WATER RESEARCH 41 (2007) 531–542

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Retention of inorganic arsenic by coryneform mutant strains

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ARTICLE INFO

Article history:
Received 12 June 2006
Received in revised form
19 October 2006
Accepted 7 November 2006

Keywords:
Arsenate
Arsenite
Corynebacteria
Mutant strains
Isotherm
Kinetics
Electrothermal atomic absorption spectrometry
Slurry sampling

ABSTRACT

The natural resistance mechanisms of corynebacteria to respond to the environments containing high levels of arsenic were successfully adopted to develop inexpensive and selective extractants for submicrogram amounts of arsenic. Kinetic and equilibrium characteristics were evaluated, and a preliminary exploration of the capability of these strains to be used for arsenic speciation was also made in this work. Three kinetics models were used to fit the experimental data. It was found that the pseudo-first-order kinetics model was not quite adequate to describe the retention process, while the intraparticle diffusion and the pseudo-second-order kinetics models provide the best fits. The equilibrium isotherm showed that the retention of arsenic was consistent with the Langmuir equation and that the Freundlich and Dubinin–Radushkevich models provided poorer fits to the experimental data. The maximum effective retention capacity for arsenic was about 15.4 ng As/mg biomass. The amount of arsenic retained was directly measured in the biomass by forward planning a slurry electrothermal atomic absorption spectrometric procedure.

1. Introduction

Heavy metal contamination of natural water is a major problem in industrialised areas, as they can cause a detrimental effect on environment and finally on human health. Arsenic is categorised as a heavy metal and comes into the environment as a result of the contribution of several natural sources, but mainly from human intervention, usually through mining, industrial, agricultural, medicinal, and like other activities (Langdon et al., 2003). The adverse health effects arsenic can produce in humans are well known (Duker et al., 2003). However, toxicity of arsenic differs with the chemical forms involved, resulting in a great challenge for arsenic speciation. For a better management of contamination of this metal, an adequate monitoring and control of the arsenic species levels are required.

The most advantageous large-scale continuous operations for the separation of metals are liquid–liquid extraction and solid retention (Maity et al., 2005; Daus et al., 2004; Lin and Wu, 2001). More recently, however, many efforts have been addressed on the development of new sorbents, where bacteria-based biosorption has been considered a suitable wastewater technology to remove efficiently heavy metals. In other fields, such as speciation analyses, diverse analytical instrumentation has generally been used (Oliveira et al., 2005; Fattorini et al., 2004; Rosen and Hieftje, 2004; Jain and Ali, 2000), but microorganisms, particularly bacteria and mainly those evolving resistance mechanisms to many toxic metals

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doi:10.1016/j.watres.2006.11.015

Nomenclature

C_0	concentration of arsenic in the initial sample solution ($ng\,ml^{-1}$)
C_e	concentration of arsenic in the sample solution at equilibrium ($ng\,ml^{-1}$)
E	mean free energy of retention ($kJ\,mol^{-1}$)
K_F	retention capacity (Freundlich isotherm constant) ($mg\,g^{-1}$)
K_L	instability equilibrium constant (Langmuir isotherm constant) ($ml\,ng^{-1}$)
K_S	stability equilibrium constant
k_d	Intraparticle diffusion rate ($ng\,ml^{-1}\,h^{-1/2}$)
k_1	the equilibrium rate constant of pseudo-first-order sorption (h^{-1})
k_2	the equilibrium rate constant of pseudo-second-order sorption ($mg\,ng^{-1}\,h^{-1}$)
k	constant related to retention energy ($mol^2\,kJ^{-2}$)
n	intensity of the sorbent
q_e	amount of As(III) retained per mass unit of biomass at equilibrium ($ng\,mg^{-1}$)
q_t	amount of As(III) retained per mass unit of biomass at any time t ($ng\,ml^{-1}$)
q_m	amount of As(III) retained at saturation ($ng\,ml^{-1}$)
R_L	dimensionless equilibrium parameter
R	the ideal gas constant ($kJ\,mol^{-1}\,K^{-1}$)
T	the absolute temperature (K)
t	retention time (h)
$t_{1/2}$	half-retention time (h)
ε	Polanyi potential
v_0	initial retention rate ($ng\,mg^{-1}\,h^{-1}$)

(Silver and Phung, 1996), have also been considered for preconcentration and/or separation of diverse metal species.

The strategies developed by bacteria to circumvent the toxicity of arsenic usually include: (i) decreasing in the uptake of arsenate through the phosphate uptake system (Cervantes et al., 1994), (ii) evolving peroxidation reactions through membrane lipids (Abdrashitova et al., 1986), and (iii) using the best characterized microbial arsenic detoxification pathway, which involves genetic determinants typically organized in the *ars* operon (Silver and Phung, 1996). The *ars* operon is generally constituted of either three (*arsRBC*) or five (*arsRDABC*) genes, organized into a single transcriptional unit. The three-gene system encodes the arsenic transcriptional repressor (*arsR*), an arsenite permease, (*arsB*), a membrane-located arsenite efflux pump, and an arsenate reductase (*arsC*), which converts arsenate to arsenite before arsenic is pumped out of the cell through the ArsB anion pump; the five-genes operon (*arsRDABC*) encodes, besides the above described genes, a negative regulatory protein which provides additional fine tuning (*arsD*) and an arsenic-specific ATPase (*arsA*) (Silver and Phung, 1996).

As a general rule, in bacteria, the interaction of arsenite with the repressor protein (ArsR) undergoes a conformational change of the protein and dissociates from the regulatory sequences of the *ars* operon, leading to the expression of downstream genes. Metal resistance mechanisms are clearly regulated by specific metalloregulatory proteins and the high affinity and specificity of the regulatory protein of the *ars* operon, ArsR, have been used for developing whole-cell bacterial biosensors for arsenic (Scott et al., 1997; Cai and DuBow, 1997; Ramanathan et al., 1997; Tauriainen et al., 1997), as well as for arsenic remediation (Kostal et al., 2004). In these studies, ArsR and other proteins, such as ArsB, have been fused to the bacterial luciferase genes (*lux* operon) (Cai and DuBow, 1997; Ramanathan et al., 1997; Roberto et al., 2002; Tauriainen et al., 1999) or to firefly luciferase (*luc* operon) (Tauriainen et al., 1997). Bioavailability studies of arsenic have also been tested using several bacteria-based biosensors (Tauriainen et al., 1997, 1999; Ji and Silver, 1992; Corbisier et al., 1993; Petänen and Romantschuk, 2002). In general, the best-suited host strains are native bacteria from the environment for which the analysis is though (Petänen and Romantschuk, 2003), although different recombinant plasmids have been used with similar successful results (Petänen et al., 2001).

The saprophytic soil bacterium *Corynebacterium glutamicum* is a Gram positive microorganism with a high resistance to arsenic; this resistance is associated to the presence of two arsenic detoxification operons (*ars1* and *ars2*), which are basically structured following the gene arrangement *arsR1-arsB1-arsC1-arsC1'* for *ars1* operon and *arsR2-arsB2-arsC2* for *ars2* (Ordóñez et al., 2005). The aim of this work was to evaluate the capability of four *C. glutamicum* strains, the wild-type strain (ATCC 13032) and three mutants generated by disruption of the arsenite permease genes (*arsB1*, *arsB2* and both *arsB1-arsB2*) for the retention of As(III) and As(V) species. The biosorption process was followed by forward planning a slurry sampling living electrothermal atomic absorption spectrometry (ETAAS) procedure, which allow us to measure in a direct way the arsenic retained by the living bacterial biomass.

2. Materials and methods

2.1. *Instruments and operating conditions*

A Thermo Jarrel Ash atomic-absorption spectrophotometer (SH 11), equipped with a CTF-188 graphite atomizer and Smith–Hieftje background correction, was used for the measurements. The operating parameters used were as follows: wavelength, 193.7 nm; the arsenic pulsed hollow cathode lamp, Visimax II, was used under the recommended conditions; bandwidth, 1 nm. Standard uncoated rectangular graphite tubes, and standard pyrolytic graphite-coated graphite platforms were used for atomization. A syringe was used to inject manually 10 μl of the solutions and slurries into the graphite atomizer at room temperature. The temperature