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Article

Production of Organic Mercury from Hg^0: Experiments Using Microcosms

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A produção de $Hg_{orgânico}$ em água e sedimento a partir de Hg^{2+} é um processo conhecido, embora pouco se saiba sobre a produção de $Hg_{orgânico}$ a partir do substrato Hg^0 em ambientes tropicais. Neste trabalho, usando microcosmos contendo água:sedimento seco na proporção de 6:1 e contaminado com 0,1% (m/m) de Hg^0, acompanhou-se a produção de $Hg_{orgânico}$ na água e no sedimento, sob condições aeróbias e anaeróbias. Constatou-se que esta é dependente da concentração de matéria orgânica presente no sedimento. A taxa de produção de $Hg_{orgânico}$ no sedimento foi maior no meio anaeróbio (617 μg kg^{-1} dia^{-1}) do que no aeróbio (280 μg kg^{-1} dia^{-1}). A avaliação abiótica da produção de $Hg_{orgânico}$ não foi possível devido às alterações nas características do microcosmo quando comparado ao experimento conduzido sob condições aeróbias. Conclui-se que o Hg^0 lançado no ambiente, principalmente através da atividade de garimpo, sofre uma dissolução reativa em água podendo ser um bom substrato para a produção de $Hg_{orgânico}$ em ambientes tropicais.

Transformation of inorganic to organic mercury is a process known to occur in the water column as well as in sediment. However, little is known about the fate of metallic mercury in tropical environments. In this work the production of organic mercury in water and sediment under aerobic and anaerobic conditions was demonstrated in a microcosm setup using a 6:1 water: dry sediment ratio, after a spike of 0.1% (w/w) of Hg^0. The rate of production of organic mercury in sediment was higher under anaerobic conditions (617 μg kg^{-1} day^{-1}) than that obtained under aerobic conditions (280 μg kg^{-1} day^{-1}). An attempt to evaluate the production of organic mercury in abiotic conditions was not possible due to drastic changes in the microcosm during sterilization when compared to the experimental conditions maintained under biotic conditions. It was concluded that metallic mercury, which enters the environment mainly due to gold-mining activities, undergoes a reactive dissolution in the aquatic medium, thus becoming a suitable substrate for the production of organic mercury in tropical environments.

Keywords: organic and reactive mercury, dissolved organic carbon, microcosm, metallic mercury

Introduction

Mercury is one of the most hazardous contaminants that may be present in the aquatic environment and its ecotoxicological effects are strongly dependent on the chemical species present.[1] The most toxic mercury species is methylmercury (MeHg) a compound responsible for the vast majority of human poisoning by this metal. Organometallic mercury species are neurotoxic, cause blockage of binding sites of enzymes, interfer in protein synthesis, inhibit thymidine incorporation into DNA, and tend to accumulate in sediments and in the biota, particularly fish and mollusks.[2-4]

Metallic mercury has been considered as non-reactive in water bodies due to its low solubility. The metallic form used in the *garimpos* can reach the aquatic system either by direct spills of liquid droplets, or by fugitive atmospheric emission during the roasting of the Au-Hg amalgam. Use of mercury in gold mining has resulted in an estimated total release of 100 ton $year^{-1}$, 40-45 % of which directly discharged into aquatic systems.[5] Several studies have been carried out in order to determine the environmental conditions that favor or suppress the formation of organic mercury from Hg^{2+} in the aquatic environment.[6-8] Considering that the literature about the fate of metallic mercury in the environmental system (and more specifically tropical waters and sediments) is scarce, evaluating the real impact of the release of metallic mercury is very important for understanding of the metal cycle,[9] especially in tropical areas.

Factors that control the rate of net conversion of Hg^0 to MeHg in the environment are currently the focus of several studies; nonetheless, a complete understanding has remained elusive. Nowadays it is believed that the rate

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and the extent of methylation of Hg^{2+} in waters and sediments depend upon many factors such as the mercury salt (acetate is easier to methylate than mercury chloride), the methylation agent, the chemical composition of the sediment, the oxygen concentration, the redox potential, the organic matter, the presence of inorganic and organic complexing agents, temperature, pH and, others.[1, 10, 11]

Organomercurial compounds may be present in surface waters due to natural processes, such as biomethylation of inorganic Hg,[12-14] or a consequence of human activity, since these compounds have been widely used in the past as fungicides, slimicides, or industrial catalysts. Use of organomercurials in the agriculture was banned in many parts of the world, therefore today the transformation of inorganic Hg is the predominant source of organomercurial compounds in the aquatic systems.[10]

The study presented in this paper was carried out to evaluate the potential production of organic mercury from Hg^0, using conditions that would mimic tropical microcosm, under both aerobic and anaerobic conditions.

Experimental

Mercury determination

All glassware, including Teflon bottles, were rinsed as described elsewhere.[15] The several forms of mercury were determined by using a Brooks Rand (Seattle, WA) cold vapor atomic fluorescence spectrophotometer (CVAFS), and gold coated quartz-sand packed columns for mercury pre-concentration. This research was carried out in the Environmental Chemistry Laboratory that takes part in the Mercury Quality Assurance Program sponsored by the Canadian Food Inspection Agency for Mercury in biological materials.

Determination of organic mercury in waters and sediments using MeHg as surrogate

The method for water was performed according to the procedure described by Bloom,[16] except for the fact that time of extraction was reduced to 14 h at 150 rpm in an orbital shaker. For sediments, the method used was adapted from Hintelmann,[17] where the sediment suspension was sonicated for 15 min using a 2210 Bransonic ultra sound bath (47 kHz ± 6%) at 234 W, followed by the addition of 10 mL of dichloromethane, and extracted for 14 h at 150 rpm in an orbital shaker.

In both procedures, the organic layer was transferred to a flask containing 100 mL of ultra pure water (MilliQ; Millipore Plus), and the organic layer was purged with N_2 for 2 h to remove the solvent, leaving the extracted MeHg in the aqueous phase. After this step, BrCl (1.0 mL of a 0.02 mol L^{-1} solution) was added to the sample, it was left to rest for 30 min and, finally, a 400 μL aliquot of a 30 % (w/v) $NH_2OH.HCl$ solution was added. Mercury was determined by CVAFS using a 10% (w/v) stannous chloride in 10% (v/v) HCl as the reducing agent. It is important to mention that this procedure is not specific for methyl mercury and, since the speciation of the most usual forms of organic mercury is not possible using this procedure, the term organic mercury is used instead of methyl mercury. However, all recovery tests were performed using MeHg as surrogate for the organic species.

Microcosms setup

Microcosms were assembled in acid washed 4-L glass bottles (Figure 1) using sediments and water from Calado Lake (Branco River) and Iara Lake (Negro River), both rivers from the Amazon basin. For the microcosm kept under aerobic conditions, a constant flow of Hg-free air (300 mL min^{-1}) was maintained throughout the experiment. To keep the microcosm under anaerobic condition, nitrogen was used instead of oxygen. Microcosms were incubated at 23 ± 3 ºC for a time period of 29 up to 33 days. Water samples (250 ml) were collected from both microcosms using polyethylene terephthalate (PET) bottles every other day, and the following parameters were determined: pH, E_H (redox potential), dissolved organic carbon (DOC), gaseous dissolved mercury (GDM), reactive mercury, total and organic mercury. At the beginning (day 0) and end (day 33 for aerobic and day 29 for anaerobic conditions) of the experiments, total mercury, organic mercury, and CHN were measured in the sediment.

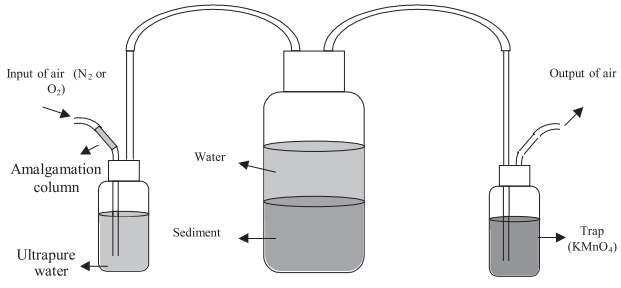

Figure 1. Experimental setup used in the microcosms.

Microcosms kept under biotic conditions

A microcosm assembled to evaluate the transformation rate of metallic mercury into the organic form was set up by incubating 500 g of wet sediment samples and 2500 g

of water sample with 0.50 g of Hg^0 (Curtin Scientific Co.). Homogenization of Hg^0 in the sediments was made by mechanical stirring with a glass rod for 2 h.

Microcosms under abiotic conditions

To evaluate the microbial role in the generation of organomercurial species, one microcosm was setup as described above, and all materials including the water and the sediment were sterilized in an autoclave for 40 min at 120 ºC and 98 kPa. The purpose of this pretreatment was to inhibit all possible microbial activity that could lead to the transformation of the metallic mercury in the microcosm.

Results and Discussion

In an earlier work,[9] the authors showed that metallic mercury is reactive in natural waters, undergoing reactive (oxidative) dissolution in two steps (equations 1 and 2). This process alters the speciation of mercuric forms, leading to the production of mercury ions, which are more toxic, more suitable to methylation, and more mobile species than metallic mercury. Evidence of reactive dissolution of elemental mercury in an aqueous medium means that the production of organic mercury from Hg^0 under aerobic condition is feasible.

$$Hg^0_{(l)} \leftrightarrow Hg^0_{(aq)} \quad (1)$$

$$Hg^0_{(aq)} \leftrightarrow Hg^{2+}_{(aq)} + 2\ e^- \quad (2)$$

Figure 2 illustrates the behavior of reactive mercury in the aqueous phase of the microcosms as a function of time. Reactive mercury is operationally defined, and represents the fraction of mercury that may be reduced by stannous chloride. Since organic species are usually not reduced by this procedure, reactive mercury can be part or, at the most, the total stock of inorganic mercury in the sample. It is observed that after 12 days (284 h), production of reactive mercury reaches a maximum, with a rate of 0.44 ng L^{-1} h^{-1} (aerobic conditions) and 0.26 ng L^{-1} h^{-1} (anaerobic conditions). Thereafter, for both ambients, the concentration drops due to a consumption process, reaching apparent steady state conditions around 80 ng L^{-1} of reactive mercury for both anaerobic and aerobic conditions. With this behaviour, it is possible to foresee that reactive dissolution of metallic mercury occurs and seems to be the limiting process in the generation of organic mercury.

Figure 3 shows the variation in the concentration of organic mercurial species in the same condition as the ones shown for reactive mercury in Figure 2. For both aerobic and anaerobic conditions, until 12 days (284 h), organic mercury is virtually absent in the aqueous phase, most likely due to absence of suitable substrates, already. After the 12th day of monitoring the microcosms, the production of organic mercury increases for both conditions, reaching a maximum at the 19th day. During these 7 day periods, the rate of organic mercury production was more intense under anaerobic conditions (2.96 ng L^{-1} h^{-1}) relative to aerobic conditions (0.71 ng L^{-1} h^{-1}). After this period of time, both systems reached steady state conditions but, interestingly, the final concentration of organic mercury obtained under anaerobic condition is about twice that observed for the aerobic microcosms.

Based upon these results, the following mechanism for the production of organic mercury from Hg^0 in tropical water bodies is proposed. Firstly, the reactive dissolution

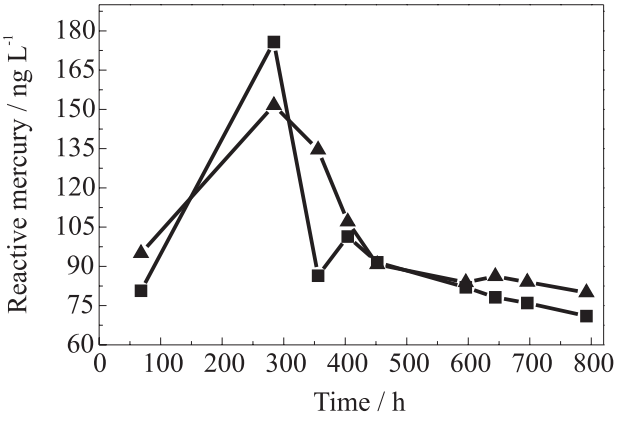

Figure 2. Variation in the concentration of reactive mercury as a function of time for the aqueous phase of the microcosms under aerobic (■), and anaerobic conditions (▲) for a 6:1 water: dry sediment ratio. The microcosms used sediments from the Iara Lake (black water).

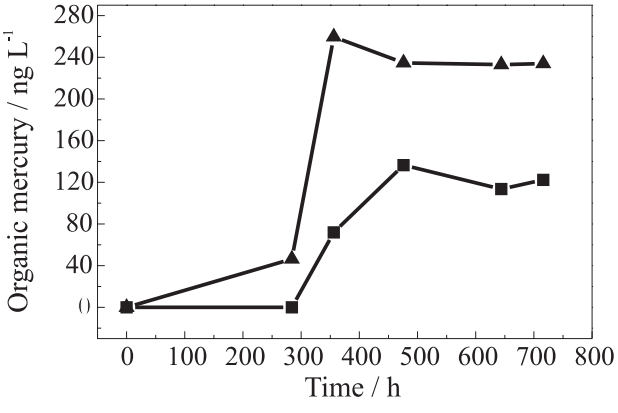

Figure 3. Variation in the concentration of organic mercury as a function of time for the aqueous phase of the microcosms under aerobic (■), and anaerobic conditions (▲) for a 6:1 water: dry sediment ratio. The microcosms used sediments from the Iara Lake (black water) .

of the metallic species produces Hg^{2+}; the concentration of this substrate builds up in the aqueous media and reaches an optimal condition for the transformation (most likely methylation) to the organic mercury species. Production of organic mercury occurs[18] according to equations 3 and 4, where both mechanisms may be occurring in the microcosms.

$$Hg^0 \xrightarrow[\text{Biotransfer of } CH_3 \text{ group}]{} (CH_3)_2Hg + CH_3Hg^+ \quad (3)$$

$$Hg^0 \xrightarrow{\text{Oxidization by } O_2} Hg^{2+} \xrightarrow[\text{group}]{\text{Chemical transfer of } CH_3} CH_3Hg^+ \quad (4)$$

Table 1 shows some key physico-chemical parameters monitored in both water and sediment of the microcosms. It is interesting to note that even on the last day, it is possible to measure both reactive and gaseous dissolved mercury. This indicates that, even though a stock of mercury is still available for methylation, the methylation/demethylation balance appears to have reached a steady state condition.

The microcosm kept under aerobic conditions presents a redox potential between + 470 and + 505 mV. For the anaerobic condition, the variation observed was between + 196 and +315 mV. Maximum methylation happens in the redox potential range between +100 mV at +200 mV, MeHg being more stable in neutral and acid conditions.[19] This may explain why the formation of organic mercury was higher under anaerobic conditions. The pH (Table 1) decreased as a function of the time, reaching the value of 5.1, at the end of the experiment. According to Compeau and Bartha,[20] an environment with values of positive redox potential and low pH values also favors methylation using Hg^{2+} as substrate.

As far as the sediment is concerned (Table 1), the concentration of $Hg_{organic}$ was similar in both aerobic and anaerobic conditions, showing that organic mercury was produced from Hg^0 under both conditions. The average rate of organic mercury production were 280 and 617 μg kg^{-1} day^{-1} for aerobic and anaerobic conditions, respectively, in a sediment with 9.5% total carbon content. It is worth mentioning that in an experiment using a sediment with an organic content of 2.5% carbon, the average rate of organic mercury production in sediment of the microcosm under aerobic conditions was 22 μg kg^{-1} day^{-1}, compared to 104 μg kg^{-1} day^{-1} obtained under anaerobic conditions. These results clearly indicate the influence of the organic matter in the production of organic mercury in sediments.

An evaluation of the microbial role in the processes of generating organic mercury was not possible due to two major points: *i*) the drastic changes in the release of organic matter caused in the sediment after autoclave sterilization, and *ii*) the contamination of the microcosms after the manipulation necessary to take water samples during the course of the experiment. The microcosm originally set under abiotic conditions was kept under these conditions for only 12 days, when reducting sulfate bacteria[21] started to be detected. In this time the DOC in the aqueous phase of this microcosm increased 333 times, compared with the same experiment that was not sterilized.

Conclusions

The results obtained using microcosms under biotic conditions indicate that Hg^0 is transformed quickly to organic mercury in both aerobic and anaerobic conditions, and that this process occurs to a higher extent in the absence of oxygen. In the Amazon region, considering the presence of large watersheds with black waters with low pH values, high concentrations of organic matter and high densities

Table 1. Variation in some measured parameters of the aqueous phase and sediment of the microcosms (biotic conditions) for the first day and final days; Final Day (day 33 for aerobic and day 29 for anaerobic conditions, respectively)

	First day	Final day	
		Aerobic condition	Anaerobic condition
		Water	
pH	6.90 ± 0.02	5.10 ± 0.01	5.80 ± 0.03
E_H/ mV	479 ± 1	481 ± 1	279 ± 1
DOC/ mg L^{-1}	1.3	1.8	13.4
Total Hg/ ng L^{-1}	< 0.02	3710 ± 162	5401 ± 323
Reactive Hg/ ng L^{-1}	< 0.02	125.2 ± 8.2	169.2 ± 7.1
Organic Hg/ ng L^{-1}	< 2.0	113.5 ± 4.7	232.9 ± 1.4
GDM[a]/ ng L^{-1}	-	40.8 ± 0.3	9.1 ± 0.2
		Sediment	
Total carbon/ %	9.5	9.5	9.5
Total Hg/ mg kg^{-1}	90.1 ± 1.0	1221 ± 87	938 ± 85
Organic Hg/ mg kg^{-1}	$5.2x10^{-3} \pm 0.1x10^{-3}$	0.50 ± 0.01	0.60 ± 0.01

[a] *Gaseous Dissolved Mercury.*

of microorganisms, all of which are the optimal conditions required for methylation, the transformation of metallic mercury to more toxic organic forms occurs. Thus, the continued use of metallic mercury may pose an additional threat to the aquatic systems in this region.

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