Mitigation of methylmercury production in eutrophic waters by interfacial oxygen nanobubbles

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Abstract

In mercury (Hg)-polluted eutrophic waters, algal blooms are likely to aggravate methylmercury (MeHg) production by causing intensified hypoxia and enriching organic matter at the sediment-water interface. The technology of interfacial oxygen (O_2) nanobubbles is proven to alleviate hypoxia and may have potential to mitigate the risks of MeHg formation. In this study, incubation column experiments were performed using sediment and overlying water samples collected from the Baihua Reservoir (China), which is currently suffering from co-contamination of Hg and eutrophication. The results indicated that after the application of O_2 nanobubbles, the %MeHg (ratio of MeHg to total Hg) in the overlying water and surface sediment decreased by up to 76% and 56% respectively. In addition, the MeHg concentrations decreased from 0.54 ± 0.15 to 0.17 ± 0.01 ng L^{-1} in the overlying water and from 56.61 ± 9.23 to 25.48 ± 4.08 ng g^{-1} in the surface sediment. The decline could be attributed to the alleviation of anoxia and the decrease of labile organic matter and bioavailable Hg. In addition, *hgcA* gene abundances in the overlying water and surface sediment decreased by up to 69% and 44% after the addition of O_2 nanobubbles, as is consistent with MeHg occurrence in such areas. Accordingly, this work proposed a promising strategy of using interfacial oxygen nanobubbles to alleviate the potentially enhanced MeHg production during algal bloom outbreaks in Hg-polluted eutrophic waters.

Key words: Mercury methylation; Algal bloom; Sediment-water interface; Anoxia remediation; Mercury microbial methylator; Mercury bioavailability

1. Introduction

As a global pollutant, mercury (Hg) can be transported across boundaries and enter aquatic ecosystems via dry and wet deposition and industrial runoff (Woerndle et al. 2018, Selin 2009). In surface waters, Hg content has been tripled due to human activities since industrialization (Lamborg et al. 2014). Inorganic Hg could be methylated to a potent neurotoxin, methylmercury (MeHg), which can cause even severer harm to organisms after bioaccumulation and biomagnification through the food chain (Harris et al. 2007). It is widely acknowledged that Hg methylation tends to occur under anaerobic conditions and is predominantly mediated by anaerobic bacteria carrying the *hgcAB* genes (Parks et al. 2013, Schaefer et al. 2011, Ullrich et al. 2001). Furthermore, organic substances, as substrate for microorganisms, can contribute to the formation of MeHg in water and sediment (Graham et al. 2012, Lambertsson and Nilsson 2006). In aquatic systems, Hg methylation rates usually reach their maximum at the oxic-anoxic interface, which also generally coincides with the sediment-water interface (Matilainen 1995, Tomiyasu et al. 2008).

Eutrophication has been a prevalent phenomenon in various lakes (Guo 2007, Copetti et al. 2016), reservoirs (De Ceballos et al. 1998, He et al. 2008), and coastal areas (Diaz and Rosenberg 2008, Soerensen et al. 2016) all over the world. It usually occurs alongside algal blooms and ends in the decomposition and deposition of them, thus leading to the state of hypoxia/anoxia and accumulation of labile organic matter on surface sediment (Conley et al. 2009b). Moreover, phytoplankton is the primary source of autochthonous organic

matter in sediments, which is generally preferred by heterotrophic bacteria, such as Hg microbial methylators (Stedmon and Markager 2005, Kritzberg et al. 2004). Hereby, sediment dominated by phytoplankton-derived organic matter has been reported to have higher Hg methylation rates (Bravo et al. 2017). As a result, eutrophication has the great potential to aggravate Hg methylation, especially at the sediment-water interface in Hg-polluted waters (Lei et al. 2019).

Owing to the substantial threats of MeHg to human health and other animals, several strategies have been reported to lower its content in surface waters (Mailman et al. 2006, Moo-Young et al. 2001, Beutel et al. 2014). It is suggested that the aeration of sediment can inhibit Hg methylation by mitigating hypoxia (Conley 2012). However, aeration by pumping can be comparatively demanding, considering the large volume of oxygen (O_2) required and the interference with natural water patterns (Conley et al. 2009a, Stigebrandt and Gustafsson 2007). In addition, capping materials such as biochar and activated carbon have been reported to decrease MeHg levels in the contaminated sediments (Gilmour et al. 2018, Gilmour et al. 2013). Nevertheless, it is inevitable for these materials to increase organic matter in the aquatic systems, which might aggravate the formation of MeHg in the long term (Liu et al. 2018a). Thus, it is of great necessity to explore an alternative strategy for MeHg remediation, especially with low-disturbance and greater stability.

Recently, interfacial O_2 nanobubbles have been reported to significantly remediate hypoxia in eutrophic waters (Shi et al. 2018, Zhang et al. 2018). Due to their miniature sizes (100–1000 nm), interfacial O_2 nanobubbles usually have long lifetimes and high gas-liquid

76 solubility (Lyu et al. 2019). They are usually loaded on natural minerals like zeolites, which
77 are hydrated aluminosilicate minerals with porous structures (Wang et al. 2018, Wang and
78 Peng 2010). With O_2 loading and a specific gravity greater than water (2.15–2.25 g cm^{-3}), O_2
79 nanobubble-loaded zeolites are capable of delivering oxygen to surface sediment areas
80 through natural settling (Osmanlioglu 2006). Considering Hg methylation tends to intensify
81 in anaerobic conditions, interfacial O_2 nanobubbles have the great potential to inhibit MeHg
82 production at the sediment-water interface in eutrophic waters. Besides, it is less likely for
83 O_2 nanobubble-loading zeolites to disturb the sediment-water interface and release organic
84 matter to the aquatic system. Accordingly, interfacial O_2 nanobubbles might provide an
85 effective solution for MeHg remediation.

86 The primary objective of this study is to investigate whether the strategy of interfacial
87 oxygen nanobubbles could mitigate MeHg production and its underpinning mechanisms for
88 the effects. To achieve this objective, we first collected samples of overlying water and
89 surface sediment from the Baihua Reservoir, a Hg-polluted eutrophic reservoir in Guizhou
90 Province, China, and built microcosms out of them. We then applied interfacial O_2
91 nanobubbles (loaded on zeolites) to the microcosms and analyzed the differences in
92 variation of %MeHg during incubation. Finally, in order to illustrate the mitigation effects of
93 O_2 nanobubbles on MeHg production, we analyzed the variations of factors that might
94 affect Hg microbial methylator activities (redox conditions and microbial substrates),
95 bioavailable Hg content (geochemical Hg fractions), and the abundance of *hgcA* gene.
96 Generally, this study proposed a new perspective for MeHg remediation in eutrophic waters.

2. Materials and methods

2.1 Sample collection

Overlying water and surface sediment samples were collected from the Baihua Reservoir (106°27' E, 26°35' N) in Qingzhen City, Guizhou Province during May, 2018. Though built to provide drinking water for local residents, the reservoir (average depth of ~13 m) has suffered from severe Hg pollution from the industrial sewage of the Guizhou Organic Chemical Plant and neighboring mines (Feng et al. 2004, Liu et al. 2012). The Guizhou Organic Chemical Plant used Hg as catalyst for acetic acid production and was reported to discharge approximately 573 tons of Hg to Baihua Reservoir from 1971 to 1985 (Yan et al. 2008). Recently, the Baihua Reservoir has been reported to be suffering from eutrophication as well (Liu et al. 2012). Overlying water samples (10 m in depth from the surface) were collected with a stainless-steel water sampler. Surface sediment (0–25 cm) samples were collected with an Ekman dredge. Once collected, the water and sediment samples were sealed in 50 L HDPE drums, transferred to the lab at 4 ℃, and stored in the dark instantly.

2.2 Incubation experiments

Samples of surface sediment and overlying water (filtered with 0.45 μm filters) were filled into 26 cylindrical plexiglass columns (6.6 cm in diameter and 110 cm in height) to establish a uniform sediment-water interface (Shi et al. 2018). Each microcosm was composed of 25 cm depth of sediment (860 mL) and 75 cm depth of overlying water (2600

117 mL) (Supplementary Information (SI), Fig. S1). All the microcosms were stabilized in the dark
118 at 25 ℃ for 1 month before further treatments. Furthermore, the 26 microcosms included
119 two background and 24 treated microcosms. The background microcosms (called the
120 Background group) were composed of collected sediment and overlying water samples
121 without any treatment, which could provide the initial information on all microcosms. The
122 characteristics of overlying water and sediment samples from the Background group were
123 listed in Table S2 and Table S3, respectively (SI).

124 The other 24 treated microcosms were divided into 4 treatment groups, namely the:
125 Control, Algae, Zeolite, and O_2 nanobubbles (O_2 NBs) group. Each group has 6 microcosms.
126 The Control group was designed to simulate the general algal level in the Baihua Reservoir.
127 The Algae, Zeolite and O_2 NBs groups were designed to simulate algae-derived organic
128 matter deposition during severe eutrophication in the Baihua Reservoir. *Pseudanabaena*
129 *limnetica*, the dominant algae species during wet periods in the Baihua Reservoir, was used
130 as the algae source in this study (Li et al. 2011). Details regarding *P. limnetica* culture and
131 calculation of the addition amount are described in the SI. In the Control group, 6 mg of
132 freeze-dried *P. limnetica* biomass (2.3 mg dry weight/L water) was added to the microcosms,
133 whereas in the Algae, Zeolite, and O_2 NBs groups, 40 mg of dry *P. limnetica* biomass (15.4
134 mg dry weight/L water) was added and then flocculated with modified soil flocculants (Zou
135 et al. 2006). After the addition and flocculation of *P. limnetica*, the O_2 NBs group was then
136 treated with 70 g O_2 nanobubble-loaded natural zeolites (2 cm in depth, 68 mL in volume)
137 (Wang et al. 2018). Details of the preparation of O_2 nanobubble-loaded natural zeolites

were elaborated in the previous study (Shi et al. 2018). Here, we provide only a summary of the method: natural zeolites underwent a cycle of a 2 h vacuum and 0.5 h O_2-loading that was repeated three times followed by equilibration in O_2 for over 12 h. For the Zeolite group, O_2 in the O_2 NBs group was replaced with nitrogen to investigate the barrier effects of zeolites. According to the previous study, O_2 loaded on zeolites in each microcosm of the O_2 NBs group was approximately 1482 mg (Wang et al. 2018).

The incubation experiments were performed over a period of 30 days at 25 ℃ in the dark (covered with black plastic films) to simulate the sediment-water interface in the long term. At intervals, dissolved oxygen (DO), oxidation reduction potential (ORP) and pH in the overlying water (2 cm above the sediment surface) were analyzed *in situ* (Tang et al. 2019). Moreover, the overlying water was sampled with a peristaltic pump (BT100-1F, LongerPump, China) and filtered with 0.22 μm filters for the analysis of Hg speciation, dissolved organic carbon (DOC), sulfate (SO_4^{2-}), and chloride ion (Cl^-). During the incubation, the background microcosms (on day 0) and two microcosms of each treatment group (on days 10, 20, and 30) were sacrificed for the analysis of Hg speciation (Hafeznezami et al. 2017), elemental (C, N, and S) content, and *hgcA* abundance in sediment (divided into layers of 0–5, 5–15, and 15–25 cm). Details on the analytical methods are provided in the SI.

2.3 Hg speciation analysis

For MeHg analysis in the overlying water samples, 30 mL of the acidified samples were added with 800 μL, 2 mol L^{-1} sodium citrate solution (Sigma-Aldrich, USA) to buffer pH. For MeHg analysis in sediments, 0.25 g sediment samples were leached with 1.5 mL, 2 mol L^{-1}

159 $CuSO_4$ and 7.5 mL, 25% HNO_3. Then the mixture was extracted with 10 mL CH_2Cl_2 (with
160 mechanical shaking) and heated at 65 ℃ for 6 h to realize back-extraction (Ji et al. 2019).
161 The concentrations of MeHg in the overlying water and sediment samples (in the back-
162 extracted solution) were analyzed using the MERX-T Automatic Methyl Mercury System
163 (Brooks Rand Laboratories, USA) following USEPA 1630 (USEPA 2001).

164 For total mercury (THg) analysis in the overlying water, 10 mL samples were oxidized
165 with 100 µL, 0.2 mol L^{-1} BrCl and left overnight. Before analysis, 40 µL, 30% NH_2·HCl were
166 added to the oxidized samples to reduce the excessive BrCl. Then 2 mL of water samples
167 were pipetted into 40 mL glass vials (Agilent Technologies, USA) with 18 mL UPW in them.
168 Finally, the THg concentrations in the overlying water samples were determined with the
169 MERX-T Automatic Total Mercury System (Brooks Rand Laboratories, USA) following USEPA
170 1631, Revision E (USEPA 2002).

171 For THg analysis in sediments, 0.02 g freeze-dried sediment samples were weighed into
172 nickel boats. The boats were then burned at 850 ℃ to reduce all Hg species to elemental
173 Hg and trapped by gold amalgam. After decomposition, Hg concentrations were
174 determined using the Leeman mercury analyzer (Leeman Labs Hydra II C, USA) according to
175 USEPA 7473 (USEPA 2007).

176 ## 2.4 DNA extraction and Real Time Quantitative PCR (qPCR)

177 The total microbial DNA was extracted from 0.25 g freeze-dried sediment samples, 1 L
178 of overlying water (filtered with 0.22 µm filter membrane), and 0.6 g freeze-dried zeolite

179 samples using the DNeasy PowerSoil Kit (QIAGEN Inc., Germany) following the
180 recommended protocol of the manufacturer. The concentrations and quality of the
181 extracted DNA were determined with a Nanodrop UV-Vis spectrophotometer (ND-2000,
182 Thermo-Fisher Scientific, USA). Then the abundance of the *hgcA* gene was quantified using
183 an iCycler iQ5 thermocycler (Bio-Rad, USA). The clade-specific degenerate primer pair for
184 Deltaproteobacteria was ORNL-Delta-HgcA (Delta-HgcA-F: GCCAACTACAAGMTGASCTWC;
185 Delta-HgcA-R: CCSGCNGCRCACCAGACRTT) (Liu et al. 2018b). The details are shown in the
186 SI.

187 ## 2.5 Quality control and statistical analysis

188 For THg analysis in sediment samples, the GSD-10 (THg content: 280 ± 40 ng g^{-1},
189 GBW07310, Institute of Geological and Geophysical Exploration, Chinese Academy of
190 Geological Sciences, China) was used as the certified reference material, and analytical
191 blanks were measured for quality control. The average THg concentration measured was
192 279.93 ± 0.03 ng g^{-1} (mean ± SD, n = 6). Limit of quantification (LOQ) was calculated
193 according to the lowest point on the standard curve, which was was 7 ng Hg in terms of
194 absolute mass. The analytical blank was under LOQ. For MeHg analysis in sediment samples,
195 we used the ERM-CC580 (MeHg content: 75.5 ± 3.7 ng g^{-1} Hg, European Reference Materials,
196 Institute for Reference Materials and Measurements, Belgium) as the certified reference
197 material and the recovery results were 97.2 ± 4.8% (mean ± SD, n = 3). LOQ was 2 pg Hg in
198 terms of absolute mass and the analytical blank was under LOQ. For Hg sequential selective
199 extraction, we used the GSD-10 as the certified reference material. Concentrations of five

fractions in GSD-10 were 1.31, 0.69, 61.76, 61.34, and 125.85 ng g^{-1}, which agreed well with the published results (Shi et al. 2005). Analytical blanks were lower than LOQ. For THg analysis in water, LOQ and analytical blank measured were 50 and 3.9 pg in terms of absolute mass, which could be converted to 2.5 and 0.19 ng L^{-1} in the water samples. For MeHg analysis in water, LOQ was 2 pg in terms of absolute mass (0.07 ng L^{-1} in water samples) and the analytical blank was under LOQ.

Statistical analysis was performed using SPSS 22.0 software. The difference between two groups throughout the incubation was analyzed using a paired-sample *t*-test after the normality test, and the independent *t* test was applied to evaluate if the difference on each sampling day. In addition, significance probability (*p*) was calculated and the difference was declared significant for $p < 0.05$. The principal component analysis (PCA) with a varimax rotated solution was applied to disentangle the combined effects of different variables (DO, ORP, DOC, SO_4^{2-}, pH, and Cl^-) attributed to the variations of %MeHg in the overlying water (SI, Table S5, Table S6, and Fig. S9).

3. Results and discussion

3.1 Mitigation of MeHg production with O_2 nanobubbles in overlying water

It has been proposed that the ratio of MeHg to THg (%MeHg) can be used as a reasonable proxy for Hg methylation rates (Schartup et al. 2012). As illustrated in Fig. 1A, the %MeHg in the overlying water varied significantly among the four treatment groups but all reached the highest on day 13. In the Algae group, the %MeHg far exceeded that in the

Control group during the incubation period, and the difference reached its peak of 1.8 times on day 1. The significant excess ($p < 0.001$) supported the hypothesis that the addition of algal biomass could enhance MeHg production (Tsui et al. 2010). More strikingly, after the addition of O_2 nanobubbles, the %MeHg (0.10–0.25%) was significantly ($p < 0.001$) reduced compared to the Algae group (0.29–0.87%), between which the largest decrement was 76% (from 0.74 to 0.18%) on day 6. The same amount of algal biomass added in these two groups could produce the equal amount of organic matter. Thus, the direct comparison of MeHg production could reflect the mitigation effects of O_2 nanobubbles. Moreover, by comparing with the Control group, the %MeHg in the O_2 NBs group decreased by up to 55% (on day 13), indicating the significant remediation of MeHg production by O_2 nanobubbles ($p < 0.01$). In addition, the distributions of MeHg concentrations in the overlying water from the four treatment groups are illustrated in Fig. 1B. Similar with the distribution of %MeHg, the concentrations of MeHg in the Algae group significantly ($p < 0.001$) exceeded those in the Control group, with the highest increase being 84% (from 0.19 to 0.35 ng L^{-1}, on day 6). Compared with those in the Algae group, MeHg concentrations from the O_2 NBs group decreased significantly ($p < 0.001$), displaying a maximum decline of 69% (from 0.54 to 0.17 ng L^{-1}) on day 16. Furthermore, there was little difference in both %MeHg and MeHg content between the Zeolite and Control groups, indicating the moderate mitigation effects of zeolite capping (without O_2 nanobubbles) on MeHg production. These results proved that interfacial O_2 nanobubbles were able to make substantial contributions to the reduction of

MeHg production in the overlying water, which could be significantly elevated in Hg-polluted waters with severe eutrophication.

Then we analyzed factors that might contribute to the variations of MeHg production in the overlying water (Fig. 2). As shown in Fig. 2A–C, the distributions of DO, ORP, and SO_4^{2-} were the same for all four treatment groups, i.e., all four groups exhibited the following sequence: O_2 NBs > Zeolite > Control > Algae. As illustrated in Fig. 2A and Table S2 (SI), the initial average DO concentration in the microcosms was 1.06 ± 0.46 mg L^{-1}, which was the typical DO concentration in surface waters suffering from severe hypoxia (Dauer et al. 1992). After the addition of the algal biomass, the DO concentrations decreased to approximately 0 mg L^{-1} and remained anoxic (< 0.2 mg L^{-1}) during the remaining incubation days. This decline might represent the natural process of hypoxia caused by the deposition and decomposition of dead algae during an algal bloom, which was reported by Funkey et al. in 2014. With the treatment of zeolites, the DO concentrations were elevated to around 0.5 mg L^{-1}, restoring the system to the Control group level. Furthermore, after the addition of O_2 nanobubbles, the DO concentrations increased to 2.83 mg L^{-1} instantly and then dropped gradually, however they remained over 1 mg L^{-1} till the end of the incubation. In addition, O_2 nanobubbles increased ORP at the sediment-water interface from −86.7 mV (the Algae group, day 1) to 1.5 mV (the O_2 NBs group, day 1), reversing the area from reduced to oxidative condition (Fig. 2B). Previous studies have shown that with the conversion of anaerobic to aerobic state, sulfide in the sediment might be oxidized to SO_4^{2-} and released from the sediment layer into the water column (Duvil et al. 2018, Zhu et al. 2017). Therefore,

261 sulfate content in the overlying water was also deemed an important proxy for redox
262 conditions (Li et al. 2010). As illustrated in Fig. 2C, the concentrations of SO_4^{2-} in the
263 overlying water from the O_2 NBs group (120.55–131.02 mg L^{-1}) significantly ($p < 0.001$)
264 exceeded those from the Algae group (104.74–111.91 mg L^{-1}), with the average daily
265 increase of 16%. Moreover, even with more algal biomass in the microcosms, the O_2 NBs
266 group still had significantly elevated content of DO, ORP, and SO_4^{2-} than the Control group.
267 These results demonstrated the remarkable anoxia remediation effects of O_2 nanobubbles.
268 In addition, by comparing the content of ORP and SO_4^{2-} in the Zeolite and Algae groups, we
269 found zeolite capping could also make a contribution to anoxia remediation in the overlying
270 water. Based on the variations of redox indexes, oxygen nanobubbles were able to provide
271 an enhanced and persistent oxidative condition, which corresponded with previous studies
272 (Shi et al. 2018, Zhang et al. 2018a). Apart from this, zeolites might act as a barrier, blocking
273 the oxygen-consuming substances (like decayed algae) from entering the overlying water.
274 This might also help remediate anoxia to a certain degree. Moreover, highly significant
275 negative correlations ($p < 0.01$) between %MeHg and the content of DO, ORP, and SO_4^{2-}
276 were observed in the overlying water (SI, Fig. S2A–C). Previous studies have declared that
277 Hg methylation tends to occur in anaerobic conditions (Ullrich et al. 2001). Accordingly,
278 anoxia remediation induced by interfacial oxygen nanobubbles could possibly explain the
279 decrease of MeHg production in the overlying water.

280 In addition, variations of DOC content in the overlying water from the four treatment
281 groups are illustrated in Fig. 2D. First of all, the DOC concentrations in the Algae group were

282 generally higher than other three groups, suggesting that the addition of algal biomass
283 could increase the content of dissolved organic matter (DOM) in the overlying water.
284 However, after the addition of O_2 nanobubbles, the DOC content in the overlying water
285 decreased significantly throughout the incubation period. Moreover, there was no
286 remarkable difference in DOC content between the Zeolite and O_2 NBs groups. This
287 indicated that whether with O_2 nanobubbles or not, zeolites could inhibit the algae-induced
288 increase in DOC, which might be related to the barrier effects of zeolite capping (Pan et al.
289 2012). The barrier effects could also be reflected from the apparent decrease of DOC
290 concentrations in the Zeolite and O_2 NBs groups on day 1. Similar to the pattern of %MeHg
291 (Fig. 1A), the DOC content in overlying water from all treatment groups reached the highest
292 on day 13, which could be related to the utilization of labile organic matter by
293 microorganisms (Chen et al. 2016). Moreover, a highly significant positive correlation ($p <$
294 0.01) was found between %MeHg and DOC in the overlying water (SI, Fig. S2D), which was
295 similar with the significant positive correlation reported between the ambient MeHg
296 concentration and the organic material content (Lambertsson and Nilsson 2006). This
297 confirmed the key role of DOM in MeHg production. Previous studies have reported the
298 potential role of DOM in Hg methylation: on the one hand, DOM was regarded as one of
299 the electron donors for Hg microbial methylators during the transformation from inorganic
300 Hg to MeHg; on the other hand, these methylators could utilize certain DOM as their living
301 substrates when engaging in Hg methylation (Jiang et al. 2018, Schaefer and Morel 2009).
302 These could further help explain the correlation between MeHg and DOC in this work. It is

303 also probable that organic matter might help transport Hg from sediments (Ravichandran
304 et al. 2004). Therefore, it is suggested that zeolite capping (in the Zeolite and O_2 NBs groups)
305 might mitigate MeHg production by inhibiting DOM from entering the overlying water,
306 therefore decreasing the activities of Hg microbial methylators. As for the increase of DOC
307 content from day 2 in the Zeolite and O_2 NBs groups, it is possible that the release of gas
308 borne on zeolites could cause the mild migration of algae from the bottom to the top of the
309 zeolite layer. Even so, during the whole incubation, the DOC concentrations in the Zeolite
310 and O_2 NBs groups were lower than those in the Algae group, indicating that the disturbance
311 was insignificant compared to the barrier effects of zeolites.

312 These results proved that interfacial O_2 nanobubbles can significantly decrease
313 both %MeHg and MeHg concentrations in the overlying water. Meanwhile, the content of
314 DO, ORP, and SO_4^{2-} was elevated, and DOC was reduced by O_2 nanobubbles. These results
315 indicated that the reduction of MeHg production might be due to the remediation of anoxia
316 as well as the decrease in labile organic matter.

317 3.2 Mitigation of MeHg production with O_2 nanobubbles in sediment

318 In an aquatic system, sediment usually has much higher MeHg levels (over three orders
319 of magnitude) and more lasting impacts on the ecosystem than the water column (Ullrich
320 et al. 2001). Therefore, the effects of O_2 nanobubbles on MeHg production in sediment
321 were the primary focus of this study.

As illustrated in Fig. 3A, the %MeHg in sediment varied with depth, treatments, and incubation time. In general, the differences in %MeHg among the four treatment groups decreased with sediment depth, and the variations were mostly revealed in the surface sediment. Moreover, the maximum %MeHg in each group was mostly observed in the surface sediment. This was in accordance with the reported results that surface sediment is a hotspot for Hg methylation (Gilmour et al. 1992). Therefore, further discussions on MeHg occurrence and the potential reasons for this occurrence should focus on surface sediment. The distribution of %MeHg in surface sediment from the four treatment groups is further illustrated in Fig. S4 (SI). Throughout the incubation period, the average %MeHg in surface sediment from the Algae group (0.71, 1.15, and 1.28% on days 10, 20, and 30, respectively) were higher than those from the Control group (0.65, 0.96, and 1.02%). This proved that massive algal deposition can indeed aggravate MeHg production in such areas. However, after the treatment with O_2 nanobubbles, MeHg production was remarkably mitigated. As shown in Fig. 3A and Fig. S4 (SI), the %MeHg in surface sediment from the O_2 NBs group was the lowest among the four groups. The daily average reduction of %MeHg in the O_2 NBs group from the Algae group was 52%, with the maximum difference being 56% (from 0.71 to 0.31%) on day 10. In addition, in comparison with the Control group, O_2 nanobubbles could still decrease %MeHg significantly by up to 52%. This demonstrated the significant mitigating effects of O_2 nanobubbles on MeHg production in surface sediment. In terms of changes over time in all treatment groups, the %MeHg in surface sediment

342 increased rapidly from day 10 to 20 (85% on average), and slowly from day 20 to 30 (9% on
343 average).

344 To give a more direct investigation on MeHg variations, we also illustrated the
345 variations of MeHg concentrations in surface sediment (Fig. 3B). After the addition of algal
346 biomass, MeHg concentrations in surface sediment from the four groups all increased
347 throughout the incubation period. Generally, the rate of increase from day 20 to 30 (20%
348 on average) was slightly lower than that from day 10 to 20 (88% on average), as was
349 consistent with the variation of %MeHg in surface sediment (Fig. 3A). The rate of increase
350 in the Algae group during the first 10 days (0.6 ng g^{-1} d^{-1}) corresponded with the reported
351 result (~0.5 ng g^{-1} d^{-1}) (Lei et al. 2019). The MeHg concentrations in the Algae group
352 experienced the largest increase (56.61 ng g^{-1} on day 30) and reached four times that of
353 those in the Background group (14.37 ng g^{-1}, SI, Table S3). By contrast, the MeHg
354 concentrations in the O_2 NBs group increased the least among the four groups, to 25.48 ng
355 g^{-1} on day 30. By comparing MeHg concentrations in surface sediment from the Algae and
356 O_2 NBs groups, we found that O_2 nanobubbles could reduce MeHg concentrations by up to
357 56%, which was similar with the decrement of %MeHg in surface sediment. Moreover, in
358 comparison with the Control group, MeHg concentrations in the O_2 NBs group also
359 decreased by 46% on average. The results of %MeHg and MeHg concentrations showed
360 that O_2 nanobubbles were capable of mitigating MeHg production, which could be
361 enhanced by algal deposition, and that surface sediment was the target area for interfacial
362 O_2 nanobubbles.

It is widely acknowledged that sulfur (especially reduced sulfide) plays an indispensable role in MeHg production (Li et al. 2019, Benoit et al. 2001). Therefore, apart from SO_4^{2-} in the overlying water (Fig. 2C), we also analyzed the total sulfur content in surface sediment. As illustrated in Fig. 4A, the S content in the O_2 NBs group (0.41–0.49%) was the lowest among the four groups, and that in the Algae group (0.47–0.56%) was the highest. The distribution of S content was significantly consistent with %MeHg in surface sediment ($p < 0.01$). In addition, a significant negative correlation ($p < 0.05$) between %MeHg in surface sediment and SO_4^{2-} concentrations in the overlying water was also observed (SI, Fig. S5). In surface waters, sulfides were reported to be mainly buried in anoxic sediments (Schippers and Jørgensen 2002). O_2 nanobubbles were likely to oxidize sulfides and produce sulfate in surface sediment. The produced sulfate might enter water column via pore water; this might lead to the the elevation of SO_4^{2-} concentrations in the overlying water (Fig. 2C) and the decrease of S content in surface sediment (Fig. 4A). According to the previous study, MeHg production in sediment would be partially weakened when SO_4^{2-} concentrations in the overlying water are above 19.2–48 mg L^{-1}, which might result from the accumulation of sulfides and the decrease of Hg bioavailability (Ullrich et al. 2001, Gilmour and Henry 1991). In this work, concentrations of SO_4^{2-} in the overlying water from the four groups were all above 100 mg L^{-1} (Fig. 2C); far beyond the optimal concentration range reported for MeHg production. To some extent, these results might help explain the decrease of MeHg production in surface sediment (Fig. 3A), which was

accompanied with the decrease of S in surface sediment and increase of SO_4^{2-} in the overlying water.

Previous studies have reported that the ratio of C and N (C/N) is a reliable indicator for the lability of organic matter mediating Hg methylation in sediment (Drott et al. 2007, Meyers 1994). In this step, the ratios of C and N content in surface sediment samples from the four treatment groups were analyzed (Fig. 4B). Among the four groups, the C/N ratios in the O_2 NBs group (12.23–13.37) were generally the highest throughout the incubation period. Sediments with higher C/N were reported to have lower content of labile organic matter, which might be due to the enhanced mineralization of organic matter under aerobic conditions (McLatchey and Reddy 1998). Accordingly, the increase of C/N ratios in the O_2 NBs group may reflect the decline of labile organic matter in surface sediment, which is the major electron donor for Hg microbial methylators. Therefore, this increase of C/N could partially lead to the decline of MeHg production in surface sediment after the addition of O_2 nanobubbles.

Sequential selective extraction has been widely applied to the analysis of Hg reactivity and bioavailability in sediments (Bloom et al. 2003, Li et al. 2019). Percentages of five fractions in surface sediment from the Background and four treatment groups are illustrated in Fig. S6 (SI). Among the five fractions, water soluble Hg (Hg-w) and human stomach acid soluble Hg (Hg-h) can readily enter overlying water and pose substantial risks to aquatic organisms after being methylated to MeHg. The percentages of these two fractions were usually combined to represent the exchangeable Hg fraction (Shi et al. 2005,

404 Li et al. 2019). Exchangeable Hg can reflect the reactive and bioavailable Hg, as is closely
405 related to MeHg production. Therefore, the percentages of Hg-w and Hg-h in surface
406 sediment samples from the Background and four treatment groups were summed and
407 illustrated in Fig. 5.

408 By comparing the Algae and O_2 NBs groups, we found that O_2 nanobubbles could
409 decrease the exchangeable Hg content (except for a slight elevation on day 10), which
410 significantly increased with the addition of algae. The maximum decline (46%) between the
411 two groups occurred on day 20, as respective exchangeable Hg content in the Algae and O_2
412 NBs groups was 5.2% and 2.8%. This decline in exchangeable Hg indicated the decrease of
413 bioavailable Hg, which might also contribute to the mitigation of %MeHg in surface
414 sediment (Fig. 3A). Moreover, with the decline of exchangeable Hg, less Hg would readily
415 enter the overlying water, and this may help explain the mitigation of %MeHg in the
416 overlying water as well (Fig. 1A). Also, it was likely that O_2 nanobubbles might partly
417 mobilize the unavailable Hg, which might be an explanation for the increase of
418 exchangeable Hg on day 10. The increase might result from the decrease of Hg-s (SI, Fig. S6),
419 which was suggested to be oxidized in oxic conditions (Chen et al. 2018). In addition, there
420 was no significant difference in exchangeable Hg content between the Control and Zeolite
421 groups. This indicated that zeolite capping could also help decrease Hg bioavailability and
422 mobility in surface sediment of waters with algal blooms.

423 According to these results, interfacial oxygen nanobubbles were able to significantly
424 mitigate MeHg production in surface sediment. After the addition of interfacial O_2

425 nanobubbles, the release of O_2 on the zeolites made surface sediment more oxidative and
426 facilitated the decrease of sulfur content, increase of the C/N ratios, and decrease of the
427 exchangeable Hg content. These results revealed that anoxia remediation, as well as the
428 decline of labile organic matter and bioavailable Hg, could contribute to the decrease of
429 MeHg production in surface sediment.

430 ## 3.3 Abundance of *hgcA* gene in different compartments of microcosms

431 Regarding the technology of interfacial O_2 nanobubbles, evaluating its effect on MeHg
432 remediation and illustrating the underpinning mechanisms are equally essential. It is widely
433 acknowledged that Hg methylation was mainly microbially mediated (Parks et al. 2013,
434 Ullrich et al. 2001). The gene of *hgcA* is a common biomarker to determine the distribution
435 of Hg microbial methylators (Liu et al. 2014, Poulain and Barkay 2013). Previous studies
436 have reported using abundances of *hgcA* to predict MeHg production (Lei et al. 2019, Liu et
437 al. 2018b). To further illustrate the mechanisms for the mitigation effect of interfacial O_2
438 nanobubbles on MeHg production, *hgcA* gene abundances in the overlying water, zeolite
439 layer, and sediment (surface, middle, and deep layers) were analyzed among the four
440 treatment groups (Fig. 6).

441 As shown in the figure, there were significant differences in *hgcA* gene abundances in
442 the overlying water and surface sediment among the four treatment groups. On days 10
443 and 20, *hgcA* abundances in the overlying water from the Algae group (2.36×10^5 and 2.69
444 $\times 10^5$ copies L^{-1}, respectively) were significantly higher ($p < 0.01$) than those in the Control
445 group (1.62×10^5 and 2.03×10^5 copies L^{-1}), suggesting that there were more Hg microbial

446 methylators after the addition of algae-derived organic matter. Nevertheless, the O_2 NBs
447 group had significantly lower *hgcA* abundances than the Control and Algae groups ($p < 0.01$);
448 this suggested the decline of the Hg microbial methylator abundance after the treatment
449 of O_2 nanobubbles. On day 20, the *hgcA* abundance from the O_2 NBs group was 0.83×10^5
450 copies L^{-1}, which was 69% lower than those in the Algae group, consistent with the
451 difference in %MeHg between the two groups (Fig. 1A). This corresponded with the
452 reported positive correlation between *hgcA* abundance and MeHg level in sediments (Lei
453 et al. 2019, Liu et al. 2014). In addition, the significant difference ($p < 0.01$) in *hgcA*
454 abundance between the two groups was also observed in surface sediment, with the
455 maximum decline being from 6.59×10^7 to 3.69×10^7 copies g^{-1} (by 44%) on day 30. This
456 might account for the decrease of %MeHg in surface sediment after the addition of O_2
457 nanobubbles (Fig. 3A). There was no significant difference in *hgcA* abundances in the middle
458 and deep sediment among the four groups, which corresponded to the similar comparison
459 results of %MeHg there. These results indicated that in the sediment, the effects of O_2
460 nanobubbles on microbial methylators mainly targeted the surface layer. Moreover, *hgcA*
461 abundances generally decreased with sediment depth, which could help explain the peak
462 of %MeHg in surface sediment (Fig. 3A). Apart from this, *hgcA* abundances in the sediment
463 were remarkably higher than those in the overlying water (by two orders of magnitude).
464 This suggested that there were more Hg microbial methylators in the sediment than the
465 overlying water, and it might help explain the relatively higher %MeHg in the sediment (Fig.

1A and Fig. 3A). These results were consistent with the notion that sediment is the hotspot for Hg methylation (Gray and Hines 2009).

According to these results, the effects of O_2 nanobubbles on *hgcA* abundances and %MeHg both were mainly revealed at the sediment-water interface of the microcosms. In addition, a significant decline ($p < 0.01$) of the *hgcA* abundance in the zeolite layer was also observed in the O_2 NBs group, demonstrating the reduction effects of O_2 nanobubbles on Hg microbial methylator abundance (Fig. 6). Studies have shown that Hg microbial methylators, especially sulfate-reducing bacteria (SRB), predominantly prefer anaerobic conditions (Benoit et al. 1999, Jensen and Jernelöv 1969). It is probable that the oxidative condition at the sediment-water interface induced by O_2 nanobubbles can inhibit the activities of SRB, and thus decrease the reduction of sulfate (Muyzer and Stams 2008). As a result, sulfate consumption and sulfide production would decline, leading to the increase of SO_4^{2-} concentrations in the overlying water and the decrease of S content in surface sediment after the addition of O_2 nanobubbles (Fig. 2C and Fig. 4A). This might help explain the significant positive correlation ($p < 0.01$) between *hgcA* gene abundance and S content in surface sediment from the four treatment groups (SI, Fig. S8).

3.4 Implications for MeHg remediation in Hg-polluted eutrophic waters

Considering the aggravated Hg pollution and the prevalent eutrophication in surface waters, the surge of MeHg content could be a worldwide environmental issue that requires more attention, especially after biomagnification and bioaccumulation (Jackson 2019, Mangal et al. 2019). From the results of the sediment-water simulation microcosms in this

study, eutrophication was demonstrated to enhance MeHg production by bringing about algal deposition and decomposition, generally leading to anoxia and rich organic matter. These results echoed the reported enhancement of Hg methylation in sediment of 10 lakes after algal biomass input (Lei et al. 2019).

To tackle the enhanced MeHg production in Hg-polluted eutrophic waters, the novel geo-engineering strategy of interfacial oxygen nanobubbles was proposed. Generally, the technology of interfacial O_2 nanobubbles was demonstrated to be effective for MeHg remediation in Hg-polluted waters with algal blooms. These nanobubbles (borne on zeolites) were proven to target the sediment-water interface, which is the most active zone for MeHg production. Moreover, there are competitive advantages of interfacial O_2 nanobubbles technology over existing MeHg remediation method. For instance, in comparison with aeration, interfacial O_2 nanobubbles were less likely to interfere with natural water patterns. Compared to the common capping materials like biochar or activated carbon, natural zeolites were not inclined to release carbon, thereby reducing the potential for Hg methylation during capping. In addition, it should be pointed out that MeHg content might also be influenced by MeHg demethylation (Zhang et al. 2018b). Without substantial solar radiation, MeHg demethylation in surface waters was predominantly microbially mediated and might be enhanced in the aerobic conditions (Whalin et al. 2007, Ullrich et al. 2001). The addition of O_2 nanobubbles was likely to stimulate MeHg demethylation as well and further decrease the MeHg content, which required further research. In a word, we demonstrated that the technology of interfacial O_2 nanobubbles could be utilized as a

promising strategy for MeHg remediation with lower disturbance and higher stability, which is of great significance for decreasing the environmental risks of MeHg in eutrophic waters. It is also probable that the descent of MeHg release from sediment to overlying water could contribute to the decline of MeHg; this requires further investigation. For the possible application to actual water bodies in the future, the long-term effects and a pilot or even commercial tests of interfacial oxygen nanobubbles, as well as the volume and adding times of zeolites (SI), should be further investigated.

4. Conclusions

Our work demonstrated the potential that interfacial oxygen nanobubbles are capable of mitigating MeHg production in the overlying water and surface sediment of Hg-polluted eutrophic waters. In the overlying water, anoxia remediation and reduction of labile organic matter may contribute to the decrease of %MeHg and MeHg concentrations. While in surface sediment, the significant decline of MeHg production could be attributed to the enhanced oxidative conditions, as well as the decrease of labile organic matter and exchangeable Hg content. Moreover, after the addition of O_2 nanobubbles, *hgcA* gene abundances decreased significantly in the overlying water and surface sediment, suggesting the reduction of Hg microbial methylators. We suggested that the technology of interfacial oxygen nanobubbles could act as a novel and effective solution for MeHg remediation in Hg-polluted eutrophic waters.

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