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Anaerobic Disposal of Arsenic-Bearing Wastes Results in Low Microbially Mediated Arsenic Volatilization

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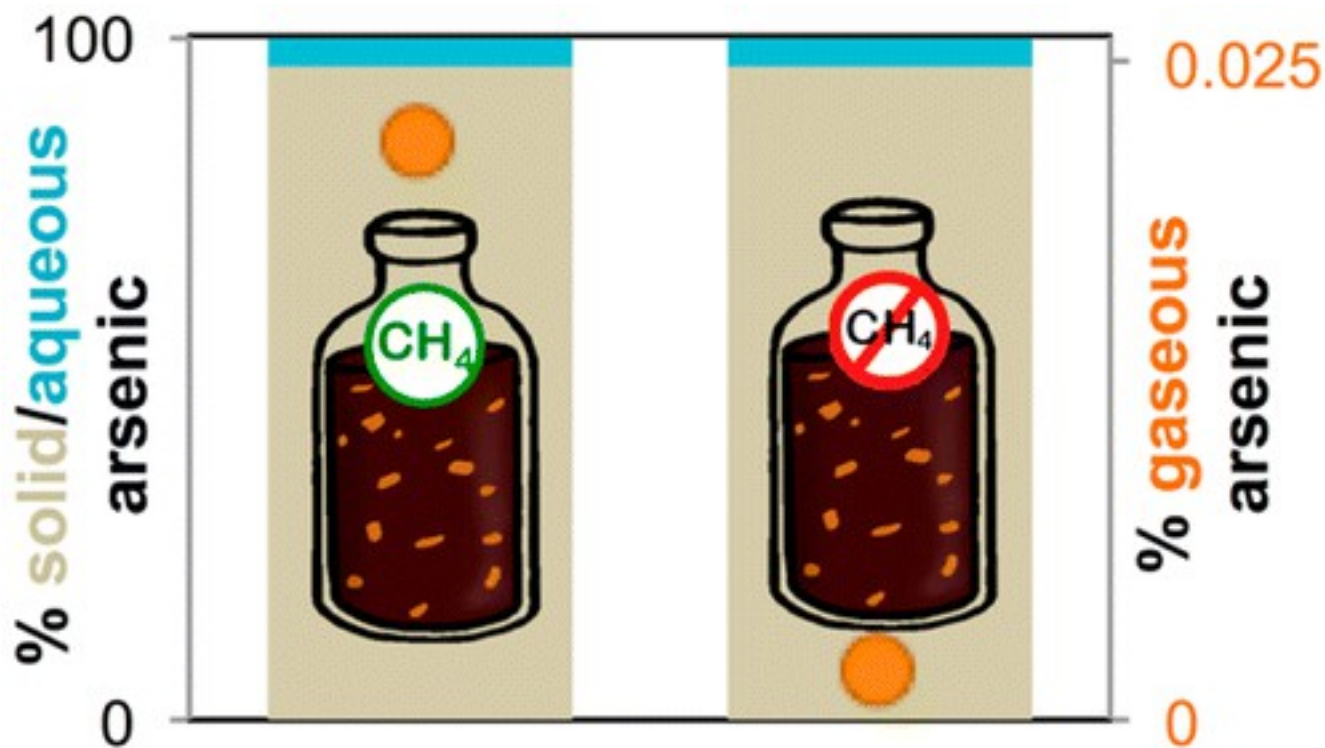
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Abstract



The removal of arsenic from drinking water sources produces arsenic-bearing wastes, which are disposed of in a variety of ways. Several disposal options involve anaerobic environments, including mixing arsenic waste with cow dung, landfills, anaerobic digesters, and pond sediments. Though poorly understood, the production of gaseous arsenic species in these environments can be a primary goal (cow dung mixing) or an unintended consequence (anaerobic digesters). Once formed,

these gaseous arsenic species are readily diluted in the atmosphere. Arsenic volatilization can be mediated by the enzyme arsenite S-adenosylmethionine methyltransferase (ArsM) or through the enzymes involved in methanogenesis. In this study, methanogenic mesocosms with arsenic-bearing ferric iron waste from an electrocoagulation drinking water treatment system were used to evaluate the role of methanogenesis in arsenic volatilization using methanogen inhibitors. Arsenic volatilization was highest in methanogenic mesocosms, but represented <0.02% of the total arsenic added. 16S rRNA cDNA sequencing, qPCR of *mcrA* transcripts, and functional gene array-based analysis of *arsM* expression, revealed that arsenic volatilization correlated with methanogenic activity. Aqueous arsenic concentrations increased in all mesocosms, indicating that unintended contamination may result from disposal in anaerobic environments. This highlights that more research is needed before recommending anaerobic disposal intended to promote arsenic volatilization.

Introduction

Human exposure to arsenic increases the risk of various cancers, skin lesions, cardiovascular problems, and other negative health outcomes.[\(1, 2\)](#) Naturally occurring arsenic contaminates groundwater used as a source for drinking water and crop irrigation in several regions of the world.[\(3, 4\)](#) Anthropogenic arsenic contamination originates from several industries, including wood processing, mining, and meat production.[\(5-7\)](#) 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,[\(8, 9\)](#) which often contain arsenic due to plant uptake of arsenic in irrigation water[\(4, 10\)](#) or as a result of arsenic containing feed additives consumed by livestock.[\(5, 11\)](#) Anaerobic lagoons have also been used for the treatment of livestock waste.[\(12\)](#) Arsenic-bearing wastes generated during drinking water treatment in developed countries are often disposed in landfills.[\(13, 14\)](#) In developing countries, anaerobic disposal options include mixing arsenic containing drinking water treatment wastes with cow dung[\(14-19\)](#) and disposal in ponds with anaerobic conditions.[\(20\)](#)

Microbial activity largely determines the fate of arsenic under anaerobic conditions. For oxidized forms of arsenic-bearing iron wastes (e.g., with As(V) and Fe(III) phases), reductive dissolution through the activity of arsenate- and iron-reducing microorganisms can lead to the undesirable

release of arsenic from solid wastes into the aqueous phase.(21-24) In contrast, the ability of microorganisms to transform arsenic into volatile arsine and methylarsine gases(25-27) has been studied as a potentially desirable outcome to remove arsenic from soil and arsenic-bearing solid wastes.(28, 29) Indeed, arsenic disposal with cow dung has been suggested based on the expectation that arsenic can be volatilized through microbial activity and transferred to the atmosphere where dilution reduces exposure risk.(17) However, the importance of microbially mediated pathways in arsenic phase transfer remains unclear. The few studies that quantified volatilization from soils and rice paddies found that less than 0.1% of total arsenic present was released from solids via volatilization.(30-32) Studies that measured gaseous arsenic release from anaerobic digesters reported volatilization between 0.3 and 32% of total arsenic.(33, 34) A few other studies reported higher amounts of volatilized arsenic.(16, 19, 35) However, these studies estimated the amount of volatilized arsenic by determining the differences between the initial amount of arsenic added and the arsenic measured in the aqueous and solid phases after incubation; they did not provide a complete mass balance of arsenic. The absence of volatile arsenic measurements calls into question whether the reported levels of volatilization in these studies resulted from microbial activity or arose from errors in different measurement techniques.

Two mechanisms for microbial arsenic methylation and volatilization have been identified.(25, 26, 36) The first mechanism occurs during methanogenesis. Cultures of methanogens have been shown to produce a range of arsine and methyl-arsine species from arsenate(37) and the demethylation of methylcobalamin has been shown to produce arsine and mono-, di-, and trimethylarsine gases from arsenite.(36) The second mechanism takes place through the activity of arsenite S-adenosylmethionine methyltransferase (ArsM) and is thought to be a detoxification mechanism found in all domains of life.(26, 27) Recently, studies have focused on quantifying the abundance of the ArsM pathway in natural environments,(29, 38, 39) and the potential of using the *arsM* gene in genetically modified microorganisms for the remediation of arsenic contaminated sites.(28, 40) The impact of methanogenesis on arsenic volatilization in natural and engineered systems has not been widely studied, even though volatilization has been observed in environments with high methanogenic activity, including rice paddies,(32) landfills,(41) and anaerobic digesters.(33, 42) To date, the relative importance of these two pathways and the activity of specific populations capable of these transformations within mixed microbial communities have not been studied.

The objectives of this study were to (1) generate a mass balance of arsenic following anaerobic disposal of arsenic-bearing ferric iron wastes to assess the distribution of arsenic among the solid, aqueous, and gas phases, (2) determine the link between methanogenic activity and arsenic

volatilization to the gas phase, and (3) characterize changes in the microbial community activity as related to the fate of arsenic to evaluate the potential for arsenic volatilization as a remediation strategy.

Materials and Methods

Mesocosm Setup 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 collected at a domestic wastewater treatment plant (Northfield Township, MI), 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) As waste and methanogenesis inhibitor 2-bromoethanesulfonate (As-BES) at 10 mM, and (4) As waste and methanogenesis inhibitor propynoic acid (As-PA) at 10 mM. The aqueous and solid phases 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 total arsenic concentration of 1.66 mg As/L in a total working volume of about 410 mL. This concentration was selected to represent a realistic ratio of cow dung to arsenic waste and to be low enough to avoid the potential impacts of arsenic toxicity. The arsenic-bearing iron waste was obtained from a pilot Electrochemical Arsenic Remediation (ECAR) system for the removal of arsenic from groundwater in West Bengal, India.[\(43\)](#) Preliminary experiments were also performed (as described in the [Supporting Information \(SI\)](#)) in which aqueous arsenite was added. To inhibit methanogenesis, two different inhibitors were selected for comparison: 2-bromoethanesulfonate (BES), a commonly used coenzyme-M analog,[\(44-46\)](#) and propynoic acid (PA), an unsaturated analog to propionate.[\(47, 48\)](#) Two inhibitors were selected to identify potential artifacts arising from nonspecific inhibition. A concentration of 10 mM for each inhibitor was selected based on previous batch studies with the same mesocosm community targeting complete inhibition of methanogenesis.[\(49\)](#) Bottles were capped, crimp sealed, and purged with N₂ 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 aqueous and solid phases.

Mesocosm Sampling

Over a 17 day incubation period, gas samples were collected to quantify gas production and characterize its composition. Sampling was performed 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).⁽³²⁾ All gas generated during the incubation passed through the trap, which was later digested to measure the amount of arsenic volatilization. The efficiency of the arsenic gas trap in monitoring arsenic volatilization was evaluated as described in the [SI](#). The detection limit for volatilized arsenic was 4 ng. At the final day of sampling, before uncapping the bottles, the headspace was purged with N₂ gas after connecting the gas trap so that the entire headspace volume was sent through the trap. For each of the four conditions (control without added arsenic, As waste, As-BES, and As-PA), one of the triplicate bottles was selected for biomass collection for molecular analyses. Biomass samples were centrifuged at 4 °C and biomass pellets were immediately frozen at –80 °C. The supernatant was filtered with Whatman no. 41 filters (GE Healthcare Life Sciences, Pittsburgh, PA) and the filtrate was acidified with nitric acid, final concentration 0.2 M. The other two mesocosms for each of the four treatments were centrifuged at 20 °C, followed by filtration through Whatman no. 41 filters. The solids collected by centrifugation and filtration were combined (solid samples). The solid samples and the filtrates (aqueous samples) were used for further chemical analyses.

Analytical Methods

Samples of gas produced were analyzed to quantify CH₄, CO₂, and N₂ using a gas chromatograph (Gow-Mac, Bethlehem, PA) coupled with a thermal conductivity detector (TCD).⁽⁵⁰⁾ Total volatilized arsenic was measured by digesting the silica beads from the gas trap first with 2 mL of 5% (w/w) nitric acid followed by 2 mL of 1% (w/w) nitric acid, and incubation at 100 °C, a procedure slightly modified from Mestrot et al.⁽³²⁾ Total arsenic in the nitric acid digestion fluid was measured with inductively coupled plasma mass spectroscopy (ICP-MS).⁽⁵¹⁾ ICP-MS instrumental error was determined to be 5% based on the variation from known standards, with a minimum measurement error of 0.8 µg/L at low concentrations. Errors reported are the larger of either the instrumental error (5%), the minimum measurement error (0.8 µg/L), or the standard deviation between triplicate samples. The detection limit for arsenic was 1.1 µg/L. The total aqueous arsenic concentration was also measured by ICP-MS. The pH of the aqueous samples was monitored using a standard probe (Mettler Toledo, Columbus, OH). Arsenic concentration in the solid samples was measured following a total digestion. Total digestions were performed at 100 °C for 2 h with 35% (w/w) nitric acid, followed by the addition of 2 mL of 15% (w/w) hydrogen peroxide and a second incubation at 100 °C for 2 h.⁽⁵²⁾ The aqueous 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 an arsenic ICP standard (Ricca Chemical, Arlington, TX).

Molecular Methods

RNA extractions from duplicate biomass samples from each treatment 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 a SuperScript VILO kit (Life Technologies, Grand Island, NY) according to the manufacturer's instructions, including an extended incubation time of 120 min to maximize yield. qPCR standards were created from a template of pooled mesocosm DNA extracts.[\(49, 53, 54\)](#) The *mcrA* and 16S rRNA genes were amplified in 20 μ L reactions containing 0.5 ng template, 500 nM primers, 0.3 mg/mL bovine serum albumin, 10 μ L Phusion High Fidelity Master Mix (NEB, Ipswich, MA), and nuclease-free water. The *mcrA* gene was amplified using the forward primer modified mlas (5'-GGYGGTGTMGNTTCACHCARTA-3')[\(49\)](#) and the reverse primer *mcrA*-rev (5'-CGTTCATBGCGTAGTTVGGRTAGT-3')[\(55\)](#). Primers for the 16S rRNA gene were F515 (5'-GTGCCAGCMGCCGCGGTAA-3') and R806 (5'-GGACTACHVGGGTWTCTAAT-3')[\(56\)](#). 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/ μ L for *mcrA* and 10^8 – 10^4 copies/ μ L for 16S rRNA amplicons).

RT-qPCR was performed using the Mastercycler Realplex Ep (Eppendorf, Hamburg, Germany) with triplicate wells for each sample and reaction volumes of 20 μ L using Fast Plus EvaGreen Master Mix (Biotium, Hayward, CA). Primer concentrations were 500 nM, except for the reverse *mcrA* primer which 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,[\(57, 58\)](#) 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[\(56\)](#) as modified by

Kozich et al.[\(59\)](#) Amplification from cDNA samples was performed with Accuprime High Fidelity 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 SequelPrepNormalization Plate Kit (Life Technologies), multiplexed and sequenced using the Illumina MiSeq, Reagent Kit V2. Sequences were processed with mothur[\(60\)](#) (version 1.33.3, Illumina MiSeq SOP accessed 11/11/2014), classified using the 16S rRNA gene taxonomy from the Ribosomal Database Project training set 9.[\(61\)](#) The sequence data generated were submitted to the DDBJ/EMBL/GenBank databases under Accession Number SRP064532. There were 222 482 paired-end reads after quality filtering. Bacterial sequences were separated and subsampled 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 resuspension in 10 µL of 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 5× 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 acrylamide (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 h 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 5× 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 h. 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 alcohol, pH 8, was used to purify the samples in a single step, followed by overnight precipitation at –20 °C with 1 µL linear acrylamide, 0.5 volumes 7.5 M ammonium acetate, and 2.5 volumes of ethanol. cDNA was resuspended and shipped to the Institute for Environmental Genomics (University of Oklahoma). About 15 ng of cDNA from each sample was amplified using the Templiphi kit (Amersham Biosciences, Piscataway, NJ) using a modified buffer[\(62\)](#) and then labeled and hybridization as previously described.[\(63, 64\)](#) The amplified DNAs were labeled with cyanine dye using the Klenow fragment and random priming and then dried using a vacufuge. The

labeled samples were then suspended in hybridization buffer containing 10% formamide and hybridized to GeoChip 5 at 67 °C for 20–22 h. Probe signal intensity was normalized using a three step process. First, the signal intensity was normalized across each array using the average signal of universal standard probes,⁽⁶⁵⁾ then across all arrays using the maximum average signal of the universal standard probes. Lastly, each probe signal was divided by the maximum mean of all probes on each array. Probes with a signal-to-ratio (SNR) < 2 and signal <100 were removed. To identify other *Bacteria* and *Archaea* that may have the *arsM* gene, 25 annotated ArsM protein sequences were downloaded from NCBI ([Table S2](#)) 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.⁽²⁷⁾

Results

Through measurements of arsenic in the gas, aqueous, and solid phases, a mass balance of arsenic was determined ([Figure 1](#)). These results included the measurements from duplicate mesocosms from the As waste treatment without methanogenic inhibitors added (sampled on day 0 and day 17) and with methanogenic inhibitors (As-BES and As-PA, sampled on day 17). The recovery of arsenic was 97–101% of the total arsenic expected based on the measurement of arsenic in the arsenic-bearing waste ($995 \pm 49.8 \mu\text{g As/g ECAR waste}$) and the mass of ECAR waste added to each bottle (0.667 g). These results showed 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 to which arsenic was added as aqueous arsenite. In those experiments, the majority of arsenic was also found in the solid phase following 11 days of incubation ([Figure S1](#)).

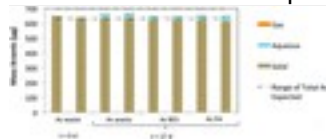


Figure 1. Arsenic mass balance in duplicate mesocosms for each treatment (ECAR waste alone and ECAR waste with inhibitors BES and PA) and the expected range of total arsenic, based on the average from total digestions of arsenic-bearing waste (range determined by 5% instrument error).

Measurements of arsenic in the aqueous phase ([Figure 2](#)) show that, immediately after mixing the arsenic-bearing wastes, cow dung, and anaerobic digester sludge, about 5 µg of arsenic had transferred to the aqueous phase. Following 17 days of incubation, the arsenic concentration in the aqueous phase had increased for all three treatments by a factor 7.2, 5.0, and 7.9 for As waste, As-BES, and As-PA, respectively. The amount of arsenic in the aqueous phase for each treatment represented <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.

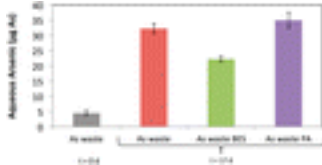


Figure 2. Mass of arsenic in the aqueous phase measured at the start of the mesocosm experiments and after 17 days of incubation with ECAR waste alone and ECAR waste with inhibitors BES or PA. Bars represent the average and standard deviation from triplicate mesocosms, except for $t = 0$, which represents the average and range of duplicate mesocosms. The average and standard deviation of the volumes for all mesocosms was 408 ± 32 mL.

As expected, the methane generation from mesocosms without methanogenic inhibitors was greatest (740 mL methane in 17 days) ([Figure 3](#)). Methane generation in both mesocosms with methanogenic 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 3](#)). Compared to the volatilization observed without inhibitors, volatilization from inhibited As waste conditions was 88% and 96% lower for BES and PA, respectively. This volatilization in the presence of methanogenic inhibitors was still above the detection limit (4 ng) and that measured in control mesocosms without added arsenic waste (4.3 ± 3.2 ng).

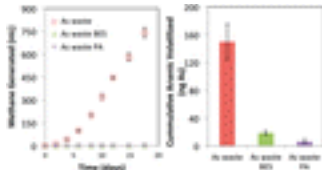


Figure 3. Methane generation over time (left) and cumulative arsenic volatilized after 17 days (right) for mesocosms with ECAR waste alone and with ECAR waste and methanogenic inhibitors BES or PA. Measurements represent the average from triplicate bottles and error bars provide standard deviations.

Results from 16S rRNA cDNA sequencing and RT-qPCR of *mcrA* expression confirm the reduced activity of methanogens in inhibited conditions ([Figure 4](#)). Methanogens comprise about 18% of the

the genera with newly identified potential *arsM* genes were not detected through 16S rRNA cDNA sequencing in these mesocosms, 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.

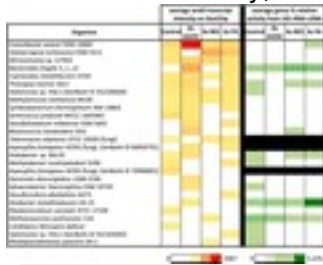


Figure 5. Heatmap of average *arsM* transcript intensity assayed by the GeoChip 5.0 (left) and the average corresponding genus % relative activity determined 16S rRNA cDNA sequencing (right) for duplicate biomass samples for each condition. Intensity ranges from 0 represented by white and 3067 represented by the darkest red for normalized *arsM* signal intensity and 0 represented by white and 3.22 represented by the darkest green for genus % relative activity. Fields for fungal groups are black to indicate that the activity is not measured through sequencing targeting bacterial and archaeal 16S rRNA cDNA.

Discussion

Methanogenic Activity Correlates with Arsenic Volatilization

Inhibition of methanogenesis reduced arsenic volatilization from anaerobic mesocosms containing anaerobic digester sludge, cow dung, and arsenic-bearing iron waste. The highest amount of arsenic volatilization occurred under conditions with the highest methane production (Pearson matrix correlation $r = 0.98$), suggesting that methanogenic activity may be responsible for arsenic volatilization. Arsenic volatilization mediated by methanogens can be due to either the enzymes involved in methanogenesis, the ArsM proteins found in methanogens, or some combination of the two pathways.

Recently, the ArsM of *Methanosarcina acetivorans* C2A was identified and characterized.

(66) Through our search of complete genomes, a potential *arsM* gene was identified in two species of *Methanosaeta*, *Methanosaeta harundinacea*, and *Methanosaeta concilii* (Table S4).

Although *Methanosaeta* spp. were the most active methanogens in the mesocosms with the most observed arsenic volatilization, since the GeoChip 5.0 microarray did not include probes for *Methanosaeta* spp. *arsM*, the data presented here could not distinguish between these two pathways. The potential for the ArsM pathway to contribute to arsenic volatilization was measured in part by the GeoChip 5.0 microarray (Figure 5). The overall activity of microbial genera with

measurable *arsM* gene expression as determined by 16S rRNA cDNA was less than 3% of the total archaeal and bacterial community activity for all genera ([Figure 5](#)). The potential for arsenic volatilization to be driven by low abundance organisms or those with previously undescribed *arsM* genes cannot be ruled out. Tracking changes in arsenic speciation in the aqueous and gaseous phases may also yield important information on the limitations to arsenic volatilization under these conditions.

Increases in Aqueous Arsenic Concentrations Were Higher than Arsenic Volatilization

The distribution of arsenic in the gaseous, aqueous, and solid phases after a 17 day incubation of arsenic waste under anaerobic conditions ([Figure 1](#)), highlights that further study of the limitations to rapid arsenic volatilization is required before implementing anaerobic disposal to promote 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 in the system ([Figure 2](#)). This release was likely facilitated through the activity of iron- and arsenate-reducing microorganisms. Other studies have also seen higher aqueous mobilization compared to volatilization. Mestrot et al. determined in a 42-day experiment that low amounts of arsenic were volatilized, that is, < 2% of the total arsenic added in the aqueous phase.[\(33\)](#) Ghosh et al. incubated arsenic-bearing ferric iron wastes under anaerobic landfill conditions for over 800 days, and determined that about 49% of the arsenic initially present was mobilized into the aqueous phase, although arsenic loss due to volatilization was not measured.[\(21\)](#)

The low levels of arsenic volatilization measured in this study are similar to a few studies that have reported arsenic volatilization in natural environments and anaerobic digesters.[\(30-33\)](#) Other studies that reported greater volatilization did not measure arsenic in the gaseous phase, but relied on the differences in aqueous and solid phase arsenic measurements to estimate gaseous arsenic production.[\(16, 35\)](#) The errors associated with arsenic measurements were not provided in these studies, so it is not possible to evaluate the reliability of these reported values. 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.

Short-Term Incubation Results to Inform Future Long-Term Waste Disposal Studies

The short time scale of this study limits the predictive ability of these results to actual disposal conditions and longer field monitoring studies would be needed in order to fully evaluate any disposal strategy. Short incubation times were selected to directly compare arsenic volatilization with and without active methanogenesis and avoid more drastic shifts in the microbial community of inhibited mesocosms under longer incubation times. Furthermore, due to the uncontained nature of

several anaerobic disposal environments (cow dung mixing, pit tanks, and pond disposal), initial transformations will play an important role in determining the fate of arsenic. When arsenic mobilization in the aqueous phase is initially much higher than volatilization, as seen here, arsenic would be more likely to be transported away from the initial disposal site after rain or flooding events. The high level of methanogenic activity supported in this study may be an overestimate of the activity in stagnant disposal environments. The 16S rRNA cDNA sequences reveal that methanogens comprise a large fraction (~18%) of the active microbial community in the mesocosms ([Figure 4](#)). However, the completely mixed conditions evaluated here would support higher levels of methanogenic activity when compared to unmixed conditions.[\(67-69\)](#) Therefore, the methanogenesis and associated arsenic volatilization observed here is likely an overestimate of what would be expected over the same time period in nonmixed disposal environments (e.g., cow dung mixed treatments, ponds, and landfills).

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Environmental Significance

The low arsenic volatilization and release of arsenic into the aqueous phase observed in this study, highlight the need for more study before volatilization of arsenic from anaerobic conditions is promoted for arsenic waste disposal. This disposal strategy bears the risk of solid-phase arsenic mobilization into water during rain and flooding events and consequent environmental contamination. Aqueous mobilization of arsenic from oxidized arsenic-bearing wastes will also be of concern for waste disposal in other anaerobic environments, including landfills, anaerobic digesters, and ponds. The small amount of volatilization observed in this study was correlated with methanogenic activity, a finding of relevance for anaerobic digester treatment of arsenic contaminated wastes where some arsenic volatilization should be expected. Even though the overall percentage of arsenic volatilization is likely to be low, volatile arsenic species may reach levels of concern depending on the volume of waste treated. Future studies should evaluate other redox environments[\(70\)](#) and microbial activities, including fungal activity,[\(71, 72\)](#) to assess whether arsenic volatilization under other conditions may provide more promising results. Such studies should include mass balances of arsenic and evaluate actual arsenic-bearing wastes given most of the previous studies have been limited to measurements of aqueous arsenic in culture media. Other disposal strategies should also be explored as volatilization is not always desirable especially under conditions where adequate gas-phase dilution cannot be achieved.

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Supporting Information

The Supporting Information is available free of charge on the [ACS Publications website](#) at DOI: [10.1021/acs.est.6b02286](https://doi.org/10.1021/acs.est.6b02286).

- Included is a further description of the gas trap efficiency experiments, results from incubations with aqueous arsenic, NCBI ArsM reference sequences, microorganisms with predicted *arsM* genes, and results from bacterial community analyses ([PDF](#))
- **PDF**
 - [es6b02286_si_001.pdf \(374.38 kB\)](#)

Anaerobic Disposal of Arsenic-Bearing Wastes Results in Low Microbially Mediated Arsenic Volatilization

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

The authors declare no competing financial interest.

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