

Organomercury Compound Determination from Microbiologically Mediated CUB Samples

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ABSTRACT

Mercury offgassing from coal utilization by-products (CUBs) is of great interest and concern, especially in applications such as agricultural use and soil stabilization. In these cases, microbiologically mediated mercury release could result from methylation reactions.

Experiments at the Energy & Environmental Research Center (EERC) were conducted in which bacterial cultures were grown in the presence of fly ash known to have relatively high mercury concentrations. Both organomercury and elemental mercury offgassing were determined. More recently, solid-phase microextraction (SPME) has been used for the determination of organomercury species in the liquid fraction of the microbiological experiments.

The EERC has developed a method of sampling gas streams and headspace gas for determination of dimethylmercury and methylmercuric chloride. A derivitization method using sodium tetraethyl, tetrapropyl, tetrabutyl, or tetraphenyl borate is carried out prior to capture with a SPME fiber coated with polydimethylsiloxane. Detection limits on the order of <25 picograms have been achieved, and lower detection limits are possible with further refinements of the method. Organomercury species have been detected in microbiologically mediated experiments using coal fly ash. Anaerobic conditions promoted greater levels of organomercury generation than aerobic conditions.

INTRODUCTION

Mercury and other air toxic elements can be present in fly ash, bottom ash, boiler slag, and flue gas desulfurization (FGD) material. The presence of mercury and other air toxic elements in coal utilization by-products (CUBs) poses a potential environmental problem depending on the stability of these elements under disposal and utilization conditions, a concern raised by state regulatory agencies¹ and citizen groups. Anticipated changes in emission regulations may impact the elements and concentrations of elements incorporated into or sorbed onto CUBs, and it is important to understand the fundamental behavior of these elements in CUBs in order to manage them in an environmentally sound manner.

Recent laboratory tasks at the Energy & Environmental Research Center (EERC) have addressed the following three areas: 1) direct leachability of air toxic constituents from CUBs, 2) vapor release of mercury from CUBs at ambient and elevated temperatures, and 3) biologically induced leachability and vapor release of mercury and other air toxic elements from CUBs. These tasks address fundamental issues critical to determining the release of these constituents over the life cycle of CUBs in a variety of management scenarios. Since the large volumes of CUBs produced annually in the United States (70 million tons of fly ash and 31 million tons of FGD material in 2003)² are managed either by disposal or utilization, the design of various tasks in this project focused on simulating potential behavior of CUBs in environments where they are typically managed. Examples of utilization scenarios with potential microbial impact include the use of fly ash in concrete, soil stabilization, or fills that could be exposed to natural waters or to microbial action and the use of FGD gypsum or fly ash as an agricultural soil amendment or mine fill where it would be exposed to natural water and, potentially, microbial action. The efforts related to biologically induced mercury release are presented in this paper.

The wide distribution and variety of microorganisms in the environment indicate that microbiological release of mercury needs to be investigated. A wide variety of specific microbe interactions can affect key elements associated with CUBs, including oxidation/reduction and alkylation/dealkylation reactions. In order for microbes to be metabolically active, a few constraints must be satisfied. In some CUB management options, these criteria are unlikely to be met, but for options where they can be met, laboratory experiments simulated appropriate scenarios.

EXPERIMENTAL

A method to determine the effect of microbes on the release of mercury and mercury compounds from CUBs was developed at the EERC since one did not previously exist. However, since most CUBs are too acidic or too alkaline for microbes when dry, a buffer was needed to neutralize the CUBs to be tested. Therefore, a slurry was used in the testing, and agitation of the slurry was necessary.

The apparatus used for this testing was improved numerous times over the course of the research; therefore, the latest setup is described here. A 24/40 standard taper 250-mL Erlenmeyer flask was fitted with an impinger inlet/outlet tube, with the inlet center shortened to 6 cm below the standard taper. Cylinder gas was passed through several sets of gold-coated quartz traps for mercury removal and admitted through a gas distribution manifold that routed the gas through 0.25-mm gas chromatography (GC) capillary tubing to each of the individual flasks. A final gold-coated quartz trap was placed directly at the inlet to each flask. A section of GC capillary tubing with a length of approximately 60 cm provided a convenient means of regulating gas flow to approximately 2 cm³/min when pressurized to between 1 and 2 psig. The gas transported mercury vapor and other volatile mercury compounds from the headspace of the flasks to a mercury vapor collection system at the outlet of the flasks, consisting of two traps. The nearest trap contained Supelco Carbotrap™, which collected

organomercury compounds. This was followed by a gold-coated quartz trap, which collected elemental mercury.

The flasks were placed on a 9-place stir plate with Teflon-coated stir bars to facilitate mixing. The experimental matrix consisted of nine flasks under aerobic conditions (using breathing-quality air) and nine flasks under anaerobic conditions (using argon). A 20-gram aliquot of CUB was placed in the flasks, and 150 mL of a phosphate buffer (with glucose) was added to create a neutral pH. The buffer system consists of L-glutamic acid, potassium phosphate monobasic, and D-(+)-glucose. This buffer system significantly reduced the previously noted analytical interferences to the solid-phase microextraction (SPME) analysis.³ When alkaline CUBs were evaluated, dilute sulfuric acid was added to neutralize the sample prior to introducing the microbes. The CUB-containing flasks also had 100 μ L of mixed bacterial culture added. The source of bacteria was a mixed bacterial inoculum from a brackish wetland. This apparatus is shown in Figure 1.

Three samples (see Table 1) were evaluated in aerobic and anaerobic glucose-fed conditions in triplicate using this latest method. Sample 03-082 was a moist FGD sample, whereas the other two samples and all previous samples were dry. The bacteria were added 4 days after the addition of the buffer and acid to allow the systems to neutralize. The system was stirred intermittently over the duration of the experiment. Vapor-phase organomercury released from the system was captured in tubes containing Supelco Carbotrap™, and elemental mercury releases were captured on gold-coated quartz traps.



Figure 1. Microbiologically mediated mercury vapor-phase collection apparatus.

Table 1. Samples Included in New Method of Microbiological Release Experiment

ID No.	Sample Type	10% Sulfuric Acid Added, mL
03-079	Fly ash	18
03-082	FGD filtercake	1.6
04-043	Fly ash	7.4

The experiments were conducted for approximately 30 days. Upon completion of the experiment, evaluations included pH, Eh, elemental and organomercury vapor releases, solution organomercury, and total trace element concentration.

After the liquid was filtered from the solid, pH and Eh values were determined. For Samples 03-079 and 03-082, the pH was higher under anaerobic conditions than aerobic; the opposite was true for Sample 04-043. Mold was noted in the Sample 04-043 aerobic flasks.

Vapor-phase elemental and organomercury releases were determined using atomic fluorescence (AF). The gold-coated quartz collection traps were desorbed for analysis by heating to approximately 500°C, and the mass of mercury released was determined using AF. The Supelco Carbotrap™ collection traps were analyzed for total mercury by heating the trap to approximately 300°C, passing the released organomercury through a tube held at about 800°C, and collecting the mercury on a gold-coated quartz trap, which was analyzed as described above.

SPME was used to determine organomercury compounds in the liquid remaining in the flasks after the experimental collection period. 5 mL of the liquid plus an aliquot of ethylmercuric chloride as an internal standard was placed into a 15-mL septum-capped nonactinic vial containing a Teflon-coated stir bar. 500 µL of a pH 4.5 acetate buffer was added followed by 200 µL of a 1% solution of sodium tetrapropylborate. SPME sampling was accomplished using a 100-µm polydimethylsiloxane-coated fiber and sampler from Supelco. The headspace was sampled for 20 minutes after which the SPME fiber was directly introduced into the GC.

A GC coupled to an AF detector was used for the separation and detection of mercury compounds captured onto the SPME fiber. The GC temperature program began at 50°C, was held at that temperature for 2 minutes, ramped at 10°C/min to 250°C, and held there for several minutes. Inlet temperature was held at 200°C. Nonpolar megabore (0.53-mm ID) columns coated with either nonpolar methylsilicone or methylsilicone with 5% phenyl content worked well for this application. Adequate separations could be achieved using 15-meter columns. Helium gas flow was optimized for each column used. The gas exiting the GC through a heated length of uncoated 0.53-mm ID fused silica capillary tubing was directed to a thermal converter held at 800°C. Just before the gas entered the thermal converter, makeup helium was added for a total gas flow of approximately 25 mL/min. These chromatographic conditions

allowed separation of elemental, dimethyl-, and diethylmercury as well as methylmercuric chloride.

Current research is directed at the use of sodium tetrabutyl borate as a derivatizing reagent for the SPME analysis. Derivatization using the tetrabutyl compound will allow the determination of both methylated and ethylated organomercury species with propylmercuric chloride as an internal standard. Unfortunately, sodium tetrabutyl borate is commercially unavailable and must be synthesized for use. This synthesis is relatively simple and involves the reaction between equivalent amounts of tributylborane and butyl chloride in the presence of two equivalents of sodium metal. The reaction mixture must be kept anhydrous and free from air.

RESULTS AND DISCUSSION

In this experiment, 3 weeks elapsed before the addition of the buffer solution; therefore, the gold-coated quartz traps were analyzed to determine the amount of elemental mercury released from the samples in the interim period (see Table 2). Sample and blank flasks generally released similar low quantities of mercury, but Sample 03-082 under aerobic conditions released significantly more elemental mercury than the other samples. It should be noted that this was a wet FGD sample that contained a significant population of sulfate-reducing bacteria. The gold-coated quartz traps were then replaced on the flasks for collection of elemental mercury over the duration of the experiment.

After only 3 days of having bacteria present, Sample 03-082 under anaerobic conditions exhibited noticeable changes. The CUB changed from a cream to a gray color, and a hydrogen sulfide smell was emitting from the flasks. The gas from these flasks was directed to flasks containing zinc acetate to capture the hydrogen sulfide being produced. 15 days after the addition of bacteria, crystal-like formations were noted on the liquid surface layer from Sample 04-043, two flasks under anaerobic and one under aerobic conditions.

Table 2. Initial Elemental Mercury Release, pg/g

ID No.	Condition	Elemental Mercury	ID No.	Condition	Elemental Mercury
03-079	Anaerobic	1.1	03-079	Aerobic	1.5
03-079	Anaerobic	0.9	03-079	Aerobic	1.3
03-079	Anaerobic	0.8	03-079	Aerobic	0.4
03-082	Anaerobic	5.5	03-082	Aerobic	193
03-082	Anaerobic	0.7	03-082	Aerobic	195
03-082	Anaerobic	3.7	03-082	Aerobic	207
04-043	Anaerobic	1.0	04-043	Aerobic	0.7
04-043	Anaerobic	0.5	04-043	Aerobic	0.7
04-043	Anaerobic	0.8	04-043	Aerobic	1.2

Upon completion of the experiment, evaluations included pH, Eh, elemental and organomercury vapor releases, and solution organomercury and total trace element concentrations. Results obtained are shown in Tables 3–5. Total trace element concentration results are not included in this paper.

A description of the contents of each sample flask upon completion of the experiment is included in Table 3. After the liquid was filtered from the solid, the pH and Eh values were determined. For Samples 03-079 and 03-082, the pH was higher under anaerobic conditions than aerobic; the opposite was true for Sample 04-043. A greater amount of mold noted in the Sample 04-043 aerobic flasks yielded a higher pH.

Vapor-phase mercury release results from the three samples evaluated under the new method are shown in Table 4, reported as pg mercury per g CUB. In some instances, the results were highly variable for the replicate flasks. The highest release of both elemental and organomercury was from Sample 03-082 under aerobic conditions. Analysis of the gold-coated quartz traps from Sample 03-082 under anaerobic conditions resulted in a white, sulfur-smelling deposit on the gold trap. Analyzing the tubes containing Supelco Carbotrap™ from Sample 03-082 aerobic condition caused a sulfur smell to be released with no deposit formed. Analyzing the tubes containing Supelco Carbotrap™ from Sample 03-082 anaerobic condition resulted in a noticeable sulfur-smelling smoke to be released and a subsequent formation of a white- to yellow-colored deposit on both the Carbotrap™ and pyrolysis tubes. The amount of vapor-phase elemental mercury released coincided with the amount of mold present in the Sample 04-043 aerobic flasks at the completion of the experiment.

Table 5 shows the results of the SPME analyses.

Results of the microbiologically mediated release experiments are under continuing review.

SPME has been used several times during the course of this research. It has been shown that derivatization of CUB sample solutions with either sodium tetraethylborate or sodium tetrapropylborate can be successfully used for the determination of organomercury species using the SPME technique. These reagents, however, are quite expensive, and their use requires careful coordination of internal standards to obtain useful results. A new reagent, sodium tetrabutylborate, is being synthesized and will be tested for use in this determination in the near future. It is believed that this reagent will minimize many of the problems associated with the current reagents.

CONCLUSIONS

The biological activity experiments were conducted in triplicate primarily because the mass of mercury collected on the gold-coated quartz and Carbotrap™ traps can only be tested once. If the amount of mercury captured on the trap over the duration of the experiment was too high for the settings used when testing on the AF instrument, the value was incomplete or lost.

Table 3. Results Including Eh, pH, and Sample Description

ID No.	Condition	Eh, mV	pH	Sample Description	ID No.	Condition	Eh, mV	pH	Sample Description
03-079	Anaerobic	164	8.23	Black layer on top of clear liquid; liquid bubbles; brown ash	03-079	Aerobic	374	8.09	Very slightly brown liquid; black and brown coatings on flask; dark brown ash
03-079	Anaerobic	179	8.30	Black layer on top of clear liquid; liquid bubbles; brown ash	03-079	Aerobic	314	8.02	Film on top of liquid; very slightly brown liquid; black and brown coatings on flask; dark brown ash
03-079	Anaerobic	189	8.45	Black layer on top of clear liquid; liquid bubbles; brown ash	03-079	Aerobic	354	7.97	Two spots of mold; film on top of liquid; very slightly brown liquid; black and brown coatings on flask; dark brown ash
03-082	Anaerobic	-176	8.39	Gray liquid and ash; H ₂ S smell; crusted bubbles on top of liquid—round on top and broken on bottom	03-082*				
03-082	Anaerobic	-161	8.55	Gray liquid and ash; H ₂ S smell; slightly crusted bubbles on top of liquid	03-082	Aerobic	39	7.89	Slightly gray liquid with gold crust on top; smelled like baby formula; gray ash
03-082	Anaerobic	-146	8.51	Gray liquid and ash; H ₂ S smell; slimy on top of liquid	03-082	Aerobic	44	7.92	Slightly yellow liquid; smelled like baby formula; gray ash
04-043	Anaerobic	164	6.85	Some carbon particles on top of clear liquid; no noticeable smell; grayish-brown ash	04-043	Aerobic	354	7.61	Layer of mold on top of slightly gray liquid confirmed by smell; grayish-brown ash
04-043	Anaerobic	154	6.95	Some carbon particles on top of clear liquid; no noticeable smell; grayish-brown ash	04-043	Aerobic	279	7.15	No mold smell; grayish liquid; carbon granules on top of liquid; grayish-brown ash
04-043	Anaerobic	149	6.81	Some carbon particles on top of clear liquid; no noticeable smell; grayish-brown ash	04-043	Aerobic	134	7.36	Patch of mold on top of gray liquid; grayish-brown ash

* One flask broke; therefore, only two samples were evaluated.

Table 4. Vapor-Phase Mercury Release Results

ID No.	Condition	Elemental Mercury, Organomercury,		ID No.	Condition	Elemental Mercury, Organomercury,	
		pg/g	pg/g			pg/g	pg/g
03-079	Anaerobic	4.1	5.7	03-079	Aerobic	14.7	9.1
03-079	Anaerobic	2.8	5.0	03-079	Aerobic	11.3	30.8
03-079	Anaerobic	5.3	6.7	03-079	Aerobic	19.8	2.5
03-082	Anaerobic	12.7	41.3	03-082*			
03-082	Anaerobic	0.7	2.6	03-082	Aerobic	486	90.0
03-082	Anaerobic	1.5	102	03-082	Aerobic	648	110
04-043	Anaerobic	1.3	1.1	04-043	Aerobic	46.5	4.5
04-043	Anaerobic	2.2	0.6	04-043	Aerobic	4.0	1.4
04-043	Anaerobic	1.9	1.0	04-043	Aerobic	9.1	1.3

* One flask broke; therefore, only two samples were evaluated.

Table 5. Solution Organomercury Results

ID No.	Condition	Methylmercury		ID No.	Condition	Methylmercury	
		Measured, pg/mL	Released, pg/g			Measured, pg/mL	Released, pg/g
03-079	Anaerobic	13	98	03-079	Aerobic	32	240
03-079	Anaerobic	15	113	03-079	Aerobic	7	53
03-079	Anaerobic	<5	<38	03-079	Aerobic	12	90
03-082	Anaerobic	*	*	03-082**			
03-082	Anaerobic	250	1875	03-082	Aerobic	18	135
03-082	Anaerobic	*	*	03-082	Aerobic	99	743
04-043	Anaerobic	31	233	04-043	Aerobic	<5	<38
04-043	Anaerobic	19	143	04-043	Aerobic	65	488
04-043	Anaerobic	18	135	04-043	Aerobic	13	98

* Samples precipitated colloidal sulfur on standing and were not analyzed.

** One flask broke; therefore, only two samples were evaluated.

During the development of a method for this experiment, some challenges were encountered. Initially, electron acceptors were added to the slurry but it was later determined that the CUB would supply these, and this approach was abandoned. A couple of CUBs were analyzed using the aerobic and anaerobic conditions. However, the collection traps did not include Carbotrap™; rather, activated carbon traps were utilized and placed after the gold-coated quartz traps. Better and more consistent results were obtained using the Carbotrap™ traps before the gold-coated quartz traps. It was determined that Carbotrap™ allows elemental mercury to pass but traps the organomercury forms. The use of derivatization reagents allows the determination of organomercuric halides by SPME. Sodium tetraethylborate or sodium tetrapropylborate can be successfully used for the determination of organomercury species using the SPME technique. The use of sodium tetrabutylborate is currently being investigated.

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