Simple Method for Introducing Elemental Mercury into Biological Growth Systems

HARVEY W. HOLM AND MARILYN F. COX

Southeast Environmental Research Laboratory, U.S. Environmental Protection Agency, Athens, Georgia 30601

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Elemental mercury can be introduced into closed aqueous growth environments and sampled therefrom without loss of elemental mercury to the atmosphere.

Elemental mercury (Hg⁰) is a common product of microbial (2, 6, 7) and chemical (1, 8) action in aquatic growth systems containing mercury. Therefore, to obtain a clearer understanding of the mercury cycling phenomenon, the fate of Hg⁰ in aquatic systems must be investigated. A closed system was developed to allow introduction of Hg⁰ into growth media, to minimize contamination from soluble oxides and bacteria present in liquid mercury, and to avoid loss of Hg⁰ by volatilization during sampling.

A 1-liter Erlenmeyer flask equipped with a 24/40 standard taper outer joint was modified to study highly volatile materials (Fig. 1). A 24/40 standard taper inner joint was converted into a glass stopper (Fig. 1, A). A small glass cup (Fig. 1, B) was attached to the stopper with a 6-inch (15.24-cm) glass rod to perform a function similar to that of the side arm of the Warburg vessel used by Horwitz (4). Liquid mercury was added to the glass cup, and the glass cup stopper was placed in the flask. The Hg⁰ volatilized and equilibrated into the cooled sterile media. Placing the flask containing the mercury on a shaker presented no problem with spilling of the mercury. The liquid mercury may remain inside for continuous exposure studies or may be easily removed from the system by replacing the holder with a sterile top.

A screw-capped, closed sampling port (Fig. 1, C) extends below the surface of the liquid to minimize loss of Hg⁰ by volatilization during sampling. Aqueous samples for chemical and biological analyses may be obtained by removing samples with a glass pipette from below the end of the tube at sampling port C.

Another feature is a port sealed with a rubber septum (Fig. 1, D) located above the level of the medium, which may be used for introducing microbial inocula and sampling the enclosed atmosphere.

Studies using the apparatus described above have shown the following. (i) Hg⁰ may be easily introduced into the aqueous phase, reaching equilibrium within 48 h. (ii) The mercury holder can be quickly replaced without loss of sufficient Hg⁰ to alter the equilibrium. (iii) Hg⁰ is stable in a basal salts medium (Table 1). (iv) Bacterial contamination is significantly decreased when Hg⁰ is added to media with this apparatus. Three out of 24 flasks were contaminated with bacteria using this system; when liquid mercury was added directly to the media, 6 out of 12 flasks were contaminated.

The closed system described may be used to investigate such processes as (i) the transformations of Hg⁰ using pure cultures of bacteria, (ii) the behavior of Hg⁰ in sediment-water systems,
### Concentration of Hg in Payne and Feisal medium (5) incubated at 25°C

| Time (h)* | Hg* (μg/liter) | Mean  | Standard deviation | No.  |
|-----------|-----------------|-------|--------------------|------|
| 0         | 55.1            | 2.3   | 10                 |      |
| 48        | 53.4            | 2.5   | 10                 |      |

* Hg* was analyzed by removing 1-ml samples from sampling port C and quantitating with a cold vapor technique without using a reducing agent (3).
* Hours represent time elapsed after removal of Hg* globule.

and (iii) the oxidation of Hg* in natural waters. The apparatus may be used in similar applications involving other volatile materials. In particular, the flask should be useful for the study of microbial transformations of volatile materials with low water solubilities, because leaving the glass cup filled with the test material inside the flask provides a continuous input of the material.

### LITERATURE CITED

1. Bongers, L. H., and M. N. Khattak. 1972. Sand and gravel overlay for control of mercury in sediments. Water Pollution Control Research Series 10980 HVA 0172, U. S. Environmental Protection Agency, Washington, D.C.
2. Furukawa, K., T. Suzuki, and K. Tonomura. 1969. Decomposition of organic mercurial compounds by mercury-resistant bacteria. Agr. Biol. Chem. 33:129-130.
3. Hatch, W. R., and W. L. Ott. 1968. Determination of submicrogram quantities of mercury by atomic absorption spectrophotometry. Anal. Chem. 40:2085-2087.
4. Horwitz, L. 1957. Observations on the affect of metallic mercury upon some microorganisms. J. Cell Comp. Physiol. 49:437-453.
5. Payne, W. J., and V. E. Feisal. 1963. Bacterial utilization of dodecyl sulfate and dodecyl benzene sulfonate. Appl. Microbiol. 11:339-344.
6. Spangler, W. J., J. L. Spigarelli, J. M. Rose, and H. M. Miller. 1973. Methylmercury: bacterial degradation in lake sediments. Science 180:192-193.
7. Summers, A. O., and S. Silver. 1972. Mercury resistance in a plasmid-bearing strain of Escherichia coli. J. Bacteriol. 112:1229-1236.
8. Toribara, T. Y., C. P. Shields, and L. Koval. 1970. Behavior of dilute solutions of mercury. Talanta 17:1025-1028.