



Mercury methylation rates of biofilm and plankton microorganisms from a hydroelectric reservoir in French Guiana

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ABSTRACT

The Petit-Saut ecosystem is a hydroelectric reservoir covering 365 km² of flooded tropical forest. This reservoir and the Sinnamary Estuary downstream of the dam are subject to significant mercury methylation. The mercury methylation potential of plankton and biofilm microorganisms/components from different depths in the anoxic reservoir water column and from two different sites along the estuary was assessed. For this, reservoir water and samples of epiphytic biofilms from the trunk of a submerged tree in the anoxic water column and from submerged branches in the estuary were batch-incubated from 1 h to 3 months with a nominal 1000 ng/L spike of Hg(II) chloride enriched in ¹⁹⁹Hg. Methylation rates were determined for different reservoir and estuarine communities under natural nutrient (reservoir water, estuary freshwater) and artificial nutrient (culture medium) conditions. Methylation rates in reservoir water incubations were the highest with plankton microorganisms sampled at −9.5 m depth (0.5%/d) without addition of biofilm components. Mercury methylation rates of incubated biofilm components were strongly enhanced by nutrient addition. The results suggested that plankton microorganisms strongly contribute to the total Hg methylation in the Petit-Saut reservoir and in the Sinnamary Estuary. Moreover, specific methylation efficiencies (%Me¹⁹⁹Hg_{net}/cell) suggested that plankton microorganisms could be more efficient methylating actors than biofilm consortia and that their methylation efficiency may be reduced in the presence of biofilm components. Extrapolation to the reservoir scale of the experimentally determined preliminary methylation efficiencies suggested that plankton microorganisms in the anoxic water column could produce up to 27 mol MeHg/year. Taking into account that (i) demethylation probably occurs in the reservoir and (ii) that the presence of biofilm components may limit the methylation efficiency of plankton microorganisms, this result is highly consistent with the annual net MeHg production estimated from mass balances (8.1 mol MeHg/year, Muresan et al., 2008a).

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1. Introduction

Mercury (Hg) is considered a major pollutant since exposures to this heavy metal have environmental and human health consequences from femto- to nanomolar concentrations (Boening, 2000; Gochfeld, 2003; Zahir et al., 2005). Its environmental cycle is highly dynamic and complex, involving reduction/oxidation or methylation/demethylation with biotic or abiotic pathways (Rocha et al., 2000; Barkay and Wagner-Döbler, 2005; Garcia et al., 2005; Celo et al., 2006; Desrosiers et al., 2006; Poulain et al., 2007). Monomethylmercury CH₃Hg⁺ (MeHg) is easily bioaccumulated and biomagnified in aquatic food chains, mainly due to fish consumption, and has serious negative effects on human health (Fréry et al., 2001; Baeyens et al., 2003; Li et al., 2008).

In French Guiana, Hg levels in hair samples of more than half of a native Amerindian people were higher than the maximum value recommended by the World Health Organization (10 µg g⁻¹), i.e. at a level that may cause neurological disorder, especially for children (Fréry et al., 2001). In Amazonia, the main mercury sources originate from gold-mining practices, including erosion of soils naturally enriched mercury (Boudou et al., 2005; Roulet et al., 1999). Great lakes and many hydroelectric reservoirs are preferential sites of MeHg production because of stratification and development of anoxic hypolimnion (Rosenberg et al., 1997; Pak and Bartha, 1998; Bellanger et al., 2004; Eckley and Hintelmann, 2006). The Petit-Saut Reservoir, on the Sinnamary River in French Guiana (South America), is a model system for studies on Hg behavior in tropical reservoirs (Coquery et al., 2003; Boudou et al., 2005; Peretyazhko et al., 2005; Peretyazhko et al., 2006; Muresan et al., 2008a). It was constructed on ancient gold-mining sites and still receives large amounts of Hg from its watershed due to gold mining (increasing soil erosion) and -extraction by amalgamation. About 15 kg Hg y⁻¹ are provided to the reservoir by the influent rivers,

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and especially by the Leblond River affected by gold-mining activities (Coquery et al., 2003). The filling of this reservoir has started in January 1994 resulting in the immersion of 365 km² of tropical rainforest and naturally Hg-rich soils. Biomass immersion has led to strong disturbances of biogeochemical cycles in both the reservoir and the Sinnamary Estuary (Richard et al., 1997; De Mérona et al., 2005).

Mercury methylation in the anoxic waters of the reservoir and in the Sinnamary Estuary downstream of the dam induces high Hg concentrations in fish representing a health risk for regional human populations depending on fish-diet (Durrieu et al., 2005; Dominique et al., 2007; Muresan et al., 2007, 2008b). In 2004/2005, the mean Hg concentrations in dorsal skeletal muscle of *Curimata cyprinoides*, a detritivorous/benthivorous fish species, were 10-fold higher in fish sampled downstream ($3400 \pm 240 \text{ ng g}^{-1}$) compared to fish sampled in the reservoir ($320 \pm 50 \text{ ng g}^{-1}$) and the MeHg fraction was close to 100% (Dominique et al., 2007). This fish species feeds by grazing immersed surfaces (rocks, tree branches and trunks, roots, etc.), recovered by an abundant slime assumed to be a biofilm. Biofilms are complex associations of multi-species microorganisms embedded in a polymeric matrix and adhering to a surface, usually in aqueous environments. This is assumed to be the preferential microbial way of life in the environment (De Beer and Stoodley, 2006). Previous work on biofilms sampled on glass sheets in the oxic water column (0–6 m depth) of the reservoir and in the Sinnamary Estuary reported high MeHg contributions to total Hg content ($28 \pm 6\%$ and $40 \pm 4\%$, respectively), suggesting that these biofilms represent a MeHg entry into the local aquatic food chain (Dominique et al., 2007). Relatively high amounts of MeHg (up to $200 \text{ ng g}^{-1} \text{ dw}$) (Dominique, 2006), suggested that biofilms in the anoxic part of the reservoir play together with the benthic communities (Coquery et al., 2003; Peretyazhko et al., 2005) a major role in Hg methylation at the reservoir scale. This is consistent with the generally observed vertical distributions of MeHg concentrations, i.e. maxima around –9 m and near the benthic interface, suggesting that these are main depth ranges of water column MeHg mobilization and/or production (Muresan et al., 2008a).

For these reasons and because several recent studies have suggested involvement of biofilms in Hg methylation/demethylation processes (Desrosiers et al., 2006; Lin and Jay, 2007), one may suppose the biofilm on the flooded dead tree trunks still standing in the reservoir lake to be a key location for Hg transformations in the Petit-Saut reservoir. Furthermore, plankton microorganisms are abundant in both

the epi- and hypolimnion of the reservoir ($\approx 5\text{--}14 \times 10^6 \text{ cells mL}^{-1}$; Dumestre et al., 2001) and there is evidence that plankton may contribute to Hg methylation in the oxic layer of the reservoir water column (Dominique et al., 2007). Nevertheless, to date there is no study on the role of plankton microorganisms and/or the biofilm attached to the dead tree trunks along the water column on Hg methylation in the anoxic water of the reservoir.

The main objective of the present work was to assess the Hg methylation potential of plankton microorganisms and the influence of biofilm components (microorganisms, organic matter, minerals) from the anoxic part of the reservoir and from the Sinnamary Estuary. For this, we used stable Hg isotope spikes followed by incubation at different time-scales in reservoir and estuary water (natural nutrient conditions) and in culture medium (artificial nutrient conditions). The use of different nutrient conditions was explorative and aimed at covering a potentially wider range of bacterial activity and/or Hg methylation. The specific methylation efficiencies of plankton microorganisms in the presence or not of biofilm components were compared and their role in Hg cycling at the whole reservoir scale is discussed.

2. Materials and methods

2.1. Study area and sampling stations

The Petit-Saut dam (5°04' North, 53°03' West) was constructed on the Sinnamary River in French Guiana ~60 km upstream from its mouth to the Atlantic Ocean (Fig. 1). The Sinnamary River has an average discharge of $250 \text{ m}^3 \text{ s}^{-1}$ with important seasonal and inter-annual variability ($170\text{--}340 \text{ m}^3 \text{ s}^{-1}$) (Sissakian, 1997). The reservoir lake has a maximum depth of up to 35 m (depending on the season) and is ~80 km long, covering ~365 km² of uncleared tropical forest. A rough estimate suggests that the tree density in the submerged area is ~25 trees 100 m^{-2} , i.e. ~91 million trees at the reservoir scale and an available apparent surface for biofilm of $\sim 3 \times 10^9 \text{ m}^2$ (Dominique et al., 2007).

The reservoir water body is highly stratified with an oxic epilimnion and an anoxic hypolimnion separated by a quasi permanent oxycline located around 5–7 m depth. Average water residence time in the reservoir is ~5 months (Richard et al., 1997). Water temperature reaches ~30 °C at the surface and decreases with depth to ~25 °C at ~30 m. Downstream from the dam, the Sinnamary Estuary has an

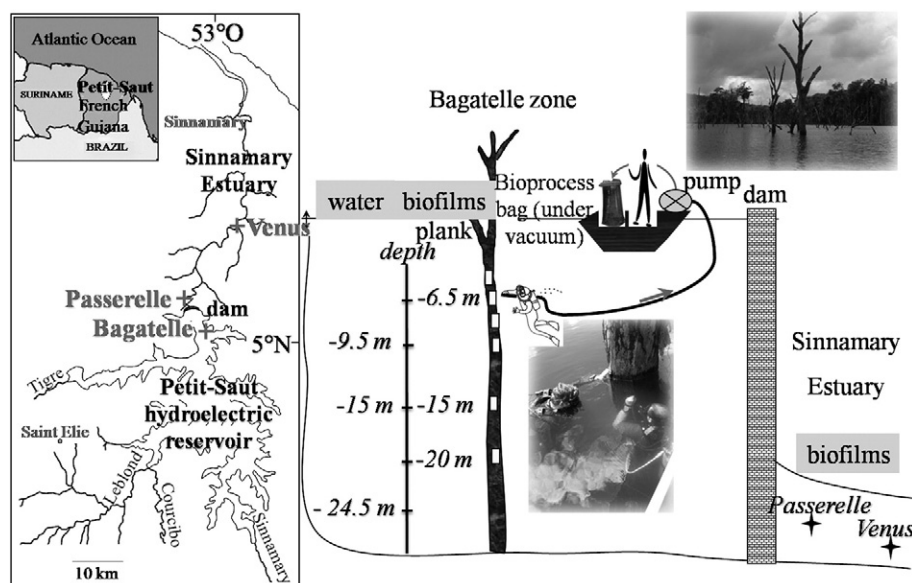


Fig. 1. Location of the Petit-Saut hydroelectric reservoir in French Guiana, location of the sampling sites in the reservoir (Bagatelle station) and in the Sinnamary Estuary (Passerelle and Venus stations), and schematic drawing illustrating the biofilm sampling procedure.

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