Applied Organometallic Chemistry (1988) 2 359–364
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0268-2605/88/02411359/$03.50

Environmental factors relating to arsenic accumulation by *Dunaliella* sp.

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Received 9 March 1988 Accepted 26 April 1988

The accumulation of arsenic by *Dunaliella* sp. was examined by using a solution containing arsenic only as a first approach to the study of arsenic recovery by aqueous systems. The accumulation of arsenic by *Dunaliella* sp. was rapid, with equilibrium established in 8 h with respect to arsenic partitioning between dissolved and particulate phase. The optimum accumulation was at pH 8.2, NaCl 20 g dm^{-3}, illumination 5000–10000 lux and temperature 22°C. Increased phosphate concentration significantly decreased the uptake of arsenic in the culture. These results suggested that accumulation of arsenic by *Dunaliella* sp. depended upon biological activity.

Keywords: arsenic, microalgae, bioaccumulation, environmental factors

INTRODUCTION

Arsenic is an element which is widely distributed in the biosphere. Marine biota maintain steep concentration gradients for arsenic with the levels in some organisms remarkably high,[1-3] and they may also biotransform arsenic to organo-arsenic compounds, i.e. methylated arsenic compounds.[4-8] Maeda[9,10] investigated the bioaccumulation of arsenic by freshwater algae which when tested were resistant to 100 mg dm^{-3} of inorganic arsenic and had a great ability to accumulate arsenic. *Dunaliella* sp. was found to tolerate exposure to 2000 mg dm^{-3} arsenic [as arsenate (AsO_4^{3-})] well when grown in an arsenic(V)-enriched medium.[11]

This report describes the effects of arsenic levels, temperature, illumination intensity and phosphate levels on growth and arsenic bioaccumulation 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.

Culture of algae

Dunaliella sp. was grown in 5 dm^3 cultures of an autoclaved medium in stationary cultures at 23°C and were constantly bubbled with air. Light was supplied by Toshiba 40 W power grove cool white lamps at intensity of 6000 lux (at the surface of the water). The medium[12] consisted of KNO_3, 72 mg; KH_2PO_4, 4.5 mg; disodium glycerophosphate, 10.5 mg; vitamin B_{12} (as cyanocobalamin), 2 μg; iron-chelated EDTA, 0.5 mg; and aged seawater, 1000 cm^3.

Determination of arsenic in algae

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

Fractionation of *Dunaliella* sp. cells

The *Dunaliella* sp. cells taking up arsenic were suspended in 20 cm^3 of distilled water with stirring at 5°C. After 120 min contact with the solution, the cells were collected by centrifugation and washed thoroughly with distilled water, and the arsenic content in the centrifuged cells was determined.

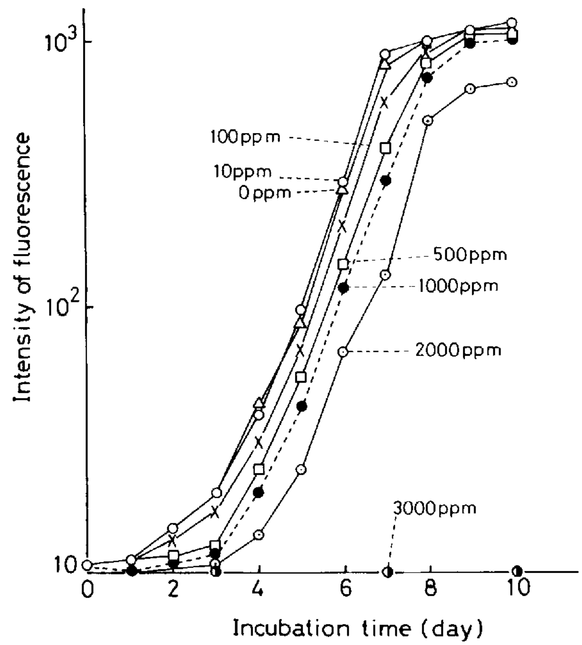

Figure 1 Effect of arsenic on the growth of *Dunaliella* sp. Growth was monitored by measuring *in vivo* fluorescence; there is a close correlation between the relative intensity of fluorescence and the biomass of *Dunaliella* sp.[17]

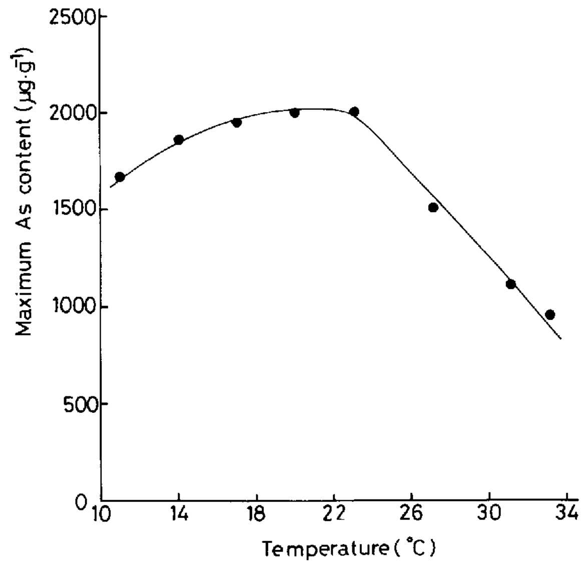

Figure 2 Effect of temperature on the uptake of arsenic by *Dunaliella* sp.

RESULTS AND DISCUSSION

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

The effect of arsenic on the growth of *Dunaliella* sp. was examined in an arsenic concentration range from 0 to 3000 mg dm^{-3}, and the results are shown in Fig. 1. The growth of *Dunaliella* sp. seemed to be unaffected by arsenic at levels ranging from 10 to 2000 mg dm^{-3}, although the growth was inhibited initially when the level of arsenic was greater 100 mg dm^{-3}. Maeda[10] reported that *Chlorella vulgaris* in a culture was unaffected by 100 mg dm^{-3} of arsenic. Since the growth of *Dunaliella* sp. was effectively unaffected by the presence of arsenic, the following accumulation experiments were mainly carried out in the presence of 1 mg dm^{-3} arsenic(V) as the sodium salt (Na_2HAsO_4).

Effect of temperature on the accumulation of arsenic by *Dunaliella* sp.

In general, the growth of algae is affected largely by changes in light energy and temperature. Therefore, the relationship between the temperature and the accumulation of arsenic by *Dunaliella* sp. was studied. The accumulation of arsenic by *Dunaliella* sp. was tested in the temperature range 10–33°C. Figure 2 shows the relationship between arsenic concentration in *Dunaliella* sp. and its incubation temperature, provided that the arsenic concentration in the medium was 1 mg dm^{-3}. In practice, *Dunaliella* sp. was added to the medium and incubated for 8 h (the optimum arsenic absorption time of *Dunaliella* sp. is 8 h[16]) and was then separated from the medium by a refrigerated centrifuge (5°C, 1500 rpm). The accumulation of arsenic by *Dunaliella* sp. was a maximum of 2000 μg g^{-1} at 22°C over 8 h. However, if the temperature rose to 22°C or more, the arsenic content then decreased linearly and reached 1100 μg g^{-1} at 33°C. (The optimum growth temperature range of *Dunaliella* sp. is 20–30°C in an arsenic-free medium.[17]) Controls with *Dunaliella* sp. cells incubated after being killed by heating to 90°C for 10 min showed no measurable uptake of arsenic.[16] These results show that the accumulation of arsenic is dependent on temperature and is greatly affected by metabolic activity in the algae.

Effect of illumination on the accumulation of arsenic by *Dunaliella* sp.

The growth of the algae depends greatly on the amount of light energy received because the algae propagate by photosynthesis. The accumulation of arsenic by

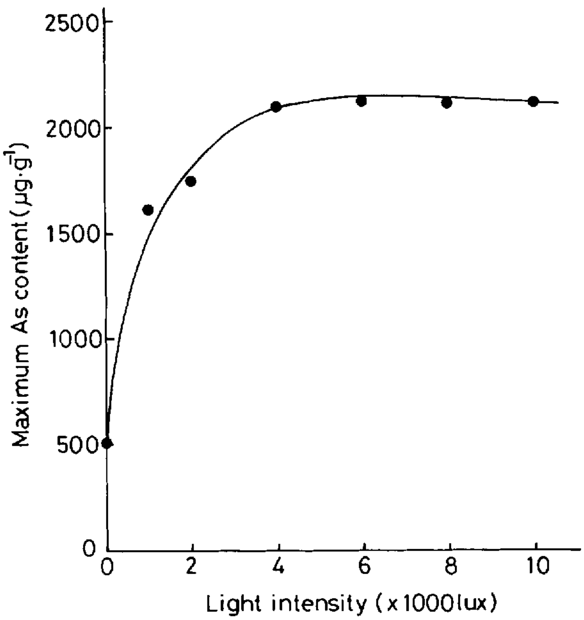

Figure 3 Effect of illumination on the uptake of arsenic by *Dunaliella* sp.

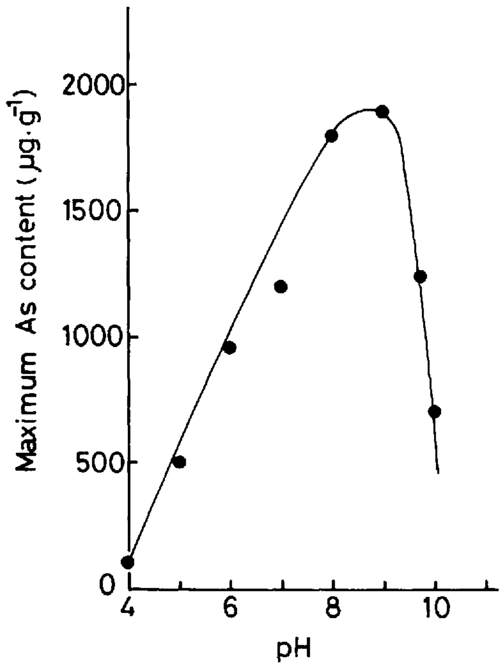

Figure 4 Effect of pH on the uptake of arsenic by *Dunaliella* sp.

Dunaliella sp. was tested in the illumination range from 0 to 1000 lux. Figure 3 showed the relationship between the accumulation of arsenic by *Dunaliella* sp. and illumination. Light intensity was adjusted to the desired level of illumination by changing the position of a fluorescent lamp (40 W). The measured illumination was the illumination at the medium surface and the arsenic concentration in the medium was 1 mg dm^{-3}. The accumulation of arsenic increased until the illumination rose to 5000 lux and was kept constant within the range 5000–10000 lux. The growth of *Dunaliella* sp. in an arsenic-free medium was unchanged within the range of optimum illumination 4000–10000 lux.[17] These results indicated that arsenic added to the medium caused a reduction in the range of optimum illumination for *Dunaliella* sp. growth.

Effect of pH on the accumulation of arsenic by *Dunaliella* sp.

The algae photosynthesize by the use of carbonate in water, so the pH of water containing algae turns alkaline due to consumption of the carbonate. The pH of seawater is 8.2. Generally, accumulation of elements by biological materials depends on the pH values of the cultures. The accumulation of arsenic in the *Dunaliella* sp. was examined in the pH range 4–10.

Figure 4 shows the relationship between the accumulation of arsenic by *Dunaliella* sp. and the pH values of the cultures. The accumulation of arsenic in *Dunaliella* sp. increased linearly to approximately 2000 μg dm^{-3} within the pH range 4–9 and at pH ≥ 9 it decreased abruptly. The optimum pH for *Dunaliella* sp. growth is 6–8 in an arsenic-free medium.[17] Thus, the optimum pH for *Dunaliella* sp. growth in the medium containing arsenic shifted to the alkaline side. In the following experiments, to approximate the usual pH of seawater, the pH of the arsenic solution was adjusted to 8.2.

Effect of sodium chloride concentration on the accumulation of arsenic by *Dunaliella* sp.

The accumulation of arsenic by *Dunaliella* sp. was tested in a sodium chloride (NaCl) concentration range from 1 to 100 g dm^{-3}. The relationship between the accumulation of arsenic by *Dunaliella* sp. and the concentration of sodium chloride in the medium is shown in Fig. 5. The accumulation of arsenic by *Dunaliella* sp. increased and reached 1700 g dm^{-3} as the sodium chloride concentration was increased from 2 to 20 g dm^{-3}. After that, it decreased abruptly and was hardly found at 100 g dm^{-3} sodium chloride. For *Dunaliella* sp. in an arsenic-free medium the optimum sodium chloride concentration for growth is from 0.1

to 0.5 mol dm^{-3}.[17] Thus, the optimum sodium chloride concentration range for growth became narrower in the medium containing arsenic than in the medium without arsenic.

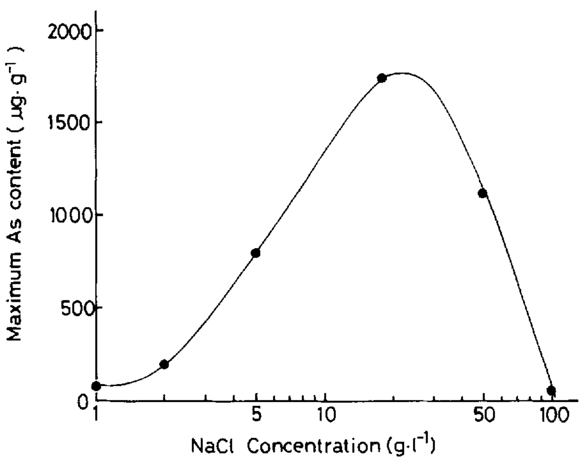

Figure 5 Effect of the concentration of sodium chloride on the uptake of arsenic by *Dunaliella* sp.

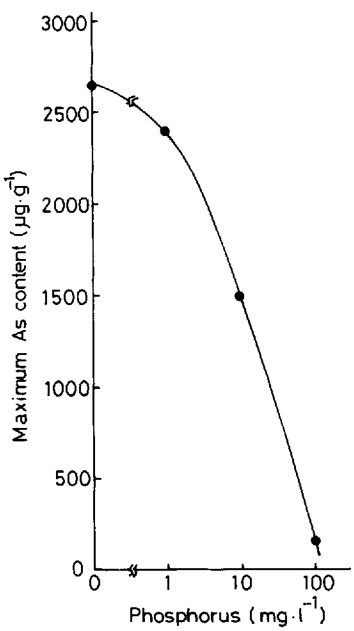

Figure 6 Effect of the concentration of phosphorus on the uptake of arsenic by *Dunaliella* sp.

Effect of phosphate (KH_2PO_4) concentration on the accumulation of arsenic by *Dunaliella* sp.

Phosphorus and arsenic belong to the same Group in the Periodic Table of the elements and resemble each other in behavior. The accumulation of arsenic by *Dunaliella* sp. was examined at a KH_2PO_4 concentration range from 0 to 100 mg dm^{-3}. Figure 6 shows the relationship between the accumulation of arsenic by *Dunaliella* sp. and the phosphorus concentration in the medium. The accumulation of arsenic decreased abruptly as the phosphorus concentration in the medium was increased.

The total arsenic concentration of *Skeletonema costatum* grown in bath cultures was also affected by phosphate additions.[3] Increased phosphate significantly decreased the uptake of arsenic in that culture and also reduced the total arsenic concentration by an order of magnitude in *Skeletonema costatum* grown under arsenic(V) enrichment.[3]

Maeda[9] has recognized, for *Chlorella vulgaris* in fresh water, a tendency similar to our experimental result and believes that phosphorus competes with arsenic. It is more likely that arsenic is taken up as arsenic(V) since the most common biological uptake pathway for arsenic is via the phosphate active transport system.

However, Matuto[18], for *Phorimidium* sp. algae, and Budd,[19] in a study on freshwater algae, believed that the accumulation of arsenic was not dependent on the concentration of phosphorus and was accomplished by an independent metabolic process.

Figure 7 shows the accumulation of phosphorus and arsenic in the growth process of *Dunaliella* sp. The accumulation of phosphorus by *Dunaliella* sp. was approximately 22% of dry algae weight within the logarithmic growth phase but decreased to approximately 10% of its logarithmic growth phase accumulation within the stationary growth phase. Arsenic was accumulated at an abnormally high concentration by *Dunaliella* sp. within the logarithmic growth phase, the same as the phosphorus, but within the stationary growth phase the concentration of arsenic decreased to 30% or less of that within the logarithmic growth phase. Within the logarithmic growth phase, the arsenic is accumulated in the forms of arsenic(V) (78.2%), arsenic(III) (18.3%), and organic arsenic (<1.0%), i.e. mostly in the form of arsenic(V), whereas within the stationary growth phase it is accumulated in the forms of arsenic(V) (66.7%), arsenic(III) (17.3%) and organic arsenic (3.3%).[16]

Andreae and Klumpp[4] also documented reduction and subsequent production of methylated arsenic for

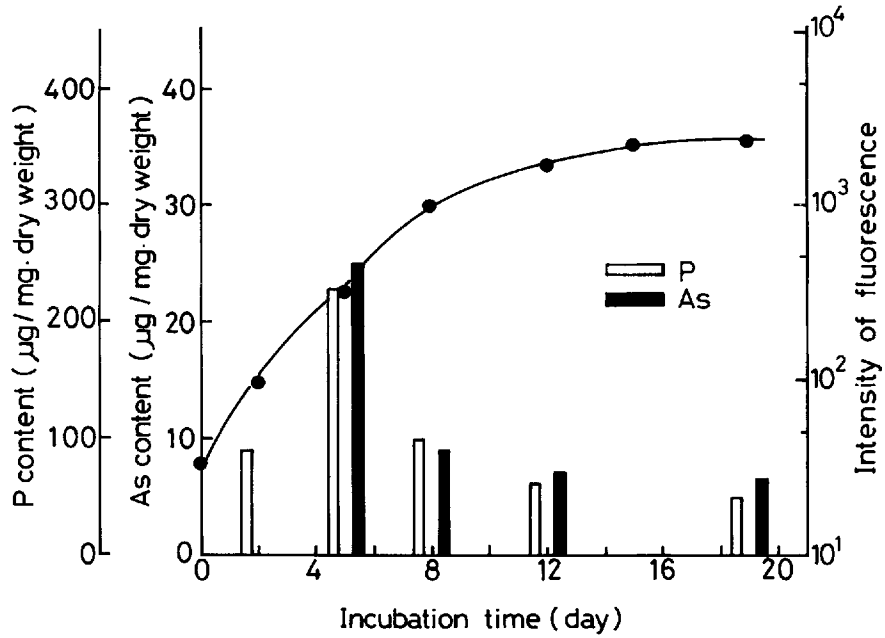

Figure 7 Time course of arsenic and phosphorus uptake by *Dunaliella* sp.: ●, growth curve; ■, arsenic in algae; □, phosphorus in algae.

several species of algae. Generally, phosphorus is accumulated as polyphosphoric acid within the logarithmic growth phase.[20] We presume that phosphorus and arsenic in *Dunaliella* sp. are metabolized by similar processes.

Distribution of arsenic taken up by *Dunaliella* sp.

To investigate the distribution of arsenic in *Dunaliella* sp. cells, the cells taking up arsenic were fractionated as described below. Deionized water (100 cm^3) was added to 5 g of powdered dry samples of *Dunaliella* sp. After standing overnight, the solution was filtered through a glass filter (Whatman GF/F, 25 mm). Water extraction was repeated once. The filtrate was concentrated with a vacuum rotary evaporator (30°C, water bath). These concentrated water extracts of algae were applied to the bottom of a gel chromatography colum[21] (Sephadex G-25 fine, 25 mm i.d. × 100 mm, 0.05 mol dm^{-3} sodium chloride, flow rate 1 cm^3 min^{-1}). About 94% of the total arsenic in *Dunaliella* sp. was extracted into water (Table 1). Oil-soluble (lipid fraction) arsenic occupied 3.8% of the entire accumulation and was present to a small extent. Wrench,[8] however, has reported that *Dunaliella* sp. contains 91% arsenic in the lipid form. From the difference between two results, we presume that the form of arsenic accumulation varied in conformity to the kind of algae. In addition, Wrench[8] also recognized that arsenic was transformed into inorganic arsenic and monomethylarsenic in *Dunaliella* sp. The arsenic in the residue (cell walls fraction) was at the same level as that in the water extracts (intracellular fraction). Thus, the ratio of arsenic in cells to total arsenic varies because the form in which arsenic exists depends on the growth environment. In future, we intend to study the change in the forms and ratios of the arsenic species in detail.

Table 1 Distribution of arsenic within cells[a]

Fraction	Arsenic (μg)	Distribution ratio (%)
Lipid	0.23	3.8
Intracellular	5.66	93.9
Cell wall	0.14	2.3

[a] The cells (dry weight: 200 mg) which absorbed arsenic were fractionated according to the procedure shown in the text.

CONCLUSION

The bioaccumulation of metals such as uranium or lithium[22,23] in algae is physical and mainly comprises chemical adsorption to the cell wall components; it does not directly affect the life cycle activity of cell. On the other hand, arsenic accumulated in *Dunaliella* sp. has an effect on the life cycle activity of cells (e.g. phosphorus uptake and its accumulation is dependent on changes in growth environmental factors). Research on the ability of marine algae to bioaccumulate arsenic is of appreciable practical importance. *Dunaliella* sp. can be used to monitor the presence of arsenic in wastewaters and can therefore serve as a biological indicator. Since algae are inexpensive to grow in continuous cultures, they offer an alternative approach to the industrial arsenic recovery processes which are presently employed.

Aknowledgements The authors wish to thank Dr Shigeru Maeda, University of Kagoshima, for his important suggestions concerning this work.

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