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Effects of sulfate-reducing bacteria on methylmercury at the sediment–water interface

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ABSTRACT

Sediment cores (containing sediment and overlying water) from Baihua Reservoir (SW China) were cultured under different redox conditions with different microbial activities, to understand the effects of sulfate-reducing bacteria (SRB) on mercury (Hg) methylation at sediment–water interfaces. Concentrations of dissolved methyl mercury (DMeHg) in the overlying water of the control cores with bioactivity maintained (BAC) and cores with only sulfate-reducing bacteria inhibited (SRBI) and bacteria fully inhibited (BACI) were measured at the anaerobic stage followed by the aerobic stage. For the BAC and SRBI cores, DMeHg concentrations in waters were much higher at the anaerobic stage than those at the aerobic stage, and they were negatively correlated to the dissolved oxygen concentrations ($r = -0.5311$ and $r = -0.4977$ for BAC and SRBI, respectively). The water DMeHg concentrations of the SRBI cores were 50% lower than those of the BAC cores, indicating that the SRB is of great importance in Hg methylation in sediment–water systems, but there should be other microbes such as iron-reducing bacteria and those containing specific gene cluster (hgcAB), besides SRB, causing Hg methylation in the sediment–water system.

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Introduction

As a trace heavy metal pollutant, mercury (Hg) is of global concern due to its large toxicity to human health. Methylmercury (MeHg) is the methylated form of Hg, which could be biomagnified in food chains due to its strong fat-solubility and bioaccumulative capacity (Celo et al., 2006). Mercury contamination in the aquatic ecosystem is a worldwide environmental issue nowadays (Clarkson, 1990; Fitzgerald and Clarkson, 1991). Although numerous studies have been conducted to understand the geochemical cycling of Hg in aquatic biota, the distribution, transformation and bioaccumulation of Hg in the aquatic ecosystem are still poorly understood.

Sediments are either major sinks of Hg in the aquatic environment, or hot spots of Hg methylation (Schäfer et al., 2010). Both inorganic Hg and MeHg in sediments are able to release into the overlying waters through desorption, diffusion and resuspension processes (Wang and Chen, 2011; Ma et al., 2015; He et al., 2016). Sediment–water interface is the critical zone for Hg methylation in lakes (Jiang et al., 2007). Methylation of Hg also occurs in the deep water column (He et al., 2015; Eckley et al., 2005). In some case, the MeHg accumulation layer transitioned from the top sediment layer in winter to the water–sediment interface in spring and then to the overlying water above sediment in summer (He et al., 2015). Hg methylation in sediments can be affected by microbial species (Hu et al., 2013;

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Schaefer et al., 2011), Hg bioavailability (Douglas et al., 2012; Han et al., 2007), temperature (Yu et al., 2012; Creswell et al., 2008), sulfur and organic speciation (Harmon et al., 2004; Mitchell et al., 2008), organic matter (Lambertsson and Nilsson, 2006; Gray and Hines, 2009; Meng et al., 2016), and redox condition (Covelli et al., 2008; Wang et al., 2012). Surface sediment usually has high organic matter content and high microbial activity. The surface or subsurface of sediments is often in anaerobic conditions, making Hg methylation more favorable (Hammerschmidt and Fitzgerald, 2004). Overall, investigation of Hg and MeHg in the sediment–water interface is of great importance for lake Hg studies.

Mercury is methylated through biological and non-biological processes, and the former seems to play more important role. In previous studies, sulfate-reducing bacteria (SRB), an anaerobic microbe, showing a high affinity for Hg in anaerobic condition (Avramescu et al., 2011), was always regarded as the main bacteria which led to the methylation of Hg in freshwater systems (Benoit et al., 1999; Avramescu et al., 2011). However, some researchers showed other anaerobic microbes such as the iron-reducing bacteria (DIRB) and those containing specific gene cluster (hgcAB), besides SRB, also have the ability for Hg methylation (Kerin et al., 2006; Gilmour et al., 2013). For example, it was reported that the *Geobacter sulfurreducens* PCA (one kind of DIRB) could methylate Hg^{2+} to MeHg (Hu, 2012). The study of Wu et al. (2011) also shows that other microbes, rather than SRB, may also act as main Hg methylators in mangrove sediments. Till now, most of the previous studies were performed under fully artificial and controlled experimental conditions, the relative importance of SRB and other microbes to Hg methylation in the natural environment remains unclear. The latest study shows that some aerobic microbes also have a strong ability to methylate mercury (Monperrus et al., 2007). Thus, the relative importance of different microbes to the methylation process in the natural environment deserves further study. In this study, *in-situ* culturing experiments were conducted for sediment cores from the Baihua Reservoir (BR). We aimed to study the variation of MeHg concentration at the sediment–water interface under different redox conditions with different microbial activities.

1. Materials and methods

1.1. Simulative experiments

Baihua Reservoir, located in Guiyang (SW China), was established in 1966. It has a surface of 14.5 km^2 and a volume of 1.91×10^8 m^3. Major rivers to the BR are Dongmenqiao, Changchong, Maicheng, Maotiao, Maixi and several small tributaries. Sediments in the BR consist of quartz, dolomite, illite, chlorite, montmorillonite, kaolinite, anatase, *etc.* (Wang et al., 2002). Organic matter concentrations in sediments were reported to be 8.08%–13.04% (Luo et al., 2014).

In June 2013, eight cores were collected from the BR, using a plexiglass sediment sampler (six cores were used in our experiment, and two cores were reserved). The plexiglass sediment sampler has a full length of 80 cm and a diameter of 8 cm. The samplers were cleaned by washing agent and 10% HCl solution prior to use. Three plastic barrels of lake water were collected, and each barrel has a volume of 20 L. The collected cores consisted of sediment of 25–35 cm long and overlying water of 45–55 cm long. All cores with sediment and water were transported to the laboratory and stored in dark conditions for future experiment. The overlying water remained clear and transparent, indicating no turbulences during sample collection and transportation process.

The cores were labeled as BAC1, BAC2, BACI1, BACI2, SRBI1 and SRBI2. Bioactivity maintained (BAC) represents sediment core with microbial activities maintained; bacteria fully inhibited (BACI) represents cores with all microbial activities fully inhibited, and sulfate-reducing bacteria inhibited (SRBI) represents cores with only the activity of SRB inhibited. Each treatment was done in duplicate, as shown in the name of each core (1 and 2 suffixes).

Before the experiment, sediment cores were pre-treated and let them have different microbial activities. No any treatment has been applied for cores BAC1 and BAC2; for cores BACI1 and BACI2, the overlying water was firstly sucked out with rubber pipette bulb and cleaned plastic hose, then 3 wt.% sodium azide (NaN_3) was added into the surface layer (0–5 cm) of the sediment for bacterial inhibition, and finally the sediment was covered by sterile lake water. For cores SRBI1 and SRBI2, the overlying water was also sucked, then 50 mmol/L sodium molybdate was added into the 0–5 cm layer of surface sediment for inhibition of SRB. All cores were exposed to anaerobic culturing for the first 20 days, and then exposed to aerobic culturing for the next 20 days. During this period, 100 mL overlying water sample (about 10 cm away from surficial sediment) was sucked out from each core every two days. The water samples were filtered by 0.45-μm PVDF membranes (Millipore), then 0.5% HCl was added to the filtered water and the samples were stored in fridge (4°C) before testing. After sampling, water with the same microbial activity level was added to each core. During anaerobic culturing, after sampling and adding more water, the overlying water were bubbled with nitrogen (15 min, 50–100 mL/min), and then closed immediately to keep anaerobic conditions. During aerobic culturing, an intermittent aeration device was utilized to keep aerobic conditions of the sediment–water interface. The whole experiment was conducted in dark at 26°C.

1.2. Sample detection

Dissolved methyl mercury (DMeHg) concentrations in water samples were determined using the standard distillation-ethylation-GC separation-CVAFS technique (Bloom, 1989; US EPA, 2001). A 45-mL aliquot of acidified sample was placed in a fluoropolymer distillation vessel and the distillation was carried out at 125°C under Hg-free N_2 flow until 35 mL of water was collected in the receiving vessel. The sample collected was adjusted to pH 4.9 with an acetate buffer and the Hg in the sample was ethylated in a closed 200-mL bubbler by the addition of sodium tetraethyl borate. The ethyl analog of CH_3Hg, $CH_3CH_3CH_2Hg$ was separated from solution by purging with N_2 onto a Tenax trap. The trapped $CH_3CH_3CH_2Hg$ was then thermally desorbed, separated from other mercury species by an isothermal gas chromatography (GC) column, decomposed to Hg(0) in a pyrolytic decomposition column (700°C) and

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