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# Accumulation of Arsenic(III) by *Chlorella vulgaris*

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**Arsenic occurs naturally in the environment and also through agricultural and industrial pollution. Since arsenic species show different toxicities, it is important to be able to separate them. Methods using microorganisms are being applied increasingly to remove metal ions and different metal species from aqueous solutions. Accumulation of As(III) by *Chlorella vulgaris* algae was studied, including various factors that influence on accumulation capacity, e.g. pretreatment of the algae (live, dry and lyophilized algae), temperature (4, 22, 37 and 100 °C), pH and exposure time of the algae to arsenic solutions. The pH appears to be the most critical factor, probably due to the species presenting different charges with pH variation. For arsenic species determination, hydride generation atomic absorption spectrometry (HG–AAS) was employed.** 

**Keywords: arsenic; *Chlorella vulgaris*; HG–AAS; accumulations**

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## INTRODUCTION

Metal uptake by microorganisms has been studied for some years.[1] The ability of algae to accumulate trace metals by biosorption is quite rapid and frequently selective.[1–4] Biosorptive interactions may occur with either living or dead microorganisms and do not necessarily require biological activity. Metal accumulation generally comprises an initial stage, rapid and reversible binding of the metal to the cell wall surface layers and, in some cases, a subsequent transport of the metal across the membrane.

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Arsenic is an interesting element to study and has an special importance because its compounds are widely used and their toxicity depends very much on their molecular form, the inorganic species being more toxic than the organic ones. Different instrumental methods have therefore been proposed for arsenic species separation. On the other hand, *Chlorella vulgaris* has been observed to accumulate total arsenic;[5–7] its cell wall has a complex chemical composition, with quantities of polysaccharides, uronic acids and proteins,[8] so this alga offers a range of potential binding sites for metal ions; this fact makes possible separation of metal species. In this paper we present a study of arsenite accumulation by *Chorella vulgaris* in order to separate this species from the others: arsenite has special importance since it is the most toxic arsenic species. This study could open new pathways to detoxification or even to analytical arsenic speciation.

## EXPERIMENTAL

### Instrumentation

A Perkin-Elmer model 2380 atomic absorption spectrophotometer, coupled with a continuous manifold to generate arsine, was employed. A four channel peristaltic pump, Gilson mod. Miniplus 3, coupling with a gas liquid separator was used for the on-line hydride generation process. An atomic absorption spectrophotometer Perkin Elmer mod. 2380 was employed as detector. A hollow cathode lamp working at 16 mA was used as the element specific high source. Absorption was measured at the 193.7 nm line with a spectral band pass of 0.7 nm.

### Reagents

Stock standard solutions of arsenic compounds (1000 mg $l^{-1}$) were prepared by dissolving adequate quantities of $NaAsO_2$ (Panreac), $CH_3AsO$-

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$(ONa)_2{\cdot}6H_2O$ (Carlo Erba), $(CH_3)_2AsNaO_2{\cdot}3H_2O$ (Merck) and $Na_2HAsO_4{\cdot}7H_2O$ (Panreac) in ultrapure water. All other chemicals were reagent grade.

## Culture conditions

*Chlorella vulgaris* Cultures were grown in an aquarium in glass bottles of 1 litre capacity, each one with an air flow of $10\,l\,h^{-1}$, at a constant temperature of 22 °C and the cultures were illuminated for 16 h every day. The algae was harvested in two different macronutrient media; one (A) had limited phosphate and nitrate concentrations and its composition (mg $l^{-1}$) was: $NaNO_3$ 25.4; $NaHCO_3$ 15; $K_2HPO_4$ 1.04; $MgSO_4{\cdot}7H_2O$ 14.7; NaCl 5.7; $CaCl_2{\cdot}2H_2O$ 4.4; and the other (B) contained a sufficient amounts of these essential nutrients and its, composition (mg $l^{-1}$) was: $NaNO_3$ 1000; $K_2HPO_4$ 75; $KH_2PO_4$ 175; $MgSO_4{\cdot}7H_2O$ 750; NaCl 250; $CaCl_2{\cdot}2H_2O$ 250. The micronutrient medium, composition (mg $l^{-1}$) $H_3BO_3$ 186; $MnCl_2$ 264; $ZnCl_2$ 3.2; $CuCl_2$ $9 \times 10^{-3}$; $CoCl_2$ 0.7; $FeCl_3$ 96; $Na_2MoO_4{\cdot}2H_2O$ 7.2; $Na_2EDTA{\cdot}2H_2O$ 300, was added to the macronutrient media in the ratio of 3 ml (micro) per litre (macro). The media and all materials used were sterilized by autoclaving.

## Analytical procedure

**Live *C. vulgaris***

The volume of live algae to be used for the following experiments was taken from the culture growth bottle, a fixed volume of *C. vulgaris* was centrifuged at 3000 rpm for 5 min, the supernatant (containing the nutrient medium) was discarded and the wet live algae were collected. The standard solutions of the arsenic species were prepared in pure water and added to the collected live *C. vulgaris.* They were in contact at the optimum pH for a fixed time with continuous stirring. The supernatant (containing the potential arsenic species) was separated from the algae, and the arsenic species in it were determined by continuous-flow HG–AAS. The amount of algae used, expressed as dry weight, were measured by collecting the live algae, once they had been used for the study of arsenic accumulation, drying them for 6 h at 90 °C, then weighing them until a constant weight was achieved.

**Dry and lyophilized *C. vulgaris***

A volume of live algae taken from the culture growth bottles was centrifuged for the same time and at the same speed as in the previous study. The supernatant was eliminated and the centrifuged alga biomass was dried at 90 °C for 6 h. A portion of dry *C. vulgaris* was weighed in a centrifuge tube. The same procedure as for live *C. vulgaris* was followed. The same study was performed with lyophilized *C. vulgaris* and the results were similar to those obtained with the dry algae. Sonication was also used instead of stirring, without improving the results.

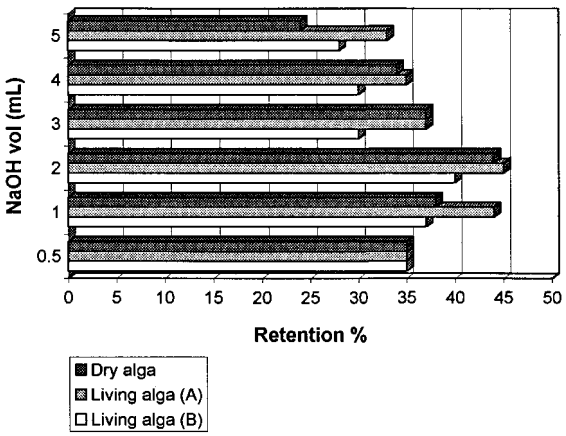


**Figure 1** Effect of volume of 1 M NaOH on retention of As(III) in live algae (A, B) and dry algae.

# RESULTS AND DISCUSSION

## Acidic/basic conditions and temperature studies to separate As(III)

The influence of pH on the ability of *C. vulgaris* to accumulate As(III) was studied in the 3–11 pH range. It was observed that As(III) was not significantly accumulated by the algae until pH = 10 was achieved. Retention therefore increased with an increase of pH (adjusted in the arsenic aqueous solution with 1 M NaOH). Since the reading at high pH using a pH-meter was not reproducible, in order to obtain good results we studied the use of different volumes of NaOH at a determined concentration: thus several different volumes of 1 M NaOH were used with 20 ml of live algae and 10 ml of As(III) solution at a concentration of 50 μg $l^{-1}$. The contact time was 15 min and the temperature was 22 °C. The results are shown in Fig. 1. It can be observed that for both live (A, B) and dry algae the optimum volume of 1 M NaOH was 2 ml.

To study the temperature effect, 20 ml of live algae were used with 10 ml of As(III) solution at a

 

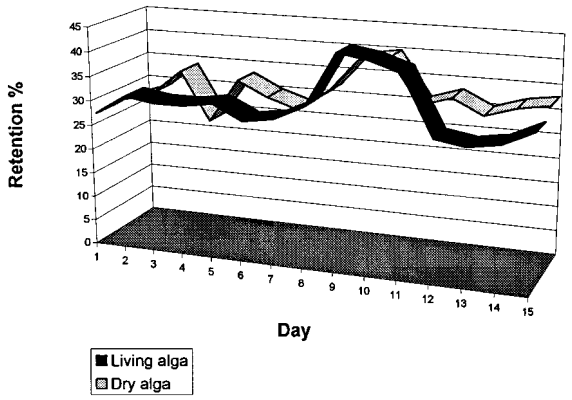


**Figure 2** Growth curves for live and dry algae, showing effect on As(III) retention.

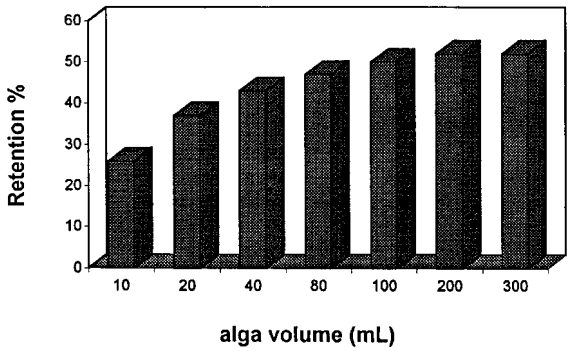


**Figure 3** Influence of volume of live algae (A) on As(III) retention.

concentration of 50 μg $l^{-1}$ 15 min of contact time at different temperatures. The accumulation of As(III) obtained at 4, 22, 37 and 110 °C was 18, 43, 40 and 24% respectively. At 110 °C the results were similar to those obtained for the dry algae, because at that temperature it is assumed that the algae are then dead. The highest As(III) accumulation occurred between 22 and 37 °C, where the algae maintain all its living characteristics. This temperature study has no purpose for dry algae, because they have already been dried at high temperature.

## Ageing and growth of live and dry *C. vulgaris*

Ageing and growth of algae were studied to investigate the influence of these parameters on As(III) accumulation: 10 ml of 50 μg $l^{-1}$ As(III) solution and 2 ml, of 1 M NaOH were employed at 22 °C for 15 min. The results showed that with algae ageing there were no significant changes, although growth curves for both live and dry *C. vulgaris* showed increased accumulation between the ninth and tenth days (see Fig. 2).

## Volume of *C. vulgaris*

**Live** *C. vulgaris*

Various volumes of algae whose densities were expressed as dry weight, were studied to determine the accumulation capacity for each of the live *C. vulgaris* (A, B); the compositions of the nutrient media are described above under 'Culture conditions'. When live *C. vulgaris* with limited phosphate and nitrate concentrations (A) was used, accumulation increased with the algae volume. The highest accumulation, 52%, was obtained from 100 ml (equivalent to 8 mg dry weight) of live algae and it remained constant at higher algae volumes (see Fig. 3). Although live *C. vulgaris* with a sufficient amount of these essential nutrients (B) showed that 20 ml of live algae (equivalent to 3 mg dry weight) were enough to obtain the highest accumulation, 10, 20 and 40 ml of live algae gave 30, 43 and 40% accumulation respectively.

It can be seen that live algae grown with medium A, with limited concentrations of nutrients, phosphate and nitrate, appeared to have a higher accumulation than B. Since $AsO_3^{3-}$ has a chemical similarity to $PO_4^{3-}$ and $NO_3^-$, it is possible that replacement of those nutrients by $AsO_3^{3-}$ could occur.

## Dry *C. vulgaris*

It was proved that for various amounts of dry algae (5, 10 and 15 mg), obtained as described, above under 'Analytical procedure' there were no differences in As(III) accumulation: 5, 10 and 15 mg of dry algae produced an accumulation of 42, 41 and 40% respectively.

This situation is probably due to the existence of different accumulation mechanisms: one in which the binding of the arsenic species to the cell surface occurs by adsorption and another mechanism consisting of As(III) transport through the cell membrane. Since the latter needs biological activity, our conclusion is that it might not occur when dry algae are employed.

## Contact time

As(III) accumulation experiments with different contact times were performed with the A and B live algae and the dry algae (Table 1). Similar results

**Table 1** Effect of contact time on the retention of live (A, B) and dry algae

| Contact time | Retention (%) Live algae (A,B) | Dry algae |
|---|---|---|
| 5 min | 24 | 23 |
| 15 min | 41 | 38 |
| 30 min | 41 | 38 |
| 1 h | 42 | 41 |
| 2 h | 45 | 40 |
| 8 h | 45 | 44 |
| 16 h | 48 | 48 |
| 24 h | 47 | 45 |
| 48 h | 45 | 44 |

were obtained for all these experiments. It was observed that with increasing contact time there were no significant improvements, so 15 min was enough to achieve the best As(III) accumulation.

## As(III) concentration – dependent uptake

The rate of As(III) uptake by *C. vulgaris* was investigated by using 100 ml of live (A) algae on 5 mg of dry algae in 10 ml of aqueous solutions at various As(III) concentrations (25, 50 and 100 $\mu g\, l^{-1}$), which were added to the algae. It appears that the percentage accumulation of As(III) in both live (A) and dry algae cells depends on concentration; it showed saturation when the concentration was higher than 50 $\mu g\, l^{-1}$.

## As(III) separation from the other species

In order to study As(III) separation from As(V), monomethylarsenic (MMA) and dimethylarsenic (DMA), the accumulation for all these species was studied using the optimal conditions: 100 ml of living algae (A) on the ninth day of growth, 10 ml of each arsenic species at a concentration of 25 $\mu g\, l^{-1}$ at 22 °C, with 15 min contact time, 2 ml of 1 M NaOH, and continuous stirring. As can be seen in Fig. 4 an accumulation of 52% was obtained for As(III) and under these conditions none of the arsenic species was accumulated by *C. vulgaris.*

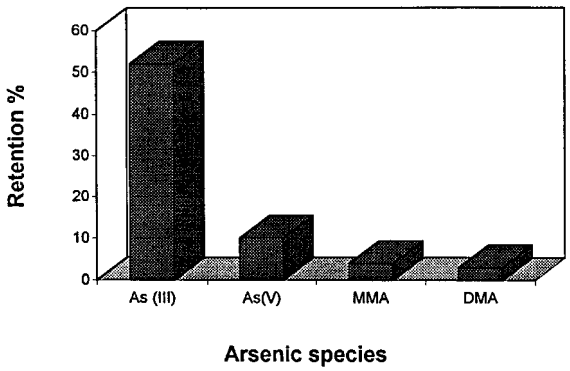


**Figure 4** Retention of various arsenic species by *C. vulgaris.*

## CONCLUSION

This study demonstrates that *C. vulgaris* can be used to obtain a reproducible and selective separation of about 50% of As(III). Various factors that influence on accumulation capacity were studied and the acid/basic condition appears to be the most critical factor; a high basic pH in aqueous arsenic solutions was necessary to obtain significant As(III) accumulation and no accumulation of the other species was observed.

Other factors studied have shown no significant influence on the accumulation, except the different algae culture nutrient media and the pretreatment of the algae; the best accumulation was obtained when live algae were used with limited essential nutrients (medium A) giving on accumulation of As(III) of 52%.

Although total separation of As(III) is not reached under these conditions this study is part of a larger project in which we will try to improve the accumulation. Perhaps the most important conclusion from this study is that the use of algae could open new pathways in metal speciation and detoxification.

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