Reduction and Bacterial Adsorption of Dissolved Mercuric Ion by Indigenous Bacteria at the Oak Ridge Reservation Site

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Abstract

Mercury exists in various forms in the environment and the indigenous bacteria mediated processes have the potential to be used for mercury remediation. In this study, two mixed cultures of indigenous bacteria at the Oak Ridge Reservation site (*i.e.,* ORR soil culture and ORR sediment culture) were selected to study the microbial mediated mercuric reduction under an aerobic condition as well as mercury adsorption onto bacterial surfaces. PCR analysis was performed to provide insights into the microbial community. The mercuric volatilizing experiment demonstrated the mercuric reducing capacity for both ORR cultures, in which the *Pseudomonas* genus was the dominating Hg^0 producer. The investigation of the impact of the sole carbon source revealed the energy-dependent characteristics of the mercuric reduction in this study. Namely, the mercuric reduction was nearly not impacted by the type of carbon source but positively related to the energy that a unit amount of substrate could provide. The study also indicated that the mercury adsorption competed with the reduction. According to the fitting of the Langmuir isotherm, the ORR soil culture was found to have a higher mercury adsorption capacity (i.e., 67.5 mg Hg/g dry biomass) than the ORR sediment culture (i.e., 53.1 mg Hg/g dry biomass). The negative correlation between the reduced mercury mass and adsorbed mercury mass was identified for both ORR cultures.

Keywords:

Adsorption, Mercury, Microbial Community, Reduction.

1 Introduction

Mercury is a naturally occurring element and ubiquitously exists in the environment in various forms including elemental mercury (Hg^0), inorganic mercury salts (*i.e.,* mercuric Hg^{2+} and mercurous Hg_2^{2+}), and organic mercury compounds (*e.g.,* methylmercury CH_3Hg^+, ethylmercury $C_2H_5Hg^+$, and dimethylmercury $(CH_3)_2Hg$). Mercury and most of its compounds are highly toxic to humans even at a low dosage (Zahir et al., 2005). The health effects of mercury depend on its form: The elemental mercury vapor can quickly penetrate the blood-brain barrier and primarily cause brain damage once it is inhaled. The inorganic mercury salts accumulate in its major target organ, kidney, and would cause more acute symptoms such as abdominal pain, vomiting, and bloody diarrhea. The organic mercury is widely distributed throughout the human body and would react with sulfhydryl groups and therefore interfere with DNA transcription, protein synthesis, and central nervous system development (Zahir et al., 2005; Fernandes Azevedo et al., 2012; Park and Zheng, 2012).

Due to its high toxicity and wide impact, mercury is considered a chemical of global concern. Elemental mercury is the dominant form of mercury emission, accounting for more than 90% of the total mercury in the atmosphere (Gonzalez-Raymat et al., 2017). It can remain in the atmosphere for up to 1.7 years, which allows its long-distance travel from the emission sources (Ariya et al., 2015). Elemental mercury is returned to the earth's surface by wet and dry deposition in more soluble forms of inorganic mercury salts. Most of the deposited mercury would complex with organic matters and reside in the soil and sediment pool (Skyllberg et al., 2003; Selin, 2009). Under specific natural conditions, mercury methylation can occur with the help of suitable abiotic methyl donors (e.g., methyl iodide and humic substances) or biotic methylator (e.g., sulfate-reducing bacteria and iron-reducing bacteria) (Celo et al., 2006; Gilmour et al., 2013). Compared

to other mercury species, methylmercury is more concerned since it easily bioaccumulates and biomagnifies in food webs and directly posts threats to the health of humans and other predators (Lavoie et al., 2013). Through global cycling, mercury could travel a long distance in the atmosphere, lithosphere, and hydrosphere, and therefore has a broad impact on human society and ecosystems at local and global scopes. Further driven by the presence of organomercury, the health risk of mercury is expanded to the biosphere and anthroposphere (Selin, 2009; Beckers and Rinklebe, 2017).

Mercury is persistent in the environment. The mercury concentrations in contaminated soil and water body can reach as high as 9,000 mg Hg/kg and 27 μg Hg/L, respectively (Wang et al., 2012; Gworek et al., 2016). In the U.S., mercury is reported as a contaminant at around 50% of the Superfund sites in different forms (Bigham et al., 2017). At the U.S. Department of Energy (DOE) managed Oak Ridge Reservation (ORR) site, the area around its Y-12 National Security Complex was heavily contaminated by mercury due to the extensive amount of mercury (i.e., 108,000 to 212,000 kg) released from 1950 to 1963 (Brooks and Southworth, 2011). Since the site was placed on the U.S. National Priorities List in 1989, a lot of efforts have been made to investigate and remediate the mercury contamination at the ORR site (Southworth et al., 2000; Han et al., 2006; Dong et al., 2010). The mercury concentration in the surface water of East Fork Poplar Creek, where the mercury-contaminated wastewater from Y-12 Complex was discharged to, was successfully reduced by 85% from around 2 μg Hg/L in the 1980s and was close to the cleaning goal (i.e., 0.2 μg Hg/L) (Brooks and Southworth, 2011). However, there is still a larger mercury inventory remaining in the creek sediments (i.e., 334 kg), which continue to post threats to uncontaminated areas at the ORR site (Demers et al., 2018).

In the environment, plenty of bacteria can survive under highly mercury-contaminated conditions. And the microbial activities of these indigenous bacteria (e.g., mercury adsorption, reduction, and methylation) can be used for the onsite mercury remediation. For example, some bacteria strains (e.g., *Bacillus cereus* and *Drepanocladus revolvens*) have high mercury adsorption capacity and could bioaccumulate the mercury in aquatic conditions (Sari and Tuzen, 2009; Sinha et al., 2012). Some aerobic bacteria and archaea (e.g., *Bacillus cereus* and *Pseudomonas putida*) can reduce soluble mercury ions to elemental Hg^0 by mercuric reductase (*merA*) protein and *mer* operon functions (Ariya et al., 2015). And the microbial reduction of mercury could detoxicate contaminated sites and recovery mercury resources simultaneously (Nascimento and Chartone-Souza, 2003). Moreover, some bacteria strains (e.g., Methanogens) could demethylate methylmercury, which significantly reduced the risk of bioaccumulation in food webs (Oremland et al., 1991; Bolan et al., 2013). While polymerase chain reaction (PCR) technology is a popular method to provide insight into the microbial community. Numerous studies have used next-generation high throughput, amplicon-based sequencing of 16S ribosomal RNA (16S rRNA) gene regions to study the composition of the mercury transformation-related microbial communities from a variety of habitats including terrestrial and aquatic ecosystems (Christensen et al., 2019; Song et al., 2019).

In this study, two major mechanisms for microbial mercury remediation, namely mercury reduction and adsorption, were investigated with two mixed cultures of ORR indigenous bacteria. The objectives of this study include (1) evaluation of mercury adsorption on ORR site cultures by adsorption isotherm simulation; (2) quantification of microbial mercuric reduction and characterization of the impact of carbon sources on the reduction; and (3) evaluation of the impact of the microbial community on mercuric reduction with PCR analysis. This study clearly

107 demonstrated the mercury remediation potential of indigenous bacteria at the ORR site, revealed
108 the energy-dependent characteristic of the microbial mercuric reduction under the aerobic
109 condition, and evidenced the competition between the mercury adsorption and the reduction.

2 Method

2.1 Bacterial Cultures

The two applied mixed cultures were derived from indigenous bacteria in the soil and sediment samples collected from the mercury-contaminated ORR site. The mixed cultures contained mercury resistant strains due to pre-exposure to mercury. They were cultured according to the method adapted from previous studies (Davis et al., 2005; Li et al., 2019a). Specifically, 2 grams of nutrient broth (Remel, Thermo scientific) was added to 1 L of tap water and the mixture was pre-sterilized by an autoclave (Tuttnauer) under 121°C and 20 psi for 20 minutes. Then, 0.5 gram of ORR soil or sediment sample was added to 100 mL of the sterilized media. The culture flask was cultivated at 30°C and shaken on an orbital shaker (Model 1000, VWR) at a constant rate of 120 rpm. After 4 days, 2 mL of the supernatant (with soil or sediment particles settling down) was collected and grown in another culture flask under the same condition.

2.1 Mercury adsorption experiment

Mercury adsorption on two ORR mixed cultures was investigated following the method described in the previous study (Li et al., 2019b). Specifically, bacterial biomass in 10 mL of culture was harvested by centrifuging at 2000×g for 25 minutes. Then the harvested biomass was re-suspended in the same volume of mercuric chloride solution with concentrations ranging from 2 to 1,200 mg Hg/L. The mixture was shaken on a digital rotator (Glas Col) at a constant rate of 40 rpm for 12 hours to reach equilibrium. The mixture was then centrifuged at 2000×g for 25 minutes, after which mercuric concentration in the supernatant was measured using an inductively coupled plasma mass spectrometry (iCAP RQ ICP-MS, Thermo Scientific). Every measurement

was replicated three times for quality control. Mercury adsorbed on bacteria was calculated based on mass balance:

$$q = (C_0 - C_e) \times \frac{V}{M} \quad (1)$$

where q is the adsorbed mercury in the unit of mg Hg/g dry biomass, C_0 and C_e are initial and equilibrium mercuric concentrations in the unit of mg Hg/L, V is the volume of the mixture in the unit of L, and M is the dry mass of bacterial biomass in the unit of g.

The obtained mercury adsorption results were fitted with two adsorption isotherms, including the Freundlich and the Langmuir isotherms (Ayawei et al., 2017). And the least-squares method was used to estimate isotherm parameters.

$$q = K_F C_e^{\frac{1}{n}} \quad (2)$$

$$q = \frac{bQ_{max}C_e}{bC_e + 1} \quad (3)$$

where K_F and n are Freundlich isotherm constants, Q_{max} and b are Langmuir isotherm constants.

Control experiment for mercury adsorption was conducted following the same protocol described in this section without adding ORR soil and ORR sediment cultures.

2.2 Microbial mercury volatilizing experiment

The microbial mercuric reduction by two ORR mixed cultures was investigated by a volatilizing experiment. Figure S1 depicts the schematic set-up for the experiment. The bacterial culture was diluted 100 times by deionized and sterilized water (18.2 mΩ, milli-Q) and 250 mL diluted volume was added to a 500 mL glass batch reactor. Then mercuric stock solution (i.e., 1,000 mg Hg/L) was added to the reactor to reach a mercuric concentration around 1,000 μg Hg/L. The reactor was wrapped with aluminum foil to prevent possible photochemical reactions. The

produced Hg^0 in the reactor headspace was flushed out by nitrogen gas (Airgas) every 0.5 or 1 hour at a constant flow rate of 25 mL/min and was then oxidized and collected in two mercury trapping solutions (i.e., 0.05 M $KMnO_4$ with 5% H_2SO_4) which were connected to the top of the reactor (Kritee et al., 2007). All glassware was sterilized by the autoclave under 121°C and 20 psi for 20 minutes before the experiment.

After a certain time (i.e., 0.5 to 12 hours), an aqueous sample was collected from the bottom outlet of the reactor to quantify the dissolved mercuric concentration in the reaction solution. At the same time, two mercury traps were replaced and the trapping solution was sampled to quantify the amount of produced Hg^0 within the time interval. The mercuric concentrations were measured by the ICP-MS (iCAP RQ ICP-MS, Thermo Scientific). Every measurement was replicated three times for quality control. Based on the mercuric concentration in the traps, the rate of mercury removal (due to the production of Hg^0) was calculated by the following equation:

$$Rate = \frac{C_{T1\,i} \times V_T + C_{T2\,i} \times V_T}{\Delta t} \quad (4)$$

where, $C_{T1\,i}$ and $C_{T2\,i}$ are i^{th} measurements for the mercuric concentration in traps in units of μg Hg/L, V_T is the volume of the trapping solution (i.e., 100 mL), and Δt is the time interval in the unit of hour.

Based on the mercuric concentration measurements and adsorption isotherm, we estimated the mercury mass recovery at the end of each mercury volatilizing experiment by the following equation:

$$Recovery = \frac{M_D + M_R + M_A}{M_0} \quad (5)$$

where M_D, M_R, M_A, and M_0 are dissolved mercury mass in batch solution, reduced mercury mass in trapping solutions, adsorbed mercury mass on bacterial biomass, and total mercury mass at the

beginning of the experiment, respectively. They are all in the unit of μg Hg. The calculation of mercury mass species was described in the Supplementary Material.

Control experiment for mercury volatilizing was conducted following the same protocol described in this section without adding ORR soil and ORR sediment cultures. And the conditions for the volatilizing experiment were described in the Supplementary Materials (Table S1).

2.3 Impact of carbon sources

To evaluate the impact of carbon sources on microbial mercuric reduction, the volatilizing experiment was repeated with three sole carbon sources including acetate, citrate, and glucose. The stock solution of each carbon source was added to the reactor to reach a substrate concentration of 0.2 mol/L. To ensure the condition of the sole carbon source, bacterial biomass was harvest by centrifuging at 2000×g for 25 minutes and the supernatant containing nutrient broth media was discarded. Then, the harvested biomass was washed with the deionized and sterilized water (milli-Q) by repeating the centrifuging process for another 2 times.

At the end of the experiment, the acetate and citrate concentration in the reactor was measured by an ion chromatographic system (Dionex, Aquion, Thermo Scientific), while the glucose concentration in the reactor was estimated from chemical oxygen demand measurements (Dr 3900, Hach). Based on the concentrations of reduced mercury and carbon source, the efficiency of mercury removal (due to the production of Hg^0) was computed for each carbon source:

$$Efficiency = \frac{(C_{T1\,i} + C_{T2\,i}) \times V_T}{MW_{Hg} \times (C_{C0} - C_{Ci}) \times V} \quad (6)$$

where, $C_{T1\,i}$ and $C_{T2\,i}$ are i^{th} measurements for the mercuric concentration in traps in units of μg Hg/L, C_{C0} and C_{Ci} are concentrations of the carbon source at the beginning and the end of the mercury volatilizing experiment in the unit of mmol/L, MW_{Hg} is the molecular weight of mercury, and V is the volume of the reaction solution in the unit of mL.

Control experiment for the investigation of carbon sources was conducted following the same protocol described in Section 2.2 without adding ORR soil and ORR sediment cultures.

2.4 PCR Analysis

Total genomic DNA extraction from soil and water samples was performed using the PowerSoil DNA isolation kit (MO BIO Laboratories Inc.; CA, USA) according to manufacturer' protocols. The concentrations and purity of isolated DNA solution were measured using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific). The primer set 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′) was used for the V4 region of the 16S rRNA gene amplification (Caporaso et al., 2011). The PCR reaction parameters were: 95°C, 3 min; 95°C, 30 s, 55°C, 30 s, 72°C, 30 s, 25 cycles; 72°C, 5 min. After two steps of PCR amplification, qualified libraries were sequenced on the Illumina MiSeq platform using the MiSeq Reagent Nano Kit V2 (500 cycles).

The raw demultiplexed paired-end sequences in FASTQ format were processed using Quantitative Insights Into Microbial Ecology version 2 (QIIME2) software for phylogenetic analysis (Bolyen et al., 2019). After the amplicon primers removal, reads were denoised and trimmed using DADA2 (Callahan et al., 2016), and then amplicon sequence variants were classified using the sklearn QIIME feature-classifier at 100% operational taxonomic units sequence identity level with the SILVA v132 reference database.

3 Result and discussion

3.1 Mercury adsorption experiment

In the adsorption experiment, the highest mercury adsorption was observed for the ORR soil culture (i.e., 69.4 mg Hg/g), which was 25% higher than that of the ORR sediment culture (i.e., 55.5 mg Hg/g). Compared with the ORR sediment culture, the ORR soil culture reached the maximum adsorption faster when C_{eq} was less than 120 mg Hg/L, which was attributed to the difference in the bacterial surface functional groups or the adaptability to mercury exposure in the same geochemistry condition (Ngwenya et al., 2003; Li et al., 2019b).

Based on the observation, experiment results were fitted with two adsorption isotherms, including the Freundlich and the Langmuir isotherms. Two statistical parameters (i.e., correlation coefficient (R) and summed squared error (SSE)) were used to evaluate the fitting of two adsorption isotherms (Table 1).

Overall, the Langmuir isotherm had better statistical performance, with larger R (i.e., 0.938, and 0.968 for the ORS soil culture and ORS sediment culture, respectively) and smaller SSE (i.e., 674 and 213, respectively). The good fit of the Langmuir isotherm suggested that the microbial mercury adsorption can be regarded as a monolayer adsorption process (Al-Ghouti et al., 2020). A reasonable explanation is that the adsorption was mainly controlled by the complexation between mercuric ion and functional groups (e.g., carboxyl, sulfhydryl, and thioether) on the bacterial surface, which agreed well with previous studies about microbial adsorption of heavy metals (Daughney et al., 1998; Sinha et al., 2012; Li et al., 2019a). The fitting with the Freundlich isotherm led to lower R and higher SSE, which were around 2.3 times larger than those of the Langmuir isotherm fitting. Therefore, it was insufficient to describe the adsorption process in this study. According to the Langmuir isotherm fitting, the mercury adsorption capacities were 67.5

mg Hg/g and 53.1 mg Hg/g for the ORR soil culture and ORR sediment culture, respectively (Table 1), which were comparable with mercury adsorption capacities reported in previous studies (Das et al., 2007; Noghabi et al., 2007). The obtained Langmuir adsorption isotherm parameters were used to estimate mercury adsorption at the end of mercury volatilizing experiments (Supplementary Material).

3.2 Mercury volatilizing experiment

The presence of mercury in two trapping solutions indicated the mercuric reducing capacity of both ORR cultures (Figure 2). Within the experiment of 12 hours, the ORR sediment culture removed more mercury in the form of Hg^0 (i.e., 128 μg Hg) with higher removal efficiency (i.e., 52.9%) than those of the ORR soil culture (i.e., 57 μg Hg, 24.3%, respectively). The removal mainly occurred in the first 3 hours with a rapid dissolved mercuric concentration decrease in the batch reactor. Meanwhile, high mercury removal rates (due to production of Hg^0) were observed in the traps for the ORR sediment culture (i.e., as high as 59 μg Hg/hour), which was nearly 3 times of the highest mercury removal rate of the ORR soil culture (i.e., 20 μg Hg/hour). Then, the mercury removal gradually decreased and the dissolved mercuric concentration remained at a stable level.

As PCR results presented, the mercury exposure significantly altered the microbial community structure. After the volatilizing experiment, the microbial community was extremely simplified in both ORR soil and ORR sediment cultures, in which *Proteobacteria* became the most dominant bacterial phylum (i.e., increased from 40% to 77% and from 43% to 90% in the ORR soil culture and the ORR sediment culture, respectively) (Figure 3a). Correspondingly, other major phyla in the original microbial community were eliminated. At the genus level, a high percentage

of the microbial community (i.e., 78% and 85% for the ORR soil culture and the ORR sediment culture, respectively) cannot be identified for raw indigenous cultures due to the lack of information in the applied database (Figure 3b). For the microbial community of the ORR sediment culture (after mercury exposure), several major genera are known as the holder for the mercuric reductase protein (*merA*), including *Pseudomonas* (56%), *Aeromonas* (9%), and *Bacillus* (0.2%) (De et al., 2003; Zhang et al., 2012; Dash et al., 2014). Therefore, the major involved mechanism for mercury removal in the volatilizing experiment was the microbial mercuric reduction meditated by the mercury resistant (*mer*) system with aerobic mercury resistant bacteria. While the relative abundance of similar genera was less than 18% in the microbial community of the ORR soil culture, which contributed to the difference of mercuric reducing capacity between these two ORR cultures.

3.3 Impact of carbon source on mercury reduction

When sole carbon sources were used, major mercury removal still occurred in the first 3 hours (Figure 4) and the PCR results indicated a similar microbial community at the level of phylum (Figure 5a). For the ORR sediment culture, the Hg^0 production was lower (i.e., 45%, 30%, and 40% for acetate, citrate, and glucose, respectively) than that of the previous experiment with nutrient broth (53%). The maximum mercury removal rate (due to the production of Hg^0) decreased from 59 μg Hg/hour to 33 μg Hg/hour, 34 μg Hg/hour, and 36 μg Hg/hour for acetate, citrate, and glucose, respectively. Compared with the previous microbial community at the genus level (Figure 3b), the relative abundance of *Pseudomonas* decreased by more than a half (i.e., decreased from 56% to 18%, 19%, and 25% with acetate, citrate, and glucose, respectively), while the relative abundance of other possible mercury-reducing genera (i.e., *Aeromonas* and *Bacillus*) remained at a similar level. For ORR soil culture, the difference in Hg^0 production was less than 6%

for all mercury volatilizing experiments (i.e., normalizing the largest Hg^0 production difference by the average Hg^0 production), which corresponded with the comparable relative abundance in all possible mercury-reducing genera (Figure 3b and Figure 5b). This observation implied that the *Pseudomonas* was the dominating Hg^0 producer in ORR indigenous bacteria. On the other hand, ORR sediment cultures had a faster substrate consumption rate in the first 4 hours, which may contribute to more mercury reduction (Figure S2).

Previous studies have reported that several strains under the *Pseudomonas* genus have the capacity to reduced dissolved mercuric ion to elemental Hg^0 in natural or laboratory conditions. Prior research found a *P. putida* strain (i.e., SP1) isolated from a marine environment was able to volatilize and remove almost 100% of mercury in the aquatic environment (Zhang et al., 2012). Another research reported a mercuric reducing rate of 99.7% under saline condition by a *P. aeruginosa* strain (i.e., FA-2) isolated from landfill leachate (Imron et al., 2019). Although the Hg^0 removal efficiency of the ORR cultures was not as remarkable as pure strains, the ORR bacteria still have the potential for the mercury remediation since the indigenous bacteria would have minor impact on the ecosystem at contaminated sites (Cowan et al., 2011).

Among three carbon sources, both ORR cultures had more Hg^0 production with acetate (i.e., 45% and 24% for ORR sediment and ORR soil cultures, respectively), although this difference for the ORR soil culture was minor (Figure 4). However, this observation cannot be explained from the microbial community aspect, since the sole carbon sources led to a similar community structure after mercury exposure with a limited difference (Figure 5). To fully understand the results, the total amount of Hg^0 production was further normalized with the amount of consumed substrate (Figure 6). The normalized Hg^0 production was found linearly to increase with the standard Gibbs free energy provided by a unit amount of substrate (i.e., the ORR soil

304 culture and the ORR sediment culture had R^2 of 0.79 and 0.82, respectively), indicating that the
305 microbial mercuric reduction in this study was an energy-dependent process. Among three sole
306 carbon sources, acetate, even though had the lowest standard Gibbs free energy (i.e., 219.2 kJ/mol),
307 was more consumed, resulting in more mercury removal subsequently (Figure 5). Compared with
308 citrate and glucose, acetate has a shorter carbon chain and may be more bioavailable to ORR
309 cultures.

310 The explanation was also supported by the Venn diagram derived from the PCR results
311 (Figure S3). The highly overlapped microbial community at the genus level (i.e., 13 out of 19, and
312 15 out of 18 major genera in the ORR soil culture and the ORR sediment culture, respectively)
313 was observed with the amendment of three sole carbon sources. Moreover, the genera that cannot
314 utilize all the sole carbon sources only accounted for less than 0.5% in the microbial community
315 of both ORR cultures. Therefore, the difference in sole carbon sources had limited impacts on the
316 microbial diversity and population distribution, which was in agreement with the above-mentioned
317 thermodynamics analysis that the observed microbial mercury reduction was an energy-dependent
318 process in this study.

319

320 **3.4 Interaction between mercury adsorption and reduction.**

321 In the mercury volatilizing experiments, the computation of the Hg^0 production rate failed
322 in capturing the decrease of dissolved mercury concentration after the first 3 hours (Figure 2 and
323 Figure 4). It was suspected that mercury adsorption onto bacterial surfaces also played a role. At
324 the end of the experiments, the mercury mass speciation (i.e., dissolved mercury mass in batch
325 solution, reduced mercury mass in trapping solutions, and adsorbed mercury mass on bacterial
326 biomass) was estimated (Table S4). The reduced and adsorbed mercury accounted for 22% - 53%

and 6% to 11% with respect to the mass of total mercury. Overall, the mercury mass recovery was higher than 92% for both ORR cultures, indicating a reasonable mercury mass balance in the estimation. The major source of error underlined the computation of adsorbed mercury mass with the adsorption isotherm parameters, which were specific for ORR cultures before the mercury volatilizing experiment. According to the PCR results, the mercury exposure altered the microbial community for both ORR cultures (Figure 3 and Figure 5), and corresponded mercury adsorption characteristics may also change.

According to the estimation of mercury mass speciation, the total mass of reduced mercury was negatively related to the total mass of adsorbed mercury (Figure 7). Although the values of R^2 were only 0.86 and 0.67 for the ORR soil culture and the ORR sediment culture, respectively, a general trend of competition between mercury adsorption and the mercuric reduction was demonstrated. In other words, the observation suggested that once the mercuric ion was complexed with the functional groups on the bacterial surfaces, it was no longer available for the mercuric binding protein (*merP*) and subsequent mercury reduction process.

The observation is similar to those in the system of mercury-resistant bacteria and natural organic matters (Kannan and Krishnamoorthy, 2006). The complexation between mercuric ion and organic matters would limit the mercury bioavailability to bacteria since complexed mercuric ion cannot be transported across the bacterial membrane. On the other hand, mercuric ion could also be reduced to elemental Hg^0 under anaerobic conditions with sulfate-reducing or iron-reducing bacteria. Although the reduction mechanism is totally different, the inhibition of mercury adsorption (mainly complexed with thiol function groups) on the reduction has also been reported (Hu et al., 2013). The competition between mercury adsorption and reduction would significantly affect the process of mercury remediation. Under a geochemical condition favoring mercury

adsorption, the mercury containing biomass needs to be carefully treated after the bioaccumulation. On the contrary, suitable mercury vapor traps would be required to prevent Hg^0 emission and secondary contamination after long-distance travel of Hg^0.

4 Conclusion

The mercury remediation capacity of two ORR cultures was evidenced by the mercury adsorption experiment and the mercury volatilizing experiment. According to the fitting of the Langmuir isotherm, the ORR soil culture and the ORR sediment culture had a mercury adsorption capacity of 67.5 mg Hg/g dry biomass and 53.1 mg Hg/g dry biomass, respectively. On the contrary, the ORR sediment culture had better mercury reducing capacity, which removed as high as 53% of total mercury in the volatilizing experiment. PCR results indicated that the genus of *Pseudomonas* was the dominating Hg^0 producer in ORR cultures. The investigation on the difference of sole carbon sources demonstrated a limited impact on the microbial community and subsequent mercury removal, which revealed the energy-dependent characteristic of microbial mercuric reduction in this study. Furthermore, the negative correlation between the reduced mercury mass and adsorbed mercury mass indicated the competence between mercury adsorption and mercuric reduction, which may significantly affect the process of mercury remediation.

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References

Al-Ghouti, M.A. and Da'ana, D.A., 2020. Guidelines for the use and interpretation of adsorption isotherm models: A review. Journal of Hazardous Materials, p.122383.

Ariya, P.A., Amyot, M., Dastoor, A., Deeds, D., Feinberg, A., Kos, G., Poulain, A., Ryjkov, A., Semeniuk, K., Subir, M. and Toyota, K., 2015. Mercury physicochemical and biogeochemical transformation in the atmosphere and at atmospheric interfaces: A review and future directions. Chemical Reviews, 115(10), pp.3760-3802.

Ayawei, N., Ebelegi, A.N. and Wankasi, D., 2017. Modelling and interpretation of adsorption isotherms. Journal of Chemistry, 2017.

Beckers, F. and Rinklebe, J., 2017. Cycling of mercury in the environment: Sources, fate, and human health implications: A review. Critical Reviews in Environmental Science and Technology, 47(9), pp.693-794.

Bigham, G.N., Murray, K.J., Masue-Slowey, Y. and Henry, E.A., 2017. Biogeochemical controls on methylmercury in soils and sediments: Implications for site management. Integrated Environmental Assessment and Management, 13(2), pp.249-263.

Bolan, N.S., Choppala, G., Kunhikrishnan, A., Park, J. and Naidu, R., 2013. Microbial transformation of trace elements in soils in relation to bioavailability and remediation. In Reviews of Environmental Contamination and Toxicology (pp. 1-56). Springer, New York, NY.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F. and Bai, Y., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology, 37(8), pp.852-857.

Brooks, S.C. and Southworth, G.R., 2011. History of mercury use and environmental contamination at the Oak Ridge Y-12 Plant. Environmental Pollution, 159(1), pp.219-228.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. and Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), pp.581-583.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N. and Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences, 108(Supplement 1), pp.4516-4522.

Celo, V., Lean, D.R. and Scott, S.L., 2006. Abiotic methylation of mercury in the aquatic environment. Science of the Total Environment, 368(1), pp.126-137.

Christensen, G.A., Gionfriddo, C.M., King, A.J., Moberly, J.G., Miller, C.L., Somenahally, A.C., Callister, S.J., Brewer, H., Podar, M., Brown, S.D. and Palumbo, A.V., 2019. Determining the reliability of measuring mercury cycling gene abundance with correlations with mercury and methylmercury concentrations. Environmental Science & Technology, 53(15), pp.8649-8663.

Cowan, D.A., Chown, S.L., Convey, P., Tuffin, M., Hughes, K., Pointing, S. and Vincent, W.F., 2011. Non-indigenous microorganisms in the Antarctic: assessing the risks. Trends in Microbiology, 19(11), pp.540-548.

Daughney, C.J., Fein, J.B. and Yee, N., 1998. A comparison of the thermodynamics of metal adsorption onto two common bacteria. Chemical Geology, 144(3-4), pp.161-176.

Das, S.K., Das, A.R. and Guha, A.K., 2007. A study on the adsorption mechanism of mercury on *Aspergillus versicolor* biomass. Environmental Science & Technology, 41(24), pp.8281-8287.

Dash, H.R., Mangwani, N. and Das, S., 2014. Characterization and potential application in mercury bioremediation of highly mercury-resistant marine bacterium *Bacillus thuringiensis* PW-05. Environmental Science and Pollution Research, 21(4), pp.2642-2653.

Davis, K.E., Joseph, S.J. and Janssen, P.H., 2005. Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria. Applied and Environmental Microbiology, 71(2), pp.826-834.

De, J., Ramaiah, N., Mesquita, A. and Verlekar, X.N., 2003. Tolerance to various toxicants by marine bacteria highly resistant to mercury. Marine Biotechnology, 5(2), pp.185-193.

Demers, J.D., Blum, J.D., Brooks, S.C., Donovan, P.M., Riscassi, A.L., Miller, C.L., Zheng, W. and Gu, B., 2018. Hg isotopes reveal in-stream processing and legacy inputs in East Fork Poplar Creek, Oak Ridge, Tennessee, USA. Environmental Science: Processes & Impacts, 20(4), pp.686-707.

Dong, W., Liang, L., Brooks, S., Southworth, G. and Gu, B., 2010. Roles of dissolved organic matter in the speciation of mercury and methylmercury in a contaminated ecosystem in Oak Ridge, Tennessee. Environmental Chemistry, 7(1), pp.94-102.

Fernandes Azevedo, B., Barros Furieri, L., Peçanha, F.M., Wiggers, G.A., Frizera Vassallo, P., Ronacher Simões, M., Fiorim, J., Rossi de Batista, P., Fioresi, M., Rossoni, L. and Stefanon, I., 2012. Toxic effects of mercury on the cardiovascular and central nervous systems. BioMed Research International, 2012.

Gilmour, C.C., Podar, M., Bullock, A.L., Graham, A.M., Brown, S.D., Somenahally, A.C., Johs, A., Hurt Jr, R.A., Bailey, K.L. and Elias, D.A., 2013. Mercury methylation by novel microorganisms from new environments. Environmental Science & Technology, 47(20), pp.11810-11820.

Gonzalez-Raymat, H., Liu, G., Liriano, C., Li, Y., Yin, Y., Shi, J., Jiang, G. and Cai, Y., 2017. Elemental mercury: Its unique properties affect its behavior and fate in the environment. Environmental Pollution, 229, pp.69-86.

Gworek, B., Bemowska-Kałabun, O., Kijeńska, M. and Wrzosek-Jakubowska, J., 2016. Mercury in marine and oceanic waters-a review. Water, Air, & Soil Pollution, 227(10), p.371.

Han, F.X., Su, Y., Monts, D.L., Waggoner, C.A. and Plodinec, M.J., 2006. Binding, distribution, and plant uptake of mercury in a soil from Oak Ridge, Tennessee, USA. Science of the Total Environment, 368(2-3), pp.753-768.

Hu, H., Lin, H., Zheng, W., Rao, B., Feng, X., Liang, L., Elias, D.A. and Gu, B., 2013. Mercury reduction and cell-surface adsorption by *Geobacter sulfurreducens* PCA. Environmental Science & Technology, 47(19), pp.10922-10930.

Imron, M.F., Kurniawan, S.B. and Soegianto, A., 2019. Characterization of mercury-reducing potential bacteria isolated from Keputih non-active sanitary landfill leachate, Surabaya, Indonesia under different saline conditions. Journal of Environmental Management, 241, pp.113-122.

Kannan, S.K. and Krishnamoorthy, R., 2006. Isolation of mercury resistant bacteria and influence of abiotic factors on bioavailability of mercury—a case study in Pulicat Lake north of Chennai, south east India. Science of the Total Environment, 367(1), pp.341-353.

Kritee, K., Blum, J.D., Johnson, M.W., Bergquist, B.A. and Barkay, T., 2007. Mercury stable isotope fractionation during reduction of Hg (II) to Hg (0) by mercury resistant microorganisms. Environmental Science & Technology, 41(6), pp.1889-1895.

Lavoie, R.A., Jardine, T.D., Chumchal, M.M., Kidd, K.A. and Campbell, L.M., 2013. Biomagnification of mercury in aquatic food webs: a worldwide meta-analysis. Environmental Science & Technology, 47(23), pp.13385-13394.

Li, R., Ibeanusi, V., Hoyle-Gardner, J., Crandall, C., Jagoe, C., Seaman, J., Anandhi, A. and Chen, G., 2019. Bacterial-facilitated uranium transport in the presence of phytate at Savannah River Site. Chemosphere, 223, pp.351-357.

Li, R., Zhang, Z., Li, S., Tang, Y., Wei, C. and Chen, G., 2019. Cadmium–bacteria complexation and subsequent bacteria - facilitated cadmium transport in saturated porous Media. Journal of Environmental Quality, 48(5), pp.1524-1533.

Nascimento, A.M. and Chartone-Souza, E., 2003. Operon *mer*: bacterial resistance to mercury and potential for bioremediation of contaminated environments. Genetics and Molecular Research, 2(1), pp.92-101.

Ngwenya, B.T., Sutherland, I.W. and Kennedy, L., 2003. Comparison of the acid-base behaviour and metal adsorption characteristics of a gram-negative bacterium with other strains. Applied Geochemistry, 18(4), pp.527-538.

Noghabi, K.A., Zahiri, H.S., Lotfi, A.S., Raheb, J.A.M.S.H.I.D., Nasri, S.I.M.A. and Yoon, S.C., 2007. Mercury absorption by *Pseudomonas fluorescens* BM07 grown at two different temperatures. Polish Journal of Microbiology, 56(2), p.111.

Oremland, R.S., Culbertson, C.W. and Winfrey, M.R., 1991. Methylmercury decomposition in sediments and bacterial cultures: involvement of methanogens and sulfate reducers in oxidative demethylation. Applied and Environmental Microbiology, 57(1), pp.130-137.

Park, J.D. and Zheng, W., 2012. Human exposure and health effects of inorganic and elemental mercury. Journal of Preventive Medicine and Public Health, 45(6), p.344.

Rittmann, B.E. and McCarty, P.L., 2012. Environmental Biotechnology: Principles and Applications. Tata McGraw-Hill Education.

Sari, A. and Tuzen, M., 2009. Removal of mercury (II) from aqueous solution using moss (*Drepanocladus revolvens*) biomass: equilibrium, thermodynamic and kinetic studies. Journal of Hazardous Materials, 171(1-3), pp.500-507.

Selin, N.E., 2009. Global biogeochemical cycling of mercury: a review. Annual Review of Environment and Resources, 34.

Sinha, A., Pant, K.K. and Khare, S.K., 2012. Studies on mercury bioremediation by alginate immobilized mercury tolerant *Bacillus cereus* cells. International Biodeterioration & Biodegradation, 71, pp.1-8.

Song, W., Qi, R., Zhao, L., Xue, N., Wang, L. and Yang, Y., 2019. Bacterial community rather than metals shaping metal resistance genes in water, sediment and biofilm in lakes from arid northwestern China. Environmental Pollution, 254, p.113041.

Southworth, G.R., Turner, R.R., Peterson, M.J., Bogle, M.A. and Ryon, M.G., 2000. Response of mercury contamination in fish to decreased aqueous concentrations and loading of inorganic mercury in a small stream. Environmental Monitoring and Assessment, 63(3), pp.481-494.

Skyllberg, U.L.F., Qian, J.I.N., Frech, W., Xia, K. and Bleam, W.F., 2003. Distribution of mercury, methyl mercury and organic sulphur species in soil, soil solution and stream of a boreal forest catchment. Biogeochemistry, 64(1), pp.53-76.

Wang, J., Feng, X., Anderson, C.W., Xing, Y. and Shang, L., 2012. Remediation of mercury contaminated sites–a review. Journal of Hazardous Materials, 221, pp.1-18.

Zahir, F., Rizwi, S.J., Haq, S.K. and Khan, R.H., 2005. Low dose mercury toxicity and human health. Environmental Toxicology and Pharmacology, 20(2), pp.351-360.

Zhang, W., Chen, L. and Liu, D., 2012. Characterization of a marine-isolated mercury-resistant *Pseudomonas putida* strain SP1 and its potential application in marine mercury reduction. Applied Microbiology and Biotechnology, 93(3), pp.1305-1314.

511 **Table 1. Fitted adsorption isotherm parameters**

Isotherms	Parameters	ORS Soil Culture	ORS Sediment Culture
Freundlich	K_F	20.9	13.7
	n	0.189	0.214
	R	0.853	0.921
	SSE	1,540	495
Langmuir	Q_{max}	67.5	53.1
	b	0.221	0.158
	R	0.938	0.968
	SSE	674	213

512

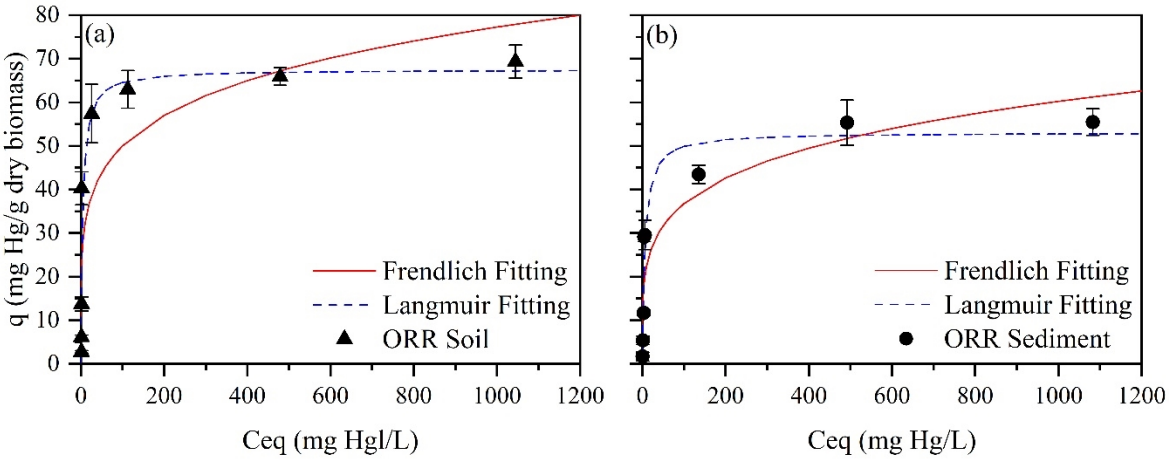
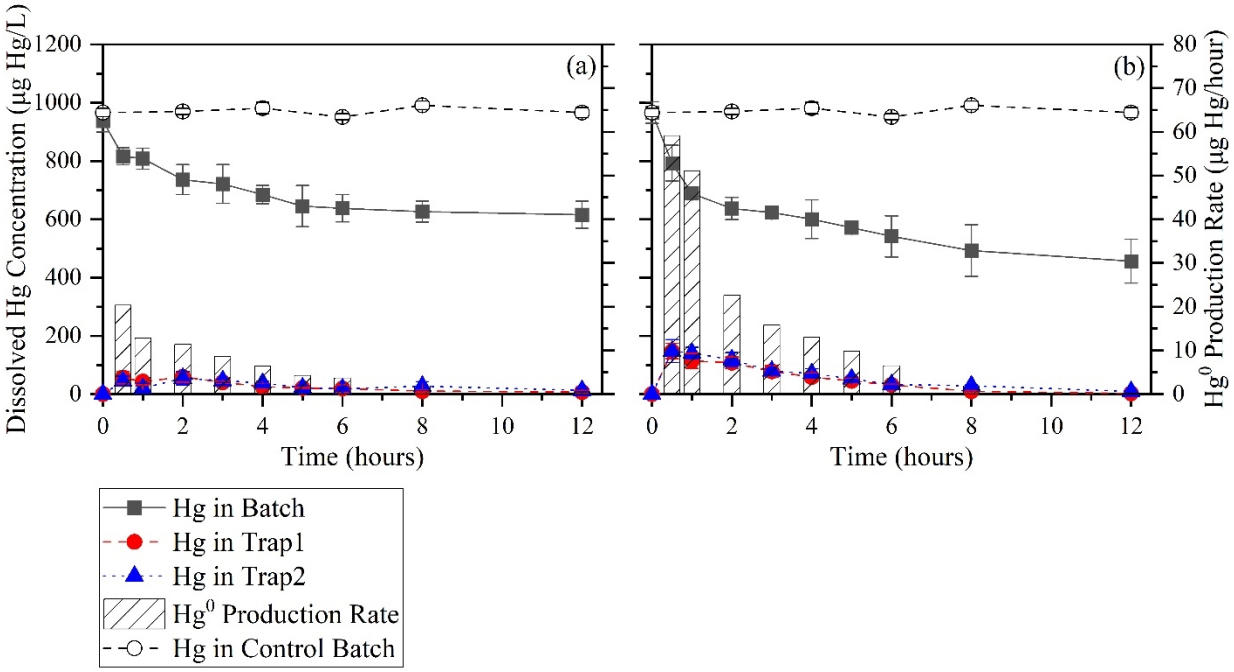
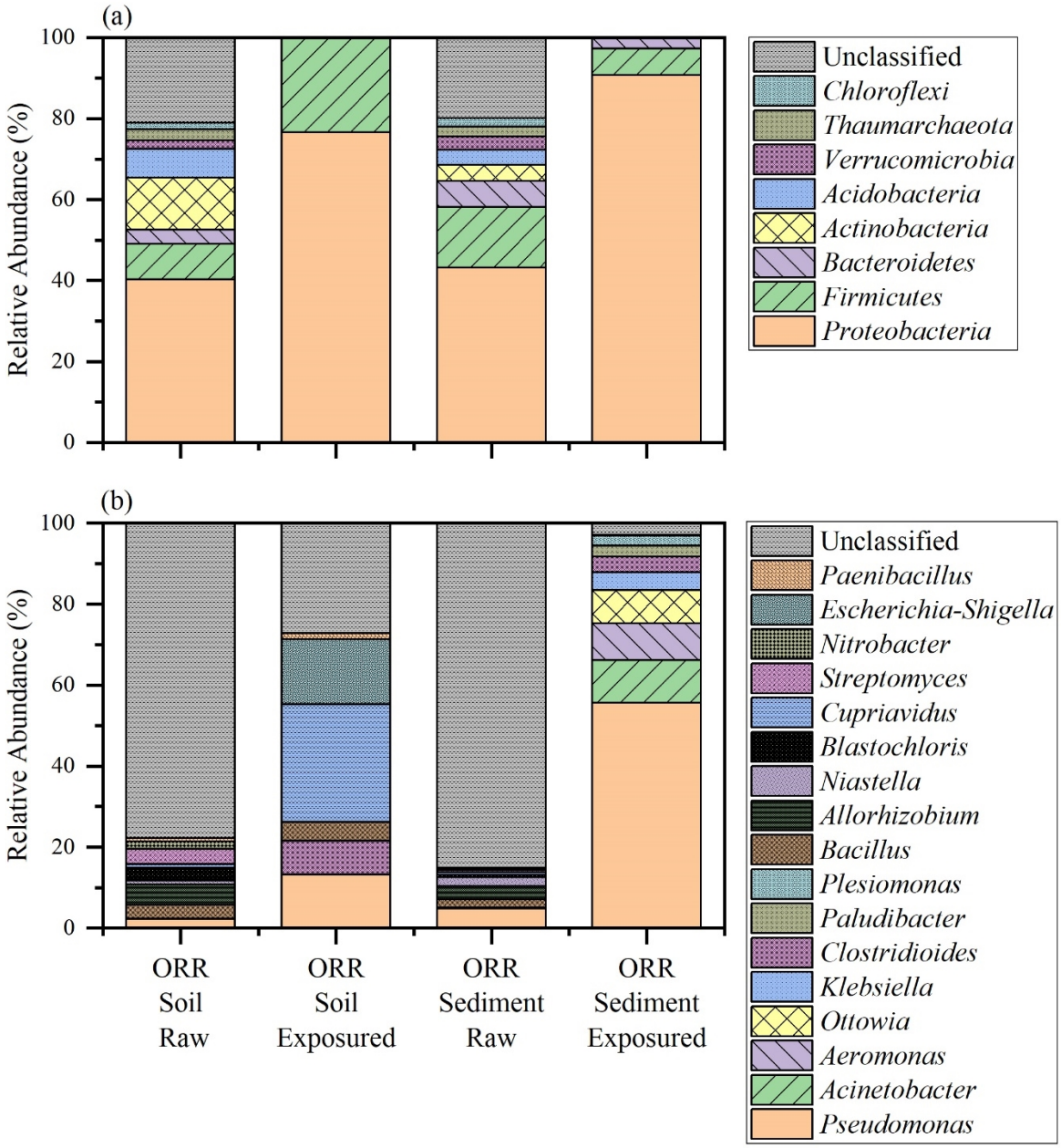


Figure 1. Mercury adsorption and isotherm fitting. (a) ORR soil culture, and (b) ORR sediment culture.



517
518 **Figure 2. Mercury removal and Hg^0 production rate. (a) ORR soil culture, and (b) ORR**
519 **sediment culture.**
520



521
522 **Figure 3. The change of microbial community due to mercury exposure. (a) Phylum level,**
523 **and (b) Genus level.**

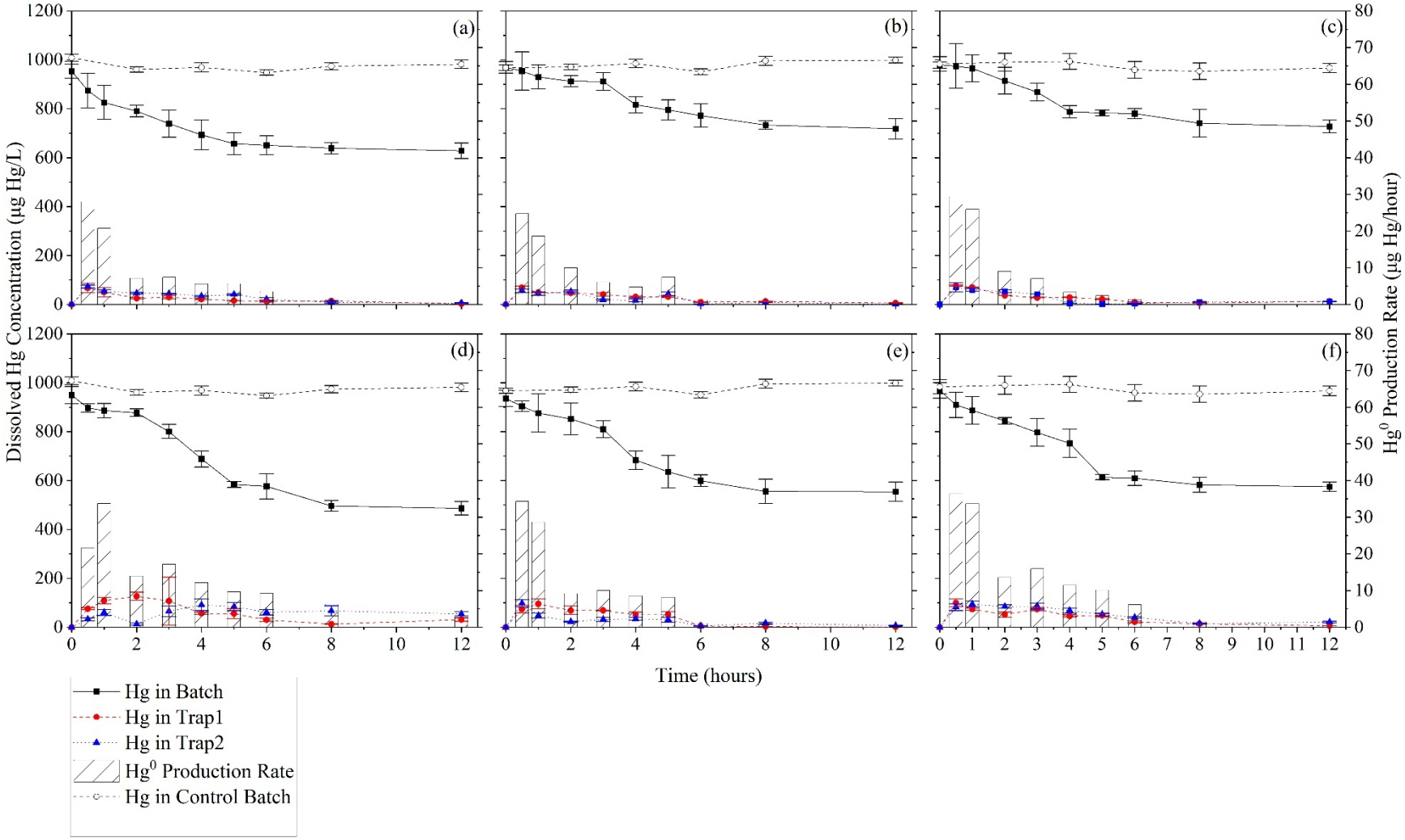
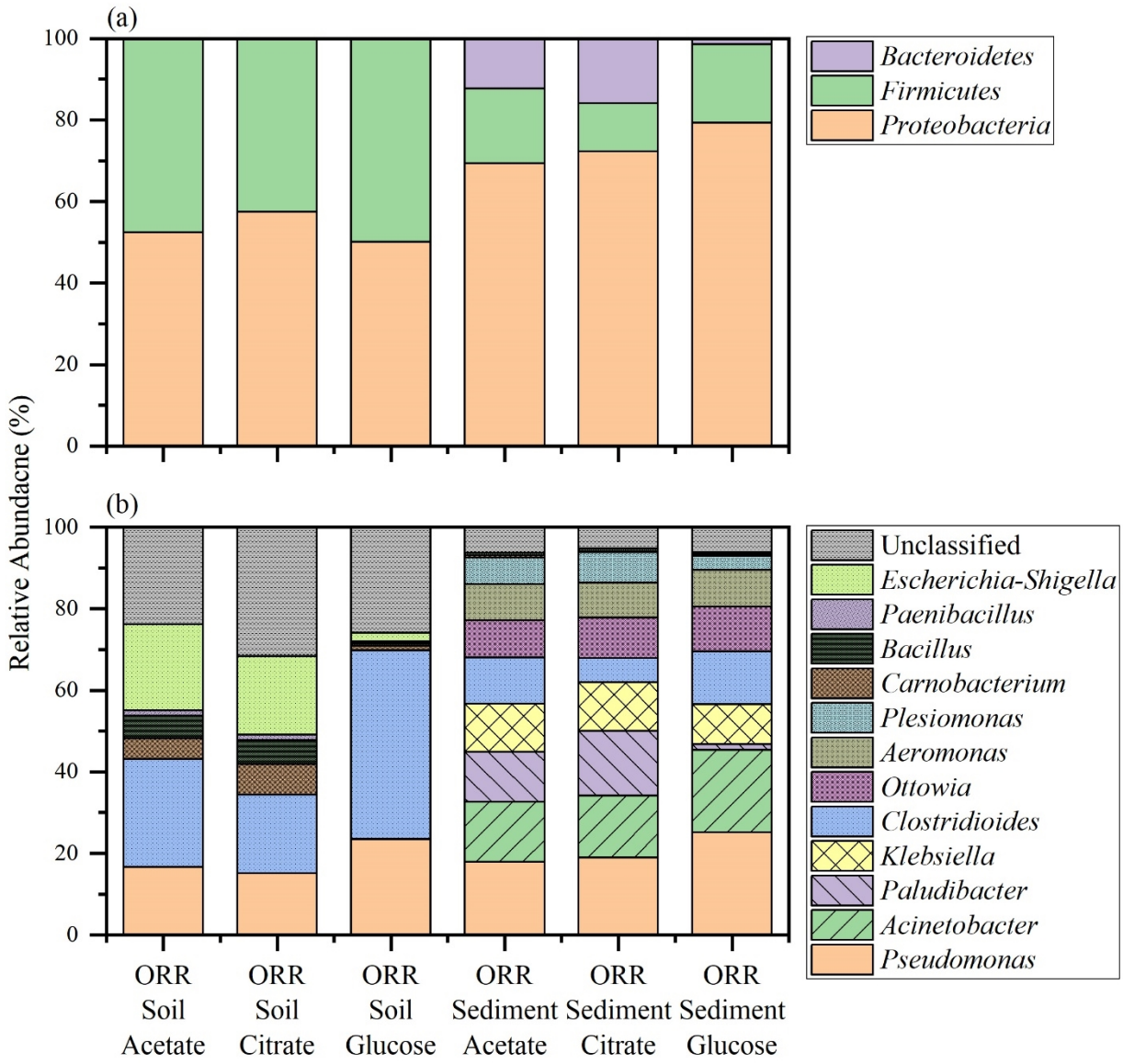
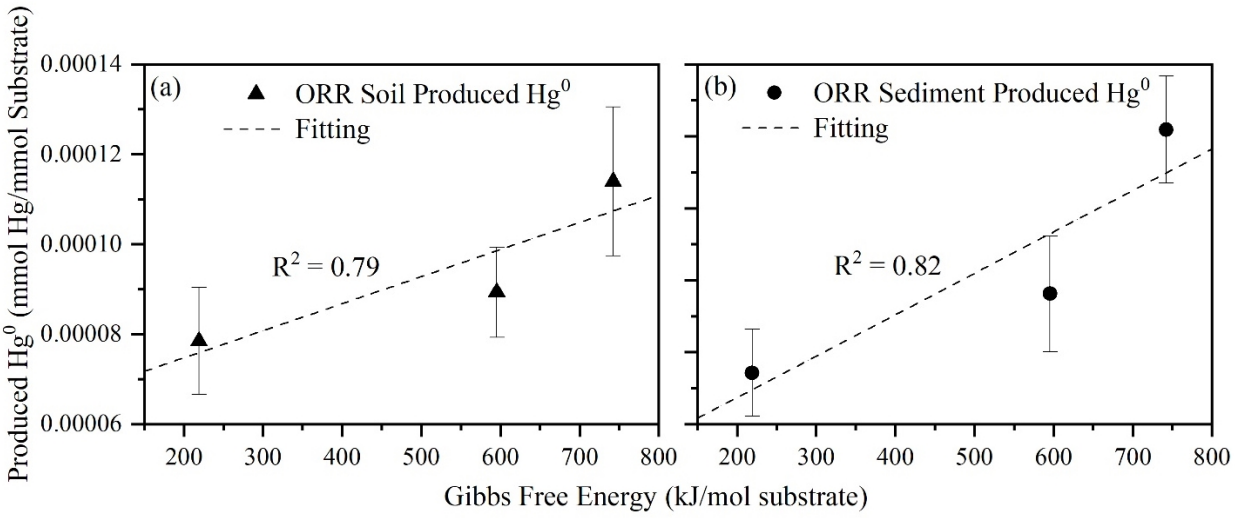


Figure 4. Mercury removal and Hg^0 production rate. (a) ORR soil culture with acetate, (b) ORR soil culture with citrate, (c) ORR soil culture with glucose, (d) ORR sediment culture with acetate, (e) ORR sediment culture with citrate, and (f) ORR sediment culture with glucose.



528
529 **Figure 5. The impact of sole carbon source on the microbial community. (a) Phylum level,**
530 **and (b) Genus level.**
531



532
533 **Figure 6. Normalized Hg^0 production. (a) ORR soil culture, and (b) ORR sediment culture.**
534 Note: The standard Gibs free energy was adopted from Rittmann and McCarty, 2012.
535

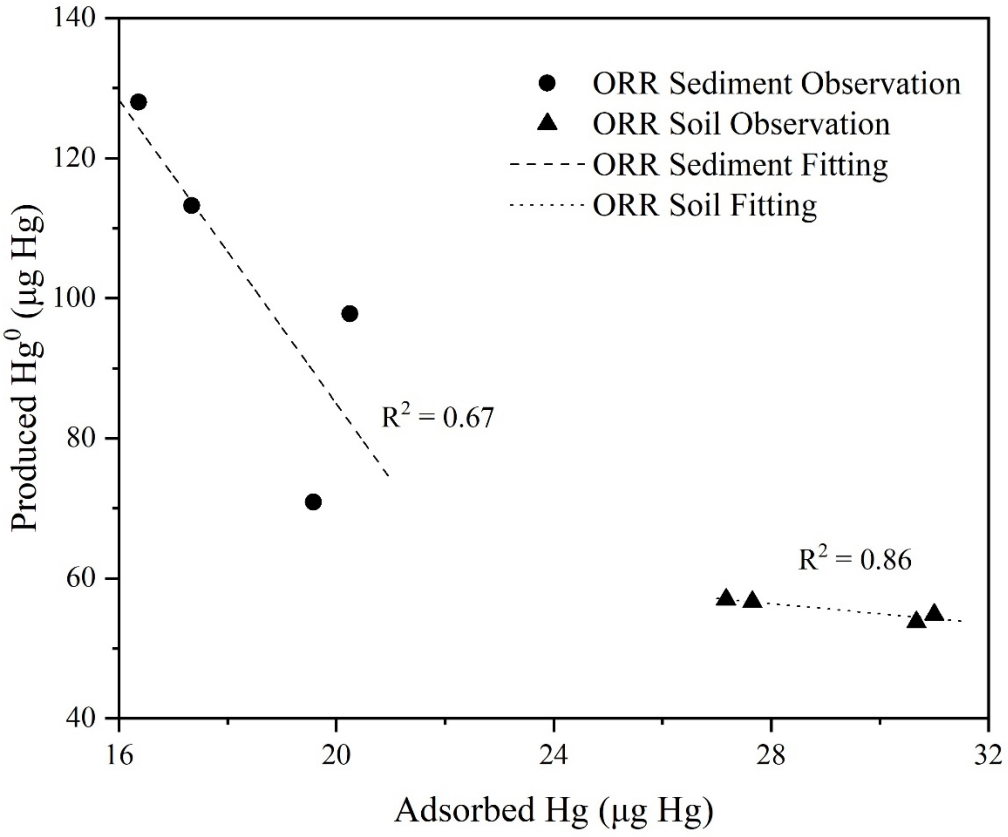


Figure 7. Competition between mercury adsorption and mercuric reduction.