APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1986, p. 110–114
0099-2240/86/010110-05$02.00/0

Vol. 51, No. 1

Methods for Measuring Specific Rates of Mercury Methylation and Degradation and Their Use in Determining Factors Controlling Net Rates of Mercury Methylation

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Received 4 June 1985/Accepted 30 September 1985

A method was developed to estimate specific rates of demethylation of methyl mercury in aquatic samples by measuring the volatile ^{14}C end products of $^{14}CH_3HgI$ demethylation. This method was used in conjunction with a $^{203}Hg^{2+}$ radiochemical method which determines specific rates of mercury methylation. Together, these methods enabled us to examine some factors controlling the net rate of mercury methylation. The methodologies were field tested, using lake sediment samples from a recently flooded reservoir in the Southern Indian Lake system which had developed a mercury contamination problem in fish. Ratios of the specific rates of methylation/demethylation were calculated. The highest ratios of methylation/demethylation were calculated. The highest ratios of methylation/demethylation occurred in the flooded shorelines of Southern Indian Lake. These results provide an explanation for the observed increases in the methyl mercury concentrations in fish after flooding.

Aquatic methyl mercury concentrations are regulated by the concurrent processes of production and degradation (e.g., see references 9 and 15). Methyl mercury can be produced biologically by microorganisms capable of metabolizing the mercuric ion as a means of detoxifying their environment (18) as well as an incorrect synthesis of other organic molecules such as amino acids (23). Other bacteria eliminate methyl mercury from their environment by converting it to methane and elemental mercury (23, 24).

Although the net methylation rate has been determined in some instances (4), we believed it more informative to examine methyl mercury production and degradation individually. The factors controlling these processes and their interactions can then be assessed. A first step involved the development of a radiochemical method which used ^{203}Hg to estimate specific rates of mercury methylation (12). In this paper we have described a method for measuring microbial degradation of methyl mercury, using [^{14}C]methyl mercuric iodide. The gaseous radioactive end products of the demethylation process were measured and a specific rate of microbial demethylation of methyl mercury was determined. When this method was used in conjunction with the radiochemical method for the measurement of mercury methylation, it enabled us to examine the factors controlling the net rate of mercury methylation in aquatic environments.

MATERIALS AND METHODS

Sampling sites. Epilimnetic sediments were collected from Lake 239 at the Experimental Lakes Area (10) in northwestern Ontario during May and June 1983. The sediments were collected with a modified Ekman grab (11). Flocculent material at the sediment-water interface (0 to 2 cm) was siphoned with a hand vacuum pump into acid-washed 500-ml polyvinyl chloride bottles.

Sediment was also collected from an isolated unnamed bay (herein referred to as Methyl Bay) on Southern Indian Lake (latitude 57°N, longitude 99°W). Southern Indian Lake was impounded in 1976 following the diversion of the Churchill River for hydroelectric development (16). The bay is relatively shallow with slumping shorelines caused by the melting permafrost. The bathymetry is unrecorded but depths >8 m occur. Three sites were sampled, two flooded inshore sites consisting of flocculent organic material overlying flooded terrestrial debris and one offshore site of lacustrine clay. The sediment was collected with a submersible pump. The intake of the pump was dragged over the surface of the sediment, and the surface material was collected in acid-washed 2-liter polyvinyl chloride bottles.

Measurement of specific rates of mercury demethylation. The rate of biological degradation of methyl mercury was measured with [^{14}C]methyl mercuric iodide (Amersham Corp.), which was stored over liquid nitrogen to prevent radiochemical decomposition of the iodide. For use, crystalline $^{14}CH_3HgI$ was dissolved in distilled, deionized water to a final concentration of 62.9 kBq (37 μg of Hg)$^{-1}$ ml^{-1}. The aqueous $^{14}CH_3HgI$ was added to each sample at a final concentration of 0.2 μg of Hg g of dry sediment^{-1}.

Naturally occurring methyl mercury was undetectable in any of the sediment used (detection level, 10 ng; 25). Total mercury was 0.35 μg g^{-1} in Lake 239 and 0.04 to 0.56 μg g^{-1} in Methyl Bay (detection limit, 5 ng; 2).

Sediment collected from each site was dispensed into three 125-ml reagent bottles: a pair of replicates and an acidified blank sample. A minimum volume of 50 ml of the sediment-water suspension was required for good replication; volumes up to 100 ml proved suitable. The sediment slurries were >95% water. Hydrochloric acid was added to stop microbial activity in blanks and in samples following incubation. Samples containing <1 g (dry weight) of sediment were killed with 1 ml of 4 mol of HCl liter^{-1}; otherwise, activity was stopped with 1 ml of concentrated HCl. The samples were incubated for 12 h at either in situ (10 to 15°C) or room (20°C) temperature.

Following the incubation period, the volatile ^{14}C produced by demethylation was stripped from the acidified samples

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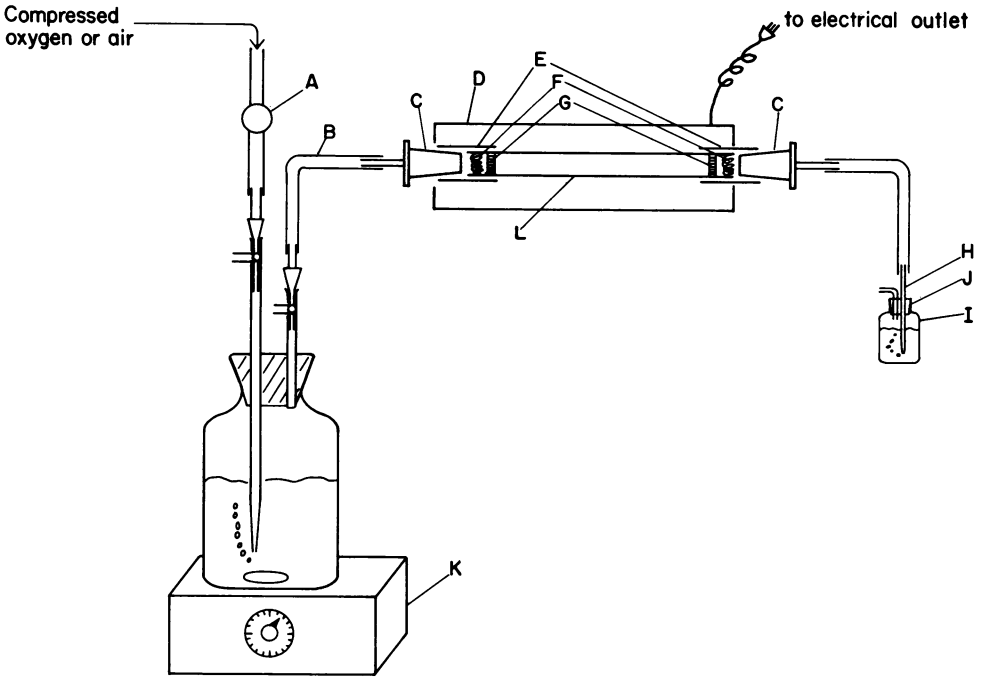

FIG. 1. Apparatus used to strip the ^{14}C end products of demethylation from sediments. A, Gas metering valve; B, glass tube; C, plastic adapter; D, tube furnace; E, silicone tubing; F, steel wool; G, quartz wool; H, disposable glass pipette; I, 20-ml glass scintillation vial with CO_2 trap; J, silicone stopper; K, magnetic stir plate; L, Vycor tubing packed with copper oxide.

(Fig. 1). The samples were stirred magnetically and bubbled with either air or oxygen (flow rate, 50 ml min^{-1}) for 1 h. The volatile ^{14}C was passed through a Vycor tube (Fisher Scientific Co.) packed with copper oxide and heated in a tube furnace at 450°C to ensure that all of the volatile ^{14}C was oxidized to $^{14}CO_2$ (furnace efficiency, >97% [20]) (Fig. 1). The $^{14}CO_2$ was collected in a carbon dioxide trap consisting of 10 ml of PCS solubilizer (Amersham), 2 ml of CO_2mMet (a carbon dioxide trapping agent; Amersham), and 2 ml of 100% methanol in a glass scintillation vial (P. M. Bower, Ph.D. thesis, Columbia University, New York, N.Y., 1981). The ^{14}C activity of the sample was determined with a liquid scintillation counter (Beckman 7000).

The rate of demethylation of methyl mercury in sediment can be calculated as nanograms of Hg demethylated per gram per hour. This in turn was used to calculate the specific rate of demethylation, i.e., the percentage of the added mercury demethylated per gram per hour.

$$\text{total nanograms of Hg demethylated} = \text{measured dpm [dpm (nanograms of Hg)}^{-1}]^{-1} \quad (1)$$

$$\text{nanograms of Hg demethylated per gram per hour} = \text{(total nanograms of Hg demethylated) (gram of dry sediment)}^{-1}\text{ (hours incubated)}^{-1} \quad (2)$$

$$\text{percent added Hg demethylated} = 100\ \text{(nanograms of Hg demethylated per gram per hour)}^{-1}\text{ (added nanograms of Hg)} \quad (3)$$

Measurement of specific rates of mercury methylation. Specific rates of mercury methylation were calculated in a manner similar to demethylation calculations (19). The specific rate of mercury methylation was measured by adding ^{203}Hg as $HgCl_2$ at a concentration of 74 kBq (2 μg of Hg)$^{-1}$ g of dry sediment^{-1} to fresh sediment samples in 125-ml glass bottles and incubating for 12 h. Methylating activity in the blanks and samples was terminated with acid as for the demethylation samples. Alkylated ^{203}Hg was extracted from the samples by using an organic extraction method (12). The ratio of specific methylation/specific demethylation (M/D) was calculated by comparing the rates for the two processes:

$$\frac{\text{percent added Hg methylated per gram per hour}}{\text{percent added Hg demethylated per gram per hour}} \quad (4)$$

Tests of the method. The first tests of this method were to determine (i) the efficiency of stripping $^{14}CO_2$ from distilled water and sediment and (ii) the effectiveness of the $^{14}CO_2$ trap. A known amount of [^{14}C]sodium bicarbonate was added to 100 ml of distilled water in the incubation vessel, and the water was acidified with 1 ml of 4 mol of HCl liter^{-1} and bubbled with oxygen for 1 h (50 ml of O_2 min^{-1}). Sodium bicarbonate was used to test the stripping efficiency because it is more soluble than methane. The trapping efficiency was measured by putting two traps in series so that volatile ^{14}C not collected in trap A would be trapped in trap B. The percent trapping efficiency was then calculated as:

$$\frac{\text{trap B} \cdot 100}{\text{trap A} + \text{trap B}} \quad (5)$$

The stripping efficiency was calculated as:

$$\frac{(\text{dpm in trap A} + \text{dpm in trap B})}{\text{original dpm}} \cdot 100 \quad (6)$$

Following these tests the stripping efficiency of sediment was tested as previously described, using sediment collected from Lake 239 instead of the distilled water.

TABLE 1. Effect of room air and nitrogen on rate of microbial methyl mercury degradation[a]

Sample	dpm	dpm − blank	Rate of demethylation (% added Hg g^{-1} h^{-1})	
Nitrogen				
1	7,880	7,500	0.014	$\bar{x}$ = 0.014
2	7,370	7,000	0.013	
Blank	390			
Room air				
1	9,870	9,550	0.018	$\bar{x}$ = 0.017
2	9,650	9,370	0.017	
Blank	320			

[a] Samples were collected from Lake 239 (average of duplicate samples).

Other experiments compared the addition of 100% Formalin and hydrochloric acid to determine which terminated activity more effectively. Variability among 14 pairs of duplicate sediment samples was calculated to establish repeatability. Time course studies were done so that a minimum but adequate incubation time would be used to determine the demethylation rate. Shorter incubation times are preferred to minimize the possibility of a change in the natural microbial population.

RESULTS

The first set of experiments was conducted to determine the efficiency of removing $^{14}CO_2$ from water and the sediment (stripping efficiency) and to evaluate how well the fluor-CO_2mMet-methanol trap collected the volatilized ^{14}C (trapping efficiency). Virtually all of the ^{14}C was stripped from the water ($\bar{x}$ = 99.7%; standard deviation = 1.2%) and sediments although the variability was greater for sediments ($\bar{x}$ = 99.5%; standard deviation = 7.3%). The fluor-CO_2mMet-methanol trap collected the volatile ^{14}C very efficiently ($\bar{x}$ = 99.7%; standard deviation = 0.4%).

A 4-mol $liter^{-1}$ amount of HCl (1 ml g of $sediment^{-1}$) was found to inhibit demethylating activity in the sediment completely (Table 1). It stopped microbial activity immediately and eliminated the need to acidify samples prior to stripping the volatile ^{14}C. It should be noted that HCl was also used to stop biological activity in samples for mercury methylation (12). Demethylation samples could be killed and stored for at least 24 h before stripping without further biological degradation of the $^{14}CH_3HgI$.

Fourteen pairs of replicate sediment samples from various lakes were analyzed for demethylating activity, and the mean percent coefficient of variation was ±8.8%.

Various concentrations of methyl mercury were added to Lake 239 sediment to examine the possible effects of methyl mercury concentration on the demethylating activity of the microorganisms (Fig. 2). Specific rates of demethylation increased linearly with concentrations up to 44 μg of Hg g^{-1}. Above this concentration the rate of increase slowed and appeared to reach a plateau. Concentrations up to 140 μg of Hg g of $sediment^{-1}$ were not inhibitory to demethylation.

Time course studies (Fig. 3) ensured that the specific rates of demethylation were linear during the incubation period. Rates were linear over a period of 24 h (r = 0.996; n = 7).

In a comparison of oxic and anoxic incubation conditions (Table 1), it was found that demethylation was significantly faster (P = 0.05) when samples were incubated with air rather than nitrogen in the headspace of the incubation vessel.

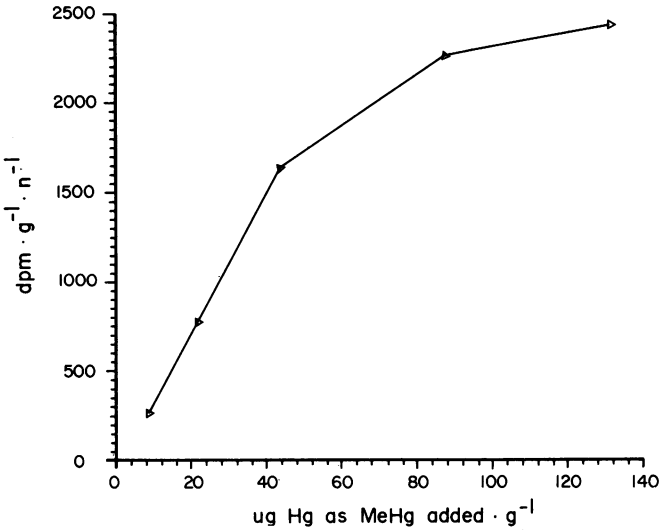

FIG. 2. Effect of various concentrations of methyl mercury on microbial demethylation in Lake 239 sediment.

The differences in both specific methylation and demethylation rates in samples collected from offshore and flooded inshore sites in the shallow bay at Southern Indian Lake were notable (Table 2). The offshore (clay-rich) sediment had low demethylation rates and almost undetectable methylation rates. The inshore (organic) sites had rapid rates of demethylation (0.184 to 0.267% added Hg g^{-1} h^{-1}). The inshore methylation rates were considerably faster than those measured in the offshore sites. The inshore sites always had higher M/D ratios than offshore sites, indicating that these sites also had higher specific rates of net methyl mercury production.

DISCUSSION

The incubation time required for measurable demethylation is short (<12 h) and there is no evidence of a change in

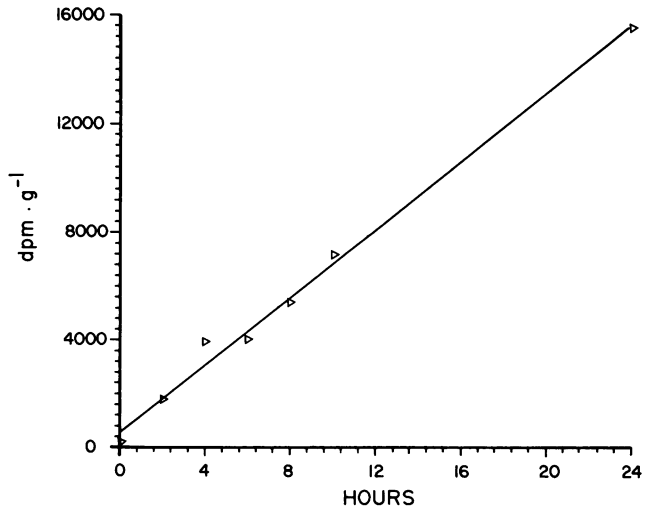

FIG. 3. Time course of [^{14}C]methyl mercury degradation (r = 0.996).

TABLE 2. Rates of biological methylation (M) and demethylation (D) of mercury by sediments collected from three stations at Methyl Bay, Southern Indian Lake, in 1983[a]

Station	19 July			1 August		
	D	M	M/D	D	M	M/D
Lake clay (5.2 m)	0.023	0.003	0.11	0.067	0.001	0.02
Organic floc (2.6 m)	0.205	0.149	0.73	0.267	0.220	0.83
Organic floc (1.7 m)	0.134	0.020	0.15	0.184	0.025	0.14

[a] As measured by added Hg methylated or demethylated per gram per hour; average of duplicate samples.

the kinetics of demethylation over this period (Fig. 3). Other methods for study of methyl mercury degradation required much longer incubation times to detect demethylation (21). The amount of [^{14}C]methyl mercury added to samples for our method is enough to overwhelm the ambient methyl mercury (0.001 to 0.02 μg of Hg g^{-1} [13]; 0.01 to 0.03 μg of Hg g^{-1} [1]), which means this is not a conventional radiotracer method; however, the amount of methyl mercury added is considerably less than has been used in previous studies (3, 21), and it does not appear to be toxic to the demethylators (Fig. 2). Because the calculated rates are mercury specific, they can be converted to absolute rates in those situations where Hg^{2+} and CH_3Hg^+ can be measured.

A major advantage of the method reported here is that it can be used in conjunction with the method developed by Furutani and Rudd (12) for the measurement of specific rates of mercury methylation; thus it is possible to assess both of the microbial processes controlling methyl mercury concentration in the environment. The specific rates of each process can be calculated in terms of the proportion of added mercury methylated or demethylated (equations 1 to 3); this allows calculation of a ratio of M/D. This ratio is a measure of the relative balance of methylating and demethylating activity. The M/D ratio may be of use in identifying potential sites of accelerated methyl mercury production, and it can be used to determine the effect of various environmental factors (e.g., oxygen) on the microflora's propensity to net methylation or demethylation.

In the past, investigators have not agreed on what effect, if any, oxygen has on net methyl mercury production (5 in 14, 15, 17). Variable rates of methyl mercury production may have been due to different demethylating capabilities of the sediment (depending on the availability of oxygen) (17). For example, we found that demethylation in Lake 239 sediments was enhanced in the presence of oxygen (Table 1). Mercury methylation studies with Lake 239 sediment (19; P. S. Ramlal, M.S. thesis, University of Manitoba, Winnipeg, 1983) found that the specific rate of mercury methylation was lower when samples contained oxygen. Therefore, it would appear that the net production of methyl mercury would be reduced when oxygen was near the sediments of the lake. While these experiments are not conclusive, they do serve to point out that the combination of the methylation and demethylation methods (12; this paper) will be useful when examining individual factors such as oxygen which contribute to the control of the net rate of mercury methylation.

As a test of using these methods together, we measured, on two occasions, the M/D ratio at one offshore and two inshore sites of Methyl Bay, an isolated bay in Southern Indian Lake. On both occasions the observed M/D ratio was consistently higher at the inshore sites where organic-rich flooded soils were being sampled. Apparently, the inshore sites favored mercury-methylating bacteria to a greater extent than demethylating organisms, although both processes were enhanced inshore. Similar results have since been found in several other sites affected by reservoirs (Ramlal, unpublished data). These data suggest that flooding of the soils during construction of a reservoir would increase the rate of net methyl mercury production by creating new, highly active sites of net methyl mercury production. These data agree with a comprehensive study of the effects of flooding on the mercury levels in fish of the Southern Indian Lake reservoir (6–8). As a result of flooding after construction of the reservoir, the mercury levels of muscle samples of northern pike (*Esox lucius*) and walleye (*Stizostedion vitreum vitreum*) increased from 0.2 to 0.3 to 0.5 to 1.0 μg g^{-1}, respectively (7), jeopardizing a large commercial fishery (6–8).

These two methods have also been successfully used to study the effects of acidification of lakes on rates of net methyl mercury production. In one recent study, it was found that acidification results in a decrease in the rate of net methyl mercury production in near-surface anoxic sediments (18). In a subsequent study it was found that acidification increased the rate of net methyl mercury production in lake water and in epilimnetic sediment whose surface was oxic (L. Xun, M.S. thesis, University of Manitoba, Winnipeg, 1985).

Although it is not possible to measure absolute rates of methylation or demethylation without knowing the biologically available concentrations of inorganic and methyl mercury, the specific rate measurements do provide the means to determine whether certain environmental perturbations will tend to increase or decrease methylating or demethylating activity in comparative time series spatial studies. When they are used together, comparisons of M/D ratios indicate how environmental disturbances affect the net rate of mercury methylation and hence the methyl mercury concentrations in aquatic environments. The M/D ratio can also be used to locate potential sites of enhanced net methyl mercury production.

ACKNOWLEDGMENTS

A. Furutani provided helpful advice and suggestions throughout the project. D. Muir confirmed the purity of the crystalline ^{14}C-labeled methyl mercuric iodide. C. Anema assisted in the collection of samples from Methyl Bay. P. Campbell, A. Furutani, R. Hesslein, J. Klaverkamp, K. E. Marshall, S. G. Lawrence, D. W. Schindler, and M. A. Turner provided valuable criticism of the manuscript.

We also thank the Canada-Manitoba Agreement on the Study and Monitoring of Mercury in the Churchill River diversion and the Canadian Department of Fisheries and Oceans for their financial support.

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