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Shigeru Maeda[a]; Seihiro Nakashima[a]; Toshio Takeshita[a]; Shiro Higashi[b]
[a] DEPARTMENT OF APPLIED CHEMISTRY FACULTY OF ENGINEERING, [b] DEPARTMENT OF BIOLOGY FACULTY OF SCIENCE, KAGOSHIMA UNIVERSITY, KAGOSHIMA, JAPAN

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Bioaccumulation of Arsenic by Freshwater Algae and the Application to the Removal of Inorganic Arsenic from an Aqueous Phase. Part II. By *Chlorella vulgaris* Isolated from Arsenic-Polluted Environment

SHIGERU MAEDA, SEIHIRO NAKASHIMA, and TOSHIO TAKESHITA

DEPARTMENT OF APPLIED CHEMISTRY
FACULTY OF ENGINEERING

SHIRO HIGASHI

DEPARTMENT OF BIOLOGY
FACULTY OF SCIENCE

KAGOSHIMA UNIVERSITY
KAGOSHIMA 890, JAPAN

Abstract

Green algae, *Chlorella vulgaris* Beijerinck var. *vulgaris*, isolated from an arsenic-polluted environment, was examined for the effects of arsenic levels, arsenic valence, temperature, illumination intensity, phosphate levels, metabolism inhibitors, heat treatment on the growth, and arsenic bioaccumulation. The following conclusions were reached from the experimental results: (a) The growth of the cell increased with an increase of arsenic(V) levels of the medium up to 2,000 ppm, and the cell survived even at 10,000 ppm; (b) The arsenic bioaccumulation increased with an increase of the arsenic level. The maximum accumulation of arsenic was about 50,000 μg As/g dry cell; (c) The growth decreased with an increase of the arsenic(III) level and the cell was cytolyzed at levels higher than 40 ppm; (d) No arsenic(V) was bioaccumulated by a cell which had been pretreated with dinitrophenol (respiratory inhibitor) or with heat. Little effect of NaN_3 (photosynthesis inhibitor) on the bioaccumulation was observed.

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INTRODUCTION

It is well known that marine algae take up inorganic arsenic compounds at high concentrations and biotransform them to organo-arsenic compounds; that is, methylated arsenic compounds (*1–4*).

Terrestrial algae are expected to have a similar ability, but few papers on the subject have been published (*5–7*). In a previous paper the authors reported some freshwater algae that preferred freshwater containing a high level of arsenic(V) and had a high resistance to arsenic (*5*). Several species of the arsenic-resistant algae were isolated and pure-cultured (*5*).

The present paper reports the experimental results for *Chlorella vulgaris* Beijerinck var. *vulgaris* thus isolated. The effects of incubating conditions (illumination, temperature, arsenic level, arsenic valence, acclimation, phosphate level, and metabolism inhibitors) on growth and the bioaccumulation of arsenic are discussed.

EXPERIMENTAL

Pure Culture of *C. vulgaris*

C. vulgaris was isolated by the method described in a previous paper (*5*) and preserved in 6 N-U-Bold Basal Medium (abbreviated 6 N-U-BBM, see Table 1) containing 100 ppm of arsenic (Na_2HAsO_4).

TABLE 1
Composition of Culture Medium (6 N-U-Bold Basal Medium)

	mg/L
Urea	540
KH_2PO_4	175
K_2HPO_4	75
$MgSO_4 \cdot 7H_2O$	75
Na_2EDTA	50
$CaCl_2 \cdot 2H_2O$	25
NaCl	25
H_3BO_3	11.4
$ZnSO_4 \cdot 7H_2O$	8.8
$FeSO_4 \cdot 7H_2O$	5.0
$CuSO_4 \cdot 5H_2O$	1.57
$MnCl_2 \cdot 4H_2O$	1.44
MoO_3	0.71
$Co(NO_3)_2 \cdot 6H_2O$	0.49

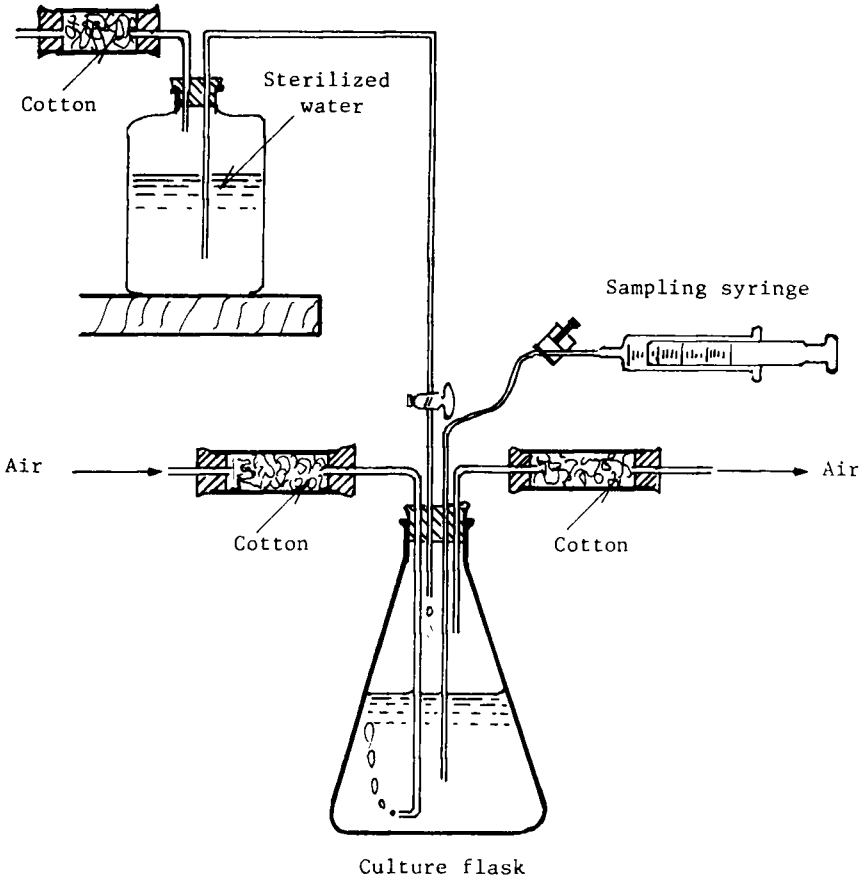

FIG. 1. Apparatus of pure culture.

The pure culture was created by the use of the apparatus shown in Fig. 1 under standardized conditions of illumination (fluorescent light, 7000 lux, 12 h/d), room temperature, and air-bubbling (2 L/min).

Determination of Growth and Arsenic Concentration of the Cell

A constant volume of the culture suspension was harvested and centrifuged, and the cell was rinsed twice with pure water and dried at 105°C for 2 h. The dry cell (5–40 mg) was put into a 100-mL porcelain crucible, 2 mL of 50% $Mg(NO_3)_2$ was added, and the mixture was heated at

60°C for 12 h and then at 550°C for 6 h. After cooling, the ashed sample was dissolved with 5 mL of 10 *N* HCl by heating at 50°C for 30 min on a hot plate.

The arsenic concentration was determined by colorimetry using silver diethyldithiocarbamate and by electrothermal atomic absorption spectrometry via reduction and hydride generation by using a AA-Spectrometer (Japan Jarrell Ash: AA-80) and its attachments (Japan Jarrell Ash: HYD-2 and AU-1A).

RESULTS AND DISCUSSION

Effect of Arsenic(V) Level

A cell suspension (3 mL) of the stock culture was inoculated and cultured as a preliminary culture in 6 N-U-BBM (300 mL) containing the same level of arsenic as that of the test culture.

The inoculum (30 mL) was taken from the preliminary culture in its log phase and inoculated as a test culture in 6 N-U-BBM (3000 mL) in which arsenic levels varied from 0 to 10,000 ppm. The culture solution was sampled at regular intervals, and the cell concentration and the arsenic concentration in the cell were determined.

The results of cell concentration (growth) in the stationary phase and the

TABLE 2
Arsenic(V) Impact and Arsenic Bioaccumulation by *C. vulgaris*

As(V) level in medium (ppm)	Cell growth at stationary phase (g dry cell/L)	As accumulated in cell (μg As/g dry cell)	
		At log phase	At stationary phase
0	0.27	0	0
0.1	0.25	33	15
1	0.28	10	21
10	0.30	110	60
100	0.33	140	260
100[a]	0.31[a]	20,000[a]	10,000[a]
1,000	0.40	15,000	3,600
2,000	0.50	—	—
5,000	0.22	18,000	19,000
10,000	0.14	52,000	34,000

[a]Modified Detmer medium (5); the others are 6 N-U-BBM.

arsenic concentrations in the cell (bioaccumulation) for both log and stationary phases are shown in Table 2.

The following conclusions are derived from the data of Table 2. (a) The growth of *C. vulgaris* increased with an increase of the arsenic(V) level of the medium up to 2,000 ppm. (b) The growth decreased at levels higher than 2,000 ppm, but the cell survived and grew slightly even at 10,000 ppm. (c) The bioaccumulation of arsenic increased with an increase of the arsenic(V) level of the medium and seemed to be slightly higher in the log phase than in the stationary phase. (d) The bioaccumulation of arsenic was greatly affected by the medium. At the 100-ppm arsenic level, the modified Detmer medium gave a larger bioaccumulation than did 6 N-U-BBM. No reason for the difference was found.

Effect of Arsenic(III) Level

A cell suspension (10 mL) in the stationary phase in 6 N-U-BBM containing no arsenic was inoculated into a new 6 N-U-BBM (90 mL), and arsenic(III) was added at a given level. The cell concentration was adjusted at 90 mg dry cell/L medium. The cell was cultured for 10 d. The arsenic(III) impact on cell growth is summarized in Table 3.

Table 3 shows that growth seemed to be unaffected by arsenic(III) at levels up to 30 ppm, and the cell was killed by cytolysis at levels higher than 80 ppm. Depending on the arsenic valence, there was a great difference in the effects of arsenic level on growth.

TABLE 3
Arsenic(III) Impact to Growth of *C. vulgaris*

As(III) level in medium (ppm)	Cell growth at stationary phase (g dry cell/L)
10	0.78
20	0.38
30	0.54
40	0.28
50	0.27
60	0.11
70	0.14
80	0.077
90	0.083

Temperature

The optimum temperature for growth was 30°C. No cell survived at a temperature higher than 45°C. Below 15°C the cell survived, but the growth was extremely slow.

Acclimation

Table 4 shows the effect of acclimation to arsenic on arsenic accumulation. The experiment was performed in the following manner. The cell was preliminarily cultured in a medium containing arsenic at a given level and was acclimated to the arsenic for 14 d. The cell suspension (3 mL) was inoculated and cultured in a medium (300 mL) containing arsenic at a given level, and arsenic accumulation by the cell in the stationary phase was measured.

It was found that the cell acclimated to the higher level of arsenic accumulated arsenic(V) at a higher concentration (Table 4). Foster (*8*) reported the relationship between the tolerance and bioaccumulation of copper ion by *C. vulgaris* (same species as the present one). According to the results of Foster, the nontolerant strain was 4 times as sensitive to copper ion but accumulated 5 to 10 times more metal compared to the tolerant strain which had been acclimated to copper ion in a copper-polluted environment (*8*). These results imply that tolerance to the copper ion is attributable to copper exclusion by the cell.

On the other hand, the arsenic-tolerant strain accumulated more arsenic than the nonacclimated strain. Therefore, different mechanisms are likely to be involved in the tolerances of *C. vulgaris* to arsenic and copper.

TABLE 4
Effect of As(V) Acclimation on As(V) Accumulation

As(V) level in test-medium (ppm)	As accumulated in cell at stationary phase (μg As/g dry cell)				
		As(V) level in acclimation medium (ppm)			
	Nonacclimation	1	100	1000	5000
1	9	21	—	47	290
100	—	—	260	580	830

Illumination

The optimum illumination intensity of growth was 13,000 lux as shown in Fig. 2. However, different results regarding bioaccumulation were obtained from short-term incubation experiments as described below.

After the cell had been inoculated and cultured in 6 N-U-BBM containing no arsenic, the cell in the stationary phase was separated by centrifuging and then resuspended in the same volume of pure water. This cell suspension is defined here as the living cell suspension. After the arsenic (V) level was adjusted to 9.6 ppm, the living cell suspension was incubated for 8 h under three different illumination intensities. The experimental results are shown in Table 5.

The results of Table 5 suggest that the ripening cell accumulates arsenic(V) better in the dark than under illumination.

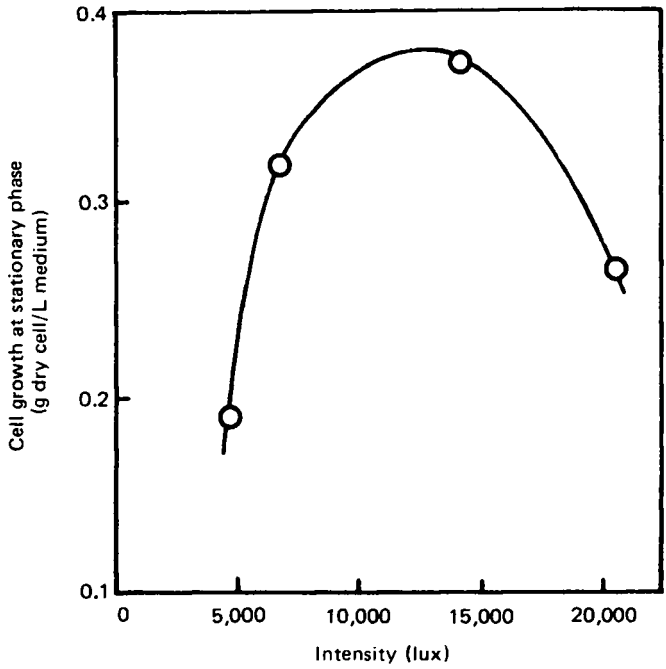

FIG. 2. Effect of illumination on cell growth.

TABLE 5
Effect of Illumination Intensity on As(V) Accumulation for Short-Term Incubation (8 h)

Illumination intensity (lux)	As accumulated in cell during 8-h incubation (μg As/g dry cell)
0	49
4000	13
7000	5

Level of KH_2PO_4

Arsenic(V) and KH_2PO_4 were added to the living cell suspension defined in the previous section. The level of arsenic(V) was adjusted at 8.5 ppm, and KH_2PO_4 was varied from 10^{-1} to 10^3 ppm. The arsenic accumulation was measured after short-term incubation (4 h, 400 lux). The results at three levels of KH_2PO_4 are tabulated in Table 6. The data in Table 6 imply that KH_2PO_4 might competitively inhibit arsenic accumulation.

NaN_3 and DNP (dinitrophenol) Treatments

Living cell suspensions (50 mL) were treated with two metabolism inhibitors, NaN_3 (photosynthesis inhibitor) and DNP (respiratory inhibitor), for 30 min at levels of 3.3 and 0.33 m*M*, respectively.

The cell was separated by centrifuging, rinsed twice with water, and the cell was resuspended in pure water (50 mL). After arsenic(V) was added and its level adjusted to 8.5 ppm, the cell suspension was incubated for 4 h under 400 lux. Arsenic accumulation results revealed that the cell treated with NaN_3 accumulated arsenic up to 56 μg As/g dry cell during 4 h of

TABLE 6
Effect of KH_2PO_4 Level on As(V) Accumulation for Short-Term Incubation (4 h)

KH_2PO_4 level in medium (ppm)	As accumulated in cell during 4-h incubation (μg As/g dry cell)
10^{-1}	49
10	36
10^3	11

incubation, but no arsenic was accumulated by the cell treated with DNP. These findings imply that arsenic accumulation by *C. vulgaris* predominantly occurs by respiratory metabolism rather than by photosynthesis metabolism.

Heat-Killed Cell

The living cell suspension was heated on a water bath at 95°C for 1 h. After arsenic(V) was added and its level adjusted to 8.5 ppm, the suspension was incubated at room temperature for 6 h. The experimental results showed that no arsenic was accumulated by the heat-killed cell.

From the above results it was found that the high resistance of *C. vulgaris* to arsenic involved its ability to bioaccumulate, and bioaccumulation occurred only by the living cell, probably in the respiratory process.

The optimum culture conditions of *C. vulgaris* for efficient removal of inorganic arsenic from the aqueous phase are now under investigation.

REFERENCES

1. T. Takeshita and S. Maeda, *Yukagaku, 29*, 75 (1980).
2. S. Maeda and T. Takeshita, *Kagaku No Ryoiki, 36*, 686 (1982).
3. S. Maeda, *Ibid., 37*, 606 (1983).
4. S. Maeda, *Bunseki*, p. 367 (1983).
5. S. Maeda, T. Kumamoto, M. Yonemoto, S. Nakajima, and T. Takeshita, *Sep. Sci. Technol., 18*, 375 (1983).
6. G. Lunde, *Acta Chem. Scand., 27*, 1586 (1973).
7. J. M. Giddings and G. K. Eddlemon, *Arch. Environ. Contam. Toxicol., 6*, 491 (1977).
8. P. L. Foster, *Nature, 269*, 322 (1977).

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