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Original Research

Adsorption and Desorption Behaviors of Hg^{2+} by *Microcystis aeruginosa*

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Abstract

The adsorption and desorption behaviors of Hg^{2+} by living and dead *Microcystis aeruginosa* in aqueous solution were investigated. The results showed that both living and dead *Microcystis aeruginosa* can effectively adsorb Hg^{2+}, the equilibrium adsorption capacity and rate increased with the increasing abundance of algae. The maximum adsorption amount of Hg^{2+} to living and dead *Microcystis aeruginosa* was 2.13×10^{-2} and 1.11×10^{-2} ng 10^{-6} cells, respectively. The adsorption processes of Hg^{2+} by living *Microcystis aeruginosa* were biosorption and bioconcentration, whereas by dead *Microcystis aeruginosa* were only biosorption. Both pseudo-first-order and pseudo-second-order kinetics model can well describe the adsorption of Hg^{2+} by living and dead *Microcystis aeruginosa*, suggesting that adsorption processes might be predominantly controlled by a combined reaction of diffusion and chemical process. The adsorption characteristics were well illustrated by both Langmuir and Freundlich isotherm model, and Langmuir model can describe adsorption characteristics better. These demonstrated that there were strong interactions between *Microcystis aeruginosa* and mercuric ion, and *Microcystis aeruginosa* adsorbed Hg^{2+} were favorable processes through monolayer adsorption predominantly. The desorption processes of Hg^{2+} by *Microcystis aeruginosa* can be divided into three stages, i.e. the desorption amount of Hg^{2+} increased quickly within 0-60 min, the rate of desorption became very slow within 60-120 min, and tended to be balanced after 120 min.

Keywords: *Microcystis aeruginosa*, mercury, adsorption, desorption

Introduction

With the rapid development of industrialization, the problem of water environment pollution, especially heavy metal pollution, has become more and more severe all over the world [1]. Mercury, a toxic heavy metal, is present naturally in ecosystem. The toxicological effects of mercury are strongly dependent on its chemical species [2]. Once inorganic mercury species transformed into methylmercury, it will pose a detrimental threat to fish-eating animals and humans because methylmercury is a potent neurotoxin and can be accumulated by aquatic biota [3]. "Minamata disease" is the notorious incidents caused by mercury contamination in the 1950s and 1960s in Japan [2].

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As mercury cannot be degraded by microorganisms and can be enriched in organisms easily, it forms a hidden risk in the environment long-termly [4]. Therefore, the treatment of mercury-containing wastewater has become a research hotspot in the recent several decades. Physicochemical and biochemical methods, such as chemical precipitation, oxidation-reduction, electrochemical, ion exchange, etc., were used to treat mercury-containing wastewater in the past [5, 6]. However, these methods had the disadvantages of high cost and/or low processing efficiency [7].

In 1980s, the process of adsorption using biosorbents, such as bacteria, fungi and algae, has demonstrated to be a sustainable alternative for the heavy metal-containing wastewater treatment which is a low cost, efficient and clean process [8, 9]. Microorganisms are target in biosorption studies due to their high surface-to-volume ratio. Thus, biosorption has a good prospect for the treatment of mercury-containing wastewater. Among these biosorbents, algae have a wide application prospect because the cell surfaces are rich in biologically active and functionalized groups, such as cellulose, pectin substance, alginic acid ammonium salt, polysaccharide, polygalactose sulphate, hydroxyl, carboxyl, and amino, which has strong absorption for heavy metal ion [10]. Cyanobacteria are attractive because their ubiquitous presence in nature and exhibit efficient metal sorption properties. Therefore, algae have obvious advantages in heavy metal adsorption compared with other biosorbents [10].

However, the adsorption and desorption behaviors of Hg(II) on phytoplankton are still unclear at present. Hg^{2+} can migrate across cell membrane and enter living algae cells by passive transport. Additionally, living algae can excrete small organic molecules, which can affect the adsorption and desorption behaviors of heavy metals ions. However, the life cycle of algae is short and dead algae do not have such physiological activity. We hypothesize that the adsorption and desorption behaviors of Hg(II) on to living and dead algae are significantly different. Laboratory experiments were designed to characterize adsorption and desorption behaviors of Hg(II) on to living and dead *Microcystis aeruginosa*, one dominant species during cyanobacterial blooms in eutrophic lakes. The adsorption kinetics and isothermal adsorption model were discussed. This study provides information about the properties of Microcystis biomass in processes of waste water decontamination, which address the knowledge gaps in application of biosorbent for heavy metal removal.

Material and Methods

Culture of Algae

Microcystis aeruginosa (Cyanobacteria) was selected as research material, which was collected from Baihua reservoir, purified and reproduced in BG-11 medium at the temperature of 25ºC, with illumination intensity of 4000 Lux and the light/dark ratio of 12:12 hr.

Algae samples in logarithmic growth period were taken and filtered with 0.45 μm filter membrane (Millpore, polyvinylidene difluoride). The membrane was washed with ultrapure water for 4-5 times to remove the residual culture solution on the surface of algae cells. The washed algae samples were divided into two groups. One group of samples was treated by water bath calefaction at 50ºC for 10 min with cell walls remained intact, and this group was called dead algae samples. The other group was called living algae samples. The concentrations of cells in both samples were diluted to 1.0×10^6, 2.0×10^6, 4.0×10^6, 8.0×10^6, and 1.0×10^7 cells mL^{-1}, respectively.

Adsorption Experiment

Each 200 mL living/dead algae solution with abundance of 1×10^6, 2×10^6, 4×10^6, 8×10^6 and 1.0×10^7 cells mL^{-1} and 20 μL mercury chloride ($HgCl_2$, 1000 ng mL^{-1}, GR) solution were mixed into a quartzose conical flask with cover, respectively. The initial concentration of Hg^{2+} in the solution was about 100 ng L^{-1}. The mixed solution was shaken at a constant speed (120 r min^{-1}) under dark condition at the temperature of 25ºC. 10 mL subsamples were taken at time phase of 20, 60, 120, 240, and 1440 min and then transferred into 10 mL centrifuge tubes. Supernatants were extracted by centrifugation at 4000 r min^{-1} for 5 min. The concentrations of total Hg^{2+} in supernatants were determined following the procedures presented in US EPA method 1631 [11].

Desorption Experiment

The algae samples were filtered by 0.45 μm filter membrane (Millpore, polyvinylidene difluoride) and transferred to a 250 mL quartzose corked conical flask when the adsorption reaction reached equilibrium. The mixed solution was shaken at a constant speed (120 r min^{-1}) under dark condition at the temperature of 25ºC. Then 10 mL solution subsamples were taken at time phase of 20, 60, 120, 240, and 1440 min and then transferred into 10 mL centrifuge tubes, centrifugation at 4000 r min^{-1} for 5 min. The concentrations of total Hg in supernatants were checked following the procedures presented in the method 1631 [11].

Data Analysis

The equilibrium adsorption capacity and adsorption rate were calculated according to formula (1) and formula (2), respectively.

$$q_e = \frac{(c_0 - c_e)V}{N} \quad (1)$$

$$w = \frac{c_0 - c_e}{c_0} \times 100\% \quad (2)$$

...where q_e is the adsorption capacity (ng 10^{-6} cells) of 10^6 algae cells to mercury at adsorption equilibrium. c_0 and c_e are the concentration (ng L^{-1}) of Hg^{2+} in the solution at initial time and equilibrium, respectively. V refers to the volume of reaction solution (L), and N is the number of algae cells (10^6 cells).

Adsorption kinetics were calculated by modeling adsorption data using the pseudo-first order (Eq. (3)) and pseudo-second order (Eq. (4)) kinetics.

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (3)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (4)$$

...where q_e is the equilibrium adsorption capacity (ng 10^{-6} cells), q_t is the adsorption capacity at time t (ng 10^{-6} cells). k_1 and k_2 are the pseudo-first-order and pseudo-second-order kinetic constant (10^6 cells ng^{-1} min^{-1}), respectively.

Langmuir (Eq. (5)) and Freundlich (Eq. (6)) isotherm models were determined for investigating the adsorption mechanisms.

$$\frac{1}{q_e} = \frac{1}{q_m b c_e} + \frac{1}{q_m} \quad (5)$$

$$\ln q_e = \ln K + \frac{1}{n} \ln c_e \quad (6)$$

...where q_m is the maximum adsorption capacity (ng 10^{-6} cells), b is Langmuir adsorption constant, K and n are Freundlich equation empirical constant.

Quality Control

All glass instruments used in the experiment made of quartz, were immersed in 25% nitric acid for 24 hr and burned in muffle furnace at 500ºC for more than 4 hr. All of the glasses were used only once after natural cooling in mercury-free environment. The quality control was followed by the method 1631 [11] and disposable gloves were used to prevent cross contamination during the whole experiment.

Results and Discussion

Adsorption Capacity and Rate of Hg^{2+}

The adsorption capacity and rate of Hg^{2+} by living and dead *Microcystis aeruginosa* were increased with the increasing of algae abundance at adsorption equilibrium (Table 1). The living algae treatment had higher adsorption capacity and rate than the treatments with dead algae samples. When the algae abundance increased from 1.0×10^6 to 1.0×10^7 cells mL^{-1}, the corresponding adsorption capacity of living algae samples to Hg^{2+} was 8.14×10^{-4}, 6.91×10^{-4}, 5.55×10^{-4}, 4.84×10^{-4}, and 5.28×10^{-4} ng 10^{-6} cells. In the same treatments with the increasing of living algae abundance, the corresponding adsorption rate was 2.74%, 3.50%, 4.66%, 6.54% and 8.14% higher than that of dead algae, respectively. These results indicated that living *Microcystis aeruginosa* had higher capacity for adsorbing Hg^{2+} than dead *Microcystis aeruginosa*.

Adsorption Kinetics

The adsorption capacity of Hg^{2+} by living and dead *Microcystis aeruginosa* increased with reaction time firstly, and then tended to be stationary when the algae abundance reached 1.0×10^7 cells mL^{-1} (Fig. 1). The adsorption rate of Hg^{2+} by living and dead *Microcystis aeruginosa* was fast within 0-5 min, and the adsorption capacity were 6.11×10^{-3} and 5.85×10^{-3} ng 10^{-6} cells, and the adsorption rates were calculated to be 1.22×10^{-3} and 1.17×10^{-3} ng 10^{-6} cells min^{-1}, respectively. The adsorption capacity and rate were slow ranging with 5 to 120 min. The adsorption capacity of living *Microcystis aeruginosa* increased from 6.11×10^{-3} to 6.79×10^{-3} ng 10^{-6} cells, and the increasing rate was 5.91×10^{-6} ng 10^{-6} cells min^{-1}. For dead algae treatments, the adsorption capacity increased from 5.85×10^{-3} to 6.42×10^{-3} ng 10^{-6} cells, and the increasing rate was 4.96×10^{-6} ng 10^{-6}

Table 1. Adsorptionn capacity and rate of Hg^{2+} by different abundance of *Microcystis aeruginosa.*

Abundance (10^6 cells mL^{-1})	Adsorption capacity (ng)		Adsorption rate (%)	
	Living	Dead	Living	Dead
1	6.09	5.93	30.5	29.7
2	8.17	7.89	40.8	39.4
4	9.97	9.52	49.8	47.6
8	12.6	11.8	63.1	59.2
10	14.0	13.0	70.1	64.9

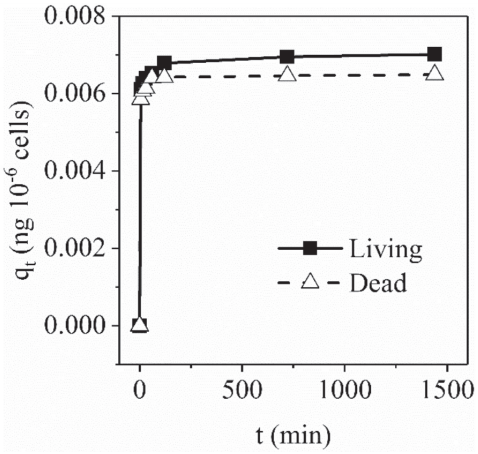

Fig. 1. Adsorption kinetics curve of Hg^{2+} by both living and dead *Microcystis aeruginosa.*

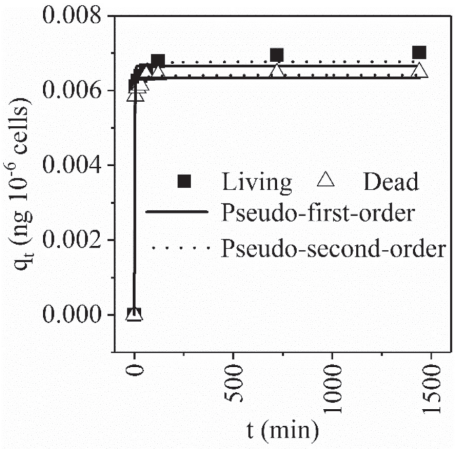

Fig. 2. Kinetic adsorption fitting of Hg^{2+} by *Microcystis aeruginosa*.

cells min^{-1}. Adsorption reaction approached equilibrium within 120-1440 min, and the adsorption rates of living and dead *Microcystis aeruginosa* were 70.14% and 64.86%, respectively. After 1440 min, the adsorption equilibrium was reached, the adsorption capacity and rate of living *Microcystis aeruginosa* was a little higher than that of dead treatments.

The kinetic characteristics and possible adsorption mechanism were described by pseudo-first-order and pseudo-second-order model (Fig. 2). For living *Microcystis aeruginosa*, the calculated values of adsorption capacity obtained by pseudo-first-order model (6.66×10^{-3} ng 10^{-6} cells), and pseudo-second-order model (6.77×10^{-3} ng 10^{-6} cells) were numerically closer to these thresholds obtained as a function of our experiments results (7×10^{-3} ng 10^{-6} cells). For dead *Microcystis aeruginosa*, we can also find the same results. In addition, the correlation coefficients of pseudo-first-order and pseudo-second-order model for both living and dead algae treatments were similar (Table 2). These results suggested that the adsorption processes of Hg^{2+} by living and dead *Microcystis aeruginosa* might be predominantly controlled by a combined reaction of diffusion and chemical process, such as complexation and ion exchange [12, 13].

Our results showed that the adsorption capacity and rate of Hg^{2+} by living *Microcystis aeruginosa* were a little higher than that of dead algae with the same abundance. In fact, the adsorption process of Hg^{2+} was different between living and dead *Microcystis aeruginosa*, which for dead algae was biosorption, whereas for living algae was biosorption and bioconcentration [10]. The biosorption process is a rapid and reversible process which is mainly composed of interaction between the functional groups on the cell wall of algae and the free metal ions in the water. The metal ions are adsorbed on the cell wall through the complexation reaction or ion exchange, which can be desorbed by other ions [14]. The viscosity of cell wall can enhance the adsorption of the metal ions. Bioaccumulation, however, is a slow and irreversible process which use the energy from algae metabolism to transport metal ions into algae cells for accumulation. The adsorption of Hg^{2+} by dead *Microcystis aeruginosa* was only a process of biosorption, while living algae included both biosorption and bioaccumulation. Therefore, the adsorption capacity and rate of Hg^{2+} by living *Microcystis aeruginosa* was a little higher than that of dead algae treatments.

Isothermal Adsorption Model

Langmuir and Freundlich isotherm adsorption model were applied to fit the adsorption process of Hg^{2+} by living and dead *Microcystis aeruginosa*. It was found that the adsorption process of Hg^{2+} by living and dead algae were well illustrated by the both models (Fig. 3). Langmuir model (living algae R^2 = 0.958 and dead algae R^2 = 0.983) can better describe the adsorption of Hg^{2+} by both living and dead *Microcystis aeruginosa* than the Freundlich model (living algae R^2 = 0.944 and dead algae R^2 = 0.966).

Table 2. Parameters of kinetic adsorption of Hg^{2+} by *Microcystis aeruginosa*.

Treatment	Pseudo -first-order kinetics			Pseudo -second-order kinetics		
	k_1(ng 10^{-6} cells·min^{-1})	q_e(ng 10^{-6} cells)	R^2	k_2(10^6 cells ng^{-1} min^{-1})	q_e(ng 10^{-6} cells)	R^2
Living algae	0.496	6.66×10^{-3}	0.988	217	6.77×10^{-3}	0.994
Dead algae	0.513	6.33×10^{-3}	0.996	284	6.41×10^{-3}	0.998

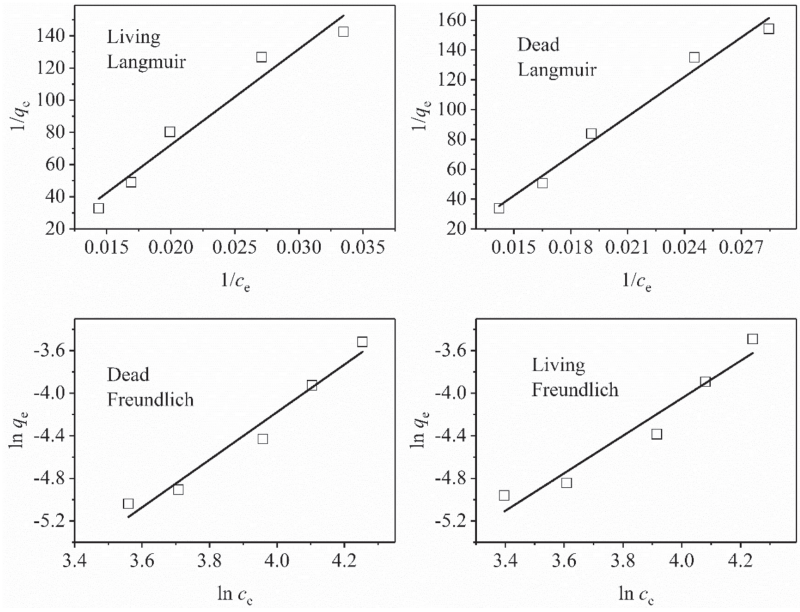

Fig. 3. The isothermal adsorption model of Hg^{2+} by living and dead *Microcystis aeruginosa*.

This suggested that the adsorption processes were mainly monolayer adsorption. According to Langmuir model, the maximum adsorption capacity of Hg^{2+} by living and dead *Microcystis aeruginosa* was 2.13×10^{-2} and 1.11×10^{-2} ng 10^{-6} cells, respectively. The respective Langmuir adsorption constant of Hg^{2+} by living and dead *Microcystis aeruginosa* was 7.88×10^{-3} and 1.02×10^{-2} L ng^{-1} (Table 3). The parameter *b* of Langmuir model for both living and dead samples were above zero, revealing that the adsorption of Hg^{2+} by living and dead *Microcystis aeruginosa* were spontaneous processes at room temperature [15]. Freundlich isotherm model can be used to elucidate adsorption and desorption of metal ion by different adsorbents, especially for fitting data calculated from highly heterogeneous adsorbents [16]. The value of *n* in Freundlich isotherm model were between 0.1 and 1, which revealed that there were strong interactions between *Microcystis aeruginosa* and mercuric ion, and that living and dead *Microcystis aeruginosa* adsorbed Hg^{2+} were favorable processes [17].

Desorption Characteristics

The same trend was observed in the desorption characteristics of Hg^{2+} by *Microcystis aeruginosa* with different abundances. The desorption amount of Hg^{2+} by living and dead *Microcystis aeruginosa* increased gradually with increasing algae abundance. At the initial stage, the amount of Hg^{2+} desorbed by dead *Microcystis aeruginosa* was lower than that by living algae. Total amount of Hg^{2+} desorbed by dead *Microcystis aeruginosa* was higher than that by living algae (Fig. 4). The treatment with 1.0×10^{7} cells mL^{-1} abundance algae was analyzed as a random example. The desorption of Hg^{2+} by living and dead *Microcystis aeruginosa* can be divided into three stages. At the first stage (0-60 min), the desorption of Hg^{2+} by living and dead *Microcystis aeruginosa* increased with the prolongation of time, and reached the maximum desorption value on living algae. In this stage, the concentration of Hg^{2+} in the solution of living algae treatment was 26.9 ng L^{-1}, and the desorption quantity was 5.38 ng. At the second stage (60-120 min), desorption quantity of living *Microcystis aeruginosa* began to decline, but the desorption of Hg^{2+} by dead *Microcystis aeruginosa* continued to increase at the rate of 0.0193 ng L^{-1} min^{-1}. At the third stage (120-1440 min), desorption quantity of living and dead *Microcystis aeruginosa* changed slowly and tended to balance. The respective Hg^{2+} concentration in the solution of living and dead algae was 26.1 ng L^{-1} and 27.5 ng L^{-1}, and corresponding desorption amount was 5.22 ng and 5.50 ng in the balance.

Table 3. Parameters of isothermal adsorption of Hg^{2+} by *Microcystis aeruginosa*.

Treatment	Langmuir model			Freundlich model		
	q_m(ng 10^{-6} cells)	*b*(L ng^{-1})	R^2	*K*(L ng^{-1})	*n*	R^2
Living algae	2.13×10^{-2}	7.88×10^{-3}	0.958	1.53×10^{-5}	0.568	0.944
Dead algae	1.11×10^{-2}	1.02×10^{-2}	0.983	2.01×10^{-6}	0.447	0.966

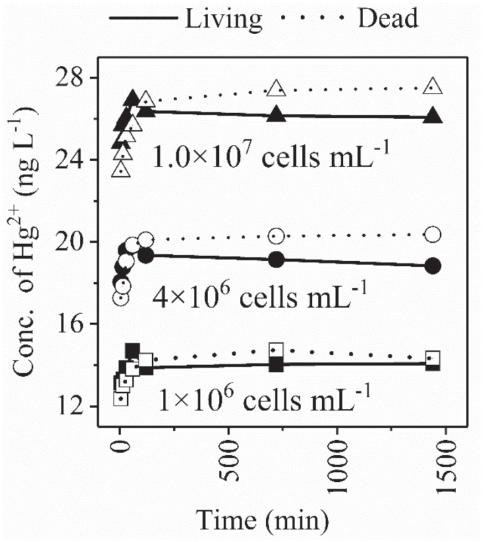

Fig. 4. Kinetic curve of desorption of Hg^{2+} by *Microcystis aeruginosa*.

Indicative Significance for Biogeochemistry of Mercury in Aquatic Environment

Algae plays an important role in the structure and function of aquatic food web, and is also the key link of mercury accumulation and biomagnification along the food chain [18, 19]. In natural water environment, the living algae float and sink periodically. The life cycle of algae is short and the dead algae will sink to the bottom of waterbody. Mercury adsorbed by living and dead algae will migrate to the bottom where the mercury methylating occurs [20]. Some studies have identified that mercury located in the surface of algae cell and the particulate matter could transform to methylmercury with the presence of bacteria [21, 22]. In addition, mercury can migrate into algae cells via passive diffusion or active transport, showing a strong ability of mercury enrichment through surface complexation, extracellular polymer coordination and other processes [23-25]. The biological concentration factor of algae for inorganic mercury is 10^5, and for methyl mercury is 1-2 orders of magnitude higher than that of inorganic mercury [14]. Thus, it's found that research about adsorption and desorption behaviors of Hg^{2+} by typical algae species is of great importance for understanding the interaction between mercury and algae.

Generally, the amount of mercury in algae cells is mostly measured by the dry weight of algae cells. The adsorption capacity of Hg^{2+} by living and dead *Microcystis aeruginosa* were given in the unit of ng 10^{-6} cells in our study. This is helpful to analyze the amount of mercury adsorbed by algae cells in the natural water environment. Take Baihua Reservoir as an example, the catchment area is 1895 km^2 with the average water depth of 10.5 m and the deepest is 45 m [26]. The average concentration of total mercury in the water is 22.4 ng L^{-1}, the average concentration of particulate mercury is 12.0 ng L^{-1} [27], and the abundance of algae is 1.32×10^6-5.31×10^8 cells L^{-1} [28]. Based on the estimation of mercury adsorption capacity (2.13×10^{-8} ng $cells^{-1}$) by *Microcystis aeruginosa* found in this study, it is assumed that the dominant algae in the water are *Microcystis aeruginosa*, and the amount of mercury adsorption by algae in the water is 38.15-15350 ng. Our results indicated that algae can affect the mercury migration of 0.13%-23.7% in the water, and even cause 23.7% of mercury in the water to enter the food chain. Therefore, the amount of mercury adsorbed by algae in the unit of ng 10^{-6} cells are of great importance in the study of biogeochemical behavior between algae and mercury [29].

Indicating Significance for Sewage Treatment

Complex formation and ion exchange are the key processes of heavy metal removal by adsorbent form aqueous solution [30]. The cell wall of algae contains polysaccharides, proteins, amino, carbonyl, carboxyl, sulfhydryl, etc. groups that can accommodate complex heavy metals on the cell surface by coordination reaction [31, 32]. Heavy metals can replace light ions with small radius such as K^+, Na^+, Ca^{2+}, etc. through ion exchange or adsorbed on the cell surface through

Table 4. Comparison of adsorption capacities of heavy metal by various algal biomass existing in literature.

Biosorbent	Metal	Capacity (mg g^{-1})	Removal rate	Reference
Tetraselmis suecaca	Cd	/	98.1%	[36]
Scenedesmus obloquus	Cu	1.8	67%	[37]
Synechocystis sp.	Pb	6.85	91.8%	[38]
Sargassum sp.	Cr(VI)	15.6	45%	[39]
Laminaria japonica (B)	Zn	32.5	55.6%	[40]
Sargassum filipendula	Cd	103.5	99.56%	[41]
Living *Microcystis aeruginosa*	Hg^{2+}	2.13×10^{-2} ng 10^{-6} cells	70.14%	Present work
Dead *Microcystis aeruginosa*	Hg^{2+}	1.11×10^{-2} ng 10^{-6} cells	64.86%	Present work

oxidation-reduction [13, 14]. The abduction of heavy metals can promote the secretion of extracellular polymers to enhance the fixation of heavy metals on the cell surface, and promote the synthesis of metallothionein, phytocomplexone, glutathione, etc. to enhance the enrichment of heavy metals by algae cells [33]. Therefore, algae cells have a strong adsorption/enrichment effect on heavy metals, which is widely applied in the removal of heavy metals in wastewater due to the characteristic of universal existence in water environment, easy expansion and cultivation, high adsorption capacity, low energy consumption and environmental friendliness [34, 35]. There are interesting examples of heavy mental adsorbed by various algae species and a comparison of adsorption capacity and remove rate were listed in Table 4. The adsorption capacity and removal rate of Hg^{2+} by living and dead *Microcystis aeruginosa* we obtained is within the general range that has been found by others, demonstrating that *Microcystis aeruginosa* is neither the worst nor the best biosorbent. This indicates that this species of algae has a certain application prospect in the removal of mercury in wastewater.

Conclusions

Microcystis aeruginosa can absorb a large amount of Hg^{2+} within a short period, the adsorption capacity and rate increased with the increase of algae abundance. The absorption capacity and rate of Hg^{2+} by living algae were higher than that of dead algae. The adsorption processes of Hg^{2+} by living *Microcystis aeruginosa* were biosorption and bioconcentration, and by dead *Microcystis aeruginosa* were only biosorption. Analysis of adsorption kinetics showed that adsorption processes might be predominantly controlled by a combined reaction of diffusion and chemical process. Langmuir and Freundlich isotherm model analysis revealed that there were strong interactions between *Microcystis aeruginosa* and mercuric ion, and *Microcystis aeruginosa* adsorbed Hg^{2+} were favourable processes through monolayer adsorption predominantly.

Desorption amount of Hg^{2+} by *Microcystis aeruginosa* increased with the increasing of algae abundance, and showed a significant temporal change characteristic. This work is beneficial to understand the biogeochemical behavior between algae and mercury, and provides theoretical evidence for the potential of the application of *Microcystis aeruginosa* in the removal of mercury in wastewater.

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Conflict of Interest

The authors declare no conflict of interest.

Reference

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