Journal of Environmental Biotechnology
Vol. 9, No. 2, 123–129, 2009

Original paper (regular paper)

Characteristics of Arsenic Removal from Aqueous Solution by *Bacillus megaterium* Strain UM-123

Munetoshi Miyatake * and Sachio Hayashi

Department of Applied Chemistry, University of Miyazaki, 1–1 Gakuen Kibanadai Nishi, Miyazaki 889–2192, Japan
TEL: +81–985–58–7316 FAX: +81–985–58–7323
E-mail: t0g205u@cc.miyazaki-u.ac.jp

(Received; 15 July, 2009/Accepted; 5 October, 2009)

Arsenic removal using microorganisms is an interesting alternative to conventional methods. *Bacillus megaterium* strain UM-123, isolated from soil in Miyazaki, has been characterized as a novel arsenic-removing bacterium. Strain UM-123 is capable of removing arsenic from culture medium supplemented with As(III) or As(V). Arsenic of 0.386 mg As/g (dry weight) was removed by strain UM-123 when the strain was grown on medium containing 5.0 mg As/l As(III) for 24 hours. In experiments of arsenic adsorption by dried cells of strain UM-123, As(V) was scarcely adsorbed by cells, while As(III) was adsorbed. The adsorption equilibrium data for As(III) are well-described by the typical Langmuir adsorption isotherm. The maximum adsorption capacity was estimated to be 0.127 mg As/g (dry weight). The ratio of As(III) adsorption by strain UM-123 was 93% or more in arsenic solutions up to 1.0 mg As/l. Arsenic concentrations in solution decreased to 0.01 mg As/l or less with initial concentrations of up to 0.2 mg As/l. These results suggest that strain UM-123 may be utilized for bioremediation of arsenic-contaminated water.

Key words: arsenic removal, arsenic adsorption, bioremediation, *Bacillus megaterium* strain UM-123

1. Introduction

Arsenic is widely distributed in the Earth's crust. It is released as a result of natural phenomena such as volcanic activity or weathering, and human activities such as extraction of fossil fuels and other resources, industrial production, or extensive use of underground water; thus, arsenic is becoming an environmental pollutant[3,14,18]. Arsenic is extremely toxic to humans, and numerous cases of serious health damage caused by arsenic have been reported worldwide in recent years. Environmental pollution by arsenic has therefore become a grave social problem[8,10]. In countries such as India and Bangladesh, where most of the drinking water comes from underground sources, water contaminated with arsenic has seriously affected the health of 57 million people. Removing arsenic from underground water has therefore become essential for ensuring the safety of drinking water, as well as for protecting aquatic environments[9].

Water contaminated with arsenic is generally purified using physico-chemical methods such as coagulation and coprecipitation, ion exchange, or adsorption, but these methods are expensive and produce secondary pollutants[20]. Furthermore, these methods are regarded as problematic as they are unable to sufficiently remove As(III) or low-concentration arsenic. The need for low-cost, environmentally friendly technologies for removing arsenic from water has recently become all the more pressing, and there is now great interest in the use of bioremediation in order to counter contamination by arsenic and other metals, with various studies being carried out in this field[2,15,19]. There is every possibility that microorganisms with the ability to remove environmental arsenic can be used for bioremediation, and there are hopes that this approach could replace or supplement existing physico-chemical methods. Microorganisms that possess mechanisms for insolubilizing and reducing the toxicity of various harmful metals have been reported, and adsorption techniques using microbial biomass are being investigated[13,16].

Adsorption of metals by microorganisms is generally believed to occur because metals carry a positive charge, while the microbe surface carries a negative charge. Arsenic is not usually adsorbed by microorganisms because in neutral aqueous solutions such as underground water, As(V) is present as negatively charged oxide ions ($H_2AsO_4^-$, $HAsO_4^{2-}$) and As(III) is present as a neutral oxide (H_3AsO_3)[3]. For arsenic to be adsorbed by microorganisms, the surface of the microorganisms must be changed by some form of pre-processing, which may be either chemical, such as the use of surfactants, or physical, such as in heat processing[4,6]. With the exception of some marine bacteria and a few genetically modified *E. coli* that have been reported previously, no bacteria have yet been found that can be used for bioremediation of arsenic, and thus the use of microorganisms for arsenic removal has not to date yielded satisfactory

results[5,11,17].

The aim of the present study was to discover a bacterium that can remove arsenic from the environment and that can be used for bioremediation without the need for pre-processing. Particular focus was placed on the effectiveness of the bacterium in removing As(III), which is highly toxic and is generally difficult to remove, and furthermore on sufficient ability to remove arsenic at low concentrations. We found that a *Bacillus megaterium* strain UM-123 isolated from soil in Miyazaki Prefecture was capable of removing arsenic. We carried out a detailed investigation of the characteristics of the arsenic-removing capacity of this strain, confirming the possibility that it could be used for bioremediation.

2. Materials and Methods

2.1. Sample strain

Bacillus megaterium strain UM-123 isolated from soil in Miyazaki Prefecture was used for the experiments. This bacterium was identified in NCIMB Japan Co., Ltd..

2.2. Experiments during bacterial culture

Nutrient broth (Nissui Pharmaceutical Co., Ltd., Japan) was used as medium. Sodium arsenite (Wako Pure Chemical Industries, Ltd., Japan) was used as an arsenic As(III) source, and disodium hydrogenarsenate (Wako Pure Chemical Industries, Ltd., Japan) was used as an arsenic As(V) source. Preculture was prepared using nutrient broth alone at 30°C for 24 hours, with agitation at 100 strokes/min. Experimental cultures were prepared by 100 ml of culture broth inoculating from the preculture at a proportion of 2.0% (v/v) into nutrient broth containing As(III) or As(V) at concentrations of 0.5-100.0 mg As/l, and culturing aerobically in 500-ml shake-flasks at 30°C for 48 hours with agitation at 100 strokes/min. The turbidity of the culture broth was measured at fixed intervals, after which bacterial cells were separated from the culture broth by centrifugation (10,000×g for 15 min), and the respective concentrations of As(III) and As(V) in the broth supernatant were measured. Harvested cells were washed twice with distilled water and then lyophilized, after which the weight was measured. The amount of arsenic removed per gram of the dry weight of cells was calculated based on the decrease in arsenic in the broth supernatant and the weight of the lyophilized cells.

In order to determine the arsenic content of the cytosol, lyophilized bacterial cells obtained from media containing 1.0 mg As/l were suspended in purified water, and suspended cells were disrupted by ultrasonication; cells thus disrupted were separated from the supernatant by centrifugation (10,000×g for 15 min), and the concentrations of As(III) and As(V) in the supernatant were measured. To determine the arsenic content of the cell wall, disrupted cells obtained by ultrasonication were washed and lyophilized, and were subjected to alkaline degradation in a 2.0 mol/l aqueous solution of NaOH at 100°C for 3 hours, after which the arsenic concentrations were measured. To determine the As(III) and As(V) content of the cell wall, arsenic was solubilized from lyophilized cells, which were obtained by ultrasonication, with the cell-wall-lytic enzyme lysozyme from chicken egg white (Wako Pure Chemical Industries, Ltd., Japan). The solution was filtered, and the concentrations of As(III) and As(V) were then measured. The sugar content of the cell wall was determined using the phenol-sulfuric acid method on the solution obtained from alkaline degradation.

Oxidation-reduction reactions of arsenic were carried out in culture broth prepared using nutrient broth alone at 30°C with agitation at 100 strokes/min. The supernatant obtained from centrifuged culture broth as an extracellular solution, and the supernatant obtained following ultrasonication as an intracellular solution were used respectively to determine the extracellular activity and intracellular activity. As(III) or As(V) solutions regulated to pH 7 were added to each supernatant to give final arsenic concentrations of 1.0 mg As/l, and were incubated at 30°C for 2 hours. The concentrations of As(III) and As(V) were then measured.

2.3. Experiments using dried cells

Bacterial cells obtained from a culture in nutrient broth alone at 30°C for 24 hours with agitation at 100 strokes/min were harvested by centrifugation (10,000×g for 15 min) and lyophilized. Lyophilized bacterial cells were used for dried cells.

The effects of pH were investigated by adding 20 g dw/l dried cells to 1.0 mg As/l solutions of As(III) or As(V) regulated at pH 2-12, and the suspensions were stirred at 30°C. At fixed intervals, cells were removed by centrifugation (10,000×g, 15 min) and the concentrations of As(III) and As(V) in the resulting supernatant were measured.

In order to investigate the effects of temperature, 20 g dw/l dried cells were added to 1.0 mg As/l solutions of As(III) regulated at pH 7 at temperatures of 30-60°C, and were stirred for 4 hours. Concentrations of As(III) and As(V) in the supernatant were then measured.

The effects of arsenic concentration were investigated by adding 20 g dw/l dried cells to solutions of As(III) of 0.02-10.0 mg As/l regulated at pH 7, and these suspensions were stirred at 35°C for 4 hours. Concentrations of As(III) and As(V) in the supernatant were then measured. The amount of arsenic adsorption was calculated based on the decrease in arsenic in the supernatant and the weight of dried cells added in solution. The equilibrium concentration of arsenic and the amount of arsenic adsorption thus determined were substituted into the Langmuir isotherm given below to determine the maximum adsorption.

Langmuir isotherm: $C_{eq}/q = 1/q_{max}K + C_{eq}/q_{max}$

where, q is arsenic adsorption (mg As/g dw), C_{eq} is the equilibrium concentration of arsenic (mg As/l), q_{max} is the maximum adsorption (mg As/g dw), and K is the equilibrium constant (l/mg As).

2.4. Analysis

The turbidity of the culture broth was measured using a spectrophotometer UV-210A (Shimadzu Co., Japan) at 600 nm, and the values were used as indicators of the level of bacterial growth. Quantitative analysis of arsenic was carried out using an atomic absorption spectrophotometer AA 6650 with an arsenic speciation pretreatment system ASA-2sp (Shimadzu Co., Japan) in order to determine the concentrations of As(III) and As(V). All experiments were carried out three times, and measurements were performed twice. All of the obtained values were in the range of ±3% from average values. All results are given as average values.

3. Results

3.1. Effects of arsenic on bacterial growth and the behavior of arsenic during culture

Changes over time in the broth turbidity at concentrations of 10.0, 50.0, and 100.0 mg As/l As(III) and As(V), when *Bacillus megaterium* strain UM-123 was cultured in medium containing arsenic, are shown in Fig. 1. In bacterial growth at each arsenic concentration, almost no differences were observed between the logarithmic growth phase of bacteria in culture with either As(III) or As(V) at concentrations of 10.0 mg As/l and culture to which no arsenic had been added, and growth reached the stationary phase in 12 hours. At a concentration of 50.0 mg As/l or more, the rate of bacterial growth was slower than in concentrations of 10.0 mg As/l, and it took 24 hours or more to reach a stationary phase. With As(V), at a concentration of 100.0 mg As/l, bacterial growth stopped after 6 hours, reaching a stationary phase, and turbidity during the stationary phase was approximately 20% of the stationary phase turbidity at concentrations of up to 50.0 mg As/l. These results indicate that As(V) inhibits the growth of strain UM-123 more than As(III).

Changes over time in the arsenic remaining in culture medium at concentrations of 0.5-100.0 mg As/l As(III) and As(V), when strain UM-123 was cultured in medium containing arsenic, are shown in Fig. 2. Fluctuations were observed in the changes in arsenic concentrations in the culture medium over time for each culture up to 10.0 mg As/l As(III) and 5.0 mg As/l As(V). At these concentrations, the ratio of arsenic remaining in the culture medium reached the lowest values at 24 hours of culture time, after which they increased. In the ratios of arsenic remaining in the culture medium at 24 hours, this value was lower for lower concentrations of arsenic added to media. When 0.5 mg As/l was added, the remaining arsenic was 45.3% of the initial concentration for As(III) and 55.2% for As(V); the ratio of As(III) remaining was thus lower than that for As(V) at the same initial concentration. The amount of arsenic removed per gram of dry weight of cells was 0.386 mg As/g dw for As(III) and 0.311 mg As/g dw for As(V), at a concentration of 5.0 mg As/l.

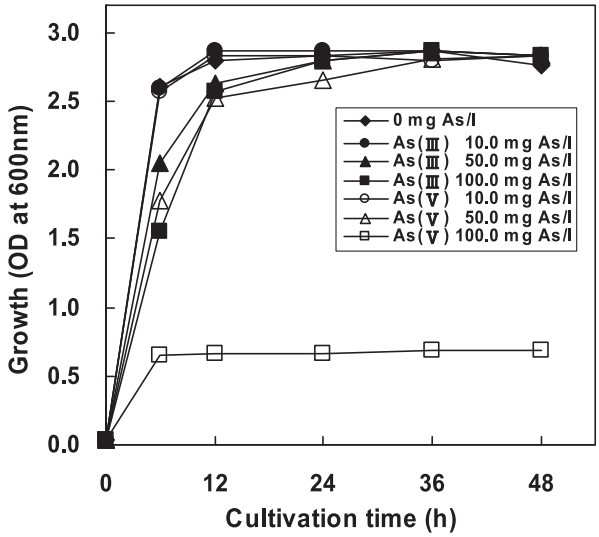

Fig. 1. Effect of arsenic concentration on cell growth of strain UM-123.

With regard to the species of arsenic remaining in the culture medium over the culture time, when As(III) was added to the media, the proportion of As(V) at the start of the culture remained largely unchanged up until 24 hours of culture time, after which it rose somewhat. On the other hand, when As(V) was added, the proportion of As(V) in the culture medium showed a rapid decrease from the start of the culture until 24 hours of culture time, after which it rose somewhat, as was the case when As(III) was added. The same changes were observed with As(III) and As(V) up to concentrations of 50.0 mg As/l; at 50.0 mg As/l As(V), the total concentrations of arsenic did not vary over time, and only the species of arsenic changed. At As(III) and As(V) concentrations of 100.0 mg As/l, no changes over time were observed in either the total arsenic concentrations or in the species of arsenic in the culture medium.

The distribution of arsenic removed from culture medium by strain UM-123 is shown in Table 1. Regardless of whether the arsenic added to the medium was As(III) or As(V), with the exception of a culture time of 6 hours, 90% or more of the arsenic removed from the culture medium was present in the cell wall, and was all As(III). Conversely, the arsenic present in the cytosol was 68-76% As(III). The amount of sugar per gram of cell wall for each culture time reached its highest value after 24 hours of culture and subsequently decreased, exhibiting the same behavior as the amount of arsenic removed from the culture medium.

3.2. Oxidation-Reduction of Arsenic by strain UM-123

Intracellular and extracellular solutions from three different culture times were used to investigate arsenic oxidation-reduction activity, and the results are shown in Table 2. When As(III) was added to the intracellular solutions, the proportion of As(V) increased from 10% to around 28%, irrespective of the culture time, indicating that oxidation of As(III) to As(V) did occur. When As(III) was added to the

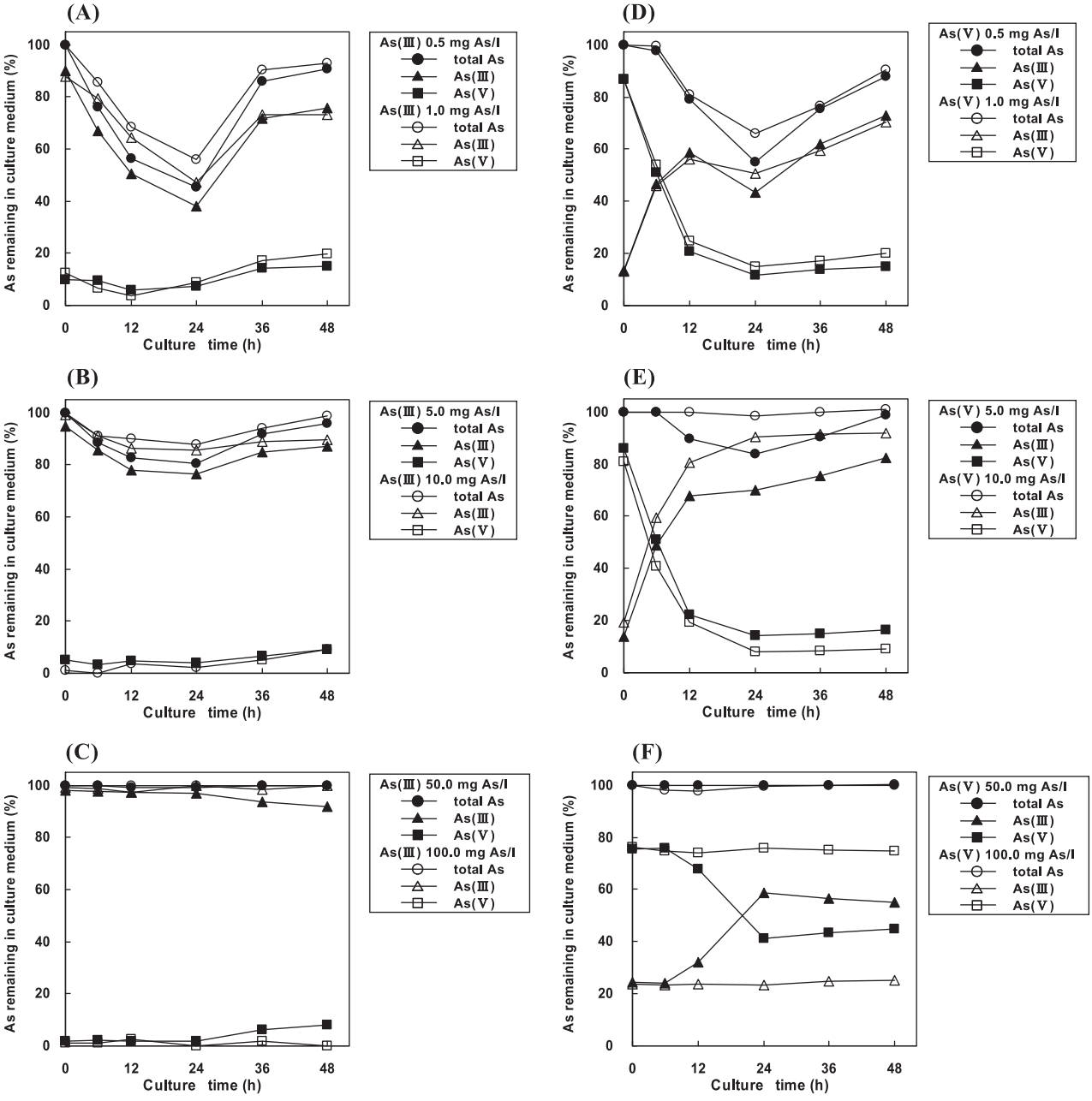

Fig. 2. Time courses of arsenic removal by strain UM-123 grown with 0.5 mg As/l and 1.0 mg As/l As(III) (A), 5.0 mg As/l and 10.0 mg As/l As(III) (B), 50.0 mg As/l and 100.0 mg As/l As(III) (C), 0.5 mg As/l and 1.0 mg As/l As(V) (D), 5.0 mg As/l and 10.0 mg As/l As(V) (E), and 50.0 mg As/l and 100.0 mg As/l As(V) (F).
Remaining arsenic in the culture broth was calculated by considering the initial arsenic concentration to be 100%.

extracellular solution, the proportion of As(V) increased to 52.2%, indicating that oxidation of As(III) to As(V) occurred, but only when the extracellular solution from a culture time of 48 hours was used. When As(V) was added to the intracellular solutions, the proportion of As(III) increased from 13% to around 78%, irrespective of the culture time, indicating that the reduction of As(V) to As(III) occurred. When As(V) was added to the extracellular solutions, no changes were observed.

3.3. Removal of Arsenic from Aqueous Solution Using Dried Cells

The changes in the adsorption ratios over time at different pH levels were investigated. The arsenic adsorption behavior differed according to pH, but under all pH conditions the maximum adsorption ratio was observed at 4 hours of contact time. In addition, in the As(III) solution, arsenic remained as As(III), with no change to As(V) observed under any of the pH conditions. Arsenic adsorption at 4 hours of contact time for each pH condition is shown in Fig. 3. Adsorption was 87.4% at pH 7, and at near-neutral

Table 1. Distribution of arsenic removed by strain UM-123 and sugar in cell wall of strain UM-123.

Culture time (h)	Cell amount (g dw/l broth)	Cytosol				Cell wall					As removal (μg As/g dw)
		As(III) (μg As/g dw)	As(V) (μg As/g dw)	Total As (μg As/g dw)	(%)[b]	As(III) (μg As/g dw)	As(V) (μg As/g dw)	Total As[a] (μg As/g dw)	(%)[b]	Sugar (mg As/g dw)	
As(III) addition											
6	2.34	5.4	2.5	7.9	12.9	50.6	—	50.6	83.1	2.64	60.9
12	2.59	2.7	1.2	3.8	3.7	96.3	—	96.3	93.3	13.83	103.2
24	2.60	2.8	1.2	4.1	2.4	162.4	—	162.4	96.1	28.73	169.0
36	2.58	2.0	0.9	2.8	2.5	108.9	—	108.9	97.0	20.31	112.3
48	2.56	1.9	0.8	2.7	6.3	38.9	—	38.9	90.9	9.84	42.8
As(V) addition											
6	2.34	0.2	0.1	0.3	15.9	1.6	—	1.6	81.1	0.09	2.0
12	2.58	3.2	1.0	4.2	5.7	67.5	—	67.5	91.3	9.92	74.0
24	2.60	3.4	1.1	4.5	3.4	124.7	—	124.7	95.1	22.29	131.2
36	2.53	2.0	0.6	2.6	2.8	89.4	—	89.4	96.5	16.76	92.7
48	2.56	1.8	0.6	2.5	6.5	34.2	—	34.2	90.0	8.72	38.0

Experimental methods are described in the text.

[a] The values measured by the method of enzyme digestion are indicated as for the amounts of total arsenic in the cell wall, and are almost the same as the values measured by the method of alkaline degradation.

[b] Percentages of total arsenic in the cytosol and cell wall were calculated by considering total arsenic removed to be 100%.

Table 2. As(V) reduction and As(III) oxidization by strain UM-123.

Culture time (h)	As(V) ratio after As(III) addition(%)[a]		As(V) ratio after As(III) addition(%)[a]	
	IS[b]	ES[c]	IS	ES
conrol[d]	9.8	9.7	13.3	13.2
12	28.4	10.8	78.6	13.4
24	27.2	11.4	78.3	13.2
48	27.9	52.2	77.8	12.9

Conditions of enzyme reactions: initial arsenic concentration, 1.0 mg As/l As(III) and As(V); pH, 7; temperature, 35℃ ; reaction time, 2 h.

[a] As(III) and As(V) ratios in the reaction solution were calculated by considering the initial total arsenic concentration to be 100%.

[b] Intracellular solution.

[c] Extracellular solution.

[d] Control experiment with purified water.

pH (pH 6, 7, 8) it was over 70%. Under acidic conditions (pH 2-5), arsenic adsorption was around 57%; under alkaline conditions, adsorption dropped sharply above pH 9 and was less than 5% at pH 12.

Arsenic adsorption under different temperature conditions is shown in Fig. 4. Adsorption was over 85% at temperatures of 30-45°C, but fell at temperatures above 50°C, and was only 20.0% at 60°C. Maximum adsorption was 93.2% at 35°C.

The effects of arsenic concentrations on arsenic adsorption were investigated, and the results are shown in Fig. 5. Adsorption of 93% or more was observed up to a concentration of 1.0 mg As/l, and up to a concentration of 0.2 mg As/l, the concentrations of arsenic in the solution decreased to less than 0.01 mg As/l. The relationship between the equilibrium concentration and the amount of arsenic adsorption is shown in Fig. 6. The amount of arsenic adsorbed increased exponentially with the concentrations of arsenic added, and the equilibrium data closely match the Langmuir isotherm. The maximum adsorption amount determined in this way was 0.134 mg As/g dw.

4. Discussion

As(V) reduction can be achieved by various bacteria possessing cytoplasmic As(V) reductase (ArsC), which is part of an arsenic resistance system, and dissimilatory As(V)-reducing bacteria (DAsRBs), which can grow by the respiratory As(V) reduction under anoxic condition[1,7,11]. ArsC is located internally within the cytoplasm in contrast to the dissimilatory As(V) reductase of DAsRBs that is located on the cell periphery. It is clear that ArsC differ both functionally and structurally from dissimilatory As(V) reductase. DAsRB *Bacillus* sp. SF-1 could not reduce As(V)

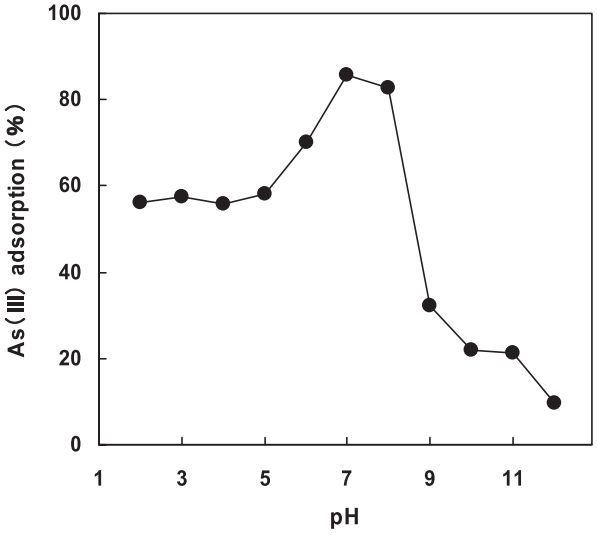

Fig. 3. Effects of pH on As(III) adsorption by dried cells of strain UM-123.
Conditions of As(III) adsorption experiments: initial As(III) concentration, 1.0 mg As/l; dried cells, 20 g dw/l; temperature, 30°C; contact time, 4 h. As(III) adsorption was calculated as a ratio of the adsorbed As(III) concentration against the initial As(III) concentration.

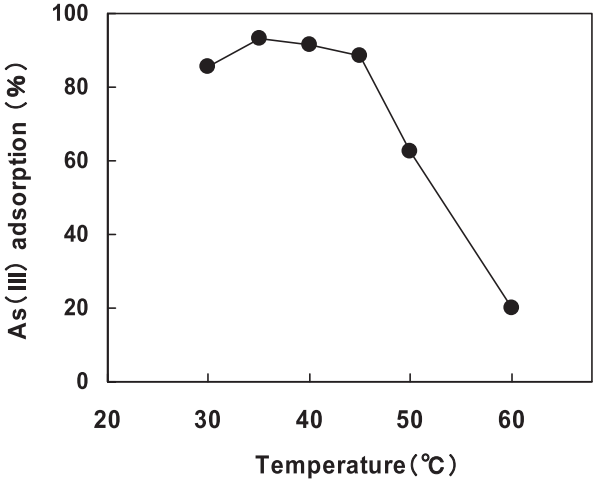

Fig. 4. Effects of temperature on As(III) adsorption by dried cells of strain UM-123.
Conditions of As(III) adsorption experiments: initial As(III) concentration, 1.0 mg As/l; dried cells, 20 g dw/l; pH, 7; contact time, 4 h. As(III) adsorption was calculated as a ratio of the adsorbed As(III) concentration against the initial As(III) concentration.

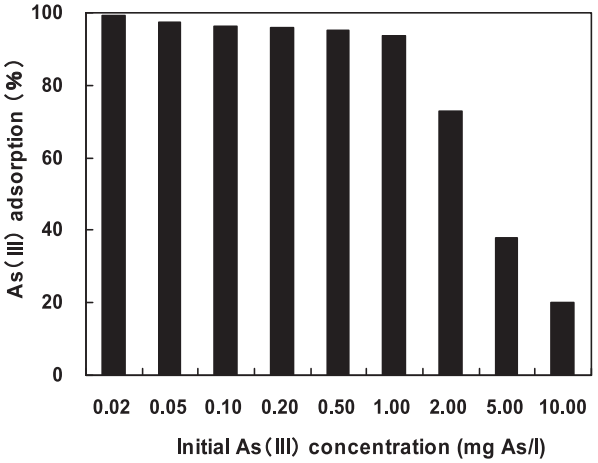

Fig. 5. Effects of arsenic concentrations on As(III) adsorption by dried cells of strain UM-123.
Conditions of As(III) adsorption experiments: dried cells, 20 g dw/l; pH, 7; temperature, 35℃ ; contact time, 4 h. As(III) adsorption was calculated as a ratio of the adsorbed As(III) concentration against the initial As(III) concentration.

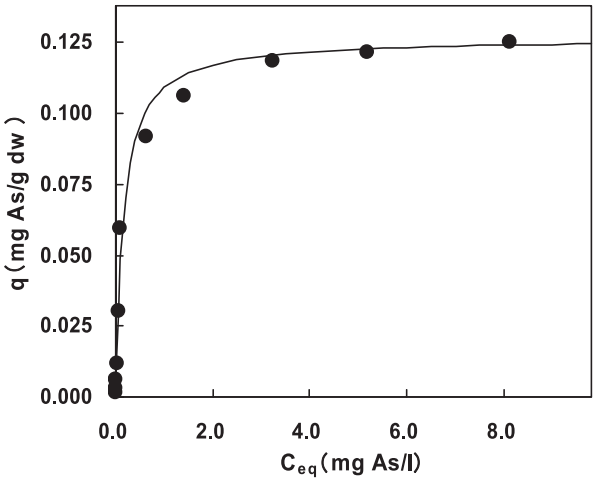

Fig. 6. Application of the equilibrium Langmuir model to As(III) adsorption on dried cells of strain UM-123.
Conditions of the As(III) adsorption experiments: dried cells, 20 g dw/l; pH, 7; temperature, 35°C; contact time, 4 h. Line showed theoretical Langmuir curve.

under strictly aerobic conditions due to the presence of oxygen, which inhibited the As(V) reduction[21]. On the other hand, strain UM-123 could reduce As(V) under strictly aerobic conditions. It was presumed that As(V) reduction by strain UM-123 was carried out by ArsC of an arsenic resistance system. In the arsenic resistance system, As(V) entering the cell via phosphate transporters is reduced to As(III) by ArsC in the cytoplasm, and then is expelled from the cell via As(III)-specific transporters. Because this process occurred in strain UM-123 similarly, the amount of As(III) in the culture medium, when As(V) was added to the medium, increased as the culture progressed.

As over 90% of the arsenic removed from the culture medium was found to be present in the cell wall as As(III), it is clear that strain UM-123 specifically adsorbs As(III) at the cell wall. Furthermore, as changes in the amount of arsenic removed from the culture medium per unit dry weight exhibited the same behavior as the changes in cell wall sugar content, it may be conjectured that sugar in the cell wall is closely involved with the adsorption of As(III). It may be further conjectured that the increase in arsenic concentrations in the culture medium from 24 hours of culture time onward was because the sugar in the cell wall either separated from the cell wall or was broken down as the culture progressed, so that the arsenic that had been adsorbed

was subsequently desorbed from the cell wall. When lyophilized bacterial cells were used, As(III) alone was specifically removed from the aqueous solution. It would therefore appear that the sequence of events resulting in As(V) removal by bacteria in cultures medium did not occur with dried cells; as lyophilized cells were unable to reduce As(V) to As(III), they were unable to remove As(V). The amount of sugar present per unit dry weight in the cell wall immediately after lyophilization was 97% of the amount during culture, while in the dried cells after the arsenic adsorption experiments it had decreased to 72% of the amount during culture. The amount of arsenic removed was also lower when dried cells rather than cultures were used, and it would appear likely that in the adsorption experiments using dried cells, the sugar in the cell wall either separated from the cell wall or was broken down.

Among the bacteria that have been reported to date, the marine bacterium *Marinomonas communis* removes the greatest quantity of arsenic, removing 2.290 mg As/g dw from cultures containing 5.0 mg As/l As(V)[17]. However, the ratio of arsenic removed is only 14.8% under these conditions, and is even lower in medium containing 0.07 mg As/l As(V). The amount of As(III) adsorbed when *M. communis* cells are used is 0.119 mg As/g dw, which is on the same order as that with strain UM-123. Moreover, even genetically modified *E. coli* bacteria, in which gene manipulation techniques can result in a high expression of ArsR, the protein regulating the *ars* operon, or can induce production of *Arabidopsis thaliana* phytochelatin, the amount of arsenic removed is 0.110-0.173 mg As/g dw, which is on the same order as that with strain UM-123[5, 11]. Strain UM-123, in several bacteria without pre-processing, is the first bacterium reported that possesses the same arsenic-removing capacity as marine bacteria and genetically modified *E. coli*. Strain UM-123 was able to remove both As(III) and As(V) from the culture medium during culture, and the ratio removed was in the region of 55% in culture medium containing 0.5 mg As/l As(III). When dried cells were used, strain UM-123 was only able to remove As(III). But by increasing the cell density to greater than when the strain was cultured, it was possible to achieve an arsenic adsorption ratio of 93% or more in solutions of As(III) of up to 1.0 mg As/l. Strain UM-123 was also able to reduce the concentrations of arsenic in solutions up to 0.2 mg As/l to 0.01 mg As/l or less, which is the environmental standard for arsenic.

Based on the present results, it appears that strain UM-123 is effective in removing As(III), which is highly toxic and difficult to remove. Furthermore, this activity is maintained even at low concentrations of arsenic. UM-123 thus appears to have sufficient potential for use in bioremediation.

References

1) Bentley, R., and T.G. Chasteen. 2002. Microbial methylation of metalloids: arsenic, antimony, and bismuth. Microbiol. Mol. Biol. Rev. 66 (2): 250-271.
2) Clausen, C.A. 2000. Isolating metal-tolerant bacteria capable of removing copper, chromium, and arsenic from treated wood. Waste Manage. Res. 18: 264-268.
3) Cullen, R.W., and K.J. Reimer. 1989. Arsenic speciation in the environment. Chem. Rev. 89: 713-764.
4) Kapoor, A., T. Viraraghavan, and D.R. Cullimore. 1999. Removal of heavy metals using the fungus *Aspergillus niger*. Bioresour. Technol. 70: 95-104.
5) Kostal J., R. Yang, C.H. Wu, A. Mulchandani, and W. Chen. 2004. Enhanced arsenic accumulation in engineered bacterial cells expressing ArsR. Appl. Environ. Microbiol. 70 (8): 4582-4587.
6) Loukidou, M.X., K.A. Matis, A.I. Zouboulis, and M. Liakopoulou-Kyriakisou. 2003. Removal of As(V) from wastewaters by chemically modified fungal biomass. Water Res. 37: 4544-4552.
7) Mukhopadhyay, R., B.P. Rosen, L.T. Phung, and S. Silver. 2002. Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiol. Rev. 26: 311-325.
8) Mushtaque, A., and R. Chowdhury. 2004. Arsenic crisis in Bangladesh. Scientific Am. Aug. 86-91.
9) Nickson, R.T., J.M. McArthur, P. Ravenscroft, W.G. Burgess, and K.M. Ahmed. 2000. Mechanism of arsenic release to groundwater, Bangladesh and West Bengal. Appl. Geochem. 15: 403-413.
10) Nickson, R., J. McArthur, W. Burgess, K.M. Ahmed, P. Ravenscroft, and M. Rahman. 1998. Arsenic poisoning of Bangladesh groundwater. Nature 395: 338.
11) Oremland, S., R., and J. F. Stolz. 2003. The ecology of arsenic. Science 300 (9): 939-944.
12) Sauge-Merle, S., S. Cuine, P. Carrier, C. Lecomte-Pradines, D.T. Luu, and G. Peltier. 2003. Enhanced toxic metal accumulation in engineered bacterial cells expressing *Arabidopsis thaliana* phytochelatin Synthase. Appl. Environ. Microbiol. 69 (1): 490-494.
13) Say, R., N. Yilmaz, and A. Denizli. 2003. Biosorption of cadmium, lead, mercury, and arsenic ions by the fungus *Penicillium purpurogenum*. Sep. Sci. Technol. 38 (9): 2038-2053.
14) Smedley, P.L., and D.G. Kinniburgh. 2002. A review of the source, behaviors and distribution of arsenic in natural waters. Appl. Geochem. 17: 517-568.
15) Srinath, T., T. Verma, P.W. Ramteke, and S.K. Garg. 2002. Chromium(VI) biosorption and bioaccumulation by chromate resistant bacteria. Chemosphere 48: 427-435.
16) Sudha, B.R., and T.E. Abraham. 2001. Biosorption of Cr (VI) from aqueous solution by *Rhizopus nigricans*. Bioresour. Technol. 79: 73-81.
17) Takeuchi, M., H. Kawahata, L.P. Gupta, N. Kita, Y. Morishita, Y. Ono, and T. Komai. 2007. Arsenic resistance and removal by marine and non-marine bacteria. J. Biotechnol. 127: 434-442.
18) Tamaki, S., and W.T. Frankenberger Jr. 1992. Environmental biochemistry of arsenic. Rev. Contam. Toxicol. 124: 79-110.
19) Tsuruta, T. 2004. Cell-associated adsorption of thorium or uranium from aqueous system using various microorganisms. Water Air Soil Pollut. 159: 35-47.
20) Wang, S., and C.N. Mulligan. 2006. Natural attenuation processes for remediation of arsenic contaminated soil and groundwater. J. Hazard. Mater. 138 (3): 459-470.
21) Yamamura, S., M. Ike, and M. Fujita. 2003. Dissimilatory arsenate reduction by facultative anaerobe, *Bacillus* sp. strain SF-1. J. Biosci. Bioeng. 96 (5): 454-460.