APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1979, p. 494-498
0099-2240/79/09-0494/05$02.00/0
Vol. 38, No. 3

Production of Arsine and Methylarsines in Soil and in Culture

C.-N. CHENG† AND D. D. FOCHT*

Department of Soil and Environmental Sciences, University of California, Riverside, California 92521

Received for publication 14 June 1979

Arsenate, arsenite, monomethylarsonate, and dimethylarsinate were added to three different soils, and the evolution of gaseous arsenical products was determined over 3 weeks. Arsine was produced in all three soils from all substrates, whereas methylarsine and dimethylarsine were produced only from methylarsonate and dimethylarsinate, respectively. At least three times more arsine than dimethylarsine was produced in soil incubated with dimethylarsinate. Resting cell suspensions of *Pseudomonas* and *Alcaligenes* produced arsine as the sole product when incubated anaerobically in the presence of arsenate or arsenite. In all instances, no trimethylarsine was observed, nor could any evidence be shown for the methylation of any arsenical substrate in soil or in culture. It was concluded that reduction to arsine, not methylation to trimethylarsine, was the primary mechanism for gaseous loss of arsenicals from soil.

Volatilization of arsenic by microorganisms was first reported in 1893 by Gosio (4). The volatile product had a characteristic garlic-like odor and eventually became known as the Gosio gas. Challenger et al. (6) likewise noted the Gosio gas formation upon addition of arsenious oxide, methylarsonate, or dimethylarsinate to cultures of *Scopulariopsis brevicaulis*. By rigorous chemical analyses, they established that the Gosio gas was trimethylarsine (TMA) and not diethylarsine as Gosio had incorrectly concluded. Challenger (5) later postulated the reduction of arsenate to TMA as follows: $AsO_4^{-3} \rightarrow AsO_3^{-3} \rightarrow (CH_3)AsO_3^{-2} \rightarrow (CH_3)_2AsO_2^{-} \rightarrow (CH_3)_3As$. The major part of this pathway was confirmed in *Methanobacterium* by McBride and Wolfe (10) except that the final product was found to be dimethylarsine (DMA) instead of TMA.

The characteristic garlic-like odor is also produced from cultures of *Trichophyton* (18), from two species of wood-rotting fungi (11), and from sewage isolates of *Candida*, *Gliocladium*, and *Penicillium* (7). On the basis of these pure culture studies, Alexander (1) proposed that the major pathway by which arsenic is lost from soil is through a series of methylation reactions which give rise to TMA as the final product. Braman (3) and Woolson (16) also found that the addition of inorganic and organic forms of arsenic gave rise to DMA and, primarily, TMA. Arsine or methylarsine were not observed.

Since arsenicals such as monosodium methylarsonate and cacodylic (dimethylarsinic) acid are used extensively as herbicides, it is important to establish the actual fate of arsenicals in soils. Arsine and methylarsines have not previously been found in nature or in culture. The following study was undertaken to investigate the nature of gaseous arsenical products produced in soil and in culture and to establish if the methylation pathway is relevant to what actually occurs in nature.

MATERIALS AND METHODS

Chemicals. Sodium arsenate (dibasic, 99% pure), sodium arsenite (95%), sodium borohydride, and TMA were obtained from Ventron Corp., Denver, Mass. Dimethylarsinic acid and disodium methylarsonate were purchased from Pfaltz & Bauer, Inc., Stamford, Conn. Carbowax 1000 and Chromosorb AW (60/80) were obtained from Applied Science Laboratories, State College, Pa.

Except for TMA, which was obtained from Ventron Corp., all other arsines were synthesized from the corresponding arsenicals as follows: arsine from arsenite, methylarsine from disodium methylarsonate, and DMA from dimethylarsinic acid. The following two methods were used to prepare the arsines: (i) the $SnCl_2$-HCl/I_2-KI procedure described by Michael (12) with an NaOH trap to remove HCl; (ii) the sodium borohydride procedure used by Andreae (2). Retention times and characterization of these prepared arsines and the standard TMA were established by gas chromotography and gas chromotography-mass spectrometry, respectively, as described below.

Soils. Three soils with fairly diverse physical and chemical properties were used in this study: (i) Hanford sandy loam (pH 5.6, 0.93% organic matter), (ii) Altamont clayey loam (pH 6.6, 2.33% organic matter), (iii) Domino silty loam (pH 7.6, 1.53% organic matter).

A 10-g sample of air-dried soil was added to a 25-ml Erlenmeyer flask. Each flask was enriched with 1%

† Present address: Department of Plant Science and Technology, North Carolina Agricultural and Technical State University, Greensboro, NC 27411.

glucose, 0.084% urea, and 160 μg of arsenic per g in the form of arsenate, arsenite, methylarsonate, or dimethylarsinate. Each sample was then flooded with 10 ml of distilled water to develop a 1-cm depth of water above the soil surface. This method was chosen because Woolson and Kearney (17) found that more arsenic was lost from dimethylarsinate-treated soils under flooded than under nonflooded conditions. After being sealed with Parafilm, the flasks were incubated at room temperature. Every 7 days, up to a period of 21 days, samples of the gas in the headspace above were removed for analysis by a gas-tight 100-μl syringe. The flasks were then flushed with ambient air, resealed, and incubated. Duplicate samples were run for all treatments.

In addition to the above treatment, a series of controls for all three soils was run in duplicate as follows: (i) 0.084% urea and 1% glucose without arsenic; (ii) 160 μg of arsenic per g without glucose and urea. To test for the loss of TMA by leakage or adsorption to the Parafilm covering, sealed flasks without soil were incubated with TMA over a 3-day period.

Analytical. Volatile arsenicals in the flask headspace were determined by a Varian 2700 gas chromatograph equipped with a flame-ionization detector and by a Finnigan 3100C mass spectrometer interfaced with a gas chromatograph. A 10% Carbowax 1000 column (2 by 3,000 mm, stainless steel) coated on Chromosorb AW (60/80 mesh) with an N_2-carrier gas flow rate of 6.0 ml/min and an oven temperature of 30°C was used for gas chromatographic analyses. Detector and injector temperatures were set at 180 and 150°C, respectively. Characterization of each volatile arsenical by gas chromatography-mass spectrometry was performed under conditions similar to gas chromatographic analyses, using He as a carrier gas, with an ionizing voltage of 70 eV in the mass spectrometer.

Cultures. The isolates used in this study were obtained from soil by enrichment in most-probable-number tubes of nitrate broth (8), streaked onto nitrate agar plates, and identified by standard taxonomic methods (4) described in a later section. Respective resting cell suspensions of *Pseudomonas* and *Alcaligenes* were prepared from 18- and 36-h cultures grown in nitrate broth (Difco Laboratories, Detroit, Mich.) containing 5 g of monosodium succinate per liter in a 5-liter fermentor (New Brunswick Scientific Co., New Brunswick, N.J.). During the first half of the incubation period, the fermentor was aerated at a rate of 2 liters/min, after which N_2 was passed through the air line at the same flow rate to create anoxic conditions during the latter half of the incubation. The cells were harvested at 10,000 × *g* for 15 min, washed, and suspended three times in 0.05 M $NaHCO_3$ buffer (pH 7.8) to give a final optical density of 0.40 when measured through an 18-mm test tube at 525 nm with a Spectronic 20 spectrophotometer (Bausch and Lomb, Inc., Rochester, N.Y.). The washed cell suspensions (25 ml) were added to 250-ml Erlenmeyer flasks, and aqueous solutions containing either arsenate or arsenite were added to give a final arsenic concentration of 50 μg/g. The flasks were fitted with gas-tight serum caps and were evacuated and flushed five times with He to a final internal pressure of 1.25 atm. The reaction mixtures were incubated at 28°C in a water bath shaker for 24 h, after which a gas sample was removed for analysis.

RESULTS

Both isolates were short gram-negative rods that would grow anaerobically only in the presence of nitrate or nitrite. They were both cytochrome oxidase and catalase positive. Neither isolate produced acid or gas from glucose, maltose, lactose, or sucrose, nor was acetylmethyl carbinol or indole produced. Hydrolysis of starch and urea was negative for both isolates. The isolate identified as *Pseudomonas* was motile by polar flagella, produced a greenish-blue diffusible pigment on agar plates, hydrolyzed gelatin, and left litmus milk unchanged. The isolate identified as *Alcaligenes* was motile by means of peritrichous flagella, produced gray to off-white colonies on agar plates, did not hydrolyze gelatin, and turned litmus milk alkaline. Arsine was the only arsenical gas produced from both cultures.

The retention times for arsines under the specified conditions described previously were 180, 210, 360, and 410 s for arsine, methylarsine, DMA, and TMA, respectively (bottom of Fig. 1); the mass spectra are shown separately in Fig. 2. The synthesized arsine and the standard TMA had mass spectra identical to that reported in the literature (13). However, no published mass spectra are currently available for methylarsine or DMA.

No arsenical gases were detected in controls to which arsenic had not been added. Only trace amounts of AsH_3 were detected in soils not enriched with urea and glucose. A 10% loss of TMA over a 3-day period was observed in the absence of soil. It could not be determined if the loss was due to leakage, adsorption, or chemical breakdown. Thus, the emissions of gaseous arsenicals reported herein are conservative.

A typical pattern of the volatile arsenicals produced from the Hanford soil with respect to arsenical substrates is shown in Fig. 1. This pattern was characteristic of the other two soils as well. Of particular interest was the observation that arsine, and not TMA, was detected in all three soils (Table 1) upon the addition of all four arsenical substrates. Moreover, methylated arsines were produced only from the corresponding methylated substrates; in other words, the number of methyl groups present in a substrate would give rise to a like number of methyl groups in the final arsine product. Thus, no evidence could be shown for methylation of any arsenical substrate in any of the three soils tested.

Because reference standards were unavailable except for TMA, we were unable to determine the yields of the synthesized arsines. Neverthe-

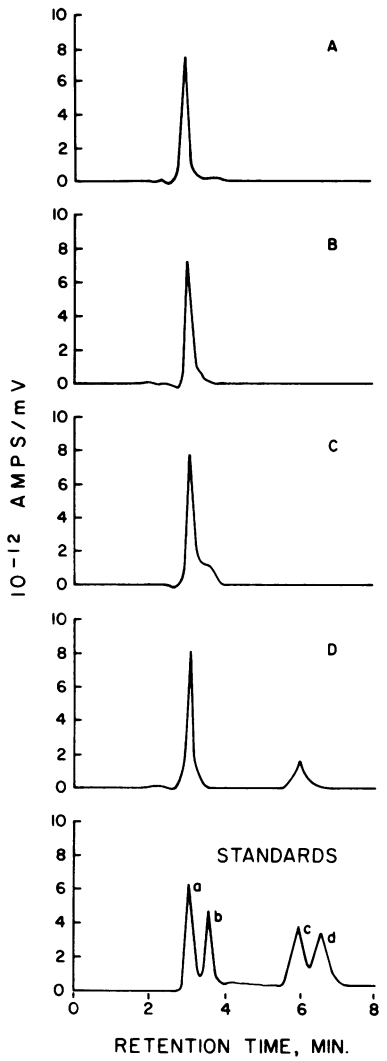

FIG. 1. *Gas chromatographic traces of gas samples taken from the Hanford soil incubated with arsenate (A), arsenite (B), methylarsonate (C), and dimethylarsinate (D). The gas chromatographic trace for standards at the bottom represents authentic samples of arsine (a), monomethylarsine (b), DMA (c), and TMA (d).*

less, an approximation can be made between the relative amounts of arsine and DMA with respect to mass emissions from soil by assuming that the recorder response of these gases to an authentic standard of TMA is the same. This assumption, of course, is not entirely correct since response of compounds to flame-ionization detection is largely dependent upon their electron-generating capacity (i.e., combustibility). Since TMA provides the most electrons per arsenic atom, whereas arsine provides the least of the volatile arsenicals, more of the latter would be required to produce a recorder response equal to the former. Thus, the mass emissions of arsenic, particularly arsine, from soil (Fig. 3) are conservative estimates when calculated from TMA standards. Furthermore, the emission of arsine with respect to DMA is probably greater than what is shown in Fig. 3, when it is considered that combustion of As^{-3} to As_2O_3 by flame ionization will require three times as much O_2 from DMA than from AsH_3, thereby yielding a higher detector response.

DISCUSSION

The production of arsine from all soils and substrates implies that some of the methylated arsenical substrates were demethylated (or oxidized and decarboxylated) first to arsenate or arsenite and then reduced to arsine since no monomethylarsine was observed from dimethylarsinate. Since demethylation of methylarsonate (14) and dimethylarsinate (17) to arsenate has been reported, inorganic arsenic would be produced in our samples no matter which methylated substrate was used and would be a source for arsine production.

The results of our study with soils and bacterial cultures were quite different from previous studies with fungal cultures (5–7, 11, 18). These differences could be due to the nature of the experimental systems. On the other hand, vigorous characterization of TMA was carried out only by Challenger et al. (6), whereas the later investigators, for the most part, assumed that the garlic-like odor was TMA and did not rigorously confirm its existence. Unfortunately, all four arsine gases have this garlic-like odor (15; personal observations). Furthermore, characterization by gas chromotography-mass spectrometry from a mixture is dependent upon adequate separation on a suitable column. We found that the FFAP column used by Cox and Alexander (7) was not suitable for our study since separation between the arsines could not be achieved. Consequently, the possibility that many of these investigators were observing arsine and not TMA cannot be excluded.

Although Woolson (16) was able to separate methyl-, dimethyl-, and trimethylamines on a Chromosorb 101 column, he was unable to detect arsine. We were also unable to separate arsine from other arsenicals using a Chromosorb 103 column, which is more suitable than Chromosorb 101 for the separation of basic gases such as ammonia and arsine. We also found that the Carbowax 20-m column used by Braman (3) was unsuitable for separation of arsine.

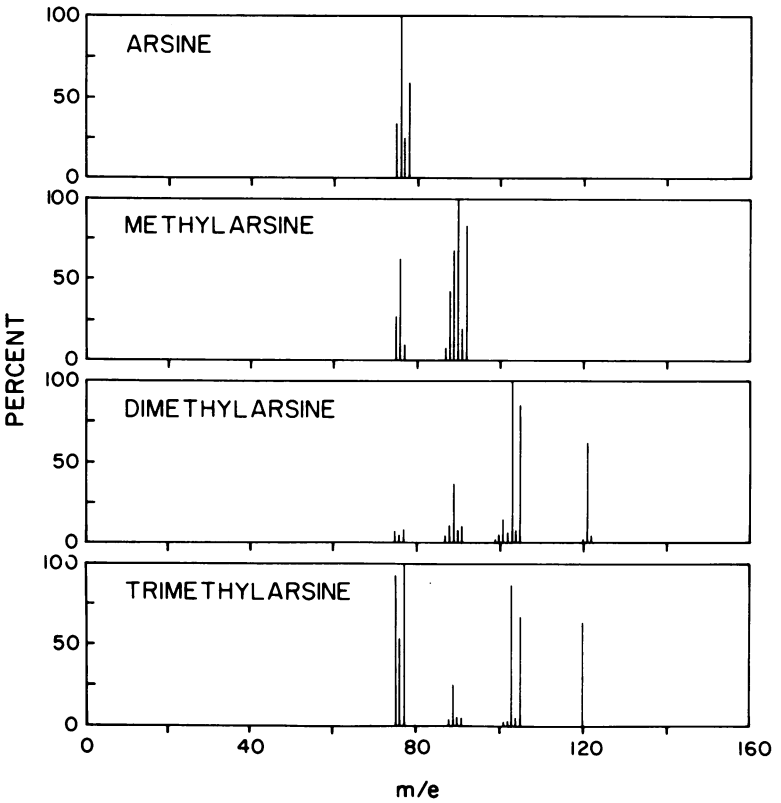

FIG. 2. *Mass spectra of gaseous arsenicals.*

TABLE 1. *Products formed (+) or not formed (−) upon addition of arsenical substrates to all three soils*

Substrate	Product			
	AsH_3	CH_3AsH_2	$(CH_3)_2AsH$	$(CH_3)_3As$
Arsenate	+	−	−	−
Arsenite	+	−	−	−
Methylarsonate	+	+	−	−
Dimethylarsinate	+	−	+	−

Our experimental procedure also differed from that of Woolson (16) in two ways. First, we measured the gases directly, whereas he continuously swept his into a KI/I_2 trap, which oxidized them to the salt. Second, our incubation period of 21 days was much shorter than his 160-day incubation and may be more indicative of processes effected by rapidly growing bacteria.

It is pertinent to note that nitrogen, phosphorus, and arsenic are in the same family of the periodic table. It is conceivable that metabolic similarity exists between these elements such as

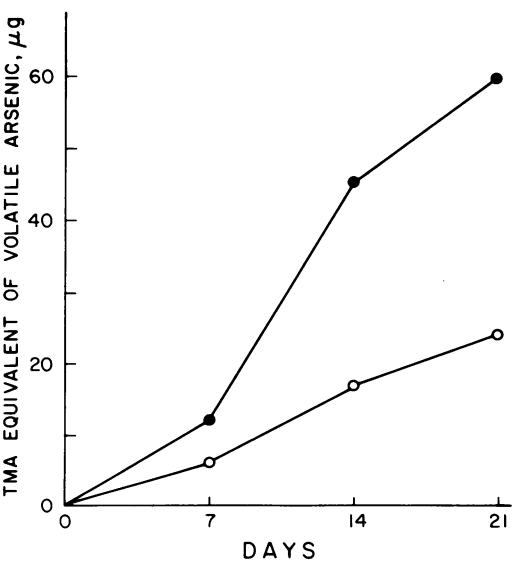

FIG. 3. *Cumulative mass emissions of arsine (●) and DMA (○) from the Hanford soil treated with dimethylarsinate. Points represent average values for two replicates.*

that which exists between selenium and sulfur. However, gaseous losses of nitrogen and phosphorus in soil do not occur via methylation reactions, although methylamines may be generated from putrefaction of organic wastes. For the most part, nitrogen is volatilized in soil as N_2 by denitrification; however, NH_3 formation through dissimilatory reduction appears to be a significant fate of NO_3^- in sediments (9). Similarly, the reduction of PO_4^{3-} and PO_3^{3-} to PH_3 may occur under extreme reducing conditions, although very little is known for certain about this process. Both isolates, *Alcaligenes* and *Pseudomonas*, reduced arsenate and arsenite to AsH_3 under anoxic conditions and did not generate any methylated arsenicals; thus, the possibility of these substrates serving as electron acceptors cannot be precluded. Further work is necessary to establish for certain if arsenate and arsenite serve as terminal electron acceptors for the same bacteria that effect reduction of other ions in soil. This may provide a more realistic assessment of the geochemical cycling of arsenic in nature.

ACKNOWLEDGMENT

This work was supported by a grant from the Kearney Foundation of Soil Science.

LITERATURE CITED

1. **Alexander, M.** 1977. Introduction to soil microbiology, 2nd ed. John Wiley & Sons, Inc., New York.
2. **Andreae, M. O.** 1977. Determination of arsenic species in natural waters. Anal. Chem. **49**:820–823.
3. **Braman, R. S.** 1975. Arsenic in the environment, p. 108–123. *In* E. A. Woolson, (ed.), Arsenical pesticides. Am. Chem. Soc. Symp. Ser. 7. American Chemical Society, Washington, D.C.
4. **Buchanan, R. E., and N. E. Gibbons (ed.).** 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
5. **Challenger, F.** 1945. Biological methylation. Chem. Rev. **36**:315–361.
6. **Challenger, F., C. Higginbottom, and L. Ellis.** 1933. The formation of organo-metalloidal compounds by microorganisms. Part 1. Trimethylarsine and dimethylarsine. J. Chem. Soc. **1933**:95–101.
7. **Cox, D. P., and M. Alexander.** 1973. Production of trimethylarsine gas from various arsenic compounds by three sewage fungi. Bull. Environ. Contam. Toxicol. **9**: 84–88.
8. **Focht, D. D., and H. Joseph.** 1973. An improved method for the enumeration of denitrifying bacteria. Soil Sci. Soc. Am. Proc. **37**:698–699.
9. **Koike, I., and A. Hattori.** 1978. Denitrification and ammonia formation in anaerobic coastal sediments. Appl. Environ. Microbiol. **35**:278–282.
10. **McBride, B. C., and R. S. Wolfe.** 1971. Biosynthesis of dimethylarsine by Methanobacterium. Biochemistry **10**:4312–4317.
11. **Merill, W., and D. W. French.** 1964. The production of arsenous gases by wood-rotting fungi. Proc. Minn. Acad. Sci. **31**:105–106.
12. **Michael, S. S.** 1977. Determination of arsenic in sulfide samples containing antimony by atomic absorption spectrometry. Anal. Chem. **49**:451–453.
13. **Stenhagen, E., S. Abramsson, and F. W. McLafferty.** 1974. Registry of mass spectral data. John Wiley & Sons, Inc., New York.
14. **Von Endt, D. W., P. C. Kearney, and D. D. Kaufman.** 1968. Degradation of monosodium methanearsonic acid by soil microorganisms. J. Agric. Food Chem. **16**:17–20.
15. **Windholz, M.,** 1976. The Merck index, 9th ed. Merck & Co., Inc., Rahway, N.J.
16. **Woolson, E. A.** 1977. Generation of alkylarsines from soil. Weed Sci. **25**:412–416.
17. **Woolson, E. A., and P. C. Kearney.** 1973. Persistence and reactions of ^{14}C-cacodylic acid in soils. Environ. Sci. Technol. **7**:47–50.
18. **Zussman, R. A., E. E. Vicher, and I. Lyon.** 1961. Arsine production by *Trichophyton rubrum*. J. Bacteriol. **81**: 157.