



Photo-induced transformations of mercury(II) species in the presence of algae, *Chlorella vulgaris*

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ABSTRACT

The effects of algae (i.e., *Chlorella vulgaris*), Fe(III), humic substances, and pH on the photoreduction of Hg(II) under the irradiation of metal halide lamps ($\lambda \geq 365$ nm, 250 W) were investigated in this paper. The photoreduction rate of Hg(II) was found to increase with the increasing concentration of algae, Fe(III), and humic substances. The cooperation action of Fe(III) and humic substances accelerated the photoreduction of Hg(II). When the initial concentration of Hg(II) was in the range of 0.0–200.0 $\mu\text{g L}^{-1}$ with initial algae concentrations 7.0×10^9 cells L^{-1} at pH 7.0, the initial photoreduction rate of Hg(II) could be expressed by the equation: $-\text{d}C_{\text{Hg(II)}}/\text{d}t = 0.65 \times [C_{\text{Hg(II)}}]^{0.39}$ with a correlation coefficient of $R = 0.9912$. The study on the photochemical process in terms of total mercury mass balance revealed that more than 40.86% of Hg(II) from the algal suspension was reduced to volatile metallic mercury. This paper discussed the photoreduction mechanism of Hg(II) in the presence of algae. This research will provide information for predicting the photoreduction of Hg(II) in the real environment. It will be helpful for understanding the photochemical transformation of Hg(II) and the formation of DGM in natural water in the presence of algae complexes. It will also be helpful for providing new methods to deal with heavy metal pollution.

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1. Introduction

Studying on heavy metal pollution is of significant importance in environmental protection and thus has been the focus of considerable research in the fields of environmental sciences. Among such pollutions, Hg(II)-containing compounds are environmentally extreme malign but the related toxicological procedure is yet still elusive. Furthermore, Hg(II)-containing compounds are very difficult to be naturally degraded to yield the products that are environmentally benign, although the evolution of such compounds can occur by various biological and/or chemical reactions, forming different chemical structures. As a consequence, some Hg(II)-containing compounds, especially those with higher risk to environment (e.g., methyl mercury), can be formed in the biological or abiotic methylation processes of inorganic mercury in natural environment [1–3]. Very recently, it has been demonstrated that more and more Hg-related incidents could result from the atmospheric deposition of Hg from anthropogenic and natural sources through long-distance atmospheric transport [4,5]. Upon evolution in natural waters, Hg undergoes an aquatic redox cycling between

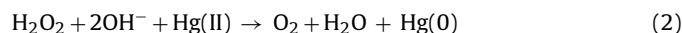
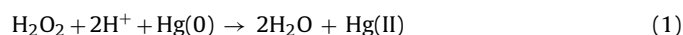
oxidized mercury, e.g., Hg(II), and metallic mercury. In this procedure, CH_3Hg^+ and $(\text{CH}_3)_2\text{Hg}$ and inorganic Hg(II) species are subject to bioaccumulation, while Hg(0) dominates dissolved gaseous mercury (DGM) that can evade back to the atmosphere [6–8].

The diel changes of DGM upon the uptake of solar radiation have been observed in natural freshwater, e.g., the Everglades, Florida [9–11] and Lake Superior, Michigan [12,13]. These findings implicate an intrinsic role of sunlight in controlling aquatic DGM dynamics. Recent researches of sunlight incubations of northern lake water in Teflon bottles further suggested that sunlight-induced photochemical reduction of Hg(II) in freshwater can induce the natural production of DGM [14–16]. Because the competition between the sunlight-driven pathway for Hg(II) substrate and the methylation pathway would reduce Hg toxic hazards in local aquatic ecosystems through removal of Hg(II) as a result of its reduction to Hg(0) [6–8], it is of significance to study the aquatic Hg biogeochemical cycling and its environmental impacts. Although the last decades witnessed the increasing efforts on the observations of sunlight-induced aquatic production of DGM in aquatic systems [12,17], the understanding of such phenomena in fundamental still remains interesting but yet challenging.

The volatilization rate of Hg is controlled by the rate of the formation of Hg(0) which is relative to the biotic and abiotic processes of Hg-based evolutions. The abiotic reduction of Hg(II) to

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Hg(0) in water can be initiated by humic substances [18]. For instance, Turner et al. [19] suggested that the abiotic reduction could account for as much as 10–70% of the volatile Hg released from some contaminated streams. It is assumed that intermediates such as semiquinones present in humic substances are involved in this case. As such, it is also indicated that the rate of such abiotic reduction can be photochemically enhanced under laboratory conditions [20]. Among the abiotic photochemical pathways involved, Schroeder et al. [21] have suggested that hydrogen peroxide (H₂O₂), mainly originating from UV-induced transformations of dissolved organic matter in surface waters [22], could act as a reducing or oxidizing agent for the Hg-based evolution, depending on the pH. The following equations have been proposed to explain these procedures:



Some biotic processes leading to the reduction of Hg(II) have also been identified in the literature. The primary mercury-detoxifying mechanism in the case of mercury-resistant bacteria was explained as the reduction of mercuric ion to volatile Hg(0) [23]. Photosynthesis may also produce Hg(II) reductants, and the rates for producing metallic Hg(0) have been observed during the course of algal blooms [6].

Although photochemical and/or photobiological processes are considered as the most important factors to affect the rate of volatile mercury producing in lake water, few experimental evidences, especially those performed under field conditions of a direct link between solar radiation and in situ DGM production, are available in the detectable literature up to date. In this context, we demonstrate the effects of sunlight and algae complexes on DGM production by simulating the conditions expected in the natural water.

There have been many publications in connection to the photoreduction of mercury(II) in aqueous solutions, but few papers have been reported on the reduction of mercury(II) in the system containing algae, Fe(III) and humic substances. This paper would focus on the photoreduction of Hg(II) in the presence of algae, Fe(III) and/or humic substances under the irradiation of metal halide lamps. The mechanisms involved in this study may also act in the observed photochemical production of DGM in the sunlit natural freshwaters [24], since algae, Fe(III) and humic substances are commonly present in natural aquatic systems. This research will provide information for predicting the photochemical reduction of mercury(II) in the real environment. It will be helpful for understanding the photochemical transformation of mercury(II) and the formation of DGM in natural water in the presence of algae complexes. It will also be helpful for providing new methods to deal with heavy metal pollution.

2. Experimental

2.1. Chemicals and reagents

HNO₃, KBr, KBrO₄, NaCl, HCl, NH₂OH·HCl, (NH₄)₂SO₄, Ca(H₂PO₄)₂·2H₂O, SnCl₂, CaSO₄·H₂O, MgSO₄·7H₂O, FeCl₃·6H₂O, CaCl₂·2H₂O, CuSO₄·5H₂O, H₃BO₃, KCl, K₂HPO₄, KH₂PO₄, MgSO₄·7H₂O, K₂Cr₂O₇, MnCl₂·4H₂O, MoO₃, NaOH, NaCl, Na₂CO₃, NaHCO₃, Na₂MoO₄·2H₂O, NaNO₃, Na₂SiO₃·9H₂O, ascorbic acid, and ferric citrate were analytical grade and obtained from Chemical Reagent Beijing Co., Ltd. Humic substances were purchased from Aldrich Chem. Co. (Milwaukee, WI, USA). Double-distilled water and N₂ (98.5%) were used in all experiments. HgCl₂ was analytical grade and denoted as Hg(II) in this context.

2.2. Preparation of algae

The algae used in the experiments were *Chlorella vulgaris* and obtained from the Wuhan Hydrobiology Institute of Chinese Academy of Sciences (Wuhan, PR China). The algae were grown in culture medium at 25 °C using 24 h light cycle in a culturing room equipped with constant temperature air-conditioner.

For *C. vulgaris*, the culture medium consisted of (NH₄)₂SO₄, [Ca(H₂PO₄)₂·2H₂O + CaSO₄·H₂O], MgSO₄·7H₂O, NaHCO₃, KCl, FeCl₃, H₃BO₃, MnCl₂·4H₂O, ZnSO₄·7H₂O, MoO₃, CuSO₄·5H₂O and soil extract (the soil extract was heated at 100 °C for 2 h from common soil and water (m/m, 1/5), and then cooled in brown bottle). The medium was adjusted to pH 7.0–7.2 by using 0.1 M Na₂CO₃. The algae were cultured in axenic medium. After staying for 12–14 days, the algae were washed prior to each measurement.

To remove the colloidal ferric hydroxide particles that might have adsorbed on the algae cells, a modified version of the procedure [25] was used in the experiments. This procedure involves washing the cells by gentle agitation for 30 min with 0.01 M aqueous ascorbic acid adjusted to pH 3.0. Then the algae were washed with double-distilled water for three times. The cell counting was carried out under inverted microscope at 400× and the density of algae (cells L⁻¹) was calculated [26]. As such, the algae were well-prepared for subsequent use. Different concentrations of algae were gained through diluting the washed algae with double-distilled water. These experiments were carried out at a room temperature of 26 ± 2 °C.

2.3. Irradiation procedure

The irradiation experiments were carried out in a cylindrical reactor (20.0 cm length, 10.0 cm diameter, 1.0 cm wall thickness) (see Fig. 1a), with a 250 W metal halide lamp (λ ≥ 365 nm, Changzhou Shangzi Lamp Co. Ltd., China) placed in cooling trap for maintaining constant temperature by water circulation, and reaction solutions pumped with N₂ at a fixed flow rate throughout the experiments. The cylindrical reactor was unsealed in the uppermost part. The cylindrical reactor was placed in a box. HCl and/or NaOH were used to adjust the pH values of solutions. The cylindrical reactor containing solutions were kept in a dark condition before and after irradiation. Different concentrations of Hg(II) and humic substances, Fe(III) or algae were mixed thoroughly and transferred into the cylindrical reactor. The light intensity at the position of the cylindrical reactor was 159,000 lx, which was detected using Digit Lux meter (TES 1332, Taiwan, China). At different time intervals during the irradiation, the samples with Hg(II) and Fe(III), humic acid or algae were taken from the cylindrical reactor. For the solution with algae, the samples were digested with HCl–HNO₃ (1+1). Then the samples and a Teflon-coated stir bar were placed in a capped and secured glass flask and heated to 90 °C in a water bath for 1 h or more upon cooling, the samples were analyzed by a cold vapor atomic absorption spectrometer (AAS). The algae-free samples were analyzed directly.

The experiments for the overall mercury mass balance were also performed to study the release of the volatile mercury from the photochemical processes in the other cylindrical reactor (20.0 cm length, 9.0 cm inside diameter, 11.0 cm outside diameter, 1.0 cm wall thickness) (see Fig. 1b). This cylindrical reactor was sealed in the uppermost part. The irradiation experiments were carried out in the cylindrical reactor, with a 250 W metal halide lamp (λ ≥ 365 nm, Changzhou Shangzi Lamp Co. Ltd., China) placed in cooling trap for maintaining constant temperature by water circulation, and reaction solutions pumped with N₂ at a fixed flow rate throughout the experiments. The mercury vapor produced upon

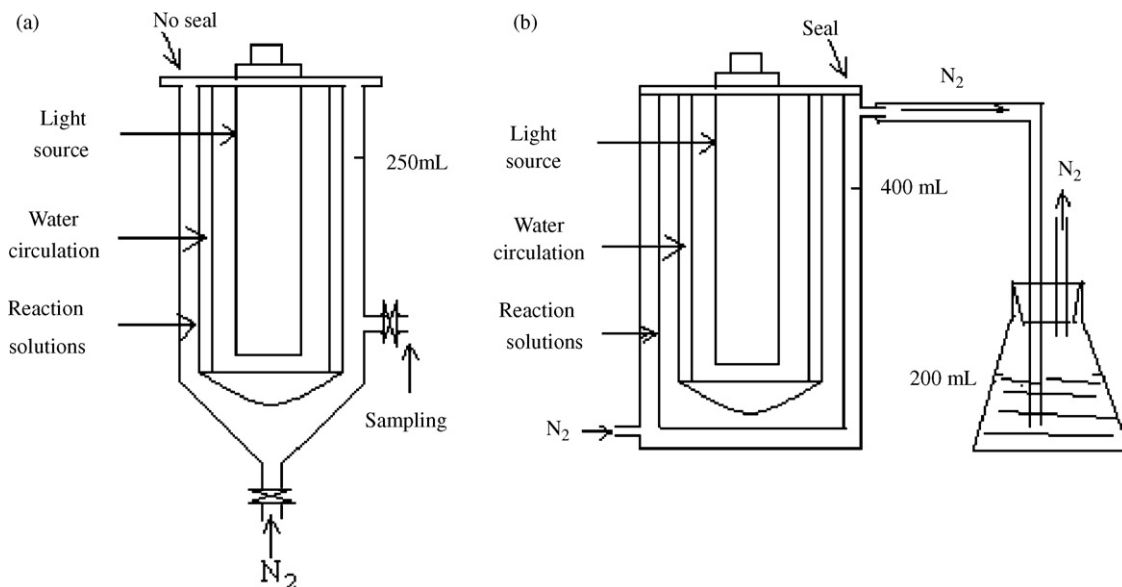


Fig. 1. Cylindrical reactors (a and b). ((a) The cylindrical reactor of irradiation experiments, (b) the cylindrical reactor of overall mercury mass balance experiments.)

irradiation was taken into an imbibing flask by N_2 that was filled with 200.0 mL $KBr-KBrO_3$ solutions and 2.0 mL H_2SO_4 .

2.4. Chemical analyses

The $Hg(II)$ concentrations were determined by a cold vapor AAS. The calibration equation for $Hg(II)$ was $A_{\text{peak area}} = 95.14C_{Hg(II)} - 6.24$ ($r = 0.992$), where $C_{Hg(II)}$ was the concentration of $Hg(II)$ in the range of 0.0–200.0 $\mu\text{g L}^{-1}$. All vitreous apparatus were dipped in HNO_3 /water (the volume ratio was 1:1) overnight to remove impurities. 3.0 mL reaction solution was transferred into a 20.0 mL reduction bottle. Then 2.0 mL HNO_3 (5%) and 2.0 mL $SnCl_2$ (10%) were added into the reduction bottle. Finally, $Hg(II)$ was determined by a cold vapor AAS.

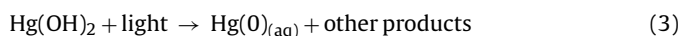
During the course of the overall mercury mass balance experiments in the presence of algae, 5 mL sample was taken from the imbibing flask at 1 h time intervals and $NH_2OH \cdot HCl-NaCl$ solution was stepwise added until the yellow color of the sample disappeared. Then the samples were stood for 5 min until clarified. Finally the samples were determined by a cold vapor AAS. All the experiments were triplicate. The results presented were the mean values with a less than 5% total analytical error.

3. Results and discussion

3.1. Photoreduction of $Hg(II)$ both in the absence and presence of algae

The experiments were carried out to study the effects of algae on the photoreduction of $Hg(II)$ in aqueous suspension at the initial algal concentration 7.0×10^9 cells L^{-1} under metal halide lamps. Fig. 2 shows the typical time series for the photochemical reduction of $Hg(II)$ in the absence and presence of *C. vulgaris*. For comparison, the corresponding control experiments were performed under a dark condition. In the presence of *C. vulgaris*, 6.6% of $Hg(II)$ disappeared from the suspension after 7 h, which is due to the slow absorption and/or reduction by the biological and thermal chemical processes [27]. Under the metal halide lamp irradiation, 13.0% of $Hg(II)$ disappeared after 7 h irradiation even in the absence of algae, suggesting the slow thermal reduction, absorption and the direct photoreduction of $Hg(II)$. The direct photoreduction of $Hg(II)$

could be responsible for the increased removal of $Hg(II)$.



In the presence of *C. vulgaris*, 27.1% of $Hg(II)$ disappeared from the suspension after 7 h irradiation under the metal halide lamps, indicating that the photolysis of *C. vulgaris* could promote the reduction of $Hg(II)$. The algae, *C. vulgaris*, might undergo photolysis to generate smaller organic molecules and free electrons that were capable to reduce $Hg(II)$. During the course of irradiation, the algae cell gave off dissolved organic matter (DOM). The structural differences in DOM could also affect the photoreduction of $Hg(II)$ [28]. The enzymatic reaction at the algae cell surface could be another possible mechanism involved in the reduction of $Hg(II)$ [29].

3.2. Effects of pH on the photoreduction of mercury(II) in aqueous solution with or without algae

The photoreduction experiment was performed in $Hg(II)$ aqueous solutions at several pH values ranging from 5 to 9. As shown in Fig. 3, it is clear that the photoreduction efficiency of $Hg(II)$ increased with the increase of pH. At pH 9, up to 14.5% of $Hg(II)$ was removed from the suspension after 7 h light irradiation. At pH

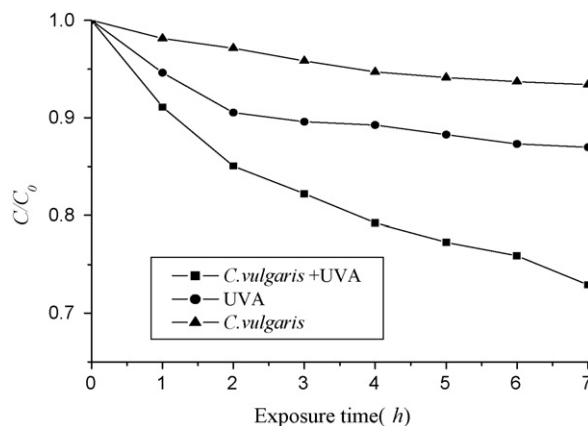


Fig. 2. Mercury(II) concentration change in the control experiments. (Light source: metal halide lamps (250 W), C_0 (Hg^{2+}) = 100 $\mu\text{g L}^{-1}$, *C. vulgaris*: 7.0×10^9 cells L^{-1} .)

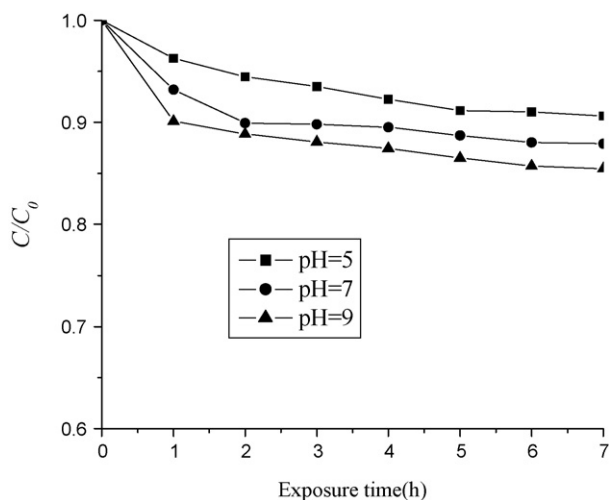


Fig. 3. Effects of pH on photoreduction of mercury(II) without algae. (Light source: metal halide lamps (250 W), C_0 (Hg^{2+}) = $100 \mu g L^{-1}$.)

7, up to 12.1% of Hg(II) was removed from the suspension after 7 h light irradiation. At pH 5, up to 9.4% of Hg(II) was removed from the suspension after 7 h light irradiation. The effects of pH demonstrated that $Hg(OH)_2$ could be the reactive species of Hg(II) involved in the direct photochemical reduction of Hg(II) in aqueous solution, which was in consistent with those reported in previous studies [18,20,30,31].

Experiments were also carried out to study the effects of pH on the photoreduction of Hg(II) in aqueous suspension at the initial algal concentration 7.2×10^9 cells L^{-1} under metal halide lamps. The results showed that the photoreduction of Hg(II) increased with the increase of pH value in the range of 5.0–9.0 in Fig. 4. At pH 9, 33.4% of Hg(II) was disappeared from the algal suspension after 7 h irradiation. At pH 7, 31.7% of Hg(II) was disappeared from the algal suspension after 7 h irradiation. At pH 5, 22.6% of Hg(II) was disappeared from the algal suspension after 7 h irradiation. In the aqueous suspension of *C. vulgaris*, the photochemical and biological processes are more complex. Besides that the higher concentration of $Hg(OH)_2$ at higher pH value leads to a higher photoreduction of Hg(II), the enhanced production of dissolved organic matter and free electrons also accelerated the conversion of Hg(II) to Hg(0). pH 7.0 was selected in the following experiments, because both natural water and the algal suspension have a similar pH value.

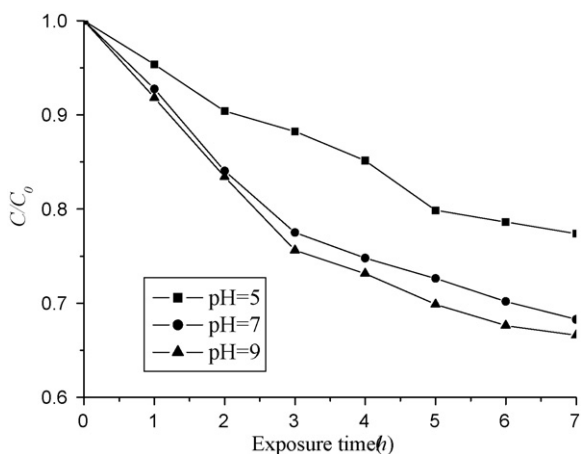


Fig. 4. Effects of pH on photoreduction of mercury(II) with algae. (Light source: metal halide lamps (250 W), C_0 (Hg^{2+}) = $100 \mu g L^{-1}$, *C. vulgaris*: 7.2×10^9 cells L^{-1} .)

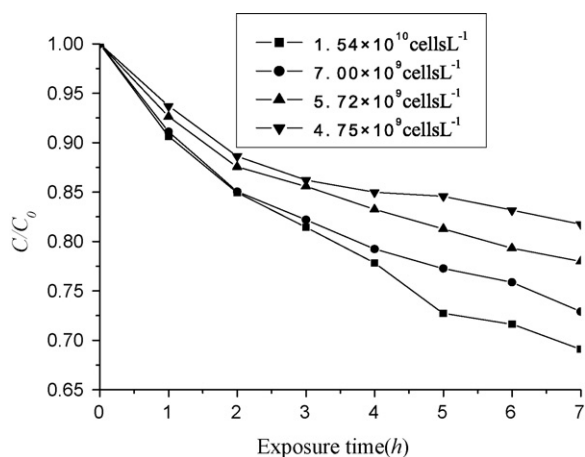


Fig. 5. Effects of algae concentrations on photoreduction of mercury(II) in solution. (Light source: metal halide lamps (250 W), C_0 (Hg^{2+}) = $100 \mu g L^{-1}$, pH 7.0.)

3.3. Effects of algae concentration on the photoreduction of mercury(II)

To test the effects of *C. vulgaris* on the photoreduction of Hg(II), suspensions of *C. vulgaris* at four initial algal concentrations, 4.75×10^9 , 5.72×10^9 , 7.00×10^9 , and 1.54×10^{10} cells L^{-1} , at pH 7.0 were illuminated under metal halide lamps up to 7 h. As shown in Fig. 5, the faster photoreduction of Hg(II) occurred with a higher algal concentration. After 7 h irradiation, 30.9% of Hg(II) was removed from the suspension under the initial algal concentration 1.54×10^{10} cells L^{-1} ; 27.1% of Hg(II) was disappeared at initial algal concentration of 7.00×10^9 cells L^{-1} ; 22.0% removal of Hg(II) at the algal concentration of 5.72×10^9 cells L^{-1} ; 18.3% removal of Hg(II) at the algal concentration of 4.75×10^9 cells L^{-1} . A higher algal concentration resulted in a faster photoproduction of free electrons and smaller dissolved organic matter. The dissolved organic matter, such as humic and fulvic acid, could serve as photosensitizers and thus accelerate the photoreduction of Hg(II) [32].

3.4. Effects of irradiation time on DOC in aqueous solution with algae

To test the effects of irradiation time on DOC in aqueous solution with algae, irradiation experiments were carried out in a cylindrical reactor (see Fig. 1a). Pretreatment algae at the initial algal

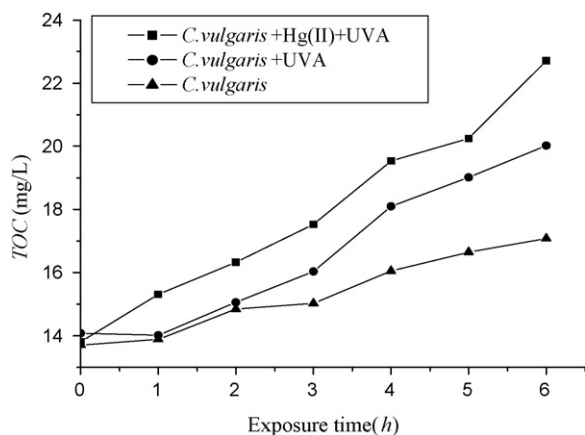


Fig. 6. Effects of irradiation time on TOC in aqueous solution with algae. (C_0 (Hg^{2+}) = $100 \mu g L^{-1}$, *C. vulgaris*: 5.6×10^9 cells L^{-1} .)

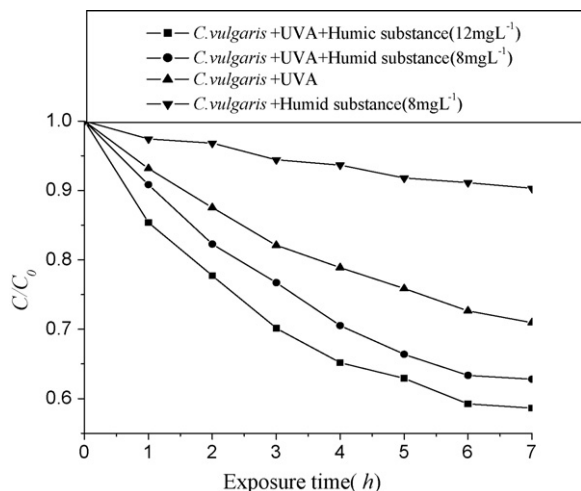


Fig. 7. Effects of humid substance on photoreduction of mercury(II) with algae. (Light source: metal halide lamps (250 W), pH 7.0, C_0 (Hg^{2+}) = $100\ \mu gL^{-1}$, $C. vulgaris$: 7.20×10^9 cells L^{-1} .)

concentration 5.60×10^9 cells L^{-1} were added the cylindrical reactor about 300 mL. Under the metal halide lamp irradiation for 6 h, 10 mL sample was taken from the cylindrical reactor every 1 h. And then, all samples were filtrated by 0.45 μm micropore filter, and carried out using Apollo 9000 TOC analysis. Thought such measure, TOC should equal to DOC in aqueous solution. Fig. 6 shows the typical irradiation time courses affecting on DOC in aqueous solution both in the presence of algae and $Hg(II)$ and in the presence of algae as well as the corresponding dark controls. In the dark, in the presence of algae, only small amount of DOC in aqueous solution with algae increased from 13.71 to 17.08 mg/L within 6 h; under the metal halide lamp irradiation, DOC in aqueous solution with algae increased from 14.08 to 20.02 mg/L within 6 h; under the metal halide lamp irradiation, in the presence of algae and $Hg(II)$, DOC in aqueous solution with algae increased from 13.81 to 22.71 mg/L within 6 h. The results showed that the concentration of DOC in aqueous solution with algae would increase under the condition of irradiation or adding $Hg(II)$. According to the process of the aqueous solution with algae after irradiation, it turns out that DOC is the concentration of dissolved organic substance in fact, so the con-

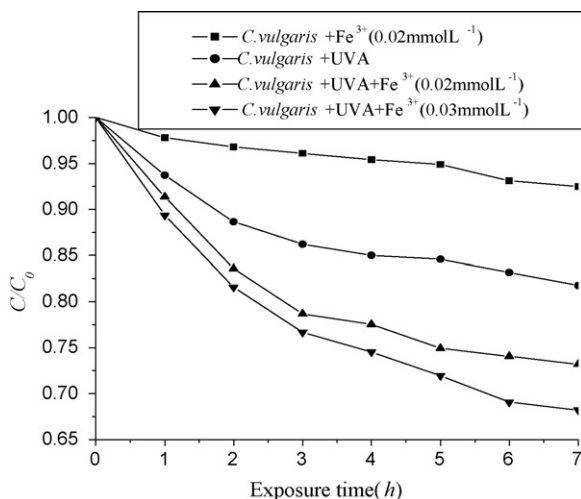


Fig. 8. Effects of Fe(III) on photoreduction of mercury(II) with algae. (Light source: metal halide lamps (250 W), pH 7.0, C_0 (Hg^{2+}) = $100\ \mu gL^{-1}$, $C. vulgaris$: 4.75×10^9 cells L^{-1} .)

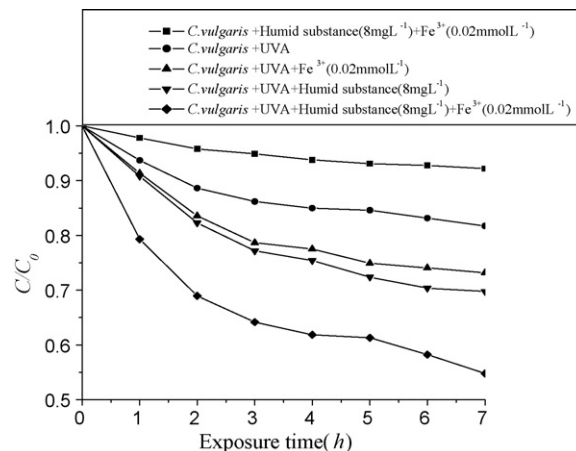


Fig. 9. Effects of humid substance and Fe(III) on photoreduction of mercury(II) with algae. (Light source: metal halide lamps (250 W), pH 7.0, C_0 (Hg^{2+}) = $100\ \mu gL^{-1}$, $C. vulgaris$: 4.75×10^9 cells L^{-1} .)

centration of dissolved organic substance would increase with the irradiation time or adding $Hg(II)$.

3.5. Effects of humic substances on the photoreduction of mercury(II) in algal suspensions

Humic substances are a ubiquitous and heterogenous group of natural organic compounds because of their important role in the fate and transport of many pollutants. Experiments were also carried out to study the effects of Humic substances on the photoreduction of $Hg(II)$ in aqueous suspension at the initial algal concentration 7.2×10^9 cells L^{-1} under metal halide lamps. In our case, Humic substances could also affect the photoreduction of $Hg(II)$ as shown in Fig. 7. In the presence of 8.0 mg/L humic substances, 9.7% $Hg(II)$ was removed from a suspension of $C. vulgaris$ stood in dark for 7 h. After 7 h irradiation, 37.2% $Hg(II)$ disappeared in the presence of 8.0 mg/L humic substances, while only 29.0% $Hg(II)$ was removed in the absence of humic substances. After 7 h irradiation, 41.4% $Hg(II)$ disappeared in the presence of 12.0 mg/L humic substances. These results indicated that humic substances could promote the reduction of $Hg(II)$ in algal suspension. Sev-

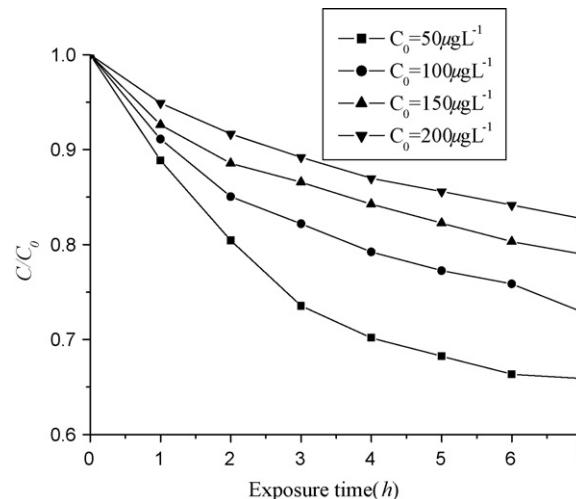
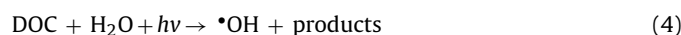


Fig. 10. Effects of initial concentrations of mercury(II) on photoreduction of mercury(II) with algae. (Light source: high-pressure mercury lamp (250 W), pH 7.0, $C. vulgaris$: 7.00×10^9 cells L^{-1} .)

eral research groups have reported that humic substances could promote the reduction of Hg(II) in aqueous solution [18,20,30,31].

3.6. Effects of Fe(III) on photoreduction of mercury(II) in algal suspensions

Experiments were also carried out to study the effects of Fe(III) on the photoreduction of Hg(II) in aqueous suspension at the initial algal concentration 4.75×10^9 cells L^{-1} under metal halide lamps. Fe(III) may also influence the photoreduction of Hg(II) as shown in Fig. 8. In the dark, 7.5% Hg(II) disappeared in the suspension of *C. vulgaris* containing 0.02 mmol/L Fe(III). After 7 h light irradiation, 18.3% of Hg(II) was removed from suspension of *C. vulgaris* without Fe(III), while 26.8% of Hg(II) disappeared in the presence of 0.02 mmol/L Fe(III). After 7 h light irradiation, 31.8% of Hg(II) was removed from suspension of *C. vulgaris* in the presence of 0.03 mmol/L Fe(III). Fe(III) could clearly accelerate the photoreduction of Hg(II) in algal suspensions under metal halide lamps irradiation. The involved abiotic mechanisms for the effects of Fe(III) on the photoreduction of Hg(II) include light-induced photochemical production of highly reducing organic free radicals through photolysis of Fe(III)-organo coordination compounds [Fe(III)-Org] and subsequent reaction of Hg(II) with the organic and inorganic free radicals formed [24,33,34]. $\cdot OH$ may also be produced in natural water by direct photolysis of DOC [35]:

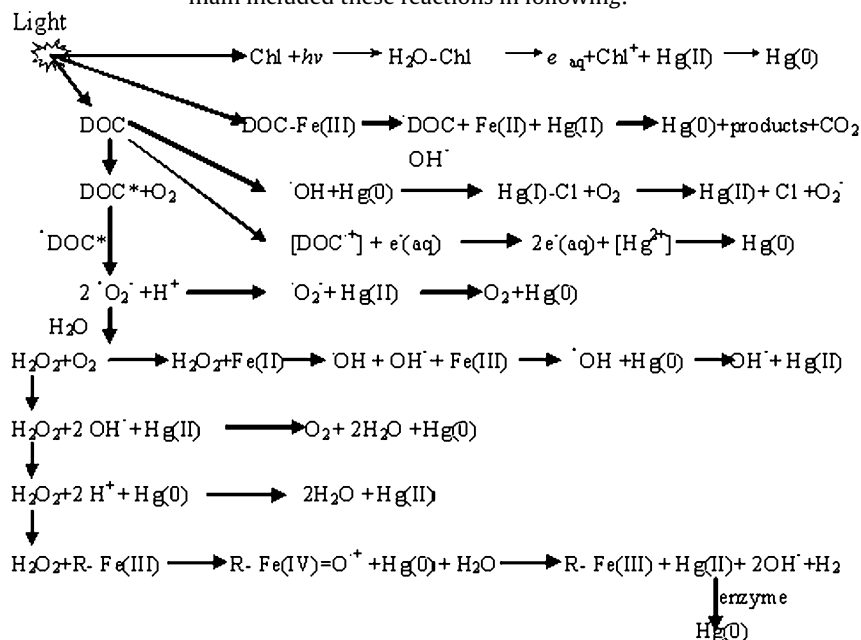


Fe(III) is highly photochemically reactive in aqueous media [36] and its role in mediating photochemical redox cycling of heavy

3.7. Effects of Fe(III) and humic substances on photoreduction of mercury(II) in algal suspensions

Experiments were also carried out to study the effects of Fe(III) and humic substances on the photoreduction of Hg(II) in aqueous suspension at the initial algal concentration 4.75×10^9 cells L^{-1} under metal halide lamps. Fe(III) and humic substances could accelerate the photoreduction of Hg(II), as shown in Fig. 9. In the dark, 7.8% Hg(II) disappeared in the suspension of *C. vulgaris* containing 0.02 mmol/L Fe(III) and 8.0 mg/L humic substances in 7 h. After 7 h light irradiation, 18.3% of Hg(II) was removed from suspension of *C. vulgaris* without Fe(III), while 26.8% of Hg(II) disappeared in the presence of 0.02 mmol/L Fe(III). 30.2% Hg(II) disappeared from suspension of *C. vulgaris* in the presence of 8.0 mg/L humic substances. 45.2% Hg(II) disappeared from suspension of *C. vulgaris* in the presence of 0.02 mmol/L Fe(III) and 8.0 mg/L humic substances. Fe(III) and humic substances could essentially accelerate the photoreduction of Hg(II) in algal suspensions under metal halide lamps irradiation. The abiotic mechanisms involved in the effects of Fe(III) and humic substances on the photoreduction of Hg(II) were similar to those proposed in previous studies [18,20,24,30,31,33,34], but those studies are simple and only study the action of Fe(III) and/or humic substances.

After analyzing above experimental results and references [31,33,41], we thought the mechanism of photochemical reduction of Hg(II) in the presence of algae should have many important photochemical and/or photobiological processes involved in algae enhancing reduction of Hg(II), including the enzymatic reduction at the algae cell surface, reduction by free electrons and dissolved organic matter produced via the photolysis of algae under the irradiation. The photochemical and/or photobiological processes were main included these reactions in following:



3.8. Effects of initial concentrations of mercury(II) on the photoreduction of mercury(II) in algal suspensions

The influence of initial Hg(II) concentration (C_0) on the photoreduction of Hg(II) was examined by irradiating the suspensions with initial algae concentrations 7.0×10^9 cells L^{-1} at pH 7.0 and initial concentrations of Hg(II) at 50, 100, 150 and 200 $\mu g L^{-1}$, respectively. As shown in Fig. 10, metal halide lamps could evidently induce photoreduction of Hg(II) in aqueous solution with algae. The pho-

metals in natural aqueous systems is well established [36–38]. Photochemical reduction of heavy metal ions [e.g., Cr(VI)] by highly reducing organic free radicals has been implicated to be one of the most important mechanisms for the redox cycling of these metals [39,40]. The reducing organic free radicals produced through photolysis of Fe(III)-organo coordination compounds.

Table 1
Photoreduction kinetics of different initial concentrations of mercury(II) in algal solution

Parameters	Concentration ($\mu\text{g L}^{-1}$)	Initial rate v_0 ($\mu\text{g L}^{-1} \text{ min}^{-1}$)	Kinetics equation
Hg(II)	50	2.92	$-\text{d}C_{\text{Hg(II)}}/\text{d}t = 0.65 \times [C_{\text{Hg(II)}}]^{0.39}$
	100	4.11	
	150	4.66	
	200	5.14	

(Light source: metal halide lamps (250 W), pH 7.0, *C. vulgaris*: 7.00×10^9 cells L^{-1} .)

Table 2
Mercury mass balance

Every term mercury	Sample 1	Sample 2	Sample 3	Sample 4
HgT ₀ (%)	100	100	100	100
LHgT ₁₁ (%)	38.98	30.52	26.35	20.80
ΔHgT_{11} (%)	40.86	47.94	52.47	54.71
ΔHgT (%)	10.86	15.49	16.78	16.73
$\Delta\text{HgT} + \Delta\text{HgT}_{11} + \text{LHgT}_{11}$ (%)	90.70	93.95	95.60	92.24

(Light source: metal halide lamps (250 W), pH 7.0, C_0 (Hg^{2+}) = $50 \mu\text{g L}^{-1}$.)

photoreduction rate of low concentration Hg(II) decreased faster than that of high concentration of Hg(II) in aqueous solution with algae. Under these conditions, with initial concentration (C_0) of Hg(II) increasing, the reduction rate decreased. The removal of Hg(II) in terms of percentage of the initial Hg(II) concentration was higher at lower initial Hg(II) concentration. In this experiment, $r_A = \text{d}[C]/\text{d}t$ (the change in C vs. t) can be measured from the slope of the exponential curve at early times. It is notable that $r_A = \text{d}[C]/\text{d}t$ must be done at the instantaneous initial velocity, because the concentration of Hg(II) was always changing. We knew that instantaneous initial velocity \approx slope at initial point. Thus a plot of r_A vs. $[C]$ could be obtained by plotting the instantaneous initial velocity of the reaction, as determined by the slope of the tangent to the curve at the initial time against the $[C]$. This plot was a straight line whose slope was equal to k , the rate constant for the reaction. After the experiment datum was fitted with a range of initial concentrations from 50 to $200 \mu\text{g L}^{-1}$ at pH 7.0, the initial photoreduction rate as listed in Table 1 was plotted as a function of initial concentration of Hg(II). The initial photoreduction rate of Hg(II) under the conditions could be expressed by the equation: $-\text{d}C_{\text{Hg(II)}}/\text{d}t = 0.65 \times [C_{\text{Hg(II)}}]^{0.39}$ with a correlation coefficient $R = 0.9912$.

3.9. Mercury mass balance on the photoreduction of mercury(II) in algal suspensions

To confirm whether the disappeared Hg(II) in the suspensions was predominantly reduced to volatile metallic mercury during the reactions, $50 \mu\text{g L}^{-1}$ mercuric chloride in the suspensions of *C. vulgaris* at three different algal concentrations (4.75×10^9 , 5.72×10^9 , 7.00×10^9 , and 1.54×10^{10} cells L^{-1}) at pH 7.0 in a new cylindrical reactor (see Fig. 1b) was exposed to the irradiation of a 250 W metal halide lamp for 11 h. The dark control was made at an algal concentration of 4.75×10^9 cells L^{-1} *C. vulgaris*. The photoreduction of Hg(II) increase with the increasing concentration of algae in consistent with the results presented in Fig. 5. To further confirm the decrease of mercury concentration in the irradiated samples, we measured the percentages of total mercury in the initial algae suspension (HgT₀), in the algae suspension after 11 h (LHgT₁₁), in the solution left in the imbibing flask after 11 h (ΔHgT_{11}) and the total mercury taken from a imbibing flask every 1 h (ΔHgT). The results for the overall mass balance studied were given in Table 2. On average, more than 90.7% of initial Hg(II) in the suspension was well balanced. The photoreduction process revealed that more than 40.86% of Hg(II) from the algal suspension was reduced to volatile metallic mercury. O'Driscoll et al. used a similar technique

to study the gross photoreduction mercury mass balance in temperate freshwater lakes and rivers if Hg(0) was bubbled out as it was formed, and found that of the total mercury available in each sample a mean of 37.8% was reduced during the experiments [42].

4. Conclusions

It was demonstrated that algae, *C. vulgaris*, could significantly accelerate the photoreduction of Hg(II) under the irradiation of metal halide lamps ($\lambda \geq 365$ nm, 250 W). The photoreduction of Hg(II) increased with the increasing concentration of algae, Fe(III) and humic substance. The cooperation action of Fe(III) and humic substances accelerated the photoreduction of Hg(II). Increasing pH value also led to a higher photoreduction rate of Hg(II) in algae suspensions. DGM increased with the increase of exposure time and then appeared to approach a steady state in the irradiated suspensions. When the initial concentration of Hg(II) was in the range of 0.0– $200.0 \mu\text{g L}^{-1}$ with initial algae concentrations 7.0×10^9 cells L^{-1} at pH 7.0, the initial photoreduction rate of Hg(II) could be expressed by the equation: $-\text{d}C_{\text{Hg(II)}}/\text{d}t = 0.65 \times [C_{\text{Hg(II)}}]^{0.39}$ with a correlation coefficient of $R = 0.9912$. Study on the overall mercury mass balance revealed that more than 40.86% of Hg(II) from the algal suspension was reduced to volatile metallic mercury. The mechanism of photoreduction of Hg(II) in the presence of algae had many important photochemical and/or photobiological processes that involved in algae enhancing reduction of Hg(II), including the enzymatic reduction at the algae cell surface, reduction by free electrons and dissolved organic matter produced via the photolysis of algae under the irradiation.

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