Chemosphere 167 (2017) 438–443

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

# Mercury methylation and sulfate reduction rates in mangrove sediments, Rio de Janeiro, Brazil: The role of different microorganism consortia

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## HIGHLIGHTS

- Biotic Hg methylation occur in mangroves but which microorganisms are involved?
- Inhibition of sulfate-reducing bacteria and prokaryotes reduced MeHg formation and SRR.
- Inhibition of methanogens either reduced or increased methylation in different sites.
- Iron amendment stimulated Hg methylation in low and inhibited in high concentrations.
- SRB are important Hg methylators but other groups (IRB, methanogens) may be involved.

## ARTICLE INFO

*Article history:*
Received 27 July 2016
Received in revised form
21 September 2016
Accepted 30 September 2016

Handling Editor: Caroline Gaus

*Keywords:*
Liquid scintillation
Radiotracer
Sulfate-reducing bacteria
Iron-reducing bacteria
Methanogens
Fungi

## ABSTRACT

Recent studies have shown Hg methylation in mangrove sediments, however, little is known about the different microorganism consortia involved. We investigated the participation of prokaryotes in general, iron-reducing bacteria-IRB, sulfate-reducing bacteria-SRB, methanogens and fungi in Hg methylation and sulfate reduction rates (SRR) in mangrove sediments using iron amendments for IRB and specific inhibitors for the other microorganisms. Sediment samples were collected from two mangrove zones, tidal flat and mangrove forest (named root sediments). Samples were incubated with $^{203}Hg$ or $^{35}SO_4^{2-}$ and $Me^{203}Hg/^{35}Sulfur$ were measured by liquid scintillation. Methylmercury (MeHg) formation was significantly reduced when SRB (87.7%), prokaryotes (76%) and methanogens (36.5%) were inhibited in root sediments, but only SRB (51.6%) and prokaryotes (57.3%) in tidal flat. However, in the tidal flat, inhibition of methanogens doubled Hg methylation (104.5%). All inhibitors (except fungicide) significantly reduced SRR in both zones. In iron amended tidal flat samples, Hg methylation increased 56.5% at 100 μg $g^{-1}$ and decreased at 500 and 1000 μg $g^{-1}$ (57.8 and 82%). In the roots region, however, MeHg formation gradually decreased in response to Fe amendments from 100 μg $g^{-1}$ (37.7%) to 1000 μg $g^{-1}$ (93%). SRR decreased in all iron amendments. This first simultaneous evaluation of Hg methylation and sulfate-reduction and of the effect of iron and inhibitors on both processes suggest that SRB are important Hg methylators in mangrove sediments. However, it also suggests that SRB activity could not explain all MeHg formation. This implies the direct or indirect participation of other microorganisms such as IRB and methanogens and a complex relationship among these groups.



## 1. Introduction

Methylmercury (MeHg) formation is an important process in mercury (Hg) biogeochemistry due to MeHg neurotoxicity and capacity to bioaccumulate in organisms and to bioamplify through food webs (Chen et al., 2008). Mercury methylation in soils and sediments is influenced by a number of factors such as Hg bioavailability, the activity of methylating bacteria, the structure of the microbial community, pH, redox potentials, availability of nutrients and electron acceptors, as well as the presence of ligands

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http://dx.doi.org/10.1016/j.chemosphere.2016.09.153
0045-6535/

and adsorbing surfaces. All these factors often interact with one another resulting in a complex system with synergistic and antagonistic effects (Ullrich et al., 2001). Biological Hg methylation has been mainly attributed to the activity of sulfate-reducing bacteria (SRB) (Compeau and Bartha, 1985; King et al., 2000), iron-reducing bacteria (IRB) (Fleming et al., 2006; Kerin et al., 2006; Schaefer and Morel, 2009) and methanogens (Hamelin et al., 2011; Yu et al., 2012).

Mangroves are diverse and very productive ecosystems that supply a variety of environmental services, such as shore line protection and refuge and nursery for a dense and economically important fauna, among many others. Despite all man-made pressures on mangroves, including Hg pollution, little is known about Hg biogeochemistry in these complex and highly dynamic coastal ecosystems. Recent studies have shown significant Hg methylation in mangroves specially in superficial sediments, and also how crab bioturbation (Correia and Guimarães, 2016) and the complex root systems of mangrove trees (Oliveira et al., 2015) both enhance this process through their effects on sediment oxygen, Eh and organic matter, among others. However, it is not yet clear which microorganisms are involved in Hg methylation in this environment. Correia and Guimarães, 2016 found a correlation between MeHg formation and sulfate reduction rates (SRR) in mangrove sediment influenced by mangrove tree roots, suggesting an involvement of SRB in the process. On the other hand, in mangrove sediments in China, there was no correlation between MeHg concentrations and the presence of SRB strains which led the authors to conclude that other microorganisms could be involved (Wu et al., 2011). Studies with specific metabolic inhibitors in freshwater sediments yielded similar findings (Correia et al., 2012; Hamelin et al., 2011).

In contrast with sulfate-reduction and methanogenesis, the role of iron-reduction in Hg methylation cannot be studied with a specific inhibitor, since none has been found or developed to date. However, iron amendments can yield useful, though indirect evidence on iron-reduction. Previous studies in river and estuarine sediments showed that iron additions in low concentrations can enhance Hg methylation but inhibits the process as iron concentrations increase (Han et al., 2008; Yu et al., 2012). The only study of iron amendment in mangrove sediments so far showed the same pattern for SRR (Attri et al., 2011).

Therefore, the objective of the present study is to analyze the involvement of certain groups of microorganisms (IRB, prokaryotes in general, SRB, methanogens and fungi) in Hg methylation and SRR in mangrove sediments of Sepetiba Bay, state of Rio de Janeiro, using iron amendments for IRB and specific metabolic inhibitors for the other groups of microorganisms.

## 2. Materials and methods

### 2.1. Sampling area

Sediment samples were collected at the moderately preserved Coroa Grande mangrove, located in Sepetiba Bay, Rio de Janeiro, Brazil (Lacerda et al., 2001; Molisani et al., 2004). GPS coordinates for the location are 22° 54′ 40.18″ S e 43° 52′45.81″ O. Samples for MeHg formation experiments were taken in October 2015 and for SRR in January 2016.

Superficial sediment from each area (0–5 cm) and local water were manually collected and placed in plastic containers and bottles and subsequently transported to the laboratory. Sediment was collected from two distinct zones of the mangrove: the tidal flat and the forested region. Sediments from the latter zone (called roots from now on) presented a dense network of roots from *Avicennia shaueriana* and *Rhizophora mangle*. All samples were stored at 4 °C until incubation.

Redox potential and pH were measured in each region with a Lutron PH-206 pH/mV/Temperature meter and sulfate concentrations were determined following Tabatabai (1974) modified by King et al., 1999. Total iron in sediment was extracted via acid digestion ($HNO_3$, HF and $HClO_4$) of 0.5 g d.w. samples in closed Teflon bombs at 150 °C to near dryness and dissolved in 0.1 M HCl. Iron concentrations in this digest were measured using a flame atomic absorption spectrophotometer (Varian AA-1475). Analytical quality control was based on the analysis of certified reference material BCSS-1 (marine sediment).

### 2.2. Methylation assays

#### 2.2.1. Incubations

Fresh sediment samples were homogenized manually and amounts equivalent to 0.5–1.0 g dry weight were placed in Teflon screw-capped 50 mL tubes filled with 20 mL of local water. Each essay included one control, killed with 1 mL 4 N HCl, and 4–5 replications. After addition of $^{203}HgCl_2$ (obtained from Eckert and Ziegler Isotope Products Laboratories, USA) and iron and/or inhibitors samples were incubated for 48 h in the dark at in situ water temperature (22–28 °C). Incubations were terminated by the addition of 1 mL 4 N HCl and samples were kept at −18 °C until extraction. Total added Hg concentrations were equivalent to 10 ng Hg $g^{-1}$ (d. w.).

#### 2.2.2. Treatments with metabolic inhibitors

All treatments contained sediment from the tidal flat or root region and included (1) control (without any inhibitor); (2) the general bacteriostatic inhibitor chloramphenicol (0.2 mM); (3) the mixture of antibiotics penicillin (300 μM) and streptomycin (172 μM); (4) sodium molybdate, a sulfate reduction inhibitor (20 mM); (5) BESA 5 mM (2-Bromoethanesulfonic acid), a methanogenesis inhibitor; (6) Chloroform (251 μM), also a methanogenesis inhibitor and (7) cyclohexamide (355 μM), an eukaryotic inhibitor. Each treatment had 4-5 replications and was acclimatized for 1 h before addition of $^{203}HgCl_2$ and incubated as described above.

#### 2.2.3. Ferric iron amendment

Approximately 0.5–1.0 g (d.w.) of sediment and 20 ml of local water were placed in Teflon screw-capped 50 ml tubes and then amended with water soluble ferric chloride stock solutions to give final concentrations of 50, 100, 250, 500 and 1000 μg $g^{-1}$ (considering the volume of solution and previously added local water). Controls without amendments were also included. Each treatment had 4-5 replications. The resulting sample slurries were acclimatized for 1 h and then spiked with $^{203}HgCl_2$. Incubations lasted 48hs in the dark and were terminated by the addition of 1 mL 4 N HCl. Samples were kept at −18 °C until extraction.

#### 2.2.4. MeHg extractions

MeHg was extracted as described in Guimarães et al. (1995). After addition of NaBr and acid $CuSO_4$ solutions, shaking and centrifuging, $Me^{203}Hg$ was extracted from the supernatant of the sediment samples with scintillation cocktail (toluene with the scintillation salts 2,5 diphenyloxazole and 1,4- bis-2(5phenyloxazolil)-benzene) and measured by beta counting on a Perkin Elmer TriCarb 2800 TR scintillation counter. The results are expressed as the percentage of total $^{203}Hg$ that was converted into $Me^{203}Hg$. The validity of the assumption that all activity in the final extract is $Me^{203}Hg$ was confirmed by back-extraction in $Na_2S$ and then in benzene, followed by separation by thin-layer chromatography and quantification by liquid scintillation (Brito and