Research Article

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Study of an adsorption method for trace mercury based on *Bacillus subtilis*

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Abstract: In order to decrease the difficulty in trace mercury determination, an adsorption method for trace mercury based on *Bacillus subtilis* cells was proposed in this article. The adsorption process was characterized by optical microscopy and SEM. The adsorption mechanism was analyzed by IR. The adsorption performance was studied by measuring the concentration of supernate and calculating the adsorption efficiency. When adsorbing Hg^{2+} , *Bacillus subtilis* cells gathered and their structure turned coarse. The IR results illustrated that functional groups bound with Hg for complexation during adsorption. *Bacillus subtilis* completed adsorption for trace Hg^{2+} in 15 min. The adsorption efficiency was maintained above 80% under low Hg^{2+} concentrations ($<200 \mu\text{g/L}$). The proposed study illustrates that *Bacillus subtilis* cells are highly efficient and easily obtained material for the adsorption of trace mercury, which shows potential to be further used in the pretreatment of trace Hg^{2+} detection.

Keywords: adsorption, *Bacillus subtilis*, trace, Hg^{2+}

1 Introduction

Heavy metal ions are serious pollutants to water environment, which have a critical influence on both aquatic organisms and human beings. Among the heavy metal ions, mercury ion is one of the most toxic ions. Mercury can be absorbed by human body through food chain or drinking water. Due to the difficulty in metabolism by the body, the absorbed mercury ions accumulate continuously [1]. The accumulated mercury ions can cause neuro

turbulence and chronic poisoning, leading to the damage of organs and even death [2]. The famous Minamata disease is the result of excessive intake of mercury polluted food and water. Because of mercury ions' extensive toxicity, the water environment quality standard for mercury ions is critical. In China, the standard limit for Hg^{2+} in surface water is two orders of magnitude lower than that of Cr^{6+} and Pb^{2+} , and is three orders of magnitude lower than that of Cu^{2+} and Zn^{2+} . Thus, sensitive, accurate, fast and convenient detection techniques for mercury are urgently needed [3].

A large number of analysis methods have been employed and studied for the determination of Hg^{2+} , including cold vapor atomic absorption spectrometry [4,5], colorimetric methods [6], fluorescence spectrometry [7], inductively coupled plasma mass spectrometry [8,9], electrochemical analysis [10–12], etc. These techniques have high sensitivity, good selectivity and low detection limit. However, they also show certain limitations, such as the high cost of instruments, the complexity of sample preparation and the complicated test procedures.

To overcome the shortcoming of a single detection technique, the combination of pre-concentration technique with detection technique shows potential in precise determination. Duan et al. [13] coupled solid phase extraction with cold vapor atomic absorption spectrometry for Hg determination, which made the established method concise and suitable for routine analysis. Seibert et al. [14] proposed a method for Hg determination using a flow injection system coupled to an inductively coupled plasma mass spectrometry (ICP-MS) instrument. The pre-concentration process included the on-line complexation with DDTP, sorption of the Hg complexes and the elution with methanol. The pre-concentration procedure and the analytical conditions can be optimized for the highest sensitivity.

In Hg pre-concentration process, sorption material determines the sensitivity and the selectivity of the method [15]. Various sorption materials have been proposed, including carbon-based materials [16], polymers [17], ionic liquid [18], biosorbents [19], etc. Among the adsorbents, biosorbents attract much attention owing to their numerous

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functional groups, good yield, small size and low cost [20,21]. Fungus, bacteria, algae and yeast are commonly used biological materials.

Bacterial biosorbents are widely distributed in the environment with a huge variety. They are cost-effective, easily cultivated and environmentally friendly. Besides, bacterial biosorbents also show high adsorption capacity and low toxicity [22–25]. Cain et al. [26] studied the biosorption of Hg^{2+} by two strains of cyanobacteria. Both of them showed excellent adsorption performance. Singh et al. [27] investigated the potential of *Brevundimonas* species IITISM22 to remove mercury. Live biomass of bacterial cells was used and the removal performance was measured. Deniz and Karabulut [28] used a coastal seaweed community composed of *Chaetomorpha* sp., *Polysiphonia* sp., *Ulva* sp. and *Cystoseira* sp. species as natural biosorbent material for the bioremediation of zinc-containing synthetic wastewater. Ibrahim [29] examined four species of red seaweeds to remove Co(II) , Cd(II) , Cr(III) and Pb(II) ions from aqueous solution. *Galaxaura oblongata* biomass was relatively more efficient to remove metal ions with mean biosorption efficiency of 84%.

Since Hg^{2+} detection is of great significance and microorganisms are good adsorption materials, it is meaningful to study biosorbents and their adsorption performance to low concentration of Hg^{2+} . In this article, an adsorption method of mercury ions based on *Bacillus subtilis* was proposed. The adsorption process was characterized by microscopy and SEM. The adsorption mechanism was analyzed based on IR measurements. The adsorption time was optimized and the adsorption performance was tested.

2 Materials and methods

2.1 Reagents and apparatus

Bacillus subtilis (No. 1.88) was obtained from the Institute of Microbiology, Chinese Academy of Sciences. The standard solution of Hg^{2+} (100 mg/L) was purchased from China National Research Centre for Certified Reference Material. NaCl, peptone and beef extract were of analytical grade and were used without further purification. Deionized water with the resistivity of 18 M Ω was used throughout the experiment. All the experiments were performed at 25°C without special illustration.

The optical microscopy images were carried out by Olympus BX51 using a 100 $\times$ Olympus objective. SEM analysis was carried out using S-4800 field emission scanning electron microscope produced by Hitachi (Tokyo, Japan).

FT-IR was measured using Spectrum One (PerkinElmer, USA) with diamond ATR accessory. ICP-MS (PerkinElmer ELAN DRC) was used for mercury determination at concentrations lower than 100 ppb. Heavy metal ion solutions with other concentrations were tested by Inductively coupled plasma optical emission spectrometer (ICP-OES) (PerkinElmer Optima 8300).

2.2 Preparation of *Bacillus subtilis* cells and Hg^{2+} solutions

B. subtilis cells were used for adsorption. The specific cultivation process of *B. subtilis* was introduced in ref. [30]. Cells in stationary phase were harvested by centrifuging at 6,000 rpm for 20 min. The cells were added to Hg^{2+} solutions at the concentration of 0.66 g/L (dry weight).

The standard solution of Hg^{2+} was diluted to 1–250 $\mu\text{g/L}$ by deionized water. To increase the ion concentration of diluted solutions, NaCl was added at the concentration of 5 g/L, which was exactly the NaCl concentration of microbe's culture medium. The prepared Hg^{2+} solutions were poured into conical flasks for subsequent adsorption.

2.3 Adsorption experiment

To observe *B. subtilis* cells' response to Hg^{2+} , the centrifuged cells of 0.66 g/L were added into 100 mL, 100 $\mu\text{g/L}$ Hg^{2+} solution. The mixture was put into a rotating shaker at 150 rpm and 37°C. At each adsorption time (0, 15, 30, 45, 90 and 180 min), 6 mL of the mixture was taken out. The taken mixture was separated by 0.45 mm filter membrane. The filtrate was sent for Hg^{2+} concentration measurement. The cells on the filter membrane were obtained by washing the filter membrane with deionized water. The collected cells were observed by optical microscopy and SEM. The IR spectrum of the cells before and after adsorption of 15 min for 100 $\mu\text{g/L}$ Hg^{2+} solution was measured.

For optimization of adsorption time, the adsorption time was subdivided to 0, 3, 7, 11 and 15 min. The adsorption efficiencies at different adsorption time were calculated. The adsorption efficiency η was calculated by the following equation.

$$\eta = \frac{C_0 - C_1}{C_0}, \quad (1)$$

where C_0 is the Hg^{2+} concentration of solution measured before adsorption. C_1 is the Hg^{2+} concentration of filtrate measured after adsorption.

Table 1: The adsorption experimental parameters

Item	Parameter
Quantity of microorganisms	0.66 g/L
pH	6
Temperature	37°C
Rotation speed	150 rpm
Base solution	5 g/L NaCl
Separation method after adsorption	0.45 μm filter membrane
Adsorption time (min)	0, 3, 7, 11, 15, 30, 45, 60, 90 and 180
Concentration of treated Hg ²⁺ solution (μg/L)	1, 5, 10, 50, 100, 150, 200 and 250

To study the adsorption performance, the adsorption efficiencies of cells to solutions with different Hg²⁺ concentrations were recorded. The parameters of the adsorption experiment are shown in Table 1.

3 Results and discussion

3.1 The morphological characteristics of *B. subtilis*

Microscope (1,000×) was used to observe the morphological characteristics of *B. subtilis* cells before and after

Hg²⁺ adsorption. *B. subtilis* cells without adsorption were used as the control group. Cells that adsorbed Hg²⁺ (100 μg/L) were used as experimental group. The adsorption time was set as 15, 30, 45, 90 and 180 min. Figure 1 shows the microscopic results. Without contacting Hg²⁺ solution, cells were plump, bacilliform and well-distributed in the observed area (Figure 1(a)). After contacting Hg²⁺ solution, cells aggregated together. Bacilliform single cells could only be seen at the edge of the aggregates. The volume of the cells was smaller than that in control group. When the adsorption time extended, the aggregation extent was enhanced and the number of cells contained in the aggregates increased (Figure 1(b)–(f)). The microscopic results indicate that *Bacillus subtilis* cells can respond to Hg²⁺ solution, which makes the following study applicable.

To further compare the cells’ morphology before and after Hg²⁺ adsorption, SEM was used for more detailed characterization. The SEM images are shown in Figure 2. The *B. subtilis* cells without adsorption had a regular edge with a plump spatial structure (Figure 2(a)). The cells were uniformly distributed. After 15 min immersion of the cells in Hg²⁺ solution, the cells aggregated and the edges of the cells were blurry (Figure 2(b)). The spatial structure was lost and the structure tended to be flat and coarse. When the immersion lasted for 90 min, the cells’ aggregation became more serious. Most cells were in piles, losing their spatial structure. Only a few cells’ edges in the margin of the aggregates could be distinguished.

Figure 1: Microscopic images of *B. subtilis* after different adsorption time, including control group (a), 15 min (b), 30 min (c), 45 min (d), 90 min (e) and 180 min (f).

Figure 2: SEM images of *B. subtilis* cells after different adsorption time, including control group (a), 15 min (b) and 90 min (c and d).

The SEM results illustrate that trace Hg^{2+} solution can cause microbes' response and that the responses are enhanced along with the adsorption time.

3.2 The IR characterization of *B. subtilis* cells

Infrared spectrum analysis can obtain the information of functional groups and chemical bonds, which plays an important role in studying the structure of organics.

Figure 3: IR spectra of *B. subtilis* cells before (a) and after (b) adsorption for 100 $\mu\text{g/L}$ Hg^{2+} solution.

Besides, the variation in the functional groups can be identified by spectra comparison. The IR spectrum of *B. subtilis* cells before and after 15 min's adsorption for 100 $\mu\text{g/L}$ Hg^{2+} solution are shown in Figure 3. The IR absorption bands and possible corresponding groups are displayed in Table 2.

As seen from the IR test results of raw cells before Hg^{2+} adsorption (Figure 3(a) and Table 2), large amounts of functional groups such as carboxyl, amino and hydroxyl existed in *B. subtilis* cells, which was in accordance with previous report [31]. After Hg^{2+} adsorption, the peaks of the spectrum showed some shifts. The peak at 3,289 cm^{-1} representing the stretching of $-\text{OH}$ groups shifted to 3,415 cm^{-1} . It indicated the improved bond length between Hg and bacterial surface. The peak at 1,635 cm^{-1} corresponding to $-\text{COO}^-$ vibration shifted to 1,641 cm^{-1} , indicating the functional groups participated in the adsorption. Before adsorption, the peaks in $-\text{SH}$ and $-\text{NH}_2$ were 2,103 and 1,560 cm^{-1} . After adsorption, the peaks shifted to 2,136 and 1,533 cm^{-1} , respectively. The peak shifts indicated that the functional groups participated in

Table 2: IR absorption bands and possible corresponding groups

Wavenumber (cm^{-1})	Functional group
3,289	$-\text{OH}$
2,103	$-\text{SH}$
1,635	$-\text{COO}^-$ and $-\text{C}=\text{O}$
1,560	$-\text{NH}$

adsorption and bound with Hg. Thus, during adsorption, complexation was considered as the main mechanism. The IR results were similar to previous studies [32,33].

3.3 Optimization of adsorption parameters

Many factors can influence the adsorption capacity of microorganisms, including microbial factors, heavy metal factors and environmental factors. The microbial factors mainly include metabolic capacity, physiological status and existing status. The heavy metal factors mainly include the species, concentration and valence of heavy metal ions. The environmental factors mainly contain pH, temperature and adsorption time. In this study, the adsorption target was Hg^{2+} and the solution concentration to be treated was at $\mu\text{g/L}$ level. Thus, the heavy metal factors could be ignored. To avoid the influence caused by microbial factors for the adsorption, critical microbial conditions (37°C , 150 rpm, 5 g/L NaCl base solution, and microbes' concentration of 0.66 g/L) were controlled during the adsorption process. The parameters were in accordance with those used in microbial cultivation process, which better improved the applicability of microorganisms to adsorption environment. Therefore, the optimization of adsorption parameters focused on the selection of adsorption time.

Adsorption time is one of the most important parameters affecting the adsorption capacity. With the increase in adsorption time, the complete sorption phenomenon is completed mainly in two steps [27]. The first step is the quick adsorption period, achieving rapid binding of metal ion to the biosorbent's surface. The main adsorption mechanism is surface adsorption process, relying on the ion exchange and complexation by the functional groups on the cells' surface. This period can be completed in a short time and the adsorption proportion can reach about 70%. The second step is the slow adsorption period. Slow intracellular diffusion is observed. Microbes consume energy to transmit heavy metal into the cell, which usually needs several hours. The above conclusion is applicable to the removal of heavy metal ions. To study the adsorption process of low concentration Hg^{2+} , the influence of adsorption time was studied in this article.

The adsorption efficiencies corresponding to different adsorption time were tested and the response curve is shown in Figure 4. In the first 15 min, the adsorption efficiency increased sharply and reached 92.96%. After 15 min, although the adsorption time increased, the adsorption efficiency remained the same as that of 15 min. This adsorption process was different from the reported two-step process. It could be explained by the Hg^{2+} concentration of

Figure 4: The optimization of adsorption time by the adsorption efficiency.

trace level. Functional groups on the surface of *B. subtilis* cells were enough for trace concentration adsorption, through which the achieved adsorption efficiency could be above 90% in 15 min. Under this circumstance, there was no need for the second stage and the adsorption could be completed quickly and efficiently. In the subsequent experiments, 15 min was used as the adsorption time.

3.4 The performance of adsorption

Adsorption efficiency is a significant characteristic for evaluating adsorption performance. The higher the adsorption efficiency is, the better the performance of adsorption is. Considering the subsequent accurate detection, another important requirement is that the adsorption efficiency should be stable. Only with stable adsorption efficiency, a trace Hg^{2+} concentration can uniquely correspond to a high Hg^{2+} concentration after pre-concentration. This uniqueness guarantees the accuracy of the subsequent detection. Thus, the adsorption efficiency of solutions with different Hg^{2+} concentrations was studied and the result is shown in Figure 5. Hg^{2+} solutions of 1–250 $\mu\text{g/L}$ were prepared for adsorption. After 15 min adsorption, 0.45 μm filter membrane was used for the separation of the cells and the supernate. The Hg^{2+} concentration of the supernate was measured and the adsorption efficiency was calculated. As seen from Figure 5, the adsorption efficiency decreased with the increase of Hg^{2+} concentration. When the Hg^{2+} concentration was lower than 200 $\mu\text{g/L}$, the adsorption efficiency remained above 80% and the efficiency differences between different Hg^{2+} concentrations were small. However, when the Hg^{2+} concentration was higher than 250 $\mu\text{g/L}$, the

Figure 5: The adsorption efficiency response curve to different Hg^{2+} concentrations.

adsorption efficiency dropped obviously, indicating that the functional sites on cell surface were not enough for Hg^{2+} . Therefore, under the experimental conditions used in this article, effective adsorption can be achieved for Hg^{2+} solution with the concentration lower than $200 \mu\text{g/L}$, which satisfies the requirement of trace Hg^{2+} adsorption.

4 Conclusion

Mercury is extremely toxic and its standard limit is at a trace level. Since Hg^{2+} detection is of great importance and microorganisms are good adsorption materials, it is meaningful to study biosorbents and their adsorption performance to low concentration of Hg^{2+} . Thus, an adsorption method of trace mercury based on *B. subtilis* was proposed in this article. The adsorption process was characterized. The adsorption mechanism was analyzed and the adsorption performance was studied. *B. subtilis* cells gathered and their structure turned coarse after Hg^{2+} adsorption. The IR test illustrated that complexation of functional groups with Hg^{2+} was the main adsorption mechanism. The adsorption for trace Hg^{2+} could be completed in 15 min. The adsorption efficiency can be maintained above 80% for solutions with low Hg^{2+} concentrations ($<200 \mu\text{g/L}$). The proposed research provides an adsorption means for trace Hg^{2+} , which is promising in the coupling with the detection method for accurate and sensitive detection of trace Hg^{2+} .

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