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Adsorption Properties and Gaseous Mercury Transformation Rate of Natural Biofilm

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Abstract Biofilms were developed on glass microscope slides in a natural aquatic environment and their mercury adsorption properties were evaluated. Results demonstrated that the biofilms contained a large number of bacterial cells and associated extracellular polymers. Mercury forms detected in the biofilms were mainly bound to residual matter and organic acids. The adsorption processes could be described by a Langmuir isotherm. The optimum conditions for adsorption of mercury to natural biofilm were an ionic strength of 0.1 mol/L, pH 6 and an optimum adsorption time of 40 min. The transformation rate was 0.79 μg gaseous mercury per gram of biofilm.

Keywords Biofilm · Mercury · Gaseous mercury · Adsorption properties

Mercury is considered a hazardous pollutant owing to its toxicity, even at low concentrations, and its non-biodegradability (Cheng et al. 2005, 2006). The transportation and bioavailability of mercury in aquatic systems are greatly affected by its binding to surfaces of solid phases and complexing with ligands in the water. Solid surfaces, including sediments, suspended particles, and associated biofilms, in freshwater systems primarily consist of organic material (such as cell debris, fecal pellets, and microorganisms), calcium carbonate, iron, manganese and aluminum oxides, and silicate minerals (Wang et al. 2002a, b; Dong et al. 2003). The behavior, distribution, transport, and fate of mercury may be significantly affected by its adsorption and remobilization on biofilms (Wang et al. 2002a). Biofilms are dynamic systems in which both adsorption and desorption processes occur at the same time. Many studies on the Pb and Cd adsorption characteristics of sediments and biofilm have been carried out using different approaches, such as additive models and the use of selective extractants and sequential extractants (Dong et al. 2001, 2002, 2003). However, there have been few reports on the mercury adsorption properties of biofilms. One study demonstrated that under mercury(II) stress, *Kluvera cryocrescens* biofilms adhering to suspended particulate matter (SPM) in the Yangtze River could reduce mercury(II) to elemental mercury, which was subsequently volatilized into the atmosphere (Wang et al. 2002a). However, little theory was proposed to explain these observations. The current study focused on the effect of environmental factors such as ionic strength, pH and time on the adsorption of mercury at the interface between a biofilm and solution. A special apparatus for gaseous mercury collection was used to simulate a biofilm ecosystem in an aquatic environment.

Materials and Methods

Pre-cleaned glass microscope slides (6.0 cm × 7.0 cm × 0.1 cm) were fixed onto polypropylene racks and submerged in a natural aquatic environment at a depth of approximately 50 cm for a period of 40 days in the fall. In each case, visible biofilms were present on the glass slides after development. This method has been used extensively to acquire representative heterogeneous assemblages in illuminated surface water (Dong et al. 2002, 2003; Wang et al. 2002b). Moreover, a previous study demonstrated significant correlations for the content of some important

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Table 1 Sequential extraction procedure

Species	Extractant	pH
Fraction I: exchangeable form	1 mol/L $MgCl_2$ (40 mL)	7.0
Fraction II: carbonate-bound form	1 mol/L NaOAc (40 mL)	5.0 (adjusted with HOAc)
Fraction III: Fe–Mn oxide-bound form	0.08 mol/L $NH_2OH \cdot HCl$ 50% (v/v) in HOAc solution	
Fraction IV: organic form (including sulfide)	0.1 mol/L HNO_3 (1 mL) and 30% H_2O_2 (5 mL)	2.0 (adjusted with HNO_3)
Fraction V: residual form	Aqua regia and $HClO_4$	

components (e.g., Fe, Mn, and Al) between biofilms collected using this method and the water body (Dong et al. 2001, 2003). Prior to submersion in the water, the glass slides and racks were pre-cleaned with detergent, soaked for 12–16 h in soap solution, washed for 30–40 min in 8 L of water containing 40 g of $KMnO_4$ and 200 mL of H_2SO_4, and then rinsed in distilled deionized water. After exposure in the field for 40 days, the glass slides with attached biofilms were transported to the laboratory for microscopic examination, extraction, and mercury adsorption experiments.

To assess the solid speciation of mercury in the biofilm samples, a sequential extraction procedure was applied. Wet biofilms (containing 2000 mg of dry matter) were placed in centrifugal tubes and extracted overnight on a mechanical shaker using 40 mL of different extractants (Table 1). Samples were then centrifuged at 2,500 rpm for 30 min. The supernatant was processed and used for mercury determination and the solid residue was subjected to subsequent extraction (Dong et al. 2001; Renneberg and Dudas 2001; Wu et al. 2002; Liu et al. 2006).

Mercury adsorption by biofilms was measured in chemically defined solutions with the same mercury concentrations. The concentration of Hg^{2+} used would have no notable effect on most aquatic microbes for short-term contact. The solutions were prepared using $HgCl_2$ in minimal mineral salts solution containing NaCl, $NaHCO_3$, Na_2CO_3, $MgSO_4$, $(NH_4)_2SO_4$ and KNO_3. The ionic strength and pH of the solution were adjusted. The pH was chosen to avoid the possible formation of Hg precipitates. Biofilms collected from the natural aquatic environment were rinsed using minimal mineral salts solution. Then three slides for each treatment were submerged in 20 mL of mercury solution in separate 100-mm round-bottom culture vessels (Dong et al. 2002, 2003). The solutions were shaken continuously at 25°C. Preliminary experiments revealed that mercury adsorption by blank glass slides was negligible. After equilibrium was reached, the slides were removed and mercury concentrations in the equilibrium solutions were measured using an AMA254 solid/liquid mercury analyzer (Milestone, Italy) with an absolute detection limit of 0.01 ng. Each sample was analyzed three times. A special apparatus (Fig. 1) was used

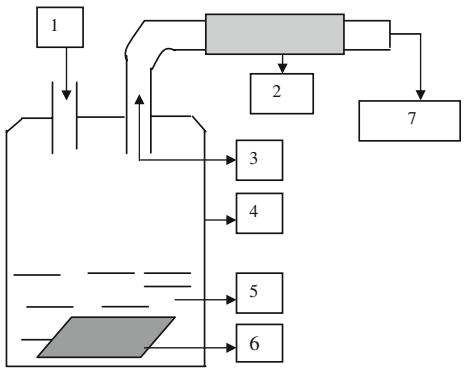

Fig. 1 Schematic diagram of the apparatus for gaseous mercury collection. (1) Vessel inlet, (2) gold sand, (3) vessel outlet, (4) reaction vessel, (5) 2 mg/L $HgCl_2$ solution, (6) natural biofilm, and (7) CD-1 gaseous sampler (Beijing Detector Instruments Ltd.)

to capture gaseous mercury transformed by the natural biofilms. A set of controlled tests was first performed without biofilm.

In this study, three slides were used for each treatment, and mean values are reported.

Results and Discussion

Natural biofilms developed in the aquatic environment were similar in appearance to those reported by Dong et al. (2003). They consisted of assemblages of microorganisms in a matrix associated with mineral deposits, and were thick and light. The biofilms were brownish green in color and contained a large number of bacterial cells and associated extracellular polymers. Some algae, including diatoms and green algae, were also observed. Metal oxides in the biofilms were determined by ICP-AES after extraction with 10% HNO_3 for 24 h (Table 2). The concentrations of metal oxides in the biofilm decreased in the order Al > Fe > Ca > Mg > K>Mn > Ti > Na > Zn > Ba > Hg. Compared to metal oxides, the concentrations of TOC in different biofilms was less variable. The coefficient of variation for measurement of metal oxides and organic material in the biofilm was <10%, indicating that biofilms were consistent from slide to slide and allowing the use of different slides for characterization of natural biofilms and

Table 2 Metal and TOC concentrations in the biofilm

Analyte	Concentration (mg/kg)	Analyte	Concentration (mg/kg)
Al	30304	Mg	9613
Ba	256	Mn	4332
Ca	15629	Na	423
Co	32.3	Ni	46.7
Cr	101.3	Pb	33.3
Cu	72.4	Sr	60.3
Fe	26223	Ti	850
K	6574	Zn	323
Li	35.2	Hg	0.56
V	55.4	TOC	108.3

measurement of mercury adsorption (Dong et al. 2001, 2003).

The distribution of mercury forms in the natural biofilm was as follows: organic form, 41.23%; exchangeable form, 29.32%; residual form, 17.2%; Fe–Mn oxide-bound form, 6.29%; and carbonate-bound form, 5.96% of the total mercury (Fig. 2). These five mercury fractions have different mobility and potential bioavailability. Organic and exchangeable forms are probably the most important in terms of environmental concerns. The exchangeable form is the most labile and can migrate in aquatic environments, and even move downward into deeper sediment. The organic form is regarded as having stronger complexation ability and thus has more limited mobility and bioavailability. Hg bound to organic matter could include methyl mercury species (mainly monomethylmercury) in spite of being of a very small proportion of total mercury in general. Organochelated Hg was observed to be strongly correlated with methylation potential and thus seems to play an important role in the biogeochemical cycle of Hg.

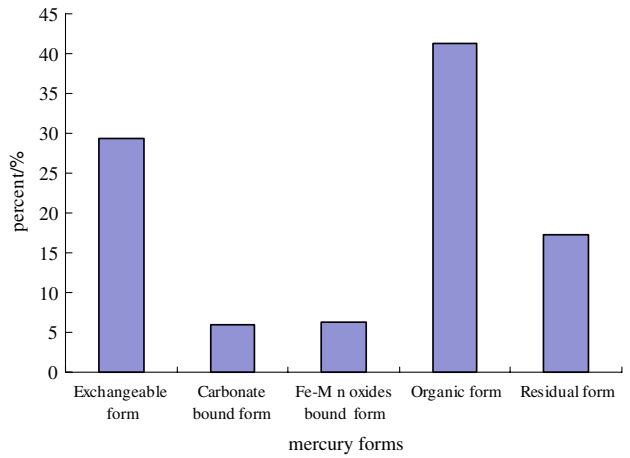

Fig. 2 Distribution of different mercury forms in natural biofilm

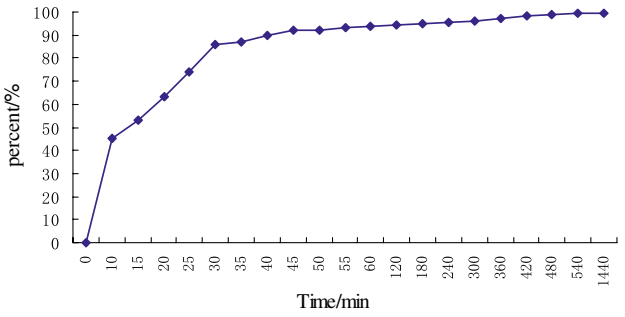

Fig. 3 Change in mercury concentrations in equilibrium solutions over time

Owing to its very low solubility, the residual form is not subject to transport and is not available for chemical or biological transformation (Wang et al. 2002a).

Figure 3 shows the change in mercury concentrations in equilibrium solution after adsorption by the biofilm over time. The ionic strength of the solution was adjusted to 0.1 M with KNO_3 and the pH was adjusted to 6.0 with dilute HCl. Figure 3 shows that mercury adsorption from the solution was up to 86% after 30 min, 90% after 40 min, 94% after 60 min, and 99.7% after 1440 min. The curve indicates that a shorter adsorption period of approximately 40 min should be suitable for selective adsorption of mercury oxides.

Mercury adsorption by the biofilm was studied over the pH range 2–11 for an initial adsorbate concentration of 2 mg/kg $HgCl_2$. The solution pH was adjusted using dilute HCl and NaOH solutions. A graphical representation of the adsorption data for the biofilm over the pH range investigated is shown in Fig. 4. A solution pH of 6.0 during adsorption led to a good result, with a maximum adsorption concentration of 9.9 μg mercury per gram of biofilm. For pH >7, mercury(II) precipitated, which did not favor adsorption by the biofilm. Thus, for pH >7, mercury adsorption by the biofilm decreased with increasing initial pH. At pH <7, mercury(II) reduction in solution was caused by the bacterial response to mercury stress. Mercury-resistant strains contain mercuric reductase, which

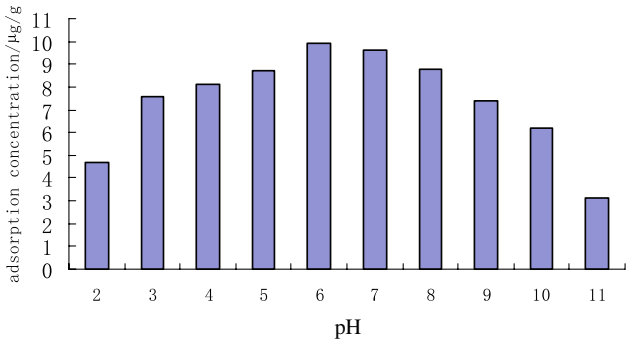

Fig. 4 Change in accumulated adsorption of mercury by the biofilm with pH

depends on donation of coenzyme II nicotinamide adenine dinucleotide phosphate acid (NADPH) as follows (Wang et al. 2002a):

$$NADP^+ + H^+ + 2e \rightarrow NAPDH, E^{0/*} = -0.32\ V \quad (1)$$

where $E^{0/*}$ is the standard redox potential (V) and NADP is nicotinamide adenine dinucleotide phosphate. Hence, the Nernst equation of the electron pair $NADP^+$/NADPH is:

$$E_n = E^0 + \frac{RT}{nF}\ln\frac{[NADP^+][H^+]}{[NADH]}, \quad (2)$$

where E_n is the electrode potential of $NADP^+$/NAPDH (V), E^0 is the standard electrode potential (V), R is the gas constant (8.314 J/L mol) and F is the Faraday constant (9.6485309×10^4 C/mol).

Apparently, the redox electrode potential of $NADP^+$/NADPH decreases with increasing pH, which is advantageous for NADPH donation. In other words, high pH is more advantageous for mercury reductase catalysis (depending on NADPH) than low pH. Thus, for pH <7, mercury adsorption by the biofilm increased with increasing initial pH.

The effect of solution ionic strength is significant for both the adsorbent and the adsorbate. Generally, since natural water contains different salts, its ionic strength is high. At high ionic strength, adsorption sites are surrounded by counterions, which partially decrease their charge and thus weaken the binding force of electrostatic interaction (Krishnan and Anirudhan 2002; Manohar et al. 2002; Feng et al. 2004; Jeon and Park 2005). To investigate the effect of ionic strength on mercury(II) adsorption by biofilm, equilibrium sorption studies were carried out using solutions of different ionic strength. The ionic strength was adjusted by addition of dilute KCl and $NaNO_3$ solutions. Figure 5 indicates that mercury oxide adsorption by the biofilm increased with increasing ionic strength up to 0.1 mol/L, corresponding to a maximum mercury concentration in the biofilm of 10.3 μg/g. Based on these results, an adsorption time of 40 min, pH 6 and ionic strength of 0.1 mol/L were chosen as the optimum conditions for mercury oxide adsorption by biofilms.

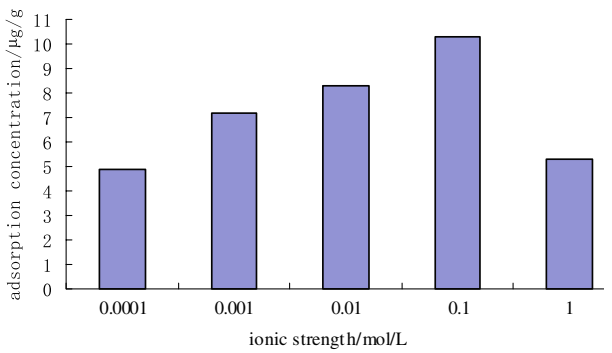

Fig. 5 Effect of ionic strength on the adsorption of mercury by biofilm

Mercury adsorption data were analyzed using Langmuir and Freundlich models to evaluate the mechanistic parameters associated with the adsorption process. Langmuir and Freundlich isotherms have been extensively used to evaluate the adsorption of organic matter and heavy metals on clays, metal oxides, soils, activated carbon, sediment, peat, etc. (Jain and Ram 1997; Loaec et al. 1997; Wang et al. 2002b). The Langmuir model assumes uniform energy of adsorption onto the surface and no transmigration of adsorbate on the surface. The linear form of the Langmuir isotherm is represented by the equation:

$$1/G = 1/G_{max} + (A/G_{max})(1/C) \quad (3)$$

where G is the amount of adsorbate adsorbed (mg/g), C is the equilibrium adsorbate concentration (mg/L), and G_{max} and A are Langmuir constants related to the maximum adsorption capacity (monolayer capacity) and energy of adsorption, respectively, which are functions of the system characteristics and time. The Langmuir parameters G_{max} and A were obtained by linearly regressing the data using Eq. 3 (Table 3). The Freundlich isotherm is represented by the equation:

$$\log G = \log K + (1/n)\log C \quad (4)$$

where K and n are Freundlich constants related to the adsorption capacity and intensity, respectively. The Freundlich parameters K and n for Hg were also obtained by linearly regressing the data using Eq. 4 (Table 3). The correlation coefficient between $1/G$ and $1/C$ ($R_L{}^2 = 0.93$) indicates that a straight line with slope A/G_{max} and intercept $1/G_{max}$ was obtained, demonstrating that Hg adsorption by the biofilm followed the Langmuir isotherm model. For a plot of $\log G$ against $\log C$, the correlation coefficient of $R_F{}^2 = 0.21$ indicates that Hg adsorption by the biofilm does not fit Freundlich isotherm model.

Mercury exists in the environment in three oxidation states, Hg(0), Hg(I) and Hg(II). For each valence state, many chemical forms can occur in solid, aqueous, and gaseous phases. Gaseous mercury includes mercury vapor, inorganic compounds (chlorides and oxides), and alkyl mercury (primarily methylmercury) (Coelho-Souza et al. 2006; Dill et al. 2006). Inorganic mercury dissolved in water is thought to exist in the form of dissociated

Table 3 Adsorption parameters and correlation coefficients calculated using Langmuir and Freundlich isotherms

Langmuir equation $1/G = 1/G_{max} + (A/G_{max})(1/C)$			Freundlich equation $\log G = \log K + (1/n)\log C$		
G_{max} (mg/g)	A (mg/L)	$R_L{}^2$	K (mg/g)	n	$R_F{}^2$
63.45	0.02	0.93	805.47	1.32	0.21

Table 4 Gaseous mercury transformed by the biofilm after 1 h of adsorption

Biofilm weight (g)	Mercury content (ng)			Transformation rate (μg/g)
	Blank	After 1 h	Gaseous	
0.7	287.6	843.7	556.1	0.79

$[HgCl_4]^{2-}$ ions. In our follow-up study, we measured the total gaseous mercury transformation rate for the biofilm (Fig. 1). The transformation rate was 0.79 μg of gaseous mercury per gram of biofilm (Table 4). A large number of bacterial cells and associated extracellular polymers were present in the biofilms. Inorganic mercury ($[HgCl_4]^{2-}$) could be transformed to methylmercury in solution by microorganisms, such as methane-producing bacteria; these contain methylcobalamin that can easily generate methylmercury from inorganic mercury ions.

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