and the dissolution of calcite and other cementitious compounds may increase the potential for arsenic release. Landfill leachate pH is typically between 4.5 and 7.5, although in some microenvironments lower pH conditions could prevail (Kjeldsen *et al.* 2002). Following the dissolution of cement

after continued exposure to low pH conditions, the activity of arsenate- and iron-reducing microbes in these environments would be expected to release arsenic from the iron wastes in the aqueous phase through reductive dissolution.

The experiments with cow dung and anaerobic digester sludge demonstrated that only very small amounts of arsenic were volatilized under anaerobic conditions. Therefore, future studies should evaluate volatilization in aerobic environments. In aerobic environments, methanogens would not be active and arsenite *S*-adenosylmethionine methyltransferase mediated conversion would be the likely pathway for arsenic volatilization. Studies comparing arsenic volatilization under aerobic and anaerobic conditions have found differences in the amounts of methylated and volatilized arsenic species, indicating that different pathways may be important depending on the redox environment (Woolson 1977, Majumder *et al.* 2013). Other studies of fungal volatilization typically report much higher volatilization, up to 30% of the total arsenic (Cernanský *et al.* 2009, Srivastava *et al.* 2011, Liu *et al.* 2013, Wang *et al.* 2014). If volatilization is determined to be an ideal remediation strategy further studies should evaluate the potential for fungal activity to be supported.

Beyond understanding the effects of redox environments, future research should also seek to understand the role of different microbial populations in arsenic volatilization. Recently, there has been interest in using genetically modified bacteria to express the *arsM* gene and support the volatilization of arsenic from contaminated sites (Liu *et al.* 2011, Chen *et al.* 2014). However, the ability for genetically modified organisms to be maintained over long time periods in natural environments or to compete with natural populations is unclear. Given that *arsM* is widely distributed across all domains of life

(Bentley and Chasteen 2002), it could be more fruitful to focus on understanding organisms that already thrive in arsenic contaminated environments and produce high amounts of volatilized arsenic. Future studies should also consider the role of fungal arsenic volatilization as pure culture studies have reported up to 30% arsenic volatilization from fungal cultures and co-cultures with bacteria (Cernanský *et al.* 2009, Srivastava *et al.* 2011, Liu *et al.* 2013, Wang *et al.* 2014). If significant volatilization could be supported given the right conditions and stimulation of specific microbial populations, the design of future disposal conditions could be improved.

The proper disposal of arsenic-bearing wastes is an important component to support the sustainable implementation of technologies for arsenic removal from drinking water. Other challenges to the provision of arsenic safe drinking water in developing communities include community interest and demand for arsenic-free water (Ahmad *et al.* 2005), long-term operation and maintenance of treatment systems (Hossain *et al.* 2005, Etmannski and Darton 2014), and the potential for multiple contaminants to co-exist in drinking water sources (Wasserman *et al.* 2006, Hug *et al.* 2011). Since the discovery of the arsenic contamination problem, many different arsenic removal technologies have been implemented in Bangladesh and India (Ahmed *et al.* 2006, Sarkar *et al.* 2010), but many systems have been abandoned (Hossain *et al.* 2005). This history of failure points to the need for new designs and techniques for implementation. More acceptable and successful solutions are likely to result from interdisciplinary evaluations of designs that consider the success of arsenic removal systems through the lenses of economic feasibility, social acceptance, and environmental sustainability.

6.4 Literature Cited

Ahmad, J., B. Goldar and S. Misra (2005). Value of arsenic-free drinking water to rural households in Bangladesh. *Journal of Environmental Management* 74(2): 173-185.

Ahmed, M. F., S. Ahuja, M. Alauddin, S. J. Hug, J. R. Lloyd, A. Pfaff, T. Pichler, C. Saltikov, M. Stute and A. van Geen (2006). Ensuring Safe Drinking Water in Bangladesh. *Science* 314(December): 1687-1688.

Ali, M. A., A. B. M. Badruzzaman, M. A. Jalil, M. F. Ahmed, M. Kamruzzaman, M. A. Rahman and A. Al Masud (2003). Fate of Arsenic in Wastes Generated From Arsenic Removal Units. Proceedings of BUET-UNU Symposium on Fate of Arsenic in the Environment, Dhaka.

Amrose, S. E., S. R. S. Bandaru, C. Delaire, C. M. van Genuchten, A. Dutta, A. DebSarkar, C. Orr, J. Roy, A. Das and A. J. Gadgil (2014). Electro-chemical arsenic remediation: Field trials in West Bengal. *Sci. Total Environ.* 488–489(0): 539-546.

Arai, Y., A. Lanzirotti, S. Sutton, J. A. Davis and D. L. Sparks (2003). Arsenic Speciation and Reactivity in Poultry Litter. *Environ. Sci. Technol.* 37(18): 4083-4090.

Bentley, R. and T. G. Chasteen (2002). Microbial methylation of metalloids: arsenic, antimony, and bismuth. *Microbiol. Mol. Biol. Rev.* 66(2): 250-250.

Bhattacharya, S., K. Gupta, S. Debnath, U. C. Ghosh, D. Chattopadhyay and A. Mukhopadhyay (2012). Arsenic bioaccumulation in rice and edible plants and subsequent transmission through food chain in Bengal basin: a review of the perspectives for environmental health. *Toxicological & Environmental Chemistry* 94(3): 429-441.

Cernanský, S., M. Kolencík, J. Sevc, M. Urík and E. Hiller (2009). Fungal volatilization of trivalent and pentavalent arsenic under laboratory conditions. *Bioresour. Technol.* 100(2): 1037-1040.

Chapman, H. D. and Z. B. Johnson (2002). Use of Antibiotics and Roxarsone in Broiler Chickens in the USA: Analysis for The Years 1995 to 2000. *Poultry Science* 81(3): 356-364.

Chen, J., G.-X. Sun, X.-X. Wang, V. d. Lorenzo, B. P. Rosen and Y.-G. Zhu (2014). Volatilization of Arsenic from Polluted Soil by Pseudomonas putida Engineered for Expression of the arsM Arsenic(III) S-Adenosine Methyltransferase Gene. *Environ. Sci. Technol.* 48(17): 10337-10344.

Clancy, T. M., R. Reddy, J. Tan, K. F. Hayes and L. Raskin (2015a). Anaerobic disposal of arsenic-bearing wastes and the impact of methanogenesis on arsenic volatilization. *in prep. for Environ. Sci. Technol*.

Clancy, T. M., A. L. Smith, R. Reddy, A. J. Pinto, K. F. Hayes and L. Raskin (2015b). Anaerobic microbial community response to methanogenic inhibitors 2-bromoethanesulfonate and propynoic acid. *in prep. for Environmental Microbiology*.

Clancy, T. M., K. V. Snyder, R. Reddy, S. E. Amrose, L. Raskin and K. F. Hayes (2015c). Evaluating the cement stabilization of arsenic-bearing iron wastes from drinking water treatment. *in prep. for Journal of Hazardous Materials*.

Etmannski, T. R. and R. C. Darton (2014). A methodology for the sustainability assessment of arsenic mitigation technology for drinking water. *Sci. Total Environ.* 488–489(0): 505-511.

Hossain, M. A., M. K. Sengupta, S. Ahamed, M. M. Rahman, D. Mondal, D. Lodh, B. Das, B. Nayak, B. K. Roy, A. Mukherjee and D. Chakraborti (2005). Ineffectiveness and Poor Reliability of Arsenic Removal Plants in West Bengal, India. *Environ. Sci. Technol.* 39(11): 4300-4306.

Hug, S. J., D. Gaertner, L. C. Roberts, M. Schirmer, T. Ruettimann, T. M. Rosenberg, A. B. M. Badruzzaman and M. Ashraf Ali (2011). Avoiding high concentrations of arsenic, manganese and salinity in deep tubewells in Munshiganj District, Bangladesh. *Appl. Geochem.* 26(7): 1077-1085.

Kjeldsen, P., M. Barlaz, A. Rooker, A. Baun, A. Ledin and T. Christensen (2002). Present and Long-Term Composition of MSW Landfill Leachate: A Review. *Crit. Rev. Environ. Sci. Technol.* 32(4): 297-336.

Leist, M., R. J. Casey and D. Caridi (2000). The management of arsenic wastes: problems and prospects. *J. Hazard. Mater.* 76(1): 125-138.

Liu, S., Y. Hou and G. Sun (2013). Synergistic degradation of pyrene and volatilization of arsenic by cocultures of bacteria and a fungus. *Front. Environ. Sci. Eng.* 7(2): 191-199.

Liu, S., F. Zhang, J. Chen and G. Sun (2011). Arsenic removal from contaminated soil via biovolatilization by genetically engineered bacteria under laboratory conditions. *J. Environ. Sci.* 23(9): 1544-1550.

Majumder, A., K. Bhattacharyya, S. Kole and S. Ghosh (2013). Efficacy of indigenous soil microbes in arsenic mitigation from contaminated alluvial soil of India. *Environmental Science and Pollution Research*: 1-9.

Mestrot, A., W.-Y. Xie, X. Xue and Y.-G. Zhu (2013). Arsenic volatilization in model anaerobic biogas digesters. *Appl. Geochem.* 33(0): 294-297.

Nachman, K. E., P. A. Baron, G. Raber, K. A. Francesconi, A. Navas-Acien and D. C. Love (2013). Roxarsone, Inorganic Arsenic, and Other Arsenic Species in Chicken: A U.S.-Based Market Basket Sample. *Environ. Health Perspect.* 121(7): 818-824.

Ravenscroft, P., H. Brammer and K. Richards (2009). Arsenic pollution: a global synthesis, Wiley-Blackwell.

Sarkar, S., J. E. Greenleaf, A. Gupta, D. Ghosh, L. M. Blaney, P. Bandyopadhyay, R. K. Biswas, A. K. Dutta and A. K. Sengupta (2010). Evolution of community-based arsenic removal systems in remote villages in West Bengal, India: Assessment of decade-long operation. *Water Res.* 44(19): 5813-5822.

Singh, T. S. and K. K. Pant (2006). Solidification/stabilization of arsenic containing solid wastes using portland cement, fly ash and polymeric materials. *J. Hazard. Mater.* 131(1–3): 29-36.

Srivastava, P. K., A. Vaish, S. Dwivedi, D. Chakrabarty, N. Singh and R. D. Tripathi (2011). Biological removal of arsenic pollution by soil fungi. *Sci. Total Environ.* 409(12): 2430-2442.

Sullivan, C., M. Tyrer, C. R. Cheeseman and N. J. D. Graham (2010). Disposal of water treatment wastes containing arsenic - a review. *Sci. Total Environ.* 408(8): 1770-1778.

Visoottiviseth, P. and F. Ahmed (2008). Technology for Remediation and Disposal. Reviews of Environmental Contamination. D. M. Whitacre. New York, Springer. 197.

Wang, P., G. Sun, Y. Jia, A. A. Meharg and Y. Zhu (2014). A review on completing arsenic biogeochemical cycle: Microbial volatilization of arsines in environment. *J. Environ. Sci.* 26(2): 371-381.

Wasserman, G. A., X. Liu, F. Parvez, H. Ahsan, D. Levy, P. Factor-Litvak, J. Kline, A. v. Geen, V. Slavkovich, N. J. Lolacono, Z. Cheng, Y. Zheng and J. H. Graziano (2006). Water Manganese Exposure and Children's Intellectual Function in Araihazar, Bangladesh. *Environ. Health Perspect.* 114(1): 124-129.

Woolson, E. A. (1977). Generation of Alkylarsines from Soil. *Weed Science* 25(5): 412-416.

Zhao, F.-J., S. P. McGrath and A. A. Meharg (2010). Arsenic as a Food Chain Contaminant: Mechanisms of Plant Uptake and Metabolism and Mitigation Strategies. *Annual Review of Plant Biology* 61(1): 535-559.

Appendices

Appendix A. Field sampling of soils near arsenic removal filters in Bangladesh

Background

For the vast majority of community scale drinking water filters for arsenic removal installed in Bangladesh, there is little or no treatment of the arsenic bearing wastes generated. Filters typically remove arsenic by sorption onto oxidized iron materials. Periodically, these filters must be backwashed to remove the accumulated insoluble arsenic-bearing iron wastes. This waste stream is commonly discharged onto the surrounding soil surface via a drain channel. There is cause for concern with this practice due to the potential for arsenic mobilization and subsequent contamination of the surrounding environment. To address these concerns, this study reports results from a collaboration with Asia Arsenic Network, a non-profit organization in Bangladesh, to investigate the effect of these disposal practices on the surrounding environment. This work was supported through the NSF-USAID program Partnerships for Enhanced Engagement in Research (PEER).

Sampling Sites and Procedures

Ten sites were selected in the Jessore district of Bangladesh to represent arsenic removal installations with two of the most common filter designs, the AIRP and SIDKO systems. The arsenic and iron removal plant (AIRP) design (Yokota *et al.* 2001) has been employed in areas with naturally high concentrations of iron in the groundwater. An AIRP first aerates water pumped from a tube well by directing water through holes in

metal trickling plates, followed by successive filtration through gravel and sand filter beds. Naturally present iron in the groundwater is oxidized and precipitates as ferric hydroxides to which arsenic is sorbed. The SIDKO filters (Ahmed 2001) remove arsenic by filtration through a gravel column followed by a ferric hydroxide based adsorbent media column.

In both designs, the filters must be periodically backwashed to remove accumulated arsenic-bearing waste solids. These wastes are often deposited on the ground nearby the filtration unit. In some filter installations, the waste backwash stream flows out through a small concrete chamber where some settling of waste solids is expected to take place. This chamber is referred to as a pit tank. However, based on field observations, this tank is quite small (typically 0.06 m^3) and its efficacy as a settling tank has not been properly evaluated. Furthermore, some pit tanks contain a plastic drain pipe either at the top or bottom of the tank that leads away from the filter to a nearby pond or ditch. Keeping in mind this variability in filter design, ten sites were chosen to include the two mentioned filter types as well as sites with and without a 'pit tank'. Five AIRP systems were chosen: AIRP sites 1, 2, and 3 had a pit tank and sites 4 and 5 did not have a pit tank. Three SIDKO filter systems were selected (S1, S2, S3). Two control sites that did not have arsenic removal filter installations were selected. Control site 1 had low groundwater arsenic concentrations (< 5 μg/L), while Control site 2 had high groundwater arsenic levels (~500 μg/L). Soil samples were collected from various sites surrounding these filters for total arsenic measurements through a nitric acid and hydrogen peroxide digestion (described in Chapter 3). Two sampling events were performed at a selection of the sites in August and April 2014. At each site, the center

point was determined as the site of waste disposal that was separate from the pit tank, if present. This often included the site of backwash waste disposal or the area nearby the pit tank where the pit tank sludge would be disposed during cleaning. Surrounding soil samples were collected from locations about five meters from the center point in four directions that did not include the direction of draining from the center point. The sampling in April was done during the dry season and included all ten sites selected. The sampling in August occurred during the rainy season and included more sampling points along the drain path which was determined visually based on what direction water from the center point would drain.

Results and Discussion

Figure A-S1summarizes the soil arsenic concentrations of samples collected from the sites in both April and August 2014. Results from sites with only one set of samples collected are shown in Figure A-S2. Arsenic levels in the surrounding soils varied from 1-8 mg/kg at different sites, though the variation between samples at any given site was smaller (standard deviations are shown as error bars for each site). These surrounding soil values represent the background concentrations in the soils and are used as a comparison to evaluate the effect of the filter and current waste management strategy.

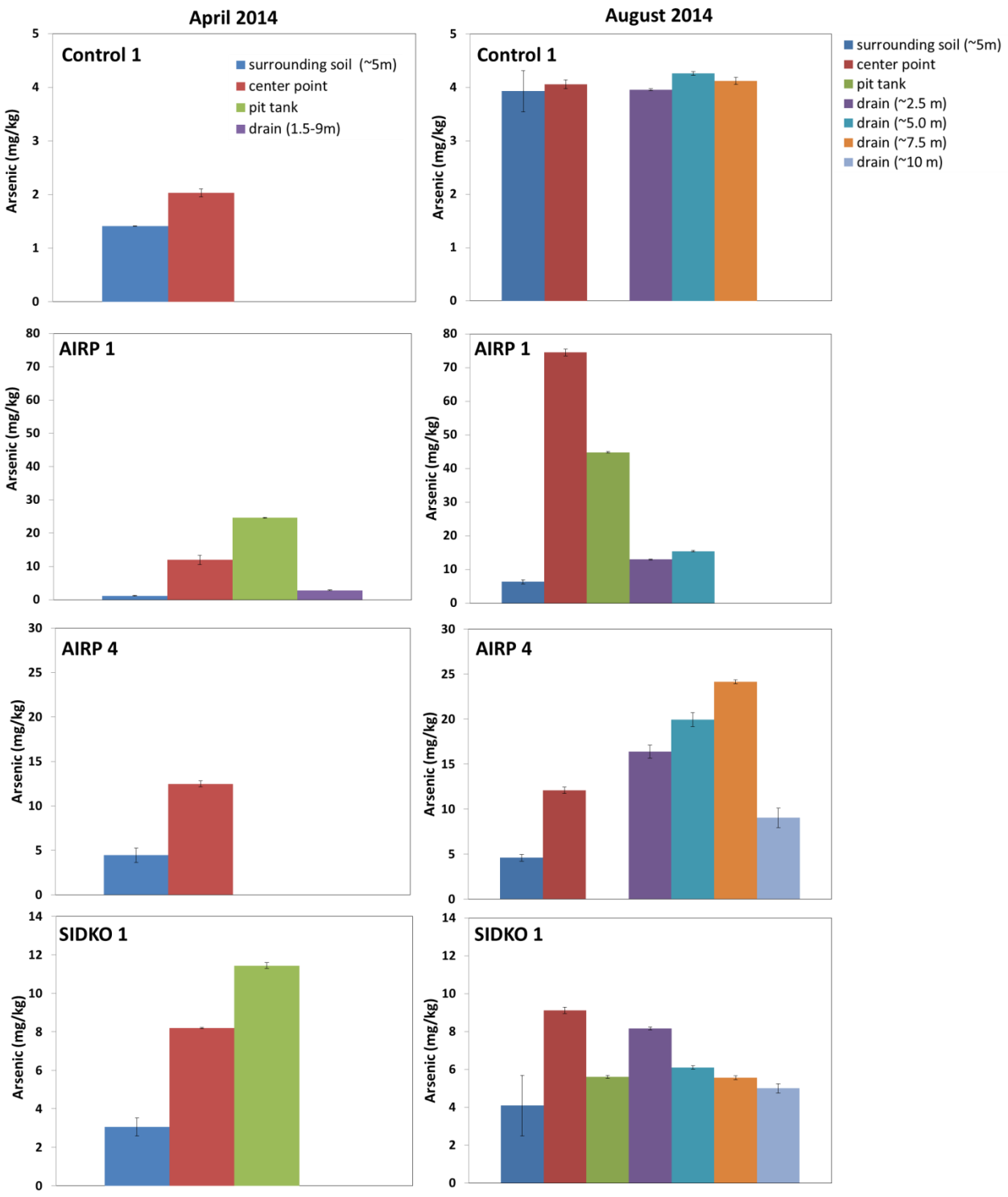


Figure A-S1. Soil arsenic concentrations (mg arsenic/kg soil) from samples taken at various locations near arsenic removal filters during two sampling events. The control samples were collected around a tube well without arsenic. Bars represent the average soil arsenic concentration and error bars the standard deviations

between triplicate soil samples from each site. Surrounding soil bars represent the average and standard deviation of four soil samples collected about 5 m from the center point at each site.

The pit tank and center point samples generally had higher arsenic levels than the surrounding soils and in a few cases this difference was very large (up to a 12 fold greater at AIRP 2) (Figure A-S2). Some drain path samples had elevated arsenic concentrations (AIRP 2, AIRP 4, SIDKO 3), while in other cases the levels were closer to the surrounding soil levels (AIRP 1, AIRP 3). Differences between arsenic concentrations measured in April and August at the same sites may be due to changes in factors such as time since the last backwash and rainfall frequency, which varied between the two samplings.

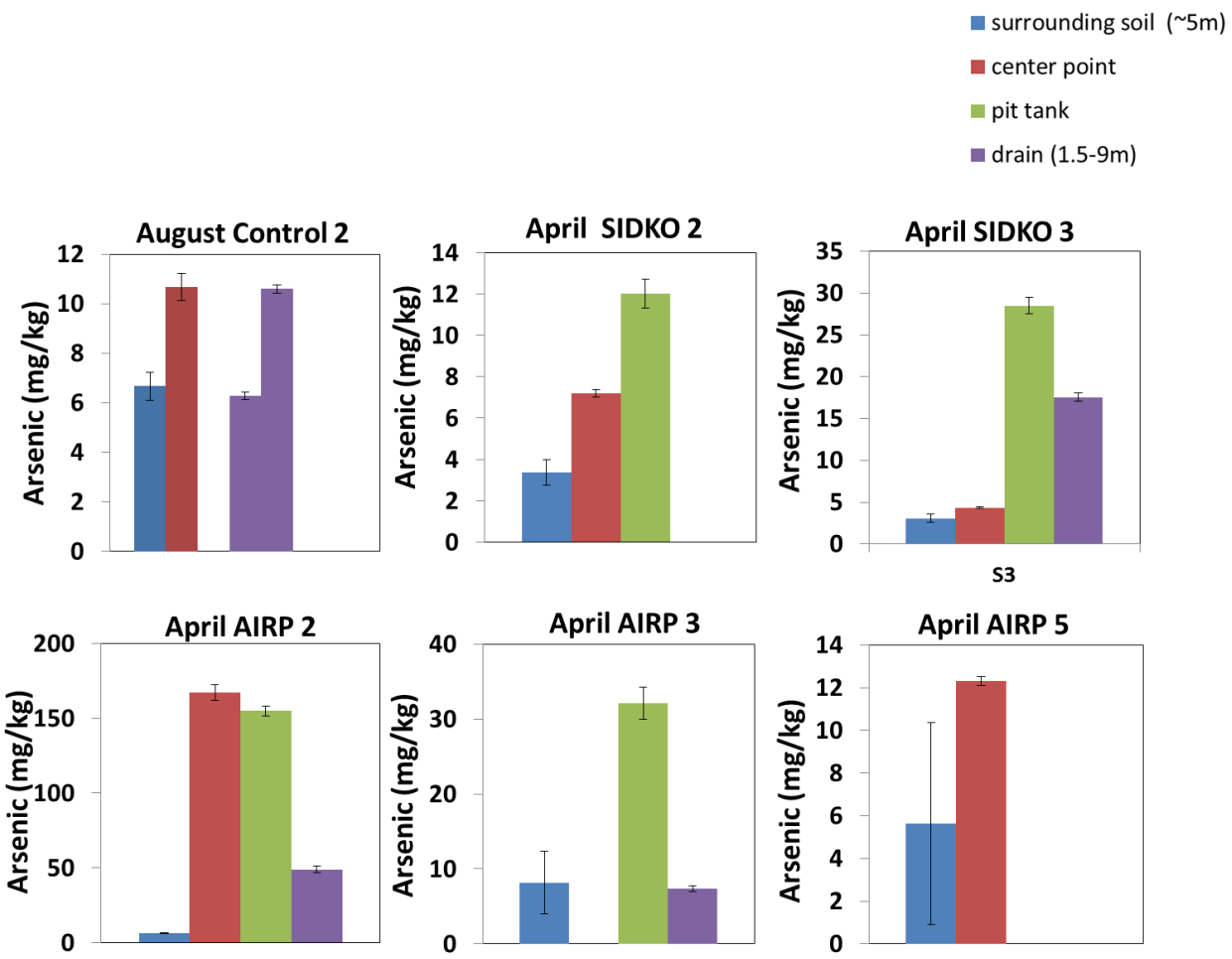


Figure A-S2. Soil arsenic concentrations from selected sites. Bars represent the average of either triplicate total digestions or for the surrounding samples four soil samples from about 5 m from the center point and the error bars represent the standard deviation.

Based on these data, it appears that current filter waste maintenance practices have a localized effect on soil arsenic concentrations. The highest soil arsenic concentrations are found in the pit tank, at the center points where waste is disposed, and along the drain path. This finding is important to consider during the design and implementation of the filters and measures to limit the spread of this waste stream should be minimized. The stability of these arsenic bearing wastes is a matter of concern and further studies are

required to understand the potential for mobilization and the relative risk these arsenic soil concentrations represent. Current studies are evaluating the potential uptake of arsenic by plants grown in the vicinity of these filters and the potential for stabilization of these arsenic-bearing wastes by incorporation into building materials such as brick and concrete.

References

Ahmed, M. F. (2001). An Overview of Arsenic Removal Technologies in Bangladesh and India**:** 251-269.

Yokota, H., K. Tanabe, M. Sezaki, Y. Akiyoshi, T. Miyata, K. Kawahara, S. Tsushima, H. Hironaka, H. Takafuji, M. Rahman, S. A. Ahmad, M. H. S. U. Sayed and M. H. Faruquee (2001). Arsenic contamination of ground and pond water and water purification system using pond water in Bangladesh. *Eng. Geol.* 60: 323-331.

Appendix B. Supplementary Information for Chapter 2

Table S1. Results of TCLP for Different Arsenic-bearing Wastes

Solids Tested	Solids Arsenic Concentration (mg/kg)	TCLP Leachate Concentration (mg/L)	Reference
Sulfide material from sulfate reducing bioreactors treating simulated mining waste	61.3	0.04	(Jong and Parry 2005)
Mine tailings (concrete stabilized)	83.8	0.18	(Choi *et al.* 2009)
	81.6	0.07	
	79.4	0.03	
	75.0	0.01	
	70.6	0.01	
	66.2	0.01	
	61.7	0.00	
	69.6	0.23	
	67.8	0.16	
	66.0	0.06	
	62.3	0.05	
	58.6	0.02	
	55.0	0.02	
	51.3	<0.004	
Mine tailings (without concrete)	88.2	1.14	(Choi *et al.* 2009)
	1.4	0.06	
	73.3	0.61	
Mine tailings	8120	0.06	(Hooper *et al.* 1998)
Slag	355	0.1	(Hooper *et al.* 1998)
Burn ash	229	0.13	(Hooper *et al.* 1998)
Laboratory synthesized ferric hydroxides	82400	2.54	(Phenrat *et al.* 2008)
	71500	1.86	(Phenrat *et al.* 2008)
	57400	1.05	(Phenrat *et*

			al. 2008)
	56000	0.4	(Phenrat *et al.* 2008)
	38400	0.26	(Phenrat *et al.* 2008)
Sludge from drinking water treatment arsenic removal plant (ferric hydroxide)	0.1	0.0067	(Banerjee and Chakraborty 2005)
	2.9	0.083	
	29.2	0.13	
	73.1	0.25	
SIDKO Adsorp® Technology (ferric hydroxide) from drinking water treatment	2025	0.225	(Hossain 2003)
Ferric chloride coagulation solids (settled dewatering pond, anaerobic) from drinking water treatment	1540	0.205	(Meng *et al.* 2001)
	935	0.283	
Ferric chloride coagulation solids (fresh solids, oxic) from drinking water treatment	638	0.078	(Meng *et al.* 2001)
Bayoxide® E33 (ferric oxide) from drinking water treatment	2150	0.021	(Stuckman *et al.* 2011)
	2140	0.001	
	7530	0.085	
	2250	0.049	
	3070	0.036	
MAGC/ALCAN top media (activated alumina based) from drinking water treatment	80.1	0.148	(Hossain 2003)
MAGC/ALCAN bottom media (activated alumina based) from drinking water treatment	72.1	0.426	(Hossain 2003)
READ-F media (hydrous cerium oxide) from drinking water treatment	258.2	0.007	(Hossain 2003)
SONO top sand from drinking water treatment	16.6	0.005	(Hossain 2003)
SONO iron filings (zero valent iron) from drinking water treatment	1.2	0.002	(Hossain 2003)
SONO bottom sand from drinking water treatment	3.5	0.004	(Hossain 2003)

References

Banerjee, G. and R. Chakraborty (2005). Management of arsenic-laden water plant sludge by stabilization. *Clean Technol. Environ. Policy* 7(4): 270-278.

Choi, W. H., S. R. Lee and J. Y. Park (2009). Cement based solidification/stabilization of arsenic-contaminated mine tailings. *Waste Manage.* 29(5): 1766-1771.

Hooper, K., M. Iskander, G. Sivia, F. Hussein, J. Hsu, M. DeGuzman, Z. Odion, Z. Ilejay, F. Sy, M. Petreas and B. Simmons (1998). Toxicity Characteristic Leaching Procedure Fails To Extract Oxoanion-Forming Elements That Are Extracted by Municipal Solid Waste Leachates. *Environ. Sci. Technol.* 32(23): 3825-3830.

Hossain, M. A. (2003). Performance Evaluation and Verification of Five Arsenic Removal Technologies. Dhaka, Bangladesh, Bangladesh Council of Scientific and Industrial Research.

Jong, T. and D. L. Parry (2005). Evaluation of the stability of arsenic immobilized by microbial sulfate reduction using TCLP extractions and long-term leaching techniques. *Chemosphere* 60: 254-265.

Meng, X., G. P. Korfiatis, C. Jing and C. Christodoulatos (2001). Redox transformations of arsenic and iron in water treatment sludge during aging and TCLP extraction. *Environ. Sci. Technol.* 35(17): 3476-3481.

Phenrat, T., T. F. Marhaba and M. Rachakornkij (2008). Leaching behaviors of arsenic from arsenic-iron hydroxide sludge during TCLP. *Journal of Environmental Engineering* 134(8): 671-682.

Stuckman, M. Y., J. J. Lenhart and H. W. Walker (2011). Abiotic properties of landfill leachate controlling arsenic release from drinking water adsorbents. *Water Res.* 45(16): 4782-4792.

Appendix C. Supplementary Information for Chapter 3

Table S2. Synthetic Rainwater Composition (Hadi *et al.* 1999)

Component	mM	mg/L
$CaCl_2$	0.012	1.3308
$NaHCO_3$	0.026	2.184
K_2CO_3	0.0015	0.2073
$MgCl_2$	0.0037	0.352277
NH_4Cl	0.05	2.6725
pH = 6.35		

Rainwater was prepared using ACS grade salts (Sigma-Aldrich).

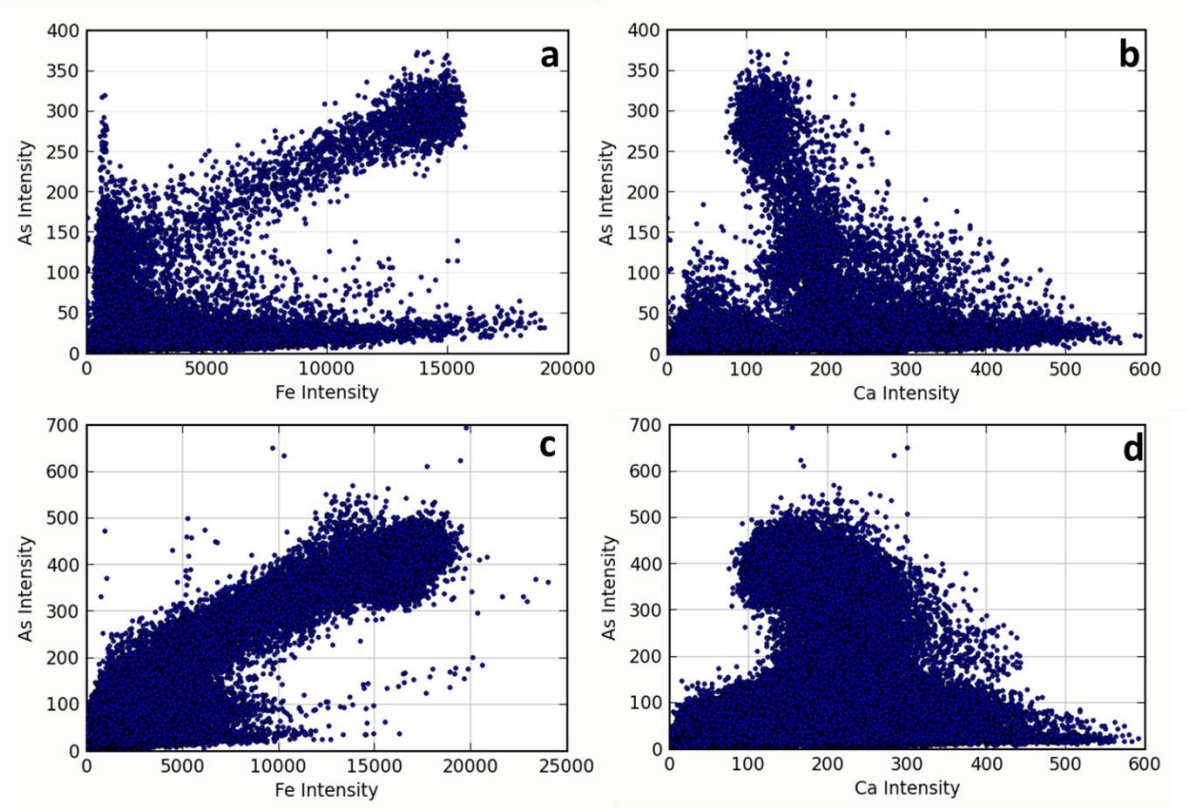


Figure S1. Comparisons of the Kα emission intensities of arsenic to iron (a,c) and arsenic to calcium (b,d) for mortar cubes from day 0 (a, b) and day 406 (c,d) from maps shown in Figure S2.

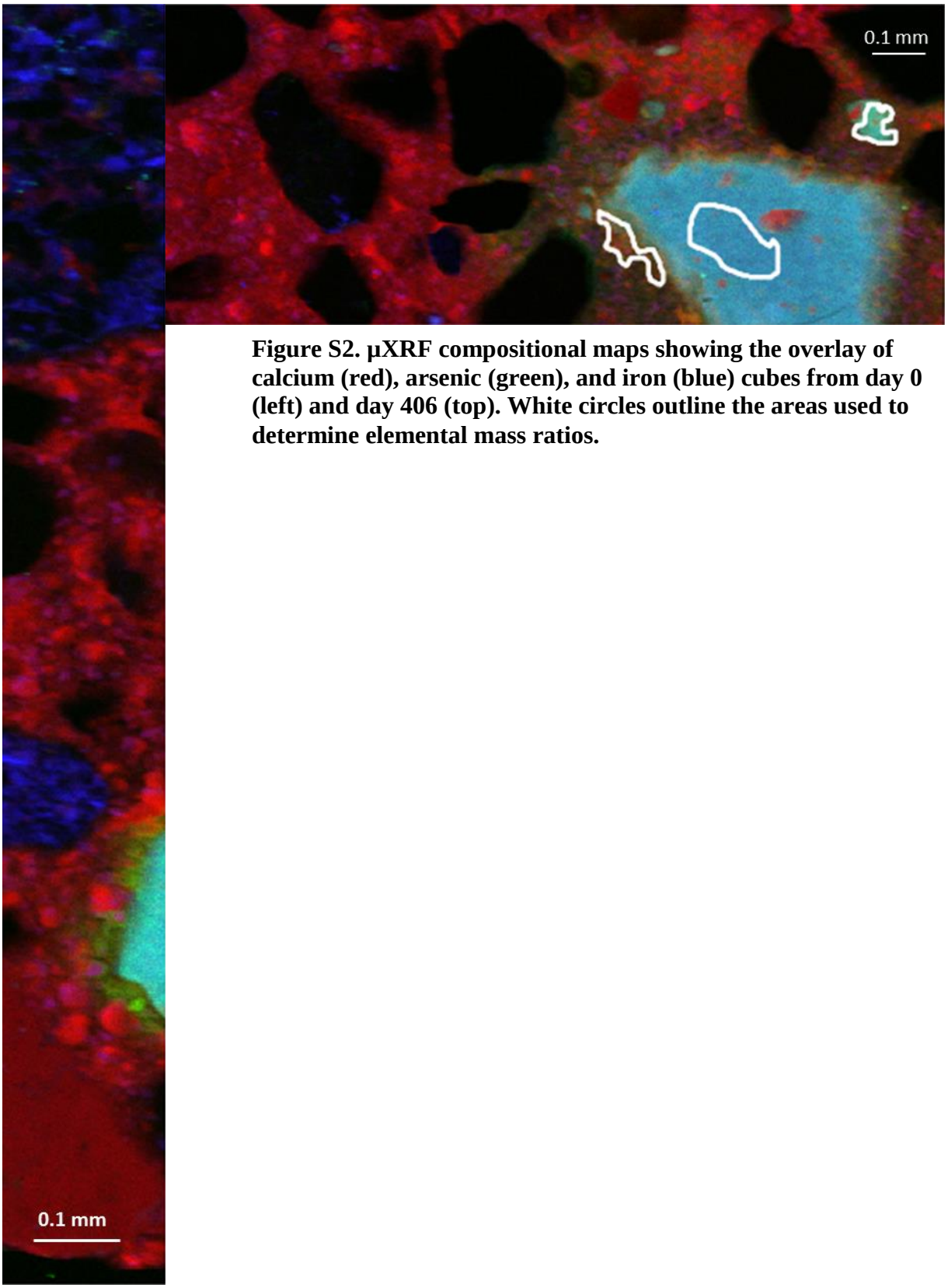


Figure S2. µXRF compositional maps showing the overlay of calcium (red), arsenic (green), and iron (blue) cubes from day 0 (left) and day 406 (top). White circles outline the areas used to determine elemental mass ratios.

Appendix D. Supplementary Information for Chapter 4

Table S3. Primer coverage of the mlas and mcrA-rev primers compared to the modified mlas and *mcrA*-rev primers used in this study for 32 methanogens for which genomes were available. Primer pair coverage is a score of the primer pair binding to the template using the sequence overlaps of the forward and reverse primers normalized to the length of the primers generated with MFE Primer 2.0 (Qu *et al.* 2012). A primer pair coverage cutoff value of 30 was used.

NCBI Reference	Taxonomy	Size (bp)	Primer Pair Coverage (%)	
			mlas/ mcrA-rev	modified mlas/ mcrA-rev
NC_015574.1	*Methanobacterium* sp. SWAN-1	472	97	97
NC_009135.1	*Methanococcus maripaludis*	469	97	97
NC_009634.1	*Methanococcus vannielii*	469	97	97
NC_009637.1	*Methanococcus maripaludis*	469	97	97
NC_009975.1	*Methanococcus maripaludis*	469	97	97
NC_003552.1	*Methanosarcina acetivorans*	490	97	97
NC_003901.1	*Methanosarcina mazei*	490	97	97
NC_014408.1	*Methanothermobacter marburgensis*	469	97	97
NC_014507.1	*Methanoplanus petrolearius*	493	97	97
NC_007355.1	*Methanosarcina barkeri*	490	97	97
NC_007681.1	*Methanosphaera stadtmanae*	469	97	97
NC_009051.1	***Methanoculleus marisnigri***	**493**	**75.8**	**97**
NC_009712.1	***Methanoregula boonei***	**472**	**75.8**	**97**
NC_015416.1	***Methanosaeta concilii***	**472**	**75.8**	**97**
NC_009515.1	***Methanobrevibacter smithii***	**472**	**<30**	**97**
NC_009464.1	***Methanocella arvoryzae***	**469**	**<30**	**97**
NC_015847.1	***Methanococcus maripaludis***	**469**	**<30**	**97**
NC_017527.1	***Methanosaeta harundinaceae***	**472**	**<30**	**97**
NC_018227.1	*Methanoculleus bourgensis*	493	97	97
NC_018876.1	*Methanolobus psychrophilus*	490	97	97
NC_019943.1	***Methanoregula formicicum***	**472**	**33**	**97**
NC_005791.1	***Methanococcus maripaludis***	**469**	**<30**	**97**
NC_023044.1	*Methanobacterium* sp. MB1	469	89	89
NC_007955.1	*Methanococcoides burtonii*	490	56	56
NC_008553.1	*Methanosaeta thermophila*	472	56	56
NC_019977.1	*Methanomethylovorans hollandica*	490	38	38
NC_008942.1	*Methanocorpusculum labreanum*	493	33	33
NC_015216.1	*Methanobacterium* sp. AL-21	466	33	33

NC_011832.1	*Methanosphaerula palustris*	472	33	33
NC_014222.1	*Methanococcus voltae*	469	33	33
NC_021355.1	*Methanobrevibacter* sp. AbM4	472	33	33
NC_020389.1	***Methanosarcina mazei***	**490**	**<30**	**33**

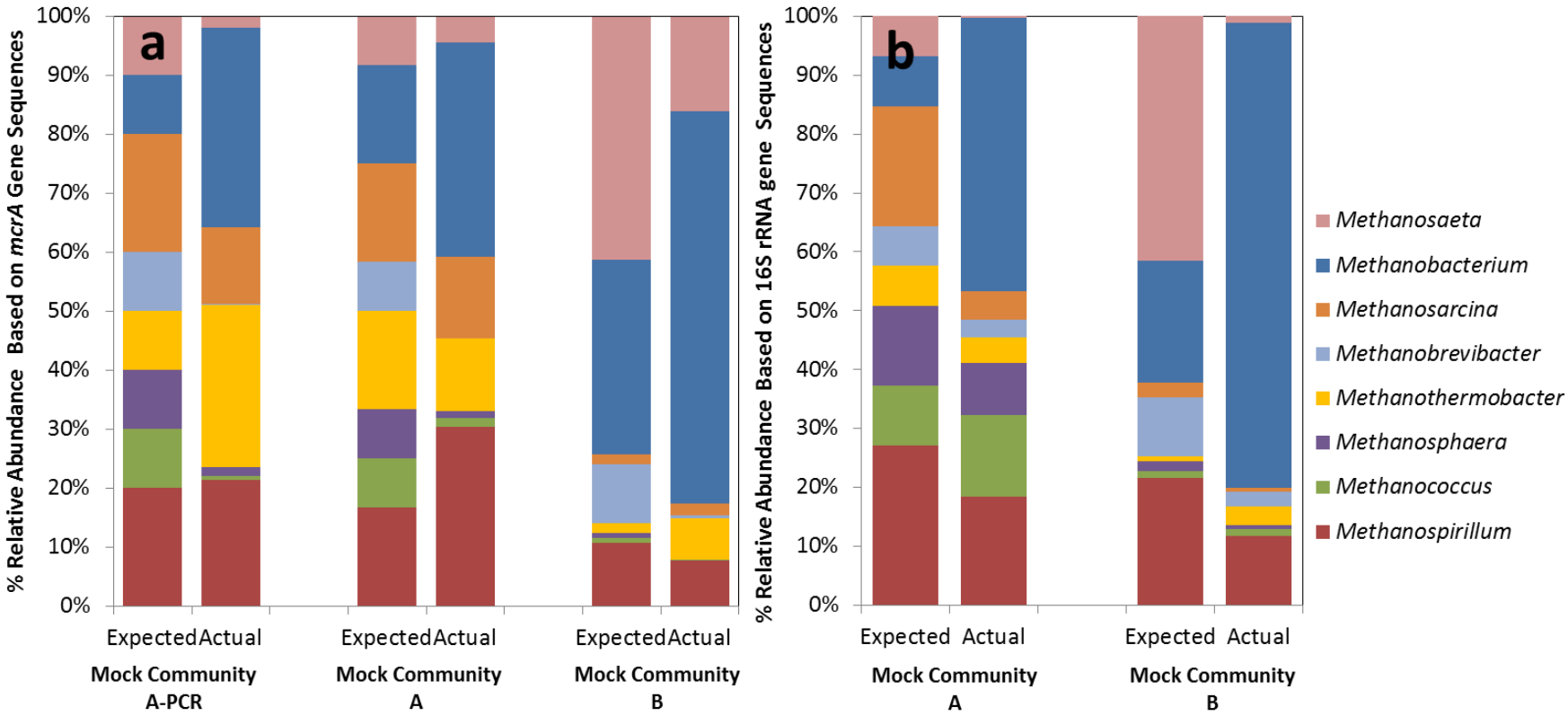


Figure S3. Comparison of expected and actual results from methanogen mock community sequencing with *mcrA* gene amplicons (a) and 16S rRNA gene amplicons (b). Mock communities A and B represent a relatively even community (A) or an uneven community (B) with relative methanogen abundances similar to what would be found in an anaerobic digester (Smith *et al.* 2013). Mock community A-PCR was generated by pooling individually amplified DNA extracts from the different strains, whereas mock communities A and B were generated by pooling DNA extracts before amplification. The expected compositions were calculated based on measured DNA concentrations extracted from 10 strains (Table S4), genome size, and gene copy number (Table S4). For the genera *Methanospirillum* and *Methanosarcina* two different strains were included from each genus (Table S4). All strains included in the mock communities were identified for each of the methods used. The *mcrA* gene based sequencing results included one sequence identified as *Methanohalophilus* and one sequence identified as *Methanoculleus*, which were excluded from the graphs.

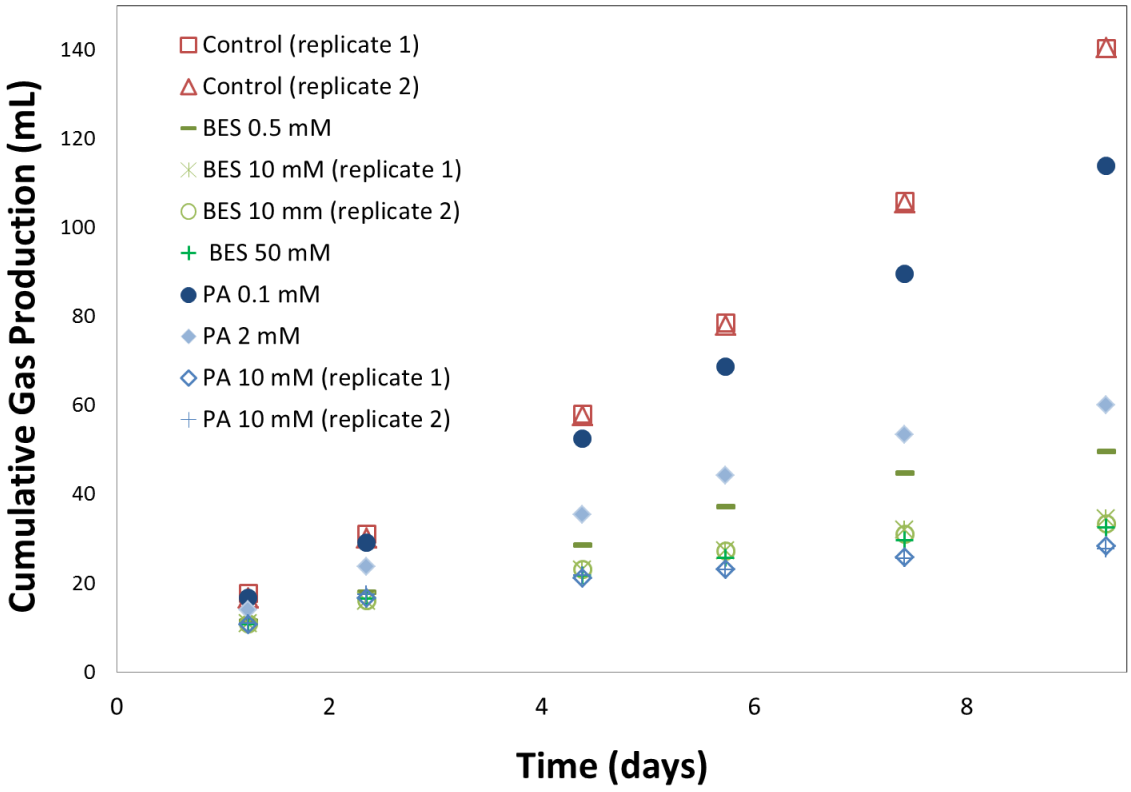


Figure S4. Cumulative gas production for all inhibitor concentrations tested including replicate mesocosms.

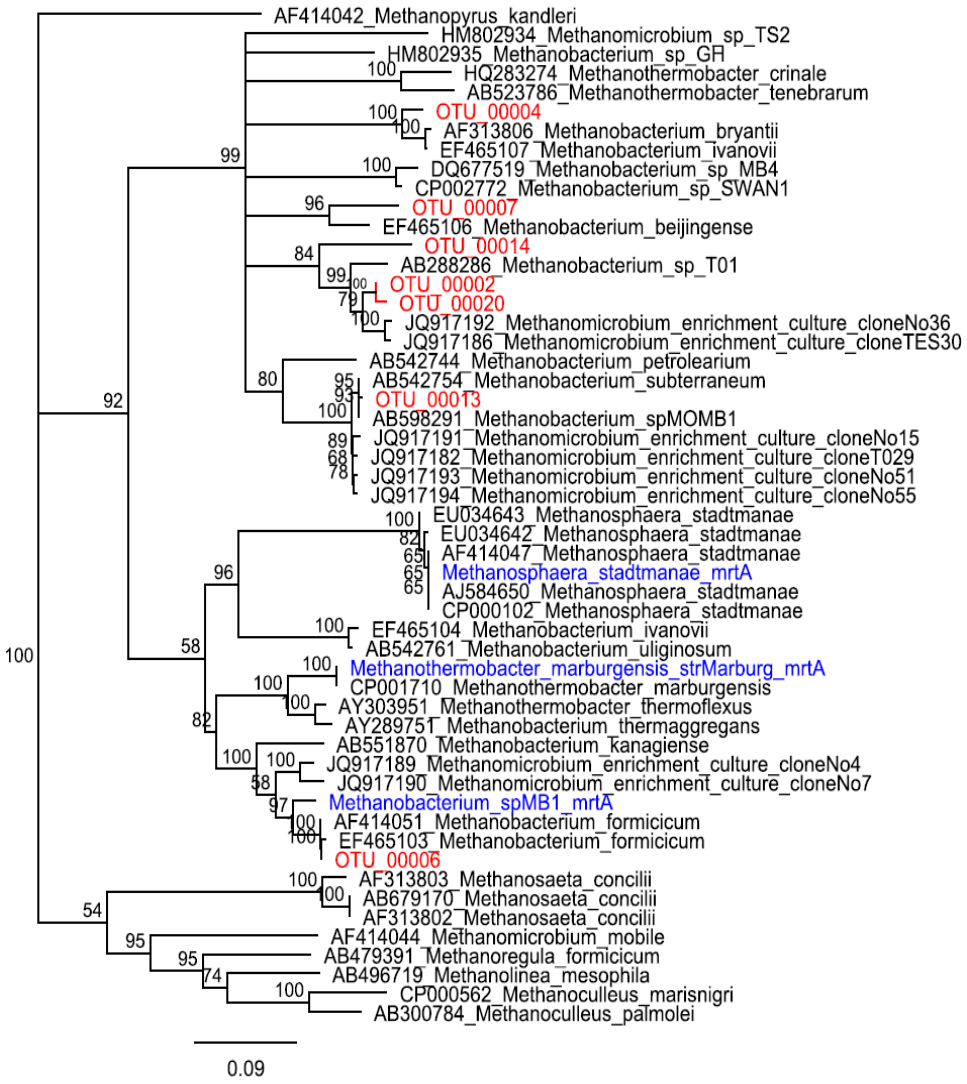


Figure S5. Neighbor-joining tree based on *mcrA* sequences showing sequences used for taxonomic identification (black), *mrtA* sequences (blue), and representative sequences from OTUs identified as *Methanobacterium* and *Methanomicrobium* (red). *Methanopyrus kandleri* was selected as the out group. The *mrtA* sequences included the only *mrtA* sequences annotated in NCBI.

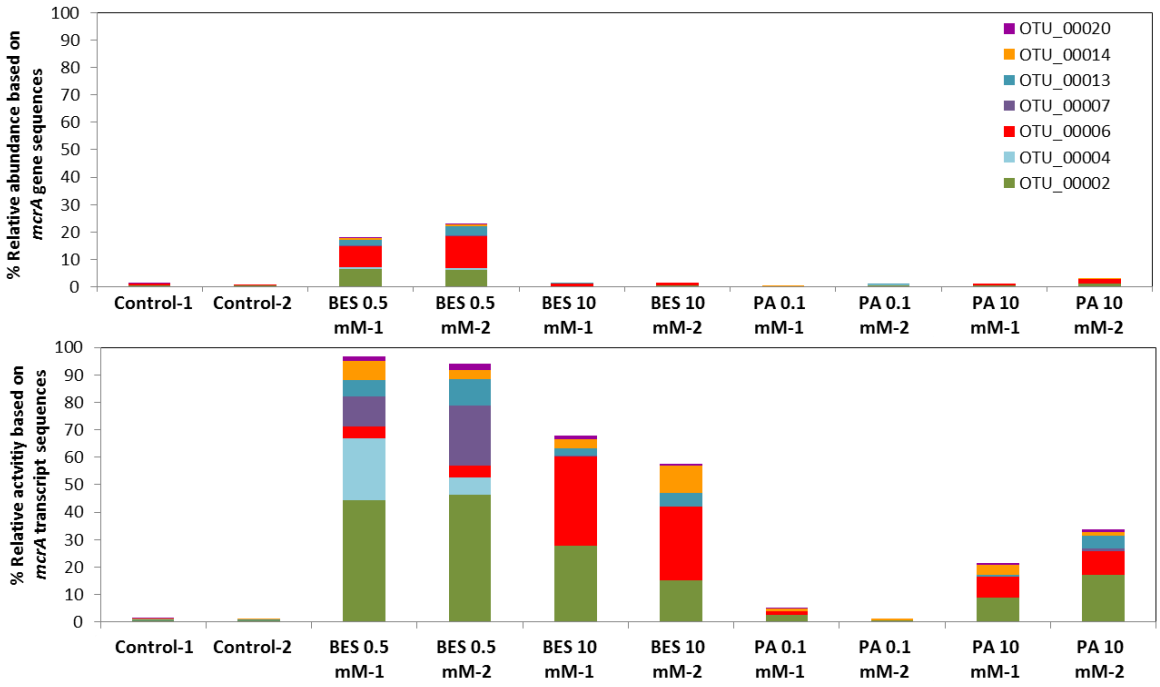


Figure S6. Relative abundance (top) and activity (bottom) of OTUs classified as *Methanobacterium* and *Methanomicrobium* based on *mcrA* gene and *mcrA* transcript cDNA sequences. Results from duplicate biomass samples from each mesocosm are shown.

Table S4. Strains of methanogenic archaea used to create mock communities.

Strain ID	Organism name	16S rRNA copy number	*mcrA* gene copy number
DSM-862	*Methanobacterium bryantii*[‡]	2.5	2
DSM-861	*Methanobrevibacter smithii*	2	1
DSM-2067	*Methanococcus maripaludis*	3	1
DSM-3671	*Methanosaeta concilii*	2	1
DSM-2834	*Methanosarcina acetivorans*	3	1
DSM-800	*Methanosarcina barkeri*	3	1
DSM-3091	*Methanosphaera stadtmanae*	4	1
DSM-1101	*Methanospirillum hungatei*	4	1
DSM-864	*Methanospirillum hungatei*	4	1
DSM-1053	*Methanothermobacter thermautotrophicus*	2	2

[‡] Gene copy numbers for *Methanobacterium bryantii* were estimated based on the complete genomes of *Methanobacterium* sp. AL and *Methanobacterium* sp. SWAN.

Primer sequences were used to search for matching sequences in the complete genomes of strains included in the mock communities. Complete genomes were downloaded from the Joint Genome Institute and NCBI. Mismatches to primers are highlighted in red. There were no mismatches to the 16S rRNA gene forward and reverse primers used. Two copies of the *mcrA* gene are present in *Methanobacterium* and *Methanothermobacter*.

Table S5. Comparison of primer match with sequences from methanogens included in mock community

Organism	mcrA-forward primer (modified mlas primer (Steinberg and Regan, 2009)) **5′-GGYGGTGTMGGNTTCACHCARTA-3′**
Methanospirillum hungatei	GGTGGTGTCGGATTTACCCAGTA
Methanobacterium sp. AL	GGTGGTGTAGGTTTCACACAGTA
Methanobacterium sp. AL	GGAGGAGTAGGATTCACTCAGTA
Methanobacterium sp. SWAN 1	GGCGGTGTCGGTTTCACCCAGTA
Methanobacterium sp. SWAN 1	GGTGGTGTTGGATTCACACAGTA
Methanobrevibacter smithii	GGTGGTGTAGGATTCACTCAATA
Methanothermobacter thermautotrophicus	GGTGGTGTAGGATTCACCCAGTA
Methanothermobacter thermautotrophicus	GGTGGTGTGGGTTTCACCCAGTA
Methanosphaera stadtmanae	GGTGGTGTAGGATTCACACAATA
Methanococcus maripaludis	GGTGGTGTAGGATTCACACAATA
Methanosaeta concilii	GGTGGTGTAGGTTTCACACAGTA
Methanosarcina acetivorans	GGTGGTGTCGGGTTCACCCAGTA
Methanosarcina barkeri	GGTGGTGTCGGATTCACACAGTA

Organism	mcrA-reverse primer (Steinberg and Regan, 2008) **5'-CGTTCATBGCGTAGTTVGGRTAGT-3'**
Methanospirillum hungatei	CGTTCATTGCGTAGTTCGGGTAGT
Methanobacterium sp. AL	CGTTCATTGCATAGTTAGGGTAGT
Methanobacterium sp. AL	CGTTCATTGCGTAGTTTGGATAGT
Methanobacterium sp. SWAN 1	CGTTCATGGCGTAGTTCGGGTAGT
Methanobacterium sp. SWAN 1	CGTTCATTGCGTAGTTAGGGTAGT
Methanobrevibacter smithii	CGTTCATTGCGTAGTTAGGGTAGT
Methanothermobacter thermautotrophicus	CGTTCATGGCGTAGTTTGGATAGT
Methanothermobacter thermautotrophicus	CGTTCATGGCGTAGTTTGGATAGT
Methanosphaera stadtmanae	CGTTCATTGCGTAGTTAGGGTAGT
Methanococcus maripaludis	CGTTCATTGCGTAGTTAGGGTAGT
Methanosaeta concilii	CGTTCATGGCGTAGTTCGGGTAGT
Methanosarcina acetivorans	CGTTCATTGCGTAGTTCGGGTAGT
Methanosarcina barkeri	CGTTCATTGCGTAGTTGGGGTAGT

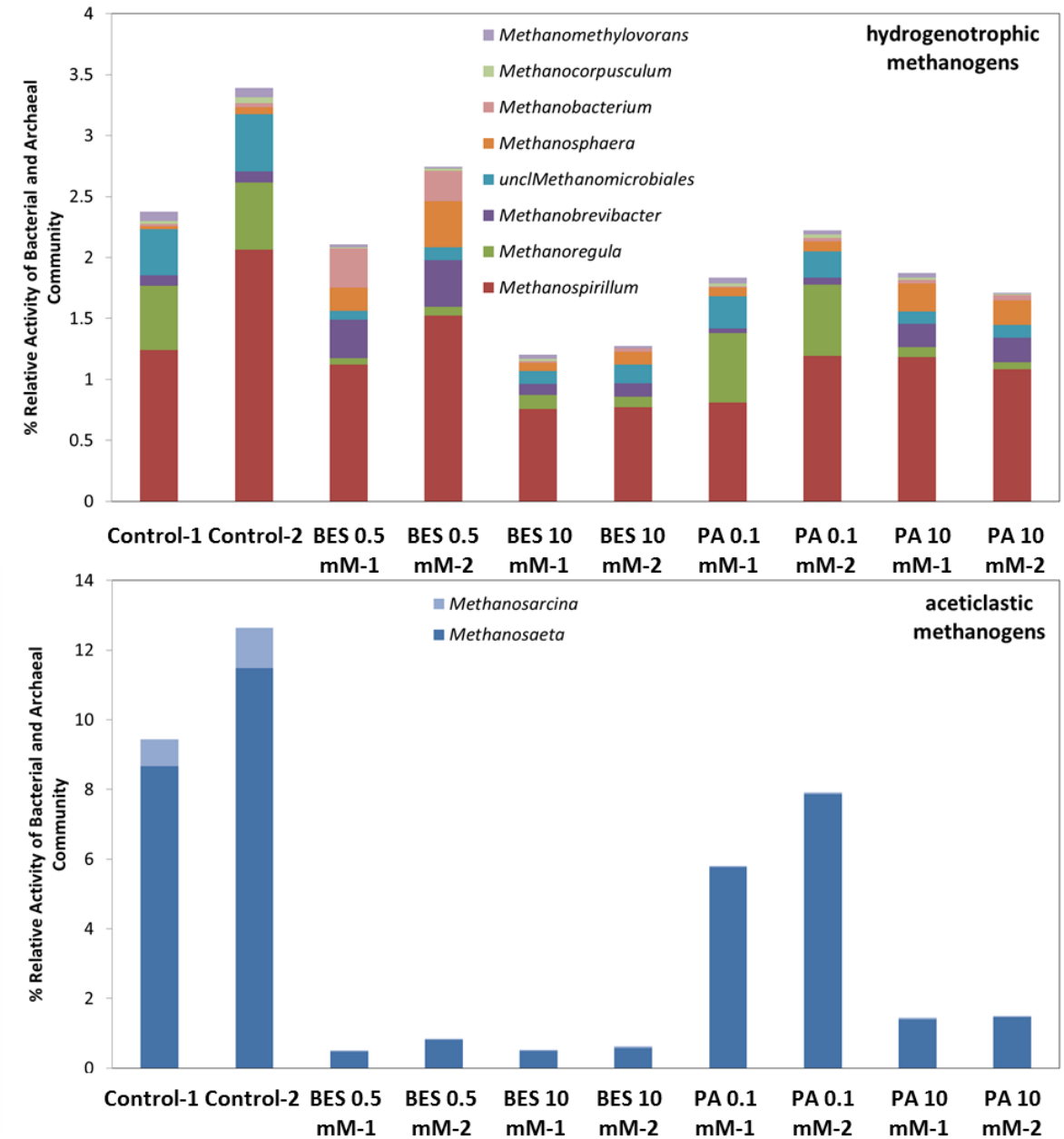


Figure S7. Relative activity of hydrogenotrophic (top) and aceticlastic (bottom) methanogens based on 16S rRNA cDNA sequencing.

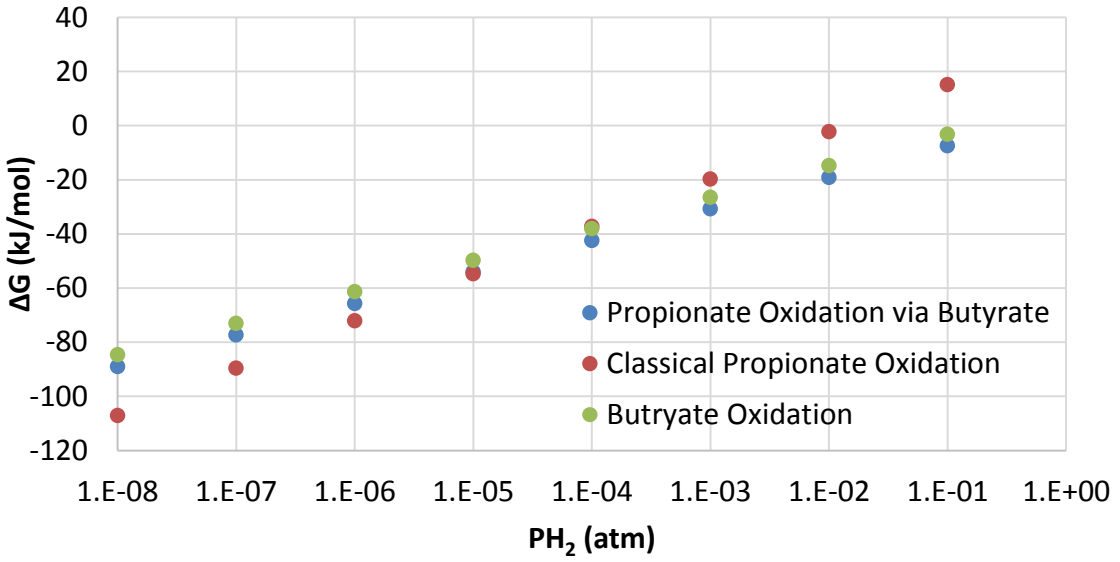


Figure S8. Gibb's free energy (ΔG) versus the partial pressure of hydrogen (P_{H2}) for "propionate oxidation via butyrate", in which propionate is dismutated by *Smithella* spp. to acetate and butyrate followed by butyrate oxidation by *Syntrophomonas* spp. (Gan *et al.*, 2012), "classical propionate oxidation", in which propionate oxidation occurs by *Smithella* spp., and "butyrate oxidation", in which butyrate oxidation occurs directly by *Syntrophomonas* spp. The $\Delta G_{reaction}$ were calculated based on the free energies of formation ($\Delta G_f°$) in (Madigan et al., 2010) and assumed to be 48,400 J/mol, 76,500 J/mol, and 48,300 J/mol for the Smithella, Classical, and Syntrophomonas Pathways, respectively, shown below. Temperature was assumed to be 31°C. Concentrations of acetate, propionate, butyrate, and bicarbonate were assumed to be 22.9, 12.2, 6.43, and 49.2 mM, respectively. A pH of 6.5 was assumed. A ΔG<0 indicates an exergonic reaction.

Propionate Oxidation via Butyrate

$$2CH_3CH_2COO^- + 2H_2O \rightarrow 3CH_3COO^- + 2H_2 + H^+$$

Classical Propionate Oxidation

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + 3H_2 + H^+$$

Butyrate Oxidation

$$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + 2H_2 + H^+$$

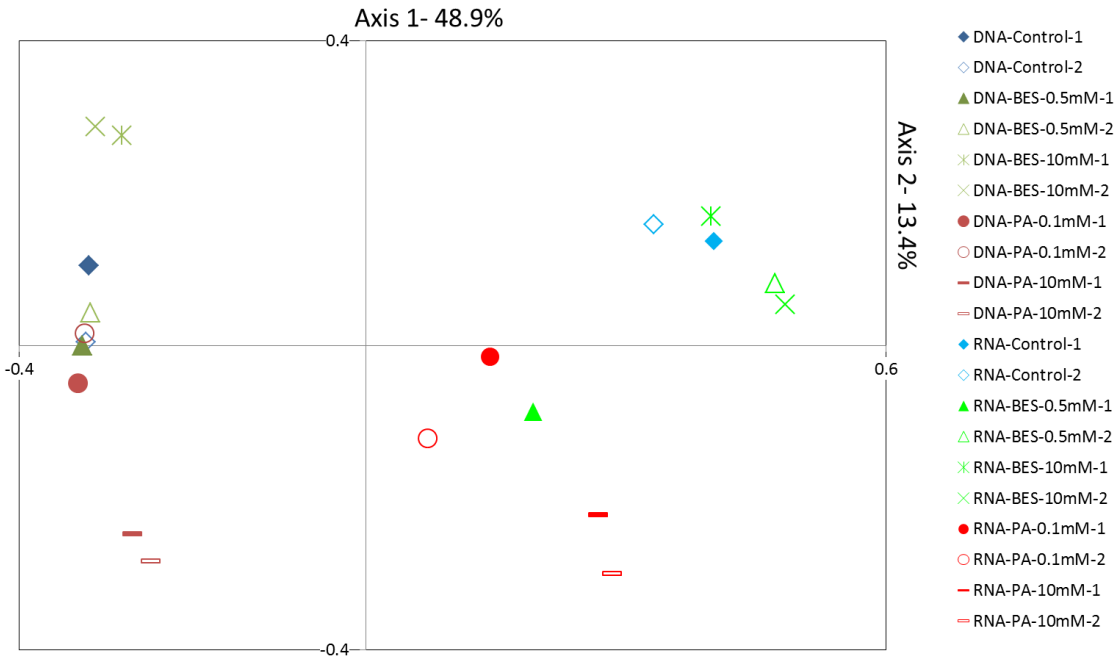


Figure S9. Principal coordinate plot of the bacterial community structure (θ_{yc}) based on OTU clustering (cutoff = 0.03) from the 16S rRNA gene and 16S rRNA cDNA sequences. Duplicate biomass samples are shown for each mesocosm.

References

Gan, Y., Q. Qiu, P. Liu, J. Rui and Y. Lu (2012). Syntrophic Oxidation of Propionate in Rice Field Soil at 15 and 30°C under Methanogenic Conditions. *Appl. Environ. Microbiol.* 78(14): 4923-4932.

Madigan, M. T., J. M. Martinko, P. V. Dunlap and D. P. Clark (2010). *Brock Biology of Microorganisms*. 13th ed. Boston, Benjamin Cummings.

Smith, A. L., S. J. Skerlos and L. Raskin (2013). Psychrophilic anaerobic membrane bioreactor treatment of domestic wastewater. *Water Res*. 47: 1655-1665.

Steinberg, L. M. and J. M. Regan (2008). Phylogenetic Comparison of the Methanogenic Communities from an Acidic, Oligotrophic Fen and an Anaerobic Digester Treating Municipal Wastewater Sludge. *Appl. Environ. Microbiol*. 74(21): 6663-6671.

Steinberg, L. M. and J. M. Regan (2009). mcrA-Targeted Real-Time Quantitative PCR Method To Examine Methanogen Communities. *Appl. Environ. Microbiol*. 75(13): 4435-4442.

Appendix E. Supplementary Information for Chapter 5

Gas trap efficiency experiment

Controlled volatilization experiments were carried out in 40 mL serum vials. The final composition of reagents added to each vial was as shown in Table S6. First an acidified solution of dimethylarsinic acid (DMAA) was prepared by adding water, DMAA stock solution, and nitric acid in that order. The vials were then sealed with a septa and crimp top. An arsenic trap (siliver nitrate impregnated silica gel) was connected to the vial with a 22 gauge needle and platinum-cured silicon tubing (Fischer Scientific). The outlet of the trap was open to the atmosphere such that any gas generated within the vial could exit through the trap. Sodium tetrahydroborate (Sigma-Aldrich, St. Louis, MO) was then added through a syringe. Immediate evolution of gas started once $NaBH_4$ was added to the mixture. The addition was done slowly while monitoring the gas evolution. Once gas evolution had stopped, the syringe was removed and the vial purged with nitrogen to force any remaining arsines in the headspace to pass through the gap trap. The residual arsenic in the reaction mixture was measured using ICPMS. The gas traps were digested as previously and analyzed using ICPMS. The arsenic volatilization was calculated by performing a mass balance on initial and final liquid concentrations. The average and standard deviation of trap recovery for triplicate tests in which about 100 ng of arsenic was volatilized was 83% ± 3.

Table S6. Composition of reagents for gas trap efficiency test

Solution	Concentration	Volume (mL)
DMAA	1 mg/L	0.2
$NaBH_4$	2% w/w	1
HNO_3	68% w/w	0.32
Water	100% w/w	8.48
Total		10

Mesocosms with aqueous arsenite

Mesocosms were prepared containing anaerobic digester sludge and cow dung as described previously. A lower total volume of 100 mL was used in 150 mL serum vials. Initially mesocosms were run for 12 days without arsenic, followed by the addition of arsenic as aqueous AsO_3 (prepared from Sigma Aldrich TraceCERT As standard) to achieve a total mass of 50 µg As in each of two replicate mesocosms. In these experiments the background matrix was not matched with an arsenic-free control and therefore an overestimate of the total arsenic may be due to matrix effects. The mesocosms were incubated for an additional 11 days at 31 °C before sampling and measurement of arsenic in the liquid and solid phases. Silver nitrate impregnated silica gel traps were digested for the measurement of arsenic volatilization.

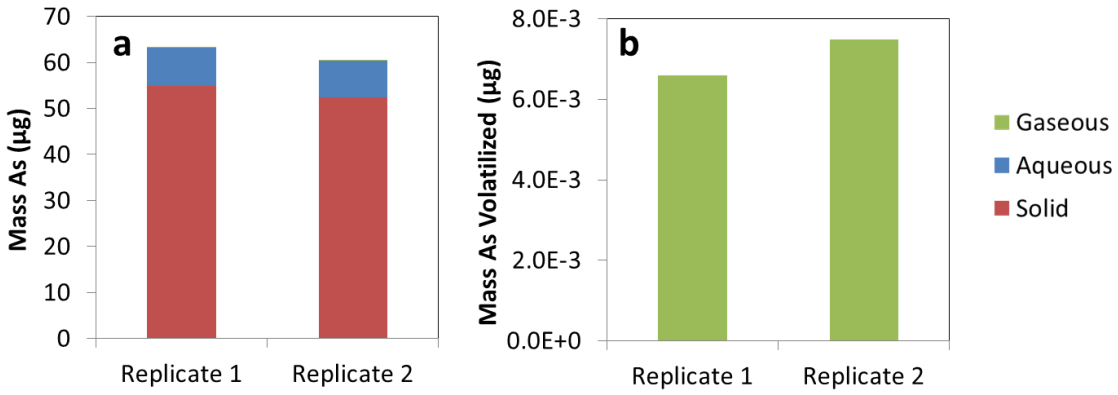


Figure S10. Arsenic distribution in solid, liquid, and gaseous phases (a) and volatilized arsenic (b) from duplicate mesocosms with arsenic added as aqueous arsenite.

Table S7. ArsM Protein Sequences from NCBI

NCBI Reference Sequence	Organism
YP_001512636.1	*Alkaliphilus oremlandii* OhILAs
YP_825656.1	*Candidatus Solibacter usitatus* Ellin6076
YP_003125722.1	*Chitinophaga pinensis* DSM 2588
YP_678125.1	*Cytophaga hutchinsonii* ATCC 33406
YP_182128.1	*Dehalococcoides ethenogenes* 195
YP_001214685.1	*Dehalococcoides* sp. BAV1
YP_308344.1	*Dehalococcoides* sp. CBDB1
YP_002457695.1	*Desulfitobacterium hafniense* DCB-2
YP_520381.1	*Desulfitobacterium hafniense* Y51
YP_001528649.1	*Desulfococcus oleovorans* Hxd3
YP_003197819.1	*Desulfohalobium retbaense* DSM 5692
YP_146445.1	*Geobacillus kaustophilus* HTA426
NP_618654.1	*Methanosarcina acetivorans* C2A
YP_305236.1	*Methanosarcina barkeri* str. Fusaro
NP_634267.1	*Methanosarcina mazei* Go1
NP_632685.1	*Methanosarcina mazei* Go1
YP_001210990.1	*Pelotomaculum thermopropionicum* SI
YP_570547.1	*Rhodopseudomonas palustris* BisB5
NP_948900.1	*Rhodopseudomonas palustris* CGA009
YP_485587.1	*Rhodopseudomonas palustris* HaA2
YP_001993051.1	*Rhodopseudomonas palustris* TIE-1
YP_446560.1	*Salinibacter ruber* DSM 13855
YP_076198.1	*Symbiobacterium thermophilum* IAM 14863
YP_002512337.1	*Thioalkalivibrio sulfidophilus* HL-EbGr7
YP_315186.1	*Thiobacillus denitrificans* ATCC 25259

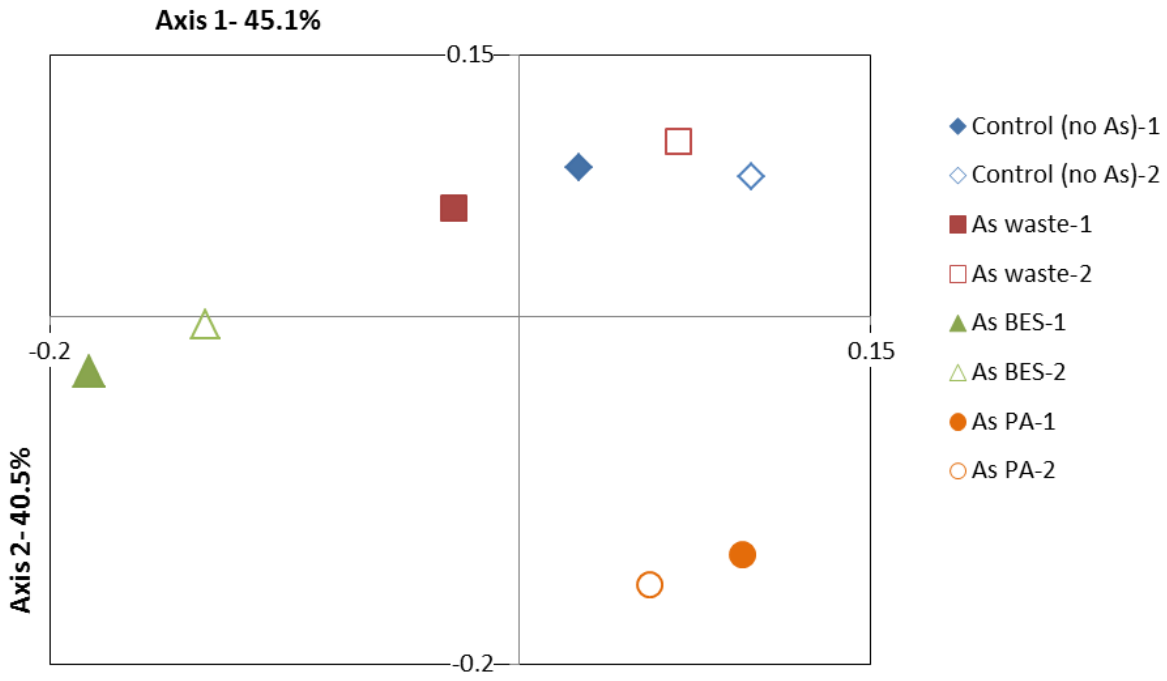


Figure S11. Principal coordinate plot of the active bacteria community structure (θ_{yc}) for duplicate biomass samples. Bacterial sequences were subsampled to a depth of 17,424 sequences per sample and grouped at the genus level. The difference in community structure between conditions was statistically significant (AMOVA, $p < 0.01$).

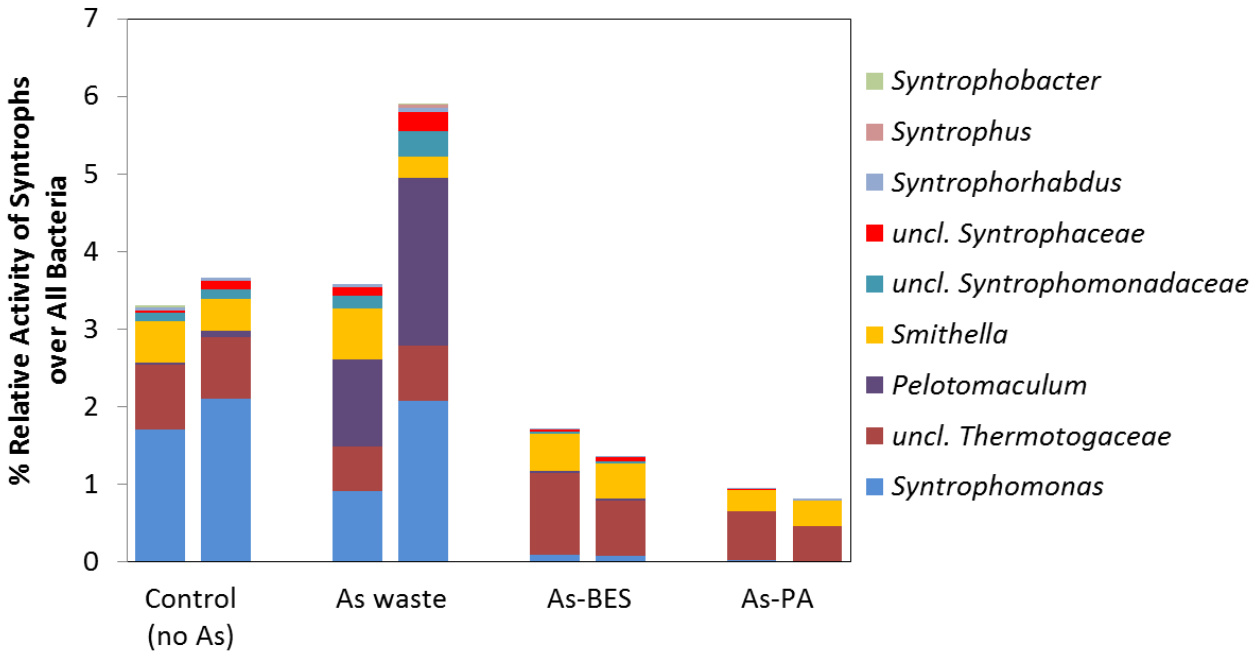


Figure S12. Percent relative activity of syntrophic bacteria over all bacteria based on 16S rRNA cDNA sequencing

The indicator analysis (Dufrêne and Legendre 1997) was performed to determine bacterial phylotypes that were significantly ($p < 0.05$) correlated with the different conditions. Results are shown in Table S9 and Table S8.

Table S8. Indicator Phylotypes for Uninhibited (Control and As waste) and Inhibited (As-BES and As-PA) Conditions

Uninhibited	**Inhibited**
uncl. *Comamonadaceae*	uncl. *Clostridiales*
Syntrophomonas	uncl. *Firmicutes*
uncl. *Syntrophomonadaceae*	uncl. *Lachnospiraceae*
uncl. *WS3*	*Acinetobacter*
Syntrophorhabdus	uncl. *Clostridia*
Acetivibrio	*Bacteroides*
uncl. *Opitutaceae*	uncl. *Rhodobacteraceae*
	Oscillibacter
	uncl. *Erysipelotrichaceae*
	uncl. *Enterobacteriaceae*
	Vitreoscilla
	Gordonibacter
	Paraprevotella
	Bilophila

Table S9. Indicator Phylotypes for Each Condition

Control (no As)	**As waste**	**As-BES**	**As-PA**
Trichococcus	uncl. *Proteobacteria*	uncl. *Flavobacteriaceae*	uncl. *Firmicutes*
	Treponema	*Acetobacterium*	*Clostridium IV*
	Zoogloea	*Erysipelothrix*	*Clostridium XlVa*
			Sedimentibacter

Table S10. *Bacteria* and *Archaea* with identified *arsM* genes

Organism	Included on GeoChip
Alkaliphilus oremlandii OhILAs	+
Aminobacterium colombiense DSM 12261	+
Anaerobaculum hydrogeniformans ATCC BAA-1850	+
Anaerophaga thermohalophila DSM 12881	+
Bacillus cereus 95/8201	+
Bacillus sp. 1NLA3E	+
Bacteroides fragilis 3112	+
Blastococcus saxobsidens DD2	+
Candidatus Nitrospira defluvii	+
Chitinophaga pinensis DSM 2588	+
Clostridium carboxidivorans P7	+
Clostridium scindens ATCC 35704	+
Conexibacter woesei DSM 14684	+
Cupriavidus metallidurans CH34	+
Cyanobium sp. PCC 7001	+
Dehalococcoides ethenogenes 195	+
Desulfitobacterium dichloroeliminans LMG P-21439	+
Desulfitobacterium hafniense DCB-2	+
Desulfitobacterium hafniense Y51	+
Desulfitobacterium metallireducens DSM 15288	+
Desulfobulbus propionicus DSM 2032	+
Desulfohalobium retbaense DSM 5692	+
Desulfotomaculum carboxydivorans CO-1-SRB	+
Desulfotomaculum gibsoniae DSM 7213	+
Desulfurivibrio alkaliphilus AHT2	+
endosymbiont of *Riftia pachyptila* (vent Ph05)	+
Finegoldia magna ATCC 53516	+
Flexistipes sinusarabici DSM 4947	+
gamma proteobacterium HTCC 5015	+
Gemmata obscuriglobus UQM 2246	+
Gemmatimonas aurantiaca T-27	+
Geobacter metallireducens GS-15	+
Halomonas sp. HAL1	+
Haloterrigena turkmenica DSM 5511	+
Hyphomicrobium denitrificans 1NES1	+
Imtechella halotolerans K1	+
Leptonema illini DSM 21528	+

Methanobacterium sp. AL-21	+
Methanosarcina acetivorans C2A	+
Methanosarcina barkeri str. Fusaro	+
Methanosarcina mazei Go1	+
Methylobacter tundripaludum SV96	+
Methylomicrobium alcaliphilum	+
Methylomonas methanica MC09	+
Niastella koreensis GR20-10	+
Nitrosomonas sp. AL212	+
Nitrosomonas sp. Is79A3	+
Nostoc sp. PCC 7120	+
Opitutus terrae PB90-1	+
Paenibacillus lactis 154	+
Paenibacillus larvae subsp. larvae BRL-230010	+
Paenibacillus polymyxa M1	+
Paenibacillus sp. HGF7	+
Pedobacter sp. BAL39	+
Pelotomaculum thermopropionicum SI	+
Rhodanobacter sp. 2APBS1	+
Rhodobacteraceae bacterium KLH11	+
Rhodomicrobium vannielii ATCC 17100	+
Rhodopseudomonas palustris BisB5	+
Rhodopseudomonas palustris DX-1	+
Serinicoccus profundi MCCC 1A05965	+
Solibacter usitatus Ellin6076	+
Sphaerobacter thermophilus DSM 20745	+
Stackebrandtia nassauensis DSM 44728	+
Symbiobacterium thermophilum IAM 14863	+
Synechocystis sp. PCC 6803	+
Syntrophothermus lipocalidus DSM 12680	+
Thermanaerovibrio acidaminovorans DSM 6589	+
Thermosediminibacter oceani DSM 16646	+
Thiobacillus denitrificans ATCC 25259	+
Thiocapsa marina 5811	+
Treponema vincentii ATCC 35580	+
Xenorhabdus nematophila ATCC 19061	+
Rhodopseudomonas palustris HaA2	-
Rhodopseudomonas palustris CGA009	-
Rubrivivax gelatinosus	-
Dehalococcoides sp. BAV1	-
Dehalococcoides sp. VS	-

Geobacillus kaustophillus HTA426	-
Salinarchaeum sp. Harcht Bsk1	-
Natronomonas moolapensis	-
Sulfuricella denitrificans skB26	-
Desulfovibrio magneticus RS-1 NC	-
Methylacidiphillum infernorum	-
Desulfosporosinus meridiei	-
Desulfococcus oleovorans	-
Desulfocapsa sulfexigens	-
Desulfotomaculum acetoxidans	-
Syntrophobacter fumaroxidans	-
Dehalogenimonas lykanthroporepellens	-
Methanoplanus petrolearius	-
Methanosaeta harundinacea	-
Methanosaeta concilii	-
Salinibacter ruber	-
Solitalea canadensis	-
Fibrella aestuarina	-
Runella slithyformis	-
Maribacter sp. HTCC 2170	-
Aequorivita sublithincola	-
Spirosoma linguale DSM74	-
Haliscomenobacter hydrossis DSM 1100	-

Reference

Dufrêne, M. and P. Legendre (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs* 67(3): 345-366.