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Accumulation of arsenic and selenium by *Dunaliella* sp.

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The unicellular marine alga, *Dunaliella* sp., was grown in seawater containing selenium and arsenic. *Dunaliella* sp. readily took up selenite from the medium over a broad range of concentrations (10^{-10}–10^{-5} mol dm^{-3}). In comparison, uptake from selenate was generally minimal. Selenite was detectable in cells within 24 h and concentrations increased gradually with time. Also iron and zinc concentrations in *Dunaliella* sp. cells were largely affected by addition of arsenic and gallium. Arsenic concentrations in cultures were largely correlated to the accumulation of arsenic by *Dunaliella* sp., but did not affect the accumulation of selenium by *Dunaliella* sp. This implied that the accumulation process of selenium was different from that of arsenic.

Keywords: Arsenic, selenium, bioaccumulation, *Dunaliella* sp., microalgae

INTRODUCTION

Marine organisms accumulate arsenic at extraordinary levels compared with land organisms.[1–3] Many researchers have investigated various kinds of marine organisms to clarify the accumulation process, the chemical form of the arsenic and the mechanism of transformation of arsenic.[4–9] The green alga *Dunaliella* sp. (oceanic product), which tolerates highly concentrated arsenic, accumulates arsenic even up to 10 000 μg g^{-1} dry cell concentrations.[10] The effect of various elements on the accumulation of arsenic by *Dunaliella* sp. and the optimum culture conditions of accumulation have already been elucidated.[11,12] In this report we describe the effects of the concentrations of arsenic and selenium in various cultures on the accumulation of arsenic and selenium by *Dunaliella* sp.

MATERIALS AND METHODS

Microalgae

Dunaliella sp. (Chlorophyceae) obtained from Hiroshima Fisheries Experimental Station, Japan (isolated by H. Takayama) was used throughout the experiments. *Dunaliella pennik* Tsukuba U, *Dunaliella england* Tsukuba U, *Dunaliella primolecta* UTEX L2355, *Dunaliella primolecta* UTEX LB1600 and *Dunaliella bioculate* UTEX LB199 were obtained from Dainippon Ink and Chemicals, Inc.

Cultures of algae

Dunaliella sp. was grown in 5-dm^3 volumes of sterile medium containing appropriate nutrient concentrations at 23°C with constant bubbling of air. Light was supplied by Toshiba 40-W power grove cool white lamps at an intensity of 6000 lux (at the surface of the liquid medium). The medium consisted of KNO_3, 72 mg; KH_2PO_4, 4.5 mg; and aged seawater, 1000 cm^3. In order to investigate the effect of arsenic concentration in the medium on selenium accumulation by *Dunaliella* sp., *Dunaliella* sp. was grown in the medium (1 dm^3) containing selenite(IV) or arsenate(V) and the selenium or arsenic concentration in *Dunaliella* after a 7-day culture was determined.

Determination of arsenic and selenium in algae

The total arsenic content in algae was measured by either silver diethyldithiocarbamate-(Ag-DDTC)spectrophotometry,[13,14] or atomic absorption spectrophotometry[15] (Jarrell Ash Co., Model AA-1 MK2) after digestion with 10 cm^3 of a mixture of concentrated acids (nitric (3 cm^3), sulfuric (2 cm^3) and perchloric (5 cm^3) acids).

Selenium in algae was determined fluorometrically with 2,3-diaminonaphthalene after digestion in nitric and perchloric acids.[16]

Determination of gallium, zinc and iron in *Dunaliella* sp.

Gallium, zinc and iron in the dry cells was determined by X-ray fluorescence analysis. Preparation of the samples was as follows: cellulose backings (4 cm diameter and 0.4 cm thickness) were prepared by pressing 6 g of cellulose powder at 2000 kg cm.$^{-2}$. Then, about 100 mg of dry cells was placed in an aluminium ring of 2 cm diameter and 0.5 cm depth on a cellulose backing. Measurements were made by X-ray fluorescence spectrometer (Kevex Co. Ltd). The appropriate instrument settings were as follows: Mo X-ray tube; tube voltage, 55 kV; tube current, 3 mA; analyzing crystal, LiF; detector, scintillation counter.

Fractionation of *Dunaliella* sp. cells

Dunaliella sp. cells taking up arsenic were suspended in 20 cm^3 of distilled water for 120 min at 5 °C, then collected by centrifugation and washed thoroughly with distilled water, and the arsenic and selenium in the cells were determined.

RESULTS AND DISCUSSION

Effect of arsenic and selenite on the growth of *Dunaliella* sp.

Dunaliella sp. cells were grown in the enriched seawater containing various concentrations of selenite and arsenic for 10 days (Fig. 1). The growth of *Dunaliella* sp. was affected by selenite(IV) addition. On the other hand, *Dunaliella* sp. was unaffected by the addition of selenite (5 mg dm^{-3} in medium) and arsenate (1–100 mg dm^{-3} in medium); the addition of both selenite and arsenate increased the growth at an average of 10% relative to the growth in blanks containing no selenite and no arsenate. However, growth in the medium containing selenite but not arsenate was depressed. These results indicate that the growth of *Dunaliella* sp. was stimulated a little by resistance of selenite and arsenate, but was impaired by selenite alone. Arsenate seems to reduce a poisonous effect of selenite.

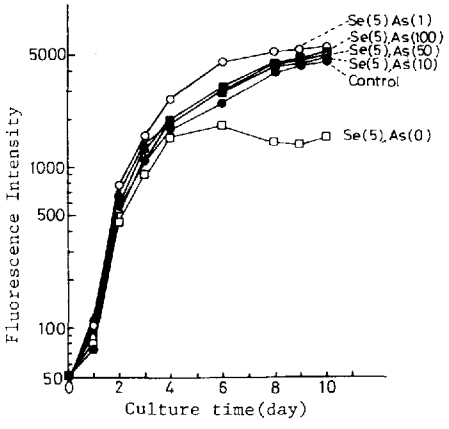

Figure 1 Effect of arsenic and selenium on the growth of *Dunaliella* sp. Growth was monitored by measuring *in vivo* fluorescence; there is a close correlation between the relative intensity of fluorescence and the biomass of *Dunaliella* sp. Concentrations of $\mu g\,cm^{-3}$ added arsenate and selenite are shown in parentheses.

Accumulation of selenium and arsenic by *Dunaliella* sp.

Dunaliella sp. was incubated in the aerated medium (selenite and selenate-enriched seawater) at 23°C under illumination of cool white fluorescent lamps at a light intensity of approximately 6000 lux. The growth of *Dunaliella* sp. was monitored by a Turner Fluorimeter. The cells were collected at the stationary growth phase by continuous centrifugation at 3000 rpm. Figure 2 shows selenium concentrations in *Dunaliella* sp. when it was grown in the culture containing selenate (Na_2SeO_3).

As compared with selenate, selenite was highly accumulated in *Dunaliella* sp. at each growth phase. Both selenite and selenate were actively

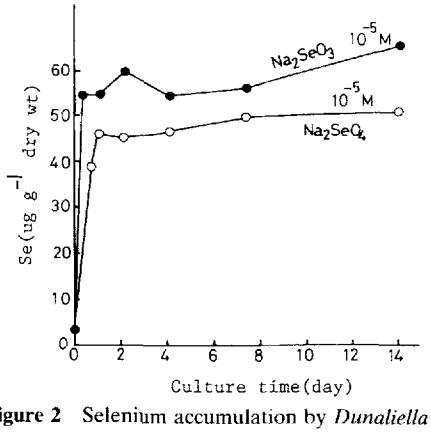

Figure 2 Selenium accumulation by *Dunaliella* sp.

Table 1 Uptake of selenite and selenate by *Dunaliella* sp. after a 7-day culture

Selenite in medium (mol dm^{-3})	Se accumulated in cell (μg Se g^{-1} dry cell)	Selenate in medium (mol dm^{-3})	Se accumulated in cell (μg Se g^{-1} dry cell)
10^{-10}	22.2	10^{-10}	12.4
10^{-9}	21.8	10^{-9}	13.9
10^{-8}	37.3	10^{-8}	16.2
10^{-7}	43.3	10^{-7}	21.2
10^{-6}	84.6	10^{-6}	30.1
10^{-5}	144.7	10^{-5}	49.8

Table 2 Amount of selenium in various species of *Dunaliella* after a 7-day culture

Selenite level in medium: 5 mg dm^{-3}

Organisms	Selenium (mg Se g^{-1} dry cell)
Dunaliella sp. (Na_2SeO_4)	33.4
Dunaliella sp. (Na_2SeO_3)	62.8
Dunaliella pennik Tsukuba U	108.7
Dunaliella england Tsukuba U	66.1
Dunaliella primolecta UTEX L2355	112.3
Dunaliella primolecta UTX LB1600	44.8
Dunaliella bioculate UTEX LB199	45.9

Table 3 Effect of selenium (Na_2SeO_3) on the accumulation of arsenic and selenium by *Dunaliella* sp. after a 7-day culture

Arsenate level in medium: 10 mg dm^{-3}

Se level in medium (mg dm^{-3})	As accumulated in cell (μg As g^{-1} dry cell)	Se accumulated in cell (μg Se g^{-1} dry cell)
0	1014	0.94
0.5	1126	32.3
1	1260	41.1
2	1199	44.5
5	822	46.0
10	874	100.6

Table 4 Effect of arsenic (Na_2HAsO_4) on the accumulation of arsenic and selenium by *Dunaliella* sp. after a 7-day culture

Selenite level in medium: 5 mg dm^{-3}

As(V) level in medium (mg dm^{-3})	As accumulated in cell (μg As g^{-1} dry cell)	Se accumulated in cell (μg Se g^{-1} dry cell)
0	10	40.0
0.5	334	35.4
1	390	33.3
2	569	35.1
5	963	41.0
10	923	45.0
50	1384	40.3

accumulated within the first one or two days, and both concentrations in *Dunaliella* sp. did not increase from the second day to the 14th day of growth. Much arsenic was accumulated during the logarithmic growth phase,[12] while the accumulation of selenium was independent of the growth phase. Vandermeulen *et al.*[18] also reported similar results. In this study selenium accumulation was examined at various concentrations of selenite or selenate. The results of selenium accumulation are shown in Table 1. Selenium content apparently increased with an increase of selenite or selenate concentration. *Dunaliella* sp. accumulated highly selenite compared with selenate. The amount of selenium in various species of *Dunaliella* are shown in Table 2. The amount of selenium accumulated varied from species to species. Of the *Dunaliella* sp. tested, *Dunaliella pennik Tsukuba U* and *Dunaliella primolecta UTEX L2355* accumulated selenium at levels of about 110 mg $kg.^{-1}$

Dunaliella sp. was grown under the optimum conditions for bioaccumulation of selenium, as follows: pH 7.5, NaCl 36 g dm^{-3}, 5000–1000 lux (luminous intensity) and 23°C.[17] Tables 3 and 4 show the experimental results of the effects of selenium and arsenic on the accumulation of arsenic and selenium by *Dunaliella* sp., respectively. With an increase of selenium(IV) concentration in the medium, selenium accumulation in *Dunaliella* sp. increased, whilst arsenic concentration in the cell had a maximum at a selenium level of 1 mg dm^{-3} (Table 3). In our previous papers the bioaccumulation of selenium, similarly to arsenic, was found to be inhibited by an increase of phosphorus concentration in the culture.[17]

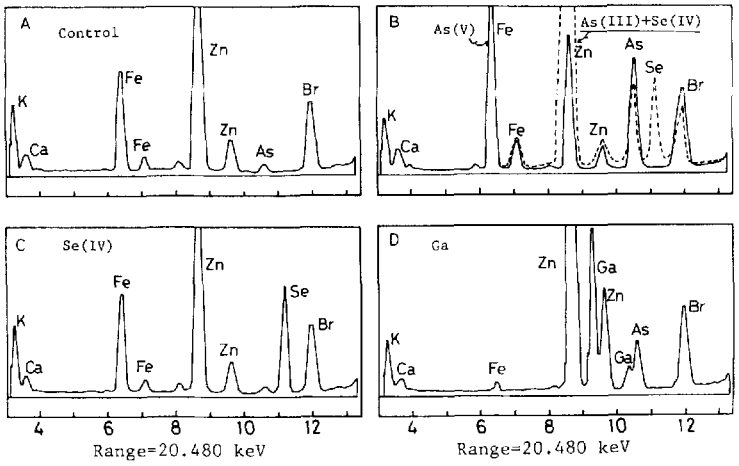

Figure 3 Effect of addition of arsenate, selenite, and gallium on the fluoresence X-ray spectrum of *Dunalliela* sp., grown for 7 days. (A) control; (B) ——, arsenate added; – – –, arsenate and selenite added; (C) selenite added; (D) gallium added.

With an increase of arsenate concentration in the medium, arsenic accumulation in *Dunaliella* sp. increased, while selenium in the cell were essentially unchanged (Table 4). These results indicated that arsenic did not have much effect on selenium accumulation.

To clarify the effect of various metal ions on the accumulation of arsenic by *Dunaliella* sp., the amounts of gallium, iron, zinc, arsenic and selenium in *Dunaliella* sp. were determined by X-ray fluorescence analysis. Figure 3 shows the fluorescence X-ray spectrum of elements in *Dunaliella*. The zinc peak decreased drastically in the culture containing arsenate, as shown in Fig. 3(B) (solid line). Iron and zinc concentrations in *Dunaliella* sp. cells were largely affected by addition of arsenic and gallium. These results suggested that there are significant differences in the mechanisms of the accumulation of elements by *Dunaliella* sp. Arsenic concentrations in cultures were largely correlated with the accumulation of arsenic by *Dunaliella* sp., but did not affect the accumulation of selenium by *Dunaliella* sp. This implies that the accumulation process of selenium is different from that of arsenic.

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