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Original Research

Biosorption of Antimony (Sb) by the Cyanobacterium *Synechocystis* sp.

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Abstract

Antimony (Sb) pollution has been of growing environmental concern. Little information is available on biosorption of Sb. In the present study, biosorption behavior and mechanisms of Sb(III) to the cyanobacterium *Synechocystis* sp. cells were investigated by batch experiments and FTIR analysis. Our study shows that *Synechocystis* sp. cells are a good adsorbent for Sb(III) with a sorption capacity of 4.68 $mg \cdot g^{-1}$ dry weight adsorbent. The isothermal sorption data can be described by the Langmuir Isotherm and the Freundlich Isotherm. Several mechanisms were involved with biosorption of Sb to *Synechocystis* sp. cells and sorption to binding sites might be dominant. The sorption kinetics followed the pseudo-second order model. The adsorbed Sb is mainly located in extracellular polysaccharides (EPS) and within the cell, and a small proportion was adsorbed onto the cell wall. The proteins and polysaccharides in EPS and the polysaccharides on the cell are the main functional groups that are responsible for adsorption of Sb to *Synechocystis* sp. cells.

Keywords: antimony, biosorption, cyanobacterium, kinetics, isotherm, FTIR

Introduction

Antimony (Sb), with a molecular weight of 121.76, belongs to subgroup 15 of the periodic table of elements. Sb is not an essential element for biological metabolisms. It is potentially toxic to organisms at very low concentrations [1]. Sufficient evidence from experimental animals have shown that Sb trioxide is carcinogenic. Sb has been listed as a prioritary pollutant by the U.S. Environmental Protection Agency [2] and the Council of the European Communities [3]. Sb is also on the list of hazardous substances under the Basel Convention concerning the restriction of transfer of hazardous waste across borders [4]. Strict drinking water standards have been set for Sb by the World Health Organization and many countries.

In southwestern China, where more than 80% of the world's total Sb reserves are deposited, aquatic environments are severely polluted by Sb due to long-term and large-scale mining of Sb ores. It was reported that the concentrations of Sb in wastewater, river waters, and polluted well water are approximately 1.33-21.79, 0.063-0.037, and 24.02-42.03 $mg \cdot L^{-1}$, respectively [5]. Sb pollution in this area has been of a great environmental concern because Sb in the aquatic environment poses great threat to the health of residents.

Unlike other heavy metals, treatment technologies of Sb-containing effluents from Sb mines are limited.

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Coagulation with alum or ferric salts and lime softening are the two convenient methods for removal of Sb from wastewater. Recently, biosorption, based on the metal binding capacities of various biological materials, has gained more attention due to its high efficiency and low cost. Biosorption behavior of heavy metals by microalgae, fungi, bacteria, and higher plant materials has been extensively studied [6-11]. However, unlike other heavy metals, limited information is available on the biosorption of Sb.

The objective of this study is to investigate the biosorption characteristics of Sb by the cyanobacterium *Synechocystis* sp. and its distribution intracellularly and extracellularly. Because *Synechocystis* sp. is a common cyanobacterial species in aquatic environments of southwestern China and it grows fast, it was selected as the test microorganism.

Materials and Methods

Culture of *Synechocystis* sp.

Synechocystis sp. strain (FACHB 898) cells were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. The cells were grown in BG-11 medium [12] at 25ºC under fluorescent white light (55 μmol photons $m^{-2} \cdot s^{-1}$).

Biosorption Experiments

Stock Sb solution was prepared by dissolving analytical grade antimony potassium tartrate in deionized water.

For the study of biosorption kinetics, 0.5 g of cyanobacterial cells were added into conic flasks containing 100 mL of 25 $mg \cdot L^{-1}$ Sb solution with pH 7.0. Two flasks were shaken at 125 rpm at 25ºC for 2 h. Aliquot amounts (2 ml) of solution were collected at 0, 5, 10, 15, 20, 30, 45, 60, 90, and 120 min. The samples were filtered through a 0.45 μm filter membrane and Sb concentrations in filtrate were determined. The kinetic experiment was conducted in duplicate. The mean values were used.

For the isothermal study, 0.5 g of cyanobacterial cells were added to flasks containing 100 mL of Sb-bearing solution of various concentrations (5, 10, 25, 50, 100 $mg \cdot L^{-1}$). Two flasks were shaken at 125 rpm at 25ºC for 2 h. Five ml of solution was collected and filtered through a 0.45 μm filter membrane. Sb concentrations in filtrate were determined. The isothermal experiment was conducted in duplicate. Mean values were used.

Intracellular and Extracellular Distribution of Sb

The residual 95 mL of cell suspension after isothermal experiment was centrifuged at 4,000 ×g for 10 min in order to collect the cells. The collected cells were resuspended in deionized water and centrifuged at 4,000 ×g for another 10 min. This step was repeated three times and then the cells were re-suspended in deionized water and again centrifuged at 20,000 ×g for 30 min. The supernatant was considered as EPS sample [13, 14]. Sb concentrations in EPS solutions were determined. The residue was considered as cells without EPS. These cells were then subjected to ultrasonolysis at 300 w mL^{-1} in an ice bath and centrifuged at 3,000 ×g for 10 min. The supernatant and the residue were considered as intracellular substances and cell walls, respectively. The intracellular substances and cell walls were further digested with a mixture of concentrated HNO_3 and concentrated HCl (HNO_3:HCl=5:3) for 2 h at 160ºC under pressure in a sealed heater. The Sb concentrations in the digested solutions were determined.

Determination of Sb Content in Solution

For the determination of Sb in solution or digest, 0.5 mL of the samples was added in a 25-mL flask with 2.5 ml of 15% m/V KI, 3% m/V ascorbic acid and 3 ml of concentrated HCl. The flask was supplemented with deionized water to 25 mL. The mixture was left to stand at room temperature for 30 min. to ensure complete reduction of Sb(V) to Sb(III) before the stibine generation [15]. The determination of total antimony was performed by the standard calibration technique [16] using an atomic fluorescence spectrometer (AFS-800, Jitian Instrument Inc., China). For each sample, three measurements were carried out.

FTIR Analysis

The EPS sample was lyophilized. About one milligram of freeze-dried EPS was mixed with 100 mg of KBr and compacted in pellet form. The FTIR spectra of EPS samples were collected using a FTIR spectrometer (Tensor 27, Bruker, German) with a resolution of 4 cm^{-1} against a KBr background. All spectra were smoothed using the Savitsky-Golay algorithm and the baseline was corrected using the "automatic baseline correct" function.

Results and Discussion

Biosorption Kinetics

We observed that biosorption of Sb by *Synechocystis* sp. was a rapid process. Nearly 50% of the Sb was adsorbed within 30 min. (Fig. 1). Equilibrium was largely approached at 1h. After 1-h adsorption, Sb concentration dropped from 25 $mg \cdot L^{-1}$ to 11.93 $mg \cdot L^{-1}$ and the amount of Sb adsorbed was 2.61 $mg \cdot g^{-1}$ adsorbent in dry weight. In order to describe the kinetics of biosorption of Sb by *Synechocystis* sp., the pseudo first-order and pseudo second-order equations were applied to fitting the sorption data, respectively.

The pseudo first-order and pseudo second-order equations were employed to model the sorption data over the entire time range. The pseudo first-order equation of Lagergren [17] is generally expressed as follows:

$$dq/dt = k_1(q_e - q) \quad (1)$$

...where q_e and q are the amount of metal sorbed per unit weight of sorbent at equilibrium and at time t, respectively

Table 1. Parameters of pseudo first-order model and pseudo second-order model for biosorption of Sb to *Synechocystis* sp.

The pseudo first-order equation			The pseudo second-order equation		
k_1 (min^{-1})	q_e ($mg \cdot g^{-1}$)	R^2	k_2 ($g \cdot mg^{-1} \cdot min^{-1}$)	q_e ($mg \cdot g^{-1}$)	R^2
0.023	11.40	0.693	0.052	2.94	0.999

(mg/g dry bead), and k_1 the rate constant of pseudo first-order sorption (l/min). Given the boundary conditions for t=0, q=0, equation (1) can be integrated to give [18]:

$$\log(q_e-q) = \log q_e - (k_1/2.303)t \quad (2)$$

If the sorption process can be described by the pseudo first-order equation, there should be a good linear relationship between $\log(q_e-q)$ and t.

It was found that Sb sorption to *Synechocystis* sp. did not follow the pseudo first-order equation (R^2=0.693, Table 1). The sorption amount at equilibrium (q_e) calculated from this equation was 11.40 $mg \cdot g^{-1}$, which was far higher than the experimental value (about 2.9 $mg \cdot g^{-1}$).

If the rate of sorption is a second order mechanism, the pseudo second-order chemisorption kinetic rate equation (3) can be used to describe the sorption kinetics [18].

$$dq/dt = k_2(q_e-q)^2 \quad (3)$$

...where k_2 is the pseudo second-order rate constant (g/mg min).

After integration and applying boundary conditions for t=0, q=0, equation (3) becomes

$$t/q = 1/(k_2 q_e^2) + t/q_e \quad (4)$$

The rate constant k_2 can be obtained from the intercept of the linearized pseudo second-order rate equation.

The sorption data was satisfactorily (R^2=0.999) represented by the pseudo second-order equation (Fig. 2). The value of qe derived from this equation was 2.94 $mg \cdot g^{-1}$ (Table 1), being close to the experimental value (about 2.9 $mg \cdot g^{-1}$).

Intraparticle Diffusion

External diffusion, intraparticle diffusion, and actual adsorption are the three stages for the adsorption process on a porous adsorbent [19]. Intraparticle diffusion will be the rate-limiting step in many cases and can be determined by using the following equation [20].

$$k_p = q_t/t^{1/2} \quad (5)$$

...where q_t is the amount of ethyl violet adsorbed at time t, ($mg \cdot g^{-1}$), k_p the intraparticle rate constant ($mg \cdot g^{-1}$ $min^{1/2}$).

If the adsorption is limited by the intraparticle diffusion process, a plot of q_t vs $t^{1/2}$ should give a straight line. Fig. 3 showed plots of of q_t vs $t^{1/2}$. The poor linearization of the data is observed over the entire sampling period, suggesting that several processes were involved in biosorption and both boundary diffusion and intraparticle diffusion occur. From the final linear step of the plot, the intraparticle diffusion coefficient (k_p) was obtained to be 0.177 ($mg \cdot g^{-1}$ $min^{1/2}$).

Isothermal Biosorption

The equilibrium of the process was described by Langmuir and Freundlich isotherms, respectively. The Langmuir Isotherm Model is a theoretical model for monolayer adsorption:

$$q = q_{max} b C_e/(1+bC_e) \quad (6)$$

...where q is the amount of metal adsorbed, mg/g (dry mass); q_{max} is the maximum metal uptake value corresponding to site saturation, mg/g (dry mass); C_e is the equi-

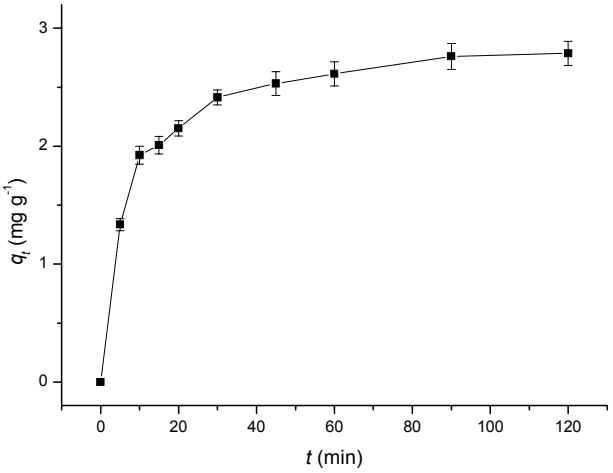

Fig. 1. The amount of Sb adsorbed by *Synechocystis* sp. with sorption time. The initial Sb concentration was 25.0 $mg \cdot L^{-1}$. Each data point represents the mean value ±S.E. of two replicates.

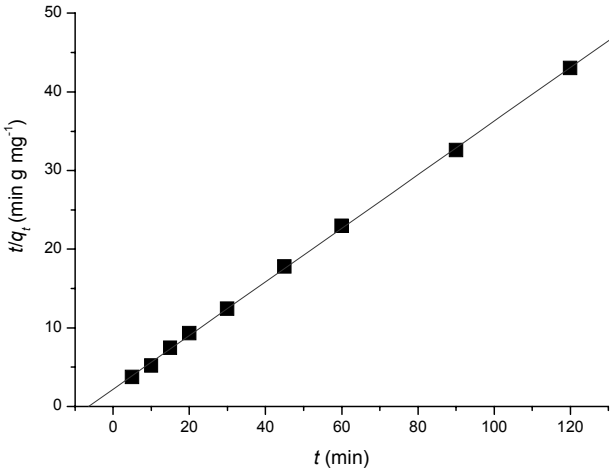

Fig. 2. The pseudo second-order fitting of the experimental data of adsorption of Sb by *Synechocystis* sp. with the initial Sb concentration of 25 $mg \cdot L^{-1}$.

Table 2. Constants of isothermal equation of Sb sorption by *Synechocystis* sp.

Langmuir Isotherm			Freundlich Isotherm		
q_m (mg·g^{-1})	b (L·mg^{-1})	R^2	K	$1/n$	R^2
4.68	0.235	0.990	1.004	0.407	0.971

librium metal concentration in solution, mg·L^{-1}; and b is the ratio of adsorption/desorption rates.

It was found that the isothermal sorption data was well fitted with the Langmuir equation (R^2=0.990) (Fig. 4a). The calculated sorption capacity (q_{max}) was 4.68 mg·g^{-1} (Table 2), indicating that *Synechocystis* sp. cells have strong sorption capacity for Sb.

The Freundlich Isotherm Model is an experimental model and it is usually expressed as follows:

$$q=kC_e^{1/n} \quad (7)$$

...where k and n are rate constants, other symbols are the same as previously described.

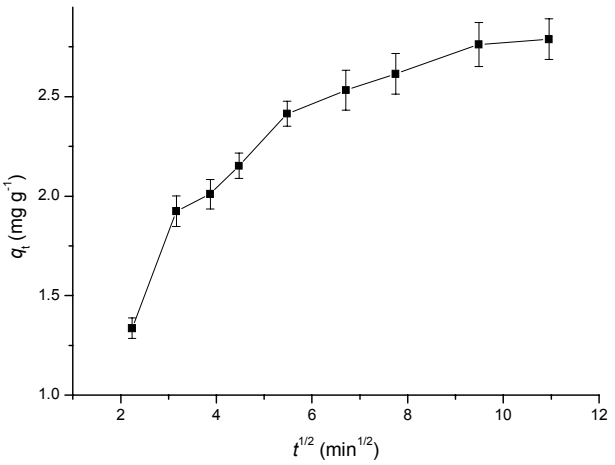

Fig. 3. q_t-$t^{1/2}$ plot of Sb sorption by *Synechocystis* sp. with an initial Sb concentration of 25 mg·L^{-1}. Each data point represents the mean value ±S.E. of two replicates.

The sorption data was well described (R^2=0.971) by the Freundlich equation (Fig. 4b). The values of K and $1/n$ were 1.004 and 0.407, respectively. The small value of $1/n$ shows that Sb can be easily adsorbed by cyanobacterial cells.

Generally, the sorption data can be better fitted with the Langmuir equation than the Freundlich equation. This means that there are several mechanisms underlying biosorption, but sorption by binding to binding sites might be dominant.

Distribution of Sb

Within the range of initial Sb concentrations of 5-100 mg·L^{-1}, 32.4-48.5% of Sb was adsorbed by EPS, 14.7-25.8% adsorbed to the cell wall, and 36.7-48.7% located intracellularly (Fig. 5). At an initial Sb concentration of 100 mg·L^{-1}, the amount of Sb adsorbed by EPS, cell wall, and cellular plasma was 1.92, 0.84, and 2.62 mg·g^{-1}. The large amounts of Sb adsorbed by EPS indicate that the higher sorption capacity for Sb might act as a protective barrier against the toxicity of Sb. On the other hand, EPS can be easily released into water and, therefore, the large amounts of Sb adsorbed by cyanobacterial cells may be shed into water with EPS again, which may ultimately reduce the degree of biosorption. The cell wall is mainly composed of polysaccharides that have a sorption capacity for Sb. Bonotto et al. [21] reported that Sb was mainly distributed intracellularly and only small proportions of Sb were immobilized on the cell wall and cellular organelles. The difference in Sb distribution between their study and the present study might be explained in that they did not consider the contribution of EPS of the microorganism.

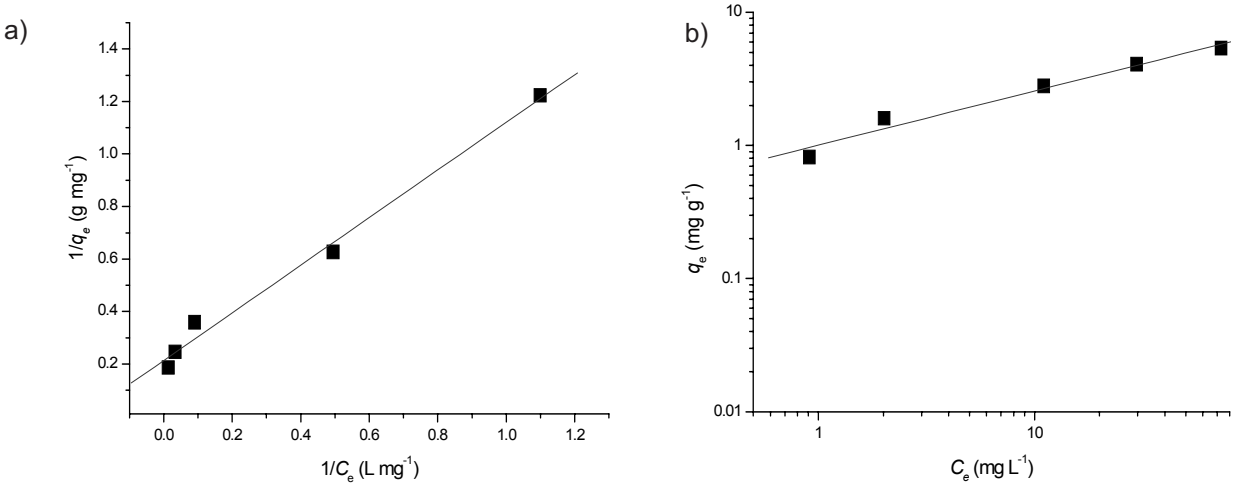

Fig. 4. Biosorption isotherm over a concentration range of 5-100 mg·L^{-1} (a) Langmuir adsorption isotherm and (b) Freundlich adsorption isotherm.

Table 3. Main functional groups observed from FTIR-spectra.

Wave number (cm^{-1})	Vibration type	Functional groups	References
3,750-3,000	Stretching vibration of OH	-OH of polysaccharides and proteins	[22, 23]
1,680-1,630	Stretching vibration of C=O and C-N	Amide I (protein peptidic bond)	[23]
1,384	Stretching vibration of C-O	Polysaccharides	[24]
1,150-1,030	C-O-C stretching	Polysaccharides	[22]
<1,000	"Fingerprint" zone	Phosphate or sulphur functional groups	[22]

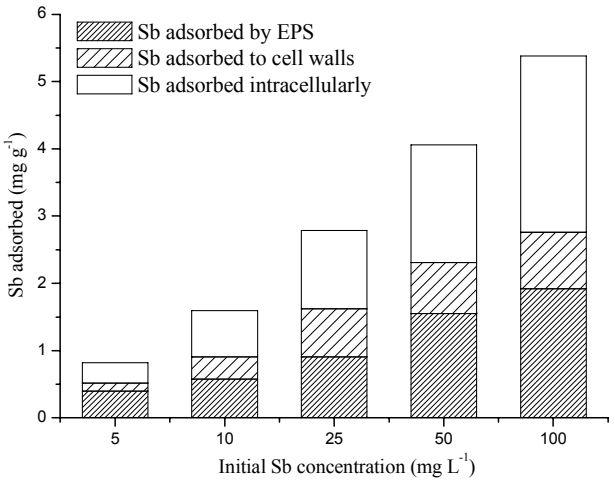

Fig. 5. Distribution of Sb in EPS, on cell wall, and within the cell of *Synechocystis* sp.

The amount of EPS the microorganism produced may significantly affect the distribution of Sb.

FTIR Analysis

Fig. 6 shows the FTIR spectra of cells with or without EPS in the absence and presence of Sb. The main functional groups observed from FTIR-spectra of cells with and without EPS before and after interaction with Sb are shown in Table 3. Broad adsorption bands at 3700-3000 cm^{-1}, representing –OH groups of the glucose and the –NH stretching of the protein and chitisan-chitosan, the weak adsorption band at 2,924 cm^{-1} of the –CH stretching, and a strong band of protein peptidic bond of Amide I group (1,680-1,630 cm^{-1}), were identified for cells with EPS or without EPS (Fig. 6a, c). Both the cells with EPS and the cells after removal of EPS had the "fingerprint zone" (<1000 cm^{-1}), indicating the possible presence of nucleic acids in EPS. On the contrary, the cells without EPS had a stronger band of C-O-C (1,150-1,030 cm^{-1}) stretching due to carbohydrate than the cells with EPS. The stronger band of carbohydrate of cells without EPS might be due to the exposure of cell walls after removal of EPS.

The addition of 100 mg L^{-1} Sb markedly changed the shape of FTIR spectra of the cells with EPS (Fig. 6a and b). The -OH groups of polysaccharides and protein (at 3,750-3,000 cm^{-1}) and protein functional groups (at 1,680-1,630 cm^{-1}), the band of C-O stretching, and the "fingerprint zone" largely disappeared after the addition of Sb, thereby indicating strong interaction of Sb with the functional groups of polysaccharides and protein. After removal of EPS from cells, the band of C-O (at 1,384 cm^{-1}) almost disappeared while the other bands were not significantly altered (Fig. 6c and d). These results indicate that polysaccharides on the cell walls were the dominant groups responsible for binding Sb.

Conclusions

Cells of *Synechocystis* sp. have a strong biosorption capacity for Sb. The isothermal sorption data can be described by the Langmuir Isotherm and the Freundlich Isotherm. Several mechanisms were involved with biosorption of Sb to *Synechocystis* sp. cells, and sorption to binding sites might be dominant. The sorption kinetics followed the pseudo second-order model. The adsorbed Sb is mainly located in EPS and within the cell, and a small proportion was adsorbed on the cell wall. The proteins and polysaccharides in EPS and the polysaccharides on the cell wall are the main functional groups that are responsible for adsorption of Sb to *Synechocystis* sp. cells.

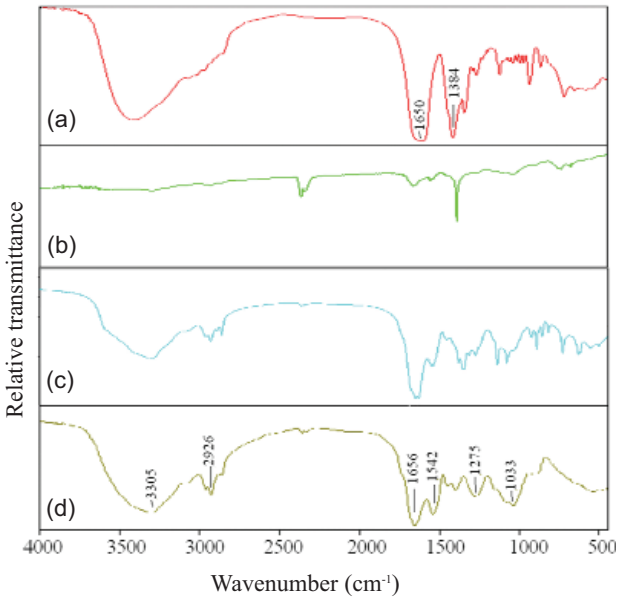

Fig. 6. FTIR spectra of *Synechocystis* sp. cells. (a) cells without the addition of Sb, (b) cells after the addition of Sb, (c) cells without EPS, (d) cells without EPS after the addition of 100 mg·L^{-1} Sb.

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