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Short Communication

Organoarsenic resistance and bioremoval of *Acidithiobacillus ferrooxidans*

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ABSTRACT

The tolerance and bioremoval of dimethylarsinic acid (DMA^V) by *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) were investigated here. The inhibitory concentration (IC) of DMA^V was determined for *A. ferrooxidans*. The effects of various parameters such as pH, contact time, initial DMA^V concentration, biosorbent dose and temperature were systematically examined to study the biosorption processes. Results indicated that Langmuir model fitted better than Freundlich model to the equilibrium data. Analysis of kinetic data showed that the biosorption processes of DMA^V involved pseudo-second-order kinetics. Thermodynamic analysis showed that the biosorption of DMA^V onto *A. ferrooxidans* was feasible, spontaneous, endothermic and chemisorptive under examined conditions. Fourier transform infrared spectroscopy (FTIR) showed the involvement of –OH, –NH and $-SO_3$ groups in the biosorption process.

1. Introduction

Arsenic is a toxic element that is ubiquitous in nature as a result of natural geologic processes and anthropogenic activities (Vaclavikova et al., 2008). Long-term exposure to arsenic can cause skin problems, circulatory problems, degenerative diseases and even cancer (Boddu et al., 2008). Therefore, the WHO and the EU adopted a new arsenic standard for drinking water at 10 μg/L (Vaclavikova et al., 2008).

DMA^V, the dominant species among the most common organoarsenic species in nature, is the predominant metabolite in urine of mammals exposed to inorganic arsenic (Thirunavukkarasu et al., 2002). Some studies (Thirunavukkarasu et al., 2002) showed that a number of lakes, estuaries and wells were found to be contaminated with DMA^V. Applications of DMA^V have included use in pesticides, herbicides, additives and therapeutic agents. Many studies (Vaclavikova et al., 2008) suggested that DMA^V was carcinogenic, genotoxic, and more toxic than originally believed.

Therefore, removal of DMA^V is of concern in addition to other arsenic species. Several studies (Thirunavukkarasu et al., 2002) have demonstrated that DMA^V removal can be achieved by various adsorbents. However, the existing technologies are often expensive, environment unfriendly and usually dependent on the concentration of the waste. These disadvantages have limited their further developments. Recently, research attention has been focused on biosorption (or bioadsorption) methods for the treatment of contaminated water (Vijayaraghavan and Yun, 2008). Although, information on the biosorption of inorganic arsenic is widely available (Vijayaraghavan and Yun, 2008), very little information is available for the biosorption of organoarsenic using biosorbent material. Currently, the biosorption process using microbial biomass can be of great interest, because of the marked tolerance towards metals and other adverse conditions such as low pH of these biological materials (Zafar et al., 2007). Among the Gram-negative bacteria, *Acidithiobacillus ferrooxidans* offers interesting characteristics due to its great use in biohydrometallurgical processes (Baillet et al., 1997). It has been reported that *A. ferrooxidans* shows particular tolerance to several heavy metals (Leduc et al., 1997). Some work has suggested that this bacterium is tolerant to arsenic and the arsenic resistance genes had been found in the chromosome of *A. ferrooxidans* (Yan et al., 2010). Despite the potential of *A. ferrooxidans* as a biosorbent for various metals has been reported (Baillet et al., 1997), there is a lack of information about the bioremoval of organic arsenic by *A. ferrooxidans*. Besides, no study on the sensitivity and tolerance of *A. ferrooxidans* to organic arsenic has been reported so far.

The aim of this work was to investigate the growth response of *A. ferrooxidans* to DMA^V and the biosorption potential of this bacterium for removing DMA^V from aqueous solution.

2. Methods

2.1. Microorganism and growth conditions

A. ferrooxidans BY-3 (CCTCC-M203071), which was isolated from the acidic mine drainage at an copper mine in Baiyin of Gansu, China, was cultured in 9 K medium (Devasia et al., 1993). The

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bacterial population in the inoculated solutions was about 1×10^7 cells mL^{-1}.

2.2. Preparation of DMA^V solutions

Stock solutions of Dimethylarsinic acid [$(CH_3)_2AsO(OH)$, DMA^V] (Sigma, USA) were prepared by dissolving solid DMA^V in deionized water to a concentration of 1000 mg/L. The required working standards were prepared daily by diluting the stock solutions and the pH was adjusted with either 0.1 M HCl or 0.1 M NaOH. All other reagents used in this work were of analytical grade.

2.3. DMA^V tolerance

To determine the resistance to DMA^V, *A. ferrooxidans* was cultured in modified media resulting from the 9 K medium supplemented with different concentrations (8–128 mM) of DMA^V, adjusted to pH 2.0. Experiments were carried out at 30 °C on a gyratory shaker for approximately 100 h, during which the growth was monitored. Control experiments were also performed with the same 9 K media without DMA^V. The IC of DMA^V was determined following the procedure reported by Leduc et al. (1997).

2.4. DMA^V bioremoval experiments

2.4.1. Preparation of biosorbent

The biosorbent was prepared by the following method reported by Yan et al. (2010). The cellular quantification was determined by measuring the dry weight of cells, following the procedure reported by Liu et al. (2004).

2.4.2. Batch biosorption procedure

Biosorption were conducted by the following method reported by Yan et al. (2010). Experimental conditions were optimized by investigating pH (2.0–8.0), contact time (10–60 min), initial DMA^V concentration (500–3000 μg/L), biosorbent dose (2–8 g/L), and temperature (20–40 °C). Experiments were conducted at optimum pH of 2.0, contact time of 40 min and biomass dose of 2 g/L. All experiments were carried out in triplicate and the average values with their standard errors were reported in the text.

2.5. Analytical procedures

2.5.1. Growth measurements

A. ferrooxidans growth was monitored by the following method reported by Baillet et al. (1997).

2.5.2. DMA^V determination

The concentration of DMA^V in solution before and after the equilibrium was determined by the use of an inductively coupled plasma atomic emission spectrometer (ICP-AES, IRIS Advantage ER/S, Thermo Jarrell Ash, USA).

The adsorption capacity of the biomass was obtained by a mass balance equation as in Eq. (1) and the removal efficiency of arsenic ion was calculated by Eq. (2)

$$q_e = \left[\frac{C_i - C_e}{m}\right] V \quad (1)$$

$$R = \frac{C_i - C_e}{C_i} \times 100\% \quad (2)$$

where q_e and R are the equilibrium arsenic uptake capacity (μg/g) and removal efficiency (%), respectively, C_i and C_e are the initial and equilibrium arsenic concentrations (μg/L), m is dry net biosorbent weight (g), and V is the working volume of the adsorption sample (mL).

2.5.3. Fourier transform infrared analysis (FTIR)

FTIR spectroscopy (FTIR, NEXUS 670, Thermo Nicolet, USA) was employed to detect vibration frequency changes on the surface of biomass before and after DMA^V biosorption, following the procedure reported by Yan et al. (2010).

3. Results and discussion

3.1. Tolerance to DMA^V

The growth responses of *A. ferrooxidans* at different concentrations of DMA^V were investigated. At 8 and 16 mM DMA^V, no adverse effect on growth was observed between the control and the assay, the lag phase period was similar but the time necessary to oxidize 90% of initial ferrous ions was 36 h and 39 h, respectively. An increase in DMA^V concentration caused the lag phase extended. For a 32 mM DMA^V concentration, growth showed a lag phase (about 30 h) compared with that in the absence of DMA^V and the time necessary to oxidize 90% of initial ferrous ions was largely expanded up to 100 h, which was longer than the one obtained for the control (about 31 h). However, growth was completely inhibited at 64 and 128 mM DMA^V.

According to the DMA^V tolerance experiments, the IC of the DMA^V, defined as the concentration which showed a significant ($p < 0.01$) decrease in the percentage of ferrous iron oxidized when compared to an untreated control, were determined in triplicate for *A. ferrooxidans*. The data revealed that the IC of DMA^V for *A. ferrooxidans* in 9 K was 32 mM.

3.2. Effect of pH on DMA^V biosorption

One of the most important factors affecting biosorption process is the pH of solution. *A. ferrooxidans* biomasses contain amino, amide and carboxyl groups on the cell wall (Yan et al., 2010), which suggests that the biosorption process could be affected by changes in the solution pH (Sari et al., 2009). The effect of pH on the biosorption was studied at pH 2.0–8.0, and the bioremoval efficiency was found to be 90.885%, 87.525%, 72.305% and 61.733% at pH 2.0, 4.0, 6.0 and 8.0. Therefore, all experiments were carried out at pH 2.0. It was observed that bioremoval efficiency increased with decreasing pH within the range of 2.0–8.0. This was attributed to the properties of both biomass and arsenic.

In the experimental pH range of 2.0–8.0, the biomass cell walls were negatively charged and the cells became increasingly negative as the pH increased (Devasia et al., 1993). At pH range 2.0–6.2, the dominant species of DMA^V is uncharged $(CH_3)_2AsO(OH)$ (Schulman and Consulting, 2000) which can be sorbed on the sorbent by interacting with the unprotonated amino groups (Boddu et al., 2008). At higher pH values (6.2–8.0), the bioremoval efficiency dramatically decreased. In the above mentioned pH range, the predominant DMA^V is $(CH_3)_2AsO\ (O)^-$ and the net surface charge on the biosorbent becomes negative. So a repulsive force which results in decreased adsorption may develop between biosorbent and anionic adsorbate (Boddu et al., 2008). The same trend was observed by Rahaman and colleagues in relation to adsorption of iAs^V by Atlantic Cod fish scale (Rahaman et al., 2008).

3.3. Biosorption isotherm studies

Langmuir and Freundlich isotherms were applied to analyze the experimental data. The linear equations (Sari et al., 2009) of models are given in Eqs. (3) and (4), respectively