Characteristic features of *Bacillus cereus* cell surfaces with biosorption of Pb(II) ions by AFM and FT-IR

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Abstract

In order to elucidate the potential mechanisms involved in the biosorption of metal ions, atomic force microscopy (AFM) and Fourier transform infrared (FT-IR) spectroscopy were used to characterize the interaction between Pb²⁺ and *Bacillus cereus*. AFM imaging of the biomass surfaces exposed to different concentrations of lead ions solution showed a major morphological change occurred after Pb²⁺ biosorption. The FT-IR spectra indicated the binding characteristics of the lead ions involved the carboxyl, hydroxyl and amino groups in the biomass. Equilibrium biosorption experiments of Pb²⁺ were carried out to investigate the effects of pH values and the initial metal concentrations. The experimental isotherm data were then modeled using Langmuir, Freundlich, and Redlich–Peterson isotherm equations. As a result, the Redlich–Peterson model yielded the best fit of experimental data. Kinetics experiments showed the biosorption was a rapid process and the pseudo-second-order model was successfully applied to predict the rate constant of biosorption.

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1. Introduction

Bacteria are ubiquitous in a wide range of geologic environments, from surface waters, groundwater aquifers and soil systems, to mid-ocean hydrothermal vents, deep sedimentary basins and geothermal hot springs [1–6]. In the water-rock systems, bacteria can conduct adsorption reaction due to their extremely high surface area to volume ratio and the presence of specific cell wall functional groups [4–8]. Heavy metal pollution in waters has been the most pervasive environmental issues nowadays. Natural waters can easily be polluted by metal ions as a result of their release by industrial plants or mining activities [9–12]. The interactions between microorganisms and metals in water have attracted a significant attention in recent years [13–21]. In order to explain the biological phenomenon, researchers have applied different kinds of chemical and physical ways to investigate the interactions between the biomass and the heavy metals, such as potentiometric and conductomet-

ric titration [22,24,26,27], Fourier transform infrared (FT-IR) [22,24–30], X-ray photoelectron spectroscopy (XPS) [28,29], transmission electron microscopy (TEM) [23,29], electron dispersive spectroscopy (EDS) [29], environmental scanning electron microscopy (ESEM) [30] and extended X-ray absorption fine structure (EXAFs) [30], etc. However, a better understanding of the relationship between the structures and their functionality still remains a challenge. Generally, quantitative and qualitative information on physical properties can be obtained by electron microscopy techniques, X-ray photoelectron spectroscopy, infrared spectroscopy and contact angle measurements [31]. For most of these methods, we must preprocess the biomass cell specimen prior to examination (staining and drying), which may seriously compromise the validity of the analysis. Thus, a powerful nondestructive tool has been a need to investigate microbial surfaces at high resolution.

During recent years, atomic force microscopy (AFM) has been increasingly used in the structural studies of biological systems [32–35]. For example, due to the high sensitivity of the AFM probes and the precise positional capability of the instrument, AFM offers the possibility of obtaining highly resolved spatial measurements, thus enables quantitative measurements

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of the sample surface in real space at extremely high resolution [35]. It can provide three-dimensional images of the surface ultrastructure with molecular resolution, in real time, with minimal sample preparation and experiments can be executed under physiological conditions. Mild treatment of biological samples minimizes artifacts in images or force measurements [31,35–37]. More importantly, imaging and force measurements can be carried out in buffer solutions representative of natural biological environments [35], thus providing new insights into the structure–functional relationship of microbial surfaces. The applications of AFM have been rapidly proliferated and well documented during the last 10 years, which have made inroads into almost all branches of studies on surface and interfacial processes.

Understanding the function of microbial cell surfaces requires the knowledge of their structural and physical properties. In the present study, we applied AFM and FT-IR techniques to characterize the *Bacillus cereus* biomass surfaces for the study of their heavy metal adsorption mechanisms. The morphological features of the microbial surfaces in relation to the impacts by heavy metal Pb^{2+} and characteristics in different pretreatment processes were investigated, combining with the results of FT-IR spectra analysis to offer reasonable explanation for the adsorption phenomenon between biomass and heavy metals.

2. Materials and methods

2.1. Bacterial growth and preparation

Fresh natural water was collected from Guanting Reservoir, Hebei province, China. The natural water was diluted with sterilized distilled deionized (DDI) water, and an aliquot (0.1 mL) was spread on nutrient agar and cultivated in an incubator at 30 °C for 3 days. The isolated colonies were identified by the institute of microbiology, Chinese Academy of Sciences. The identified species of *B. cereus* is a gram-positive bacterium.

The biomass *B. cereus* cells were cultured in 600 mL of nutrient broth for 72 h with growth condition (pH 7.2–7.5, temperature 30 °C, stirring speed 160 rpm). Then the bacterial was harvested by centrifugation at 7000 rpm for 30 min, rinsed four to six times in distilled deionized (DDI) water. The biomass was freeze-dried at –50 °C and reduced pressure over 24 h with a freeze dryer (ALPHA 1-2 LD, Germany, CHRIST).

2.2. Biosorption experiments

All biosorption experiments were performed by the batch technique, using a non-complexing reagent of 0.01 M $NaNO_3$ as the background electrolyte to buffer ionic strength (unless otherwise specified) and plastic test tubes as reaction vessels. The biosorption isotherms of *B. cereus* were carried out to determine the equilibrium relationship between the metal sorption by the sorbent and the final metal concentration in the aqueous phase. In the biosorption experiments, an appropriate amount

of *B. cereus* cell stock suspension was added to plastic tubes with a range of lead concentration (from 5 to 200 mg/L) and different pH (3.0–6.0), then the mixture were agitated on a rotary shaker at 150 rpm for 24 h which was more than ample time for equilibrium. After 24 h equilibration, the bacterial suspensions were separated by centrifugation and the supernatant was analyzed for dissolved metal content via flame atomic absorption spectrophotometer (AAS, Hitachi, Z-6100). Blanks without biosorbent were run simultaneously as control.

To determine the contact time required for the biosorption equilibrium experiments, the adsorption dynamics was performed. The initial concentration of lead ions was approximately 50 mg/L, and the desired amount of biomass was added into the beaker containing 1000 mL of lead solution. The suspension was agitated with a magnetic stirrer at room temperature. The pH of the solution was maintained at the desired value of 5.5 by using 0.1 mol/L HNO_3 or 0.1 mol/L $NaOH$. The sorption time was varied between 0.33 and 24 h, 5 mL aliquots sample solution was intermittently removed from the beaker and filtered through a 0.45 μm membrane filter in order to analyze the lead concentration using AAS. Control experiments were conducted without biomass to determine whether precipitation or metal loss to the reaction vessels occurred.

2.3. FT-IR analysis of the *B. cereus* biomass

Infrared spectra of the original and lead-loaded biomass were performed with a Fourier transform infrared (FT-IR) spectrometer (Thermo Nicolet, Nexus 670). A 5 mg of dried bacteria biomass was mixed and ground with 150 mg of KBr (Spectralab) in an agate mortar. The translucent disks were prepared by pressing the ground material with the aid of 8-tonnes pressure bench press. The tablet was immediately analyzed with a spectrophotometer in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} . The influence of atmospheric water and CO_2 were always subtracted.

2.4. AFM imaging of the *B. cereus* biomass

AFM imaging and measurements were performed using a NanoScope IIIa Multimode Scanning Probe Microscopy and Nanoprobe cantilevers with silicon nitride tips (Digital Instruments, Santa Barbara, CA). The AFM images were obtained by using the retrace signal in the contact mode with both deflection (or error signal) and height signal simultaneously recorded. The standard V-shaped silicon nitride (Si_3N_4) cantilevers (with integral tips, Model OTR8-35) of different stiffness and tip sharpness were used for imaging. The scanning speed and the loop gain factors were varied during the imaging process. Line scan rates were typically set at about 2.5 Hz with other scan parameters such as Scan size, Set point, Integral gain, Proportional gain etc. varied and optimized. In order to avoid tip-related artifacts, imaging was performed with a minimal force and image features were reproduced before being accepted as representative. The prepared samples were investigated by AFM within a few hours after preparation. For each experimental condition, two mica sheets were prepared and at least three images were obtained

Fig. 1. Application of the three equilibrium adsorption models: Langmuir, Freundlich and Redlich–Peterson to the Pb^{2+} biosorption at different pH (3.0–6.0) and biomass = 1.0 g/L.

for each mica sheet sample. Images were taken with online filtering and subsequently by flattening to remove the background slope.

Freshly cleaved mica surface was used as substrate through all the experiments. Before use the mica pieces were rinsed with distilled water to remove the small residual particulates, followed by a rinse with 95% ethanol and absolute alcohol. The mica sheets were allowed to air-dry in a clean culture dish under ambient conditions.

For the AFM imaging of biomass, the drop deposition method was used for the sample preparation and 20 μL of biomass suspension was employed. In this study, the surface morphology of *B. cereus* cells was investigated with biosorption of heavy metals in variety of metal ion solutions (metal concentration 0, 10, 20 and 50 mg/L; pH 5.5; ionic strength 0.01 mol/L). After biosorption experiments, the residual lead ions were removed by centrifugation and the bacteria adsorbent was rinsed two times with ultra pure water at the same ionic strength and pH, and finally re-suspended in 50 mL of ultra pure water for the AFM sample preparation. For comparison, the raw bacteria were also immersed into 0.001 mol/L EDTA or 0.1 mol/L HNO_3 for the removal of the background impurity bound on the cell surface to release the occupied acidic or binding sites. Bacteria under the aforementioned conditions were taken for the AFM observation.

3. Results and discussion

3.1. Biosorption isotherm

Experimental isotherm points for Pb^{2+} ions sorption on *B. cereus* biomass at different initial pH (3.0–6.0) are plotted in Fig. 1. The analysis of the isotherm data is important to develop an equation which accurately represents the results and could be used for design purpose. In this study, three equilibrium models most commonly used in the literature were selected to describe the biosorption data, namely the Langmuir, the Freundlich and the Redlich–Peterson isotherm equations [38–45].

The Langmuir isotherm is often used to describe sorption of a solute from a liquid solution and it can be represented by the equation:

$$q_e = \frac{Q_{\max} b C_e}{1 + b C_e} \quad (1)$$

where b is the Langmuir parameter (affinity constant of metal-active site species) (L/mg), $Q_{\max}$ the maximum adsorption capacity (mg/g), C_e the equilibrium concentration of heavy metal in the solution (mg/L) and q_e is the equilibrium heavy metal concentration on the biosorbent (mg/g).

The Freundlich isotherm is an empirical equation and it assumes that adsorption occurs on heterogeneous surface of

Table 1

Parameters calculated from the three equilibrium models: Langmuir, Freundlich, and Redlich–Peterson for the biosorption of Pb^{2+} at different pH (3.0–6.0)

Models	pH			
	3.0	4.0	5.0	6.0
Langmuir				
b	0.0205 ± 0.0061	0.0373 ± 0.0104	0.0582 ± 0.0124	0.122 ± 0.029
$Q_{\max}$	47.7 ± 6.7	51.2 ± 5.6	54.8 ± 3.6	72.0 ± 4.9
R^2	0.940	0.931	0.960	0.942
Freundlich				
K	2.69 ± 1.08	6.13 ± 2.16	8.91 ± 1.60	19.1 ± 4.5
n	1.84 ± 0.33	2.42 ± 0.52	2.70 ± 0.33	3.51 ± 0.81
R^2	0.851	0.789	0.927	0.756
Redlich–Peterson				
A	0.782 ± 0.135	1.15 ± 0.14	4.45 ± 1.67	6.34 ± 1.26
B	0.00190 ± 0.00354	0.000920 ± 0.001200	0.150 ± 0.128	0.0409 ± 0.0269
n	1.44 ± 0.35	1.66 ± 0.24	0.874 ± 0.104	1.17 ± 0.11
R^2	0.970	0.978	0.965	0.959

solid and in multilayer sorption manner. It is expressed by the following equation:

$$q_e = KC_e^{1/n} \quad (2)$$

where n is the Freundlich constant related to the energy of adsorption and K is a constant indicative of adsorption capacity.

The Redlich–Peterson isotherm contains three parameters and combines the features of the Langmuir and the Freundlich isotherms. It can be described in the following form:

$$q_e = \frac{AC_e}{1 + BC_e^\beta} \quad (3)$$

where A and B are the constant of the Redlich–Peterson isotherm (L/mg) and β is the exponent in Redlich–Peterson isotherm ($0 < \beta < 1$).

The non-linear regression analysis was carried out with the use of software OriginPro 7.0. Table 1 presents the isotherm parameters calculated for biosorption of Pb^{2+} on *B. cereus* biomass. The higher adsorption of Pb^{2+} on the biomass is obvious with an increase of pH. The fitted results indicate that the Redlich–Peterson model provided the highest R^2 regression coefficient. The biosorption reaction thus can be well approximated by the Redlich–Peterson isotherm. The adsorption data with respect to simulation curves are given in Fig. 1.

3.2. Kinetic analysis

Kinetics of biosorption is one of the most important characteristics to describe the solute sorption rate which in turn controls the residence time of biomass sorption at the solid–solution interface. Based on sorption capacity, Ho et al. [12,46–48] presented a pseudo-second-order sorption rate expression to show how the rate depends on the sorption capacity on solid phase but not the sorbate concentration. We here applied the pseudo-second-order rate law for the experimental data to predict the biosorption kinetics. The kinetic rate equation can be written as follows:

$$\frac{dq_t}{dt} = k(q_c - q_t)^2 \quad (4)$$

Integrating this for the boundary conditions $t=0$ to $t=t$ and $q_t=0$ to $q_t=q_t$, gives:

$$\frac{1}{q_c - q_t} = \frac{1}{q_c} + kt \quad (5)$$

Eq. (5) can be rearranged to obtain a linear form as follows:

$$\frac{t}{q_t} = \frac{1}{kq_c^2} + \frac{1}{q_c}t \quad (6)$$

where q_c is the amount of lead ions sorbed at equilibrium (mg/g), t the reaction time (min), q_t the amount of lead ions sorbed on the surface of the sorbent at any time t (mg/g) and k is the rate constant of pseudo-second-order sorption (g/mg min).

Using the linear form equation, Eq. (6), plotting t/q_t versus t will give a straight line (see Fig. 2, inset). The values of q_c and k can be determined from the slope and intercept of the plot, respectively.

Fig. 2 shows that cells of *B. cereus* enabled rapid adsorption of lead ions under the experimental conditions, the adsorption

Fig. 2. Model description and the actual experimental data for Pb^{2+} biosorption kinetics by *B. cereus* biomass.

rate reached to ca. 80% within 1 h. A plot of Eq. (6) for the biosorption of lead ions is also shown in Fig. 2. The results demonstrate a highly significant linear relationship for the lead sorption. The correlation coefficient ($R^2 = 0.999$) (Fig. 2) shows that experimental data are in good compliance with the pseudo-second-order rate law based on sorption capacity.

3.3. IR analysis

To determine which functional groups may contribute to the Pb^{2+} adsorption on the *B. cereus* biomass surface, the FT-IR study was carried out. As shown in Fig. 3, the FT-IR spectra of raw and metal-loaded *B. cereus* samples display a number of absorption peaks, indicating the complex nature of the biomass examined. The spectrum of raw biomass exhibits a broad absorption band between 3500 and 3200 cm^{-1} due to bonded OH stretching vibration. The peak at 2932.9 cm^{-1} is the indicator of alkyl chains CH stretching vibration. The C=O of the carboxylic groups or esters groups stretching vibration, appears at 1726.7 cm^{-1} . The typical amide I band, C=O stretching vibration, appears strongly at 1654.7 cm^{-1} . The peak at 1541.1 cm^{-1} , as known as the amide II, is contributed to a motion combining both the NH bending and the CN stretching vibration of the group C(=O)-NH- in its trans-form, and this peak usually appears as a shoulder with a moderate to strong intensity on C=O stretching vibration absorption. The absorbance peaks at 1455.6 cm^{-1} , are due to N-H bending (*cis* form), CH_2 scissoring or CH_3 antisymmetrical bending vibration and O-H deformation. The other typical amide band (amide III) located

Fig. 3. FT-IR spectra of the *B. cereus* biomass with different amounts of Pb^{2+} ions biosorption.

in 1380.8 cm^{-1} is identified, and in general, the C(=O)O^- symmetric stretching vibration in carboxylate is overlapped in this wavenumber. The C(=O)-O^- stretching vibration coupled to the OH in-plane deformation, C-N stretching vibration, all exhibit the moderate band at about 1280.08 cm^{-1} . The peaks at 1184.6 and 1056.8 cm^{-1} may be attributed to C-N stretching vibration

Fig. 4. AFM images of *B. cereus* cells at mica surface and the changes of cell surface structure under different media conditions: (a) and (b) newly cultivated bacteria cells with different batches; (c) after treated in 0.1 mol/L HNO_3 for 1 h; (d) after treated in 0.001 mol/L EDTA for overnight.

Fig. 5. AFM images of *B. cereus* cells under the exposure to different amounts of Pb^{2+} ions solutions (ion strength 0.01 mol/L; pH 5.5): (A) control blank of *B. cereus*; (B) 10 mg/L Pb^{2+} ion-exposed cells; (C) 20 mg/L Pb^{2+} ion-exposed cells; (D) 50 mg/L Pb^{2+} ion-exposed cells.

of amine groups, P–O–C links of the organic phosphated groups and P–O vibration of the ($\text{C}-\text{PO}_3^{-2}$) moiety [22,24–30,49–51].

After the contact with lead solution, a significant shift of adsorption peaks in the FT-IR spectra can be seen for the lead-laden biomass samples (Fig. 3). The peaks around 1455.6 and 1280.08 cm^{-1} gradually disappeared with the increase of Pb^{2+} ions concentration in the solution, while the peak around 1380.8 cm^{-1} became higher. Changes in the spectra are attributed to the interaction of Pb^{2+} with the carboxyl, hydroxyl and amino groups present on the surface of the biosorbent. The shift of peak at 1455.6 cm^{-1} transposing to lower frequencies up to disappearance is due to the complexation of amino and hydroxyl groups with Pb^{2+} . Another shift was observed at 1280.8 cm^{-1} to higher frequencies, corresponding to the complexation of oxygen from the carbonyl C–O bond. These band changes result in the absorbance band at 1380.8 cm^{-1} became stronger.

3.4. AFM analysis

Environmental microbes are of great concern in the field of water and wastewater treatment. Since they usually have a well-defined shape, and their wall surfaces constitute the frontier between the cells and their external environment, microbial cells play very important roles in many interfacial processes. On the other hand, the morphology of microbial cells and their

surface structures are prone to changes or even structural damage when exposed to adverse conditions. Microbial cells in the natural environments may also be subject to unfavorable changes in their surface structures with the more hazardous and toxic pollutants entering into the environment, causing the deficiency of their functions. Therefore, the knowledge of the structure and properties of microbial surfaces at higher resolution, preferably in the nanometer range, is of major importance to understand their functions and impacts in the natural environment and to efficiently exploit their potential in applied research.

The surface morphology of *B. cereus* cells under various conditions in relation to the biosorption process of heavy metals was first provided as shown in Fig. 4. It can be seen that newly cultivated *B. cereus* cells may have some slight variety in cell dimensions for different bacterial colonies (Fig. 4a and b). To compare with the cell morphology without any treatment, *B. cereus* cells treated in 0.1 mol/L HNO_3 for 1 h (Fig. 4c) or 0.001 mol/L EDTA for overnight (Fig. 4d) become more rough in their cell surface, but still preserve the integrity of their cell structure such as the shape and size, implying that the treatment with 0.1 mol/L HNO_3 or 0.001 mol/L EDTA does not cause any major damage or variety in the cell structure and probably can release only the acidic or binding sites occupied by some metal ions and the background impurity on the cell surface. However, the morphological changes of Pb^{2+} ion-exposed *B. cereus* cells

in the biosorption processes, including the size, shape and adhesive properties of the bacteria are obvious (Fig. 5). In order to further learn the changes of biomass, these cells (Fig. 5A and D) were selected to carry out the section analysis. While *B. cereus* bacteria without Pb^{2+} exposure in the control blank are rod-like in shape with a smooth surface (the dimension of these cells is about $3.876\ \mu\text{m}$ long and $1.236\ \mu\text{m}$ wide, on average, as shown in Fig. 5A), and usually found to form a larger rod-like ultra-structure with internal cells connected from end to end, they become more roughened in the surface with the cell dimension changed (the length of the bacteria cells is about $3.605\ \mu\text{m}$, the width in the section profile is $2.147\ \mu\text{m}$, respectively, on average, as shown in Fig. 5D) after the Pb^{2+} exposure and the ultra-structures mostly disconnected with the cells adhering to each other randomly. It can be seen clearly that the biomass shape has changed into a spindle-like structure after Pb^{2+} biosorption. The morphological changes of the sample can be attributed to the interactions between heavy metal and the surface of *B. cereus* cells. These agree with the results of FT-IR spectra analysis.

4. Conclusions

The AFM imaging of *B. cereus* biomass surfaces after Pb^{2+} biosorption indicates a major morphological difference on mica, the assembly structures changing from rod-like to spindle-like. The FT-IR analysis describes the chelating characteristics of lead ions coordination to the functional groups on the *B. cereus* cell surface, and the functional groups involved may include carboxyl, hydroxyl and amino groups. Kinetic studies demonstrate that the biosorption of Pb^{2+} by *B. cereus* biomass is a rapid process and follows the pseudo-second-order rate law. The adsorption isotherms under different pH conditions show that a higher pH is in favor of lead ions biosorption by the biomass, and the data are better fitted in the Langmuir and Redlich–Peterson models based on the non-linear χ^2 (R^2) values than the Freundlich model.

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