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Effect of salinity and pH on cobalt biosorption by the estuarine microalga *Chlorella salina*

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Summary. Accumulation of cobalt (^{60}Co) by the estuarine microalga *Chlorella salina* has been characterized. At cobalt concentrations ranging over 3.125–100 μM, a significant amount of cobalt was bound within 1 min. This was metabolism-independent and unaffected by incubation in light or dark conditions. This initial rapid phase of biosorption was followed by a slower phase of uptake which was apparently active and inhibited by incubation in the dark, or by the uncoupler dinitrophenol and the respiratory and photosynthetic inhibitor potassium cyanide in the light. For cells suspended in 10 mM Taps pH 8, cobalt biosorption followed a Freundlich adsorption isotherm. However, in the presence of 0.5 M NaCl, biosorption deviated from the Freundlich model because of competition by Na^+. Cobalt biosorption was decreased by increasing concentrations of Na^+, decreasing pH and the presence of Cs^+, Li^+, Rb^+, Zn^{2+}, Mn^{2+} and Sr^{2+} (added as chlorides). This was a result of competition between Co^{2+} and the other cations, including H^+, for available binding sites on the cell wall and was confirmed by increased desorption of cobalt by solutions of low pH or high salinity. Increasing cell density resulted in increased removal of cobalt from solution but decreased the specific amount of cobalt taken up by the cells.

Key words: Biosorption – Cell density – *Chlorella salina* – ^{60}Co – pH – Salinity

Introduction

With the development of nuclear power and the widespread use of radioisotopes in medicine and other industries, levels of radionuclides in the world's oceans and estuaries have increased (Clark 1989). The fate of these radionuclides after release in waste and the possibility of accumulation in the biota to harmful levels have become a matter for great pubic concern (Camplin et al. 1986; Fisher et al. 1988; Mann et al. 1988; Clark 1989). ^{60}Co is a radionuclide found in waste streams from nuclear power installations and is frequently released into the sea (Ashley et al. 1987). It is also used extensively in medicine as a tracer and in radiotherapy (Greenwood and Earnshaw 1984) and is finally dumped at sea. ^{60}Co has a half-life of 63.2 months and therefore significant amounts can remain active in the natural environment for long periods. Non-radioactive cobalt is itself toxic at high levels and is used widely in the paint industry and in the production of stellite alloys (Greenwood and Earnshaw 1984); cobalt-containing waste again finds its way via rivers into marine environments.

Little information is known about cobalt accumulation by estuarine microalgae, which could be an important step in its further assimilation into other organisms via estuarine and marine food chains. Coleman et al. (1971), Nakajima et al. (1981) and Mahan et al. (1989) have shown that cobalt can be taken up by freshwater species of *Chlorella*; the ability of microalgae to accumulate other radionuclides/metals from aqueous solution is well documented (see Gadd 1988 and Reed and Gadd 1990 for reviews). However, there are no studies which examine the mechanism of cobalt uptake in detail or assess those abiotic factors that can affect it in either freshwater or marine microalgae.

In this study we have investigated cobalt uptake by *Chlorella salina* Kufferath, a green microalga commonly isolated from estuarine environments and also frequently associated with domestic sewage effluent (Wong et al. 1979). In addition, the effects of salinity, pH, competing ions and cell density on accumulation have been investigated.

Materials and methods

Organism and growth conditions. Axenic cultures of *Chlorella salina*, Kufferath CCAP 211/25 (obtained from Dr G. Russell, Department of Evolutionary and Environmental Biology, University of Liverpool, P. O. Box 147, Liverpool, L69 3BX) were grown at

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22° C in 100 ml MN medium which comprised: 0.04 g $MgSO_4 \cdot 7H_2O$, 0.02 g $CaCl_2 \cdot 2H_2O$, 0.75 g $NaNO_3$, 0.02 g K_2HPO_4, 0.03 g citric acid, 0.003 g ferric ammonium citrate, 0.0005 g Na_2EDTA, 0.02 g Na_2CO_3 and 10 ml trace metal mix A5 [2.86 g H_3BO_3, 1.81 g $MnCl_2 \cdot 4H_2O$, 0.222 g $ZnSO_4 \cdot 7H_2O$, 0.039 g $Na_2MoO_4 \cdot 2H_2O$, 0.079 g $CuSO_4 \cdot 5H_2O$, 0.0494 g $Co(NO_3)_2 \cdot 6H_2O$ in 1 l distilled water] in 1 l 75% (vol./vol. distilled water) filtered sea water (Waterbury and Stanier 1978). For some experiments, *C. salina* was grown in 100 ml BG11 medium which comprised: 1.5 g $NaNO_3$, 0.04 g K_2HPO_4, 0.075 g $MgSO_4 \cdot 7H_2O$, 0.036 g $CaCl_2 \cdot 2H_2O$, 0.006 g citric acid, 0.006 g ferric ammonium citrate, 0.001 g Na_2EDTA, 0.02 g Na_2CO_3, 1 ml trace metal mix A5 (see above) in 1 l distilled water (Allen 1968). Both media were adjusted to pH 8 with tetramethylammonium hydroxide and autoclaved (120° C, 15 min) before being inoculated to approximately 2×10^5 cells ml^{-1}. Cultures were incubated in 250-ml conical flasks with rotary incubation at 150 cycles min^{-1}, at 23° C and with a photon fluence irradiance, incident on the surface of the flasks, of 12 μmol photon $m^{-2}\,s^{-1}$ provided by white fluorescent tubes.

Cobalt uptake. Cultures in the exponential phase of growth (approximately 20-day incubation) were harvested by centrifugation (5 min, 1000 *g*). The supernatant was removed and the cells were then washed once with 10 mM 3-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-amino}-1-propanesulfonic acid (Taps) with or without 0.5 M NaCl, and adjusted to pH 8 with solid tetramethylammonium hydroxide. Cells were again centrifuged (5 min, 1000 *g*) and resuspended in fresh buffer to a density of approximately 4×10^6 cells ml^{-1} unless otherwise stated. For ^{60}Co uptake, 10-ml exponential cell suspensions were incubated in universal bottles on a magnetic stirrer in the light (30 μmol photon $m^{-2}\,s^{-1}$) at 23° C unless stated otherwise. Aliquots from a 500 μM $CoCl_2$ solution containing 4.76 μCi ^{60}Co (supplied by Amersham International as cobaltous chloride in 0.1 M HCl giving a $CoCl_2$ solution of concentration 0.119 nM) were added to give cobalt concentrations in the range 3.125–100 μM. Samples (200 μl) were taken from the cell suspensions at time intervals after the addition of the $CoCl_2 + {}^{60}Co$ cocktail and centrifuged through a layer (200 μl) comprising 40% (by vol.) Dow Corning 550 silicone oil and 60% bis 3,3,5-trimethylhexylphthalate (Fluka) in 500-μl Beckmann PRO22 plastic tubes using an Eppendorf 5412 microcentrifuge (30 s, 8000 *g*). The bottom of each tube containing the cell pellet was cut off and placed in 5 ml Ecoscint A scintillation fluid (National Diagnostics, Maville NJ) for 24 h before measuring radioactivity (at an efficiency of at least 30%) using a Packard Minaxi tri-carb 4000 scintillation counter. For calculation of specific activity, 1 ml of the remaining cell suspension was placed in the microcentrifuge (1 min, 8000 *g*), and two replicate 200-μl samples taken from the supernatant. Where desired, cells were pretreated 30 min prior to harvesting with 50 μM dinitrophenol or 100 μM potassium cyanide. Monovalent and divalent cations were added to the cell suspensions as chlorides to give final concentrations of 25 μM before ^{60}Co addition. An uptake was also performed with cells in MN media.

Effect of NaCl on cobalt uptake. ^{60}Co uptake experiments were performed in 10 mM Taps pH 8 with NaCl concentrations ranging over 0–0.5 M (approximate sea water concentration). *C. salina* grown in BG11 medium (Allen 1968), which is low in NaCl, was also used for uptake experiments. To establish whether any effects of 0.5 M NaCl on ^{60}Co uptake were osmotically related and/or due to Na^+, uptake experiments were performed using 10 mM Taps + 0.5 M KCl and 10 mM Taps + 0.25 M Na_2SO_4 both pH 8. The $CoCl_2$ concentration was 25 μM in all cases.

Effect of pH on cobalt uptake. Uptake experiments were performed with cells resuspended in the following buffers and with a $CoCl_2$ concentration of 25 μM: 10 mM Taps + 0.5 M NaCl, pH 8 and 9; 10 mM 2-(*N*-cyclohexylamino)ethanesulfonic acid (Ches) + 0.5 M NaCl, pH 9, 10 mM 4-(2-hydroxymethyl)-1-piperazineethanesulfonic acid (Hepes) + 0.5 M NaCl, pH 7 and 8; 1, 4-piperazinediethanesulfonic acid] (Pipes) + 0.5 M NaCl, pH 6 and 7; 4-morpholineethanesulfonic acid (Mes) + 0.5 M NaCl, pH 6.

Desorption of cobalt. Cell suspensions were prepared in 10 mM Taps pH 8 as previously described; 0.5 ml of the stock cobalt/^{60}Co solution was added to 9.5 ml of cell suspension to give a final $CoCl_2$ concentration of 25 μM. Cell suspensions were then incubated for at least 24 h in the light, as described above. Three 200-μl samples were then taken and measured for cobalt uptake. The remaining cells were then separated by centrifugation (5 min, 1000 *g*) and resuspended in 10 ml 10 mM Taps pH 8. Samples (200 μl) were taken in triplicate at time intervals and measured for cobalt uptake as described above. Where desired, the pH of the suspending medium was altered 30 min after resuspension to pH 6 by the addition of 250 μl 0.1 M HNO_3 or to pH 11 with 25 mg tetramethylammonium hydroxide. Where desired, NaCl and Na_2SO_4 were added 30 min after resuspension to give concentrations of 0.5 M and 0.25 M, respectively.

Chemicals and reagents. All chemicals used were of Analytical grade. Inhibitors, buffers and tetramethylammonium hydroxide were supplied by Sigma. Dow Corning 550 silicone oil was supplied by BDH (Dagenham, Essex, England) and bis(3,5,5-trimethylhexylphthalate) supplied by Fluka Chemie AG (Industriestrasse 25, CH-9470, Buchs, Switzerland).

Results

Cobalt uptake by Chlorella salina *and the influence of NaCl and other cations*

Uptake of cobalt by *C. salina* in 10 mM Taps pH 8 containing 0.5 M NaCl appeared to be biphasic. An initial phase was discernible in which approximately 0.23 nmol $(10^6 \text{ cells})^{-1}$ was accumulated within 5 min, at a rate of 0.25 nmol min^{-1} $(10^6 \text{ cells})^{-1}$, and a second slower phase in which approximately 0.57 nmol $(10^6 \text{ cells})^{-1}$ was accumulated over 7 h (Fig. 1). The initial phase was independent of light or the presence of KCN and dinitrophenol, which indicated that this accumula-

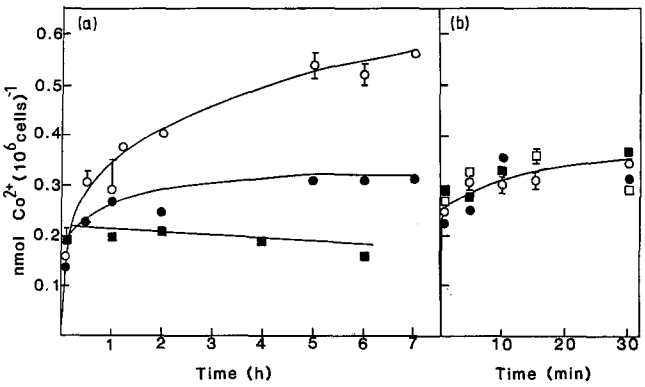

Fig. 1. Uptake of cobalt by *C. salina* at a cell density of 4×10^6 cells ml^{-1} in 10 mM Taps + 0.5 M NaCl, pH 8, with a $CoCl_2$ concentration of 25 μM at 23° C. (a) Long-term uptake isotherm; (b) short-term uptake isotherm; (○) light incubation; (●) dark incubation; (□) cells pretreated prior to incubation in the light with 100 μM KCN; (■) cells pretreated prior to incubation in the light with 100 μM dinitrophenol. Each point is a mean of three replicates, bars indicate standard error of mean and, when not shown, were smaller than the dimensions of the symbols used

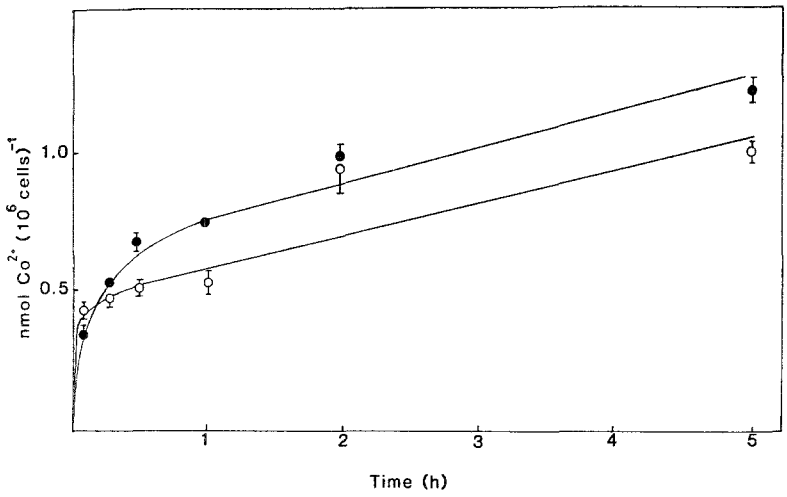

Fig. 2. The effect of resuspension medium on cobalt uptake by *C. salina* at a cell density of 4×10^6 ml^{-1} from a cobalt concentration of 50 μM at 23° C. (○) 10 mM Taps + 0.5 M NaCl, pH 8; (●) MN medium. Each point is a mean of three replicates, bars indicate standard error of mean and, when not shown, were smaller than dimensions of symbols used

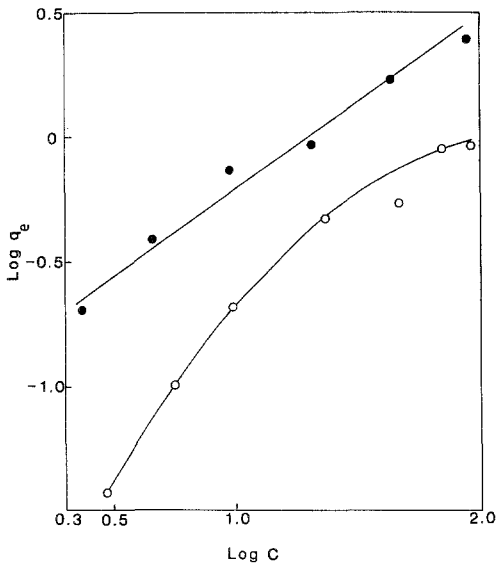

Fig. 3. Freundlich plot of cobalt biosorption by *C. salina* at a cell density of 4×10^6 ml^{-1} in (●) 10 mM Taps pH 8, or (○) 10 mM Taps + 0.5 M NaCl, pH 8, at 23° C. Each point is a mean of three replicates with a standard error not greater than 0.01. q_e = the quantity of cobalt adsorbed/mass adsorbent at a fixed temperature; C = the concentration of cobalt remaining in solution at equilibrium

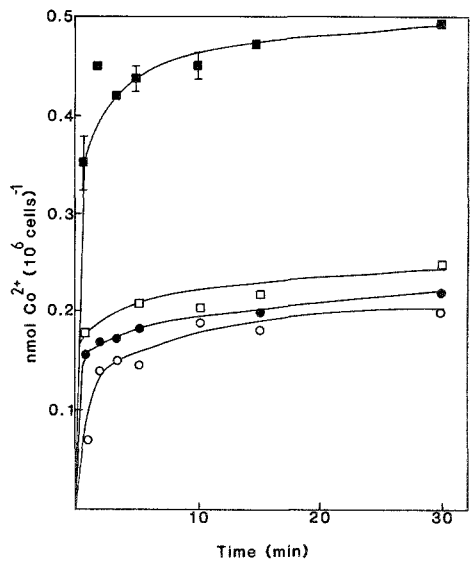

Fig. 4. Effect of Na^+ and K^+ on cobalt biosorption by *C. salina* cells at a cell density of 4×10^6 ml^{-1} in Taps pH 8, and with a $CoCl_2$ concentration of 25 μM. (■) 10 mM Taps pH 8 with no added Na^+/K^+; (□) 10 mM Taps + 0.5 M KCl, pH 8; (●) 10 mM Taps + 0.5 M NaCl, pH 8; (○) 10 mM Taps + 0.25 M Na_2SO_4, pH 8. Values are means of three replicates. Bars indicate standard errors and, when not shown, were smaller than the dimensions of the symbols used

tion of cobalt was a passive process. In contrast, the second phase was clearly dependent on light and could be inhibited by KCN and the uncoupler dinitrophenol, indicating that this uptake was an active process. Subsequent work has concentrated on the initial biosorptive phase, judged to be complete in 30 min (Fig. 1b). Uptake in MN medium was similar to that in Taps pH 8 + 0.5 M NaCl (Fig. 2). However, the initial binding phase was reduced slightly [0.43 as compared to 0.35 nmol (10^6 cells)$^{-1}$] although a slightly greater overall uptake was seen at the end of the second phase [0.93 as compared to 1.2 nmol (10^6 cells)$^{-1}$].

At all cobalt concentrations examined, biosorption of cobalt was less in Taps buffer containing 0.5 M NaCl than in NaCl-free Taps buffer (Fig. 3). A typical Freundlich adsorption isotherm (straight line; Freundlich 1926) was obtained with cells in NaCl-free Taps buffer but not with cells in Taps buffer with 0.5 M NaCl (Fig. 3). An external NaCl concentration of 0.5 M (= sea water) caused a reduction of approximately 60% in the biosorption of cobalt by the cells (Fig. 4). Such a reduction was also produced by an equivalent concentration of Na^+, as shown by the addition of 0.25 M Na_2SO_4 to the suspending medium, or by K^+, as shown by the addition of 0.5 M KCl (Fig. 4). The significant effect of Na^+ on cobalt biosorption is further illustrated in Fig. 5. Cells grown in BG11 medium (which has a low NaCl concentration of approximately 26 mM) or transferred into 10 mM Taps buffer from MN media (which has an NaCl concentration of ap-

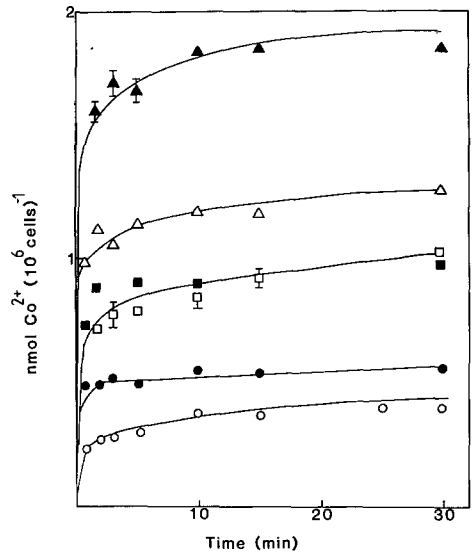

Fig. 5. Effect of growth medium, washing with distilled water and resuspension buffer, on cobalt biosorption by *C. salina* cells at an initial cell density of 4×10^6 ml^{-1} in 10 mM Taps pH 8, and a $CoCl_2$ concentration of 25 μM. (○) Cells previously grown in MN medium then transferred into Taps + 0.5 M NaCl, pH 8; (●) cells previously grown in MN medium, washed with distilled water and then transferred into Taps + 0.5 M NaCl, pH 8; (□) cells previously grown in BG11 medium then transferred into Taps + 0.5 M NaCl, pH 8; (■) cells previously grown in MN medium then transferred into Taps pH 8; (△) cells previously grown in MN medium washed with distilled water then transferred into Taps pH 8; (▲) cells previously grown in BG11 media then transferred into Taps pH 8. Values are means of three replicates. Bars indicate standard errors and, when not shown, were smaller than the dimensions of the symbols

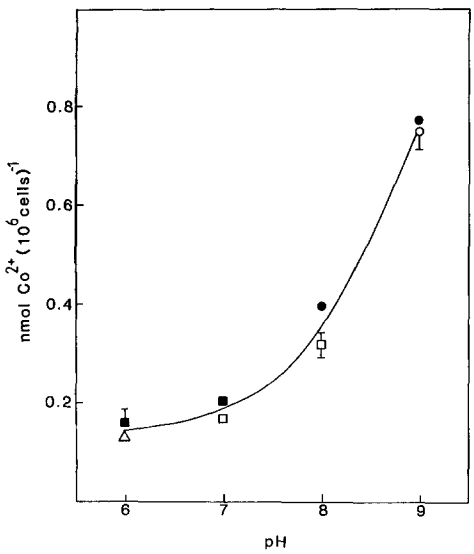

Fig. 6. Effect of pH on biosorption of cobalt by *C. salina* cultures all containing 4×10^6 cells ml^{-1} from a $CoCl_2$ concentration of 25 μM in the following buffer solutions containing 0.5 M NaCl: (○) 10 mM Ches; (●) 10 mM Taps; (□) 10 mM Hepes; (■) 10 mM Pipes; (△) 10 mM Mes. Values are means of three replicates, bars indicate standard errors and when not shown, were smaller than the dimensions of the symbols used

Table 1. Effect of monovalent cations on cobalt uptake by *C. salina* in the absence or presence of 0.5 M NaCl

Cation	Co^{2+} uptake [nmol (10^6 cells)$^{-1}$]		Reduction in uptake (%)	
	− NaCl	+ NaCl	− NaCl	+ NaCl
Cs^+	0.75	0.16	14	58
Rb^+	0.58	0.16	34	58
Li^+	0.61	0.51	23	60
K^+	1.05	0.32	0	15
Na^+	1.00	—	0	—

Cells were suspended in 10 mM Taps + 0.5 M NaCl, pH 8, to a cell density of approximately 4×10^6 cells ml^{-1}. Monovalent cations were added as chlorides to give final concentrations of 25 μM, 5 min after the $CoCl_2/^{60}Co$ cocktail was added to a concentration of 25 μM. The data are means of three replicate determinations after 30-min incubations; standard errors were less than 0.024

proximately 0.5 M) showed an approximate 150% increase in cobalt biosorption when compared to cells grown in MN media and then transferred into 10 mM Taps buffer containing 0.5 M NaCl. Washing cells grown in MN media with distilled water, which removed Na^+ from the cells, also increased cobalt biosorption by 12–30% compared to cells washed with 10 mM Taps buffer containing 0.5 M NaCl.

Other monovalent and divalent cations were in some cases shown to reduce cobalt biosorption in a similar way to Na^+. In the absence of 0.5 M NaCl, 25 μM K^+ or Na^+ showed no effect; Cs^+, Rb^+ and Li^+ reduced cobalt biosorption with Rb^+ exerting the greatest inhibition (34%; Table 1). However, in the presence of 0.5 M NaCl, Cs^+, Rb^+ and Li^+ all reduced cobalt biosorption by approximately 60%. K^+ also reduced cobalt biosorption but to a lesser extent (15%; Table 1). In the presence of 0.5 M NaCl, 25 μM Sr^{2+}, Zn^{2+} and Mn^{2+} reduced biosorption of cobalt by 20%, 17% and 2%, respectively. Other divalent cations tested Cu^{2+}, Ni^{2+}, Ca^{2+}, Mg^{2+}, and Cd^{2+}, had no effect upon cobalt biosorption. In the absence of NaCl, only 25 μM Sr^{2+} reduced cobalt biosorption by approximately 28%.

Effect of pH and cell density on cobalt biosorption

As the pH was reduced from 9 to 6, the amount of cobalt taken up by the cells after a 30-min incubation in a cobalt concentration of 25 μM decreased from 0.75 to 0.15 nmol (10^6 cells)$^{-1}$, a reduction of 80% (Fig. 6). With increasing cell density, the removal of cobalt from solution was increased. An approximate 22% removal of cobalt was achieved by a cell density of 4×10^7 cells ml^{-1}; the least removal was approximately 0.7% at a cell density of 2×10^6 cells ml^{-1} (Fig. 7a). However, as the cell density increased there was a decrease in the specific amount of cobalt taken up, when expressed/number of cells [(10^6 cells)$^{-1}$] (Fig. 7b).

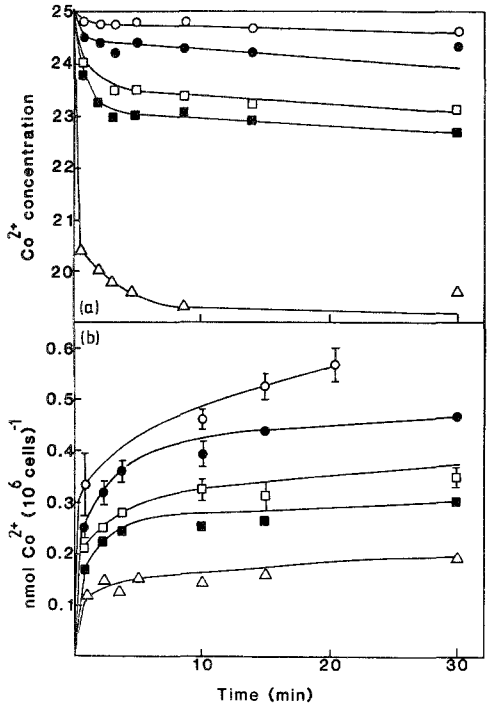

Fig. 7. The effect of cell density on (a) cobalt removal from solution and (b) biosorption of cobalt by *C. salina* cells in 10 mM Taps + 0.5 M NaCl, pH 8, with a $CoCl_2$ concentration of 25 μM. (△) 4×10^7 cells ml^{-1}; (■) 8×10^6 cells ml^{-1}; (□) 4×10^6 cells ml^{-1}; (●) 2×10^6 cells ml^{-1}; (○) 4×10^4 cells ml^{-1}. Values are means of three replicates, bars indicate standard errors and, when not shown, were smaller than the dimensions of the symbols used

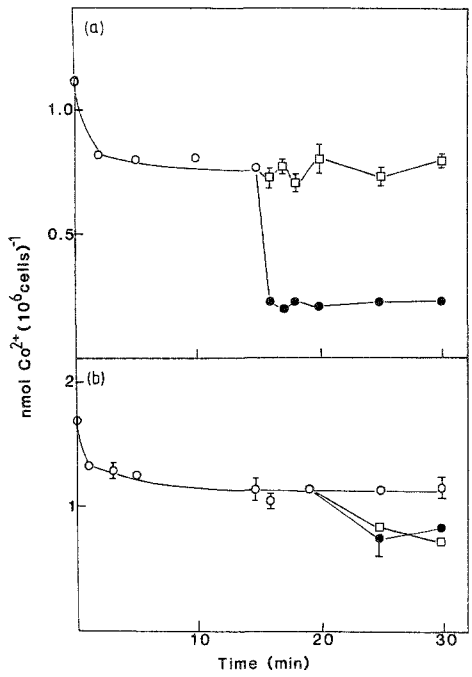

Fig. 8. Desorption of cobalt from *C. salina* preloaded by incubation in 25 μM cobalt/^{60}Co (4×10^6 cells ml^{-1}) in 10 mM Taps pH 8. (a) The effect of pH on desorption: (○) pH 8; (□) shift to pH 11; (●) shift to pH 6. (b) The effect of Na^+ on desorption; (○) control; (●) addition of NaCl to give a final concentration of 0.5 M; (□) addition of Na_2SO_4 to give a final concentration of 0.25 M. Values are means of three replicates, bars indicate standard errors and, when not shown, were smaller than the dimensions of the symbols used

Desorption of cobalt

Within 5 min after transfer of ^{60}Co-loaded cells of *C. salina* into fresh 10 mM Taps pH 8, lacking cobalt, cells lost approximately 28% of the total cobalt taken up over a 24-h incubation (Fig. 8). On reducing the pH to 6 by the addition of 4 M HCl, it was found that a further 56% of the total cobalt taken up was rapidly lost from the cells (Fig. 8a). A shift to pH 11 by the addition of tetramethylammonium hydroxide did not result in any loss of cobalt. The addition of NaCl and Na_2SO_4 to final concentrations of 0.5 M and 0.25 M, respectively, caused an approximate 25% loss of cobalt from the cells (Fig. 8b).

Discussion

Accumulation of heavy metals/radionuclides by microalgae has been described as consisting of two phases: a 'fast' phase which mainly refers to metabolism-independent binding to the cell wall (biosorption), followed by a 'slow' phase due to the simultaneous effects of growth and surface adsorption, active uptake or intracellular uptake by passive diffusion (Khummongkol et al. 1982; Gadd 1988). There is often little discrimination between these possible mechanisms in the literature (Khummongkol et al. 1982). Accumulation of cobalt by *C. salina* showed both fast and slow phases. The initial phase of uptake, which was independent of light and metabolic inhibitors, was considered to be biosorption, while the second slower phase of uptake, dependent on light and inhibited by the respiratory uncoupler dinitrophenol, was interpreted as an active uptake mechanism, as opposed to diffusion or increased binding due to growth. Trace amounts of cobalt must be taken up by the cells, since cobalt is essential for vitamin B_{12} synthesis and in the biomethylation of heavy metals by algae (Holm-Hansen et al. 1954; Brinckman and Olson 1988). An active mechanism for cobalt uptake of high affinity would be useful for *C. salina* since the average concentration of cobalt in the marine environment is approximately 0.3 nM (Clark 1989). Broda (1972) suggested active uptake of cobalt by *Chlorella fusca* although no experimental data were presented. To our knowledge, no other study has described active uptake of cobalt by a unicellular marine microalga, although there are several reports of active uptake of other heavy metals, e.g. cadmium in *Chlorella pyrenoidosa* (Hart and Scaife 1977) and zinc in *C. fusca* (Broda 1972). In this study, it was found that differences in cobalt uptake could only be detected between cells incubated in the light and those in the dark if cells had been left in 10 mM Taps buffer containing 0.5 M NaCl in the absence of light for at least 24 h before the uptake experiment. Such cells must utilise stored energy (starch

granules) during this dark starvation period which presumably could be used for an active uptake mechanism. This observation emphasises that precautions must be taken to ensure adequate dark starvation of algal cells, and also that uptake experiments in the presence of suitable inhibitors are performed before active uptake of a metal by microalgae can be confirmed.

This work has concentrated on the initial rapid phase of cobalt uptake, representing metabolism-independent biosorption. This has received little previous attention in microalgae despite it now being clear that such processes can be an important means by which heavy metals and radionuclides are transported in the marine environment (Fisher et al. 1983; Mann et al. 1988). Biosorption is also of importance if algae are considered as candidates for industrial metal removal. Many algae capable of metal biosorption to significant levels have been considered as systems for removal of metals from industrial effluent (Horikoshi et al. 1978; Greene et al. 1987; Greene and Darnall 1990; Gadd 1990). In the absence of NaCl, cobalt biosorption was found to conform to the Freundlich adsorption isotherm, which is assumed to indicate single layer adsorption (Freundlich 1926). In the presence of 0.5 M NaCl, cobalt biosorption was depressed and deviated markedly from the Freundlich model, presumably due to competition by Na^+. Other monovalent and divalent cations (Cs^+, K^+, Li^+, Rb^+, Sr^{2+}, Zn^{2+} and Mn^{2+}) also depressed cobalt biosorption to varying extents depending on the presence or absence of NaCl. Greene and Darnall (1990) explain such effects in terms of competition between ions for metal binding sites in algal biomass. Crist et al. (1981) showed that metal ion binding to the cell wall of the microalga *Vaucheria* occurred partly through an ion-exchange mechanism, with binding sites arising from amino and carboxyl groups as well as sulphates and imidazoles associated with polysaccharides and proteins in the cell wall. This ion-exchange mechanism was also used to describe cobalt biosorption by ground fragments of the brown macroalga *Ascophyllum nodosum* (Kuyucak and Volesky 1988). These authors suggested that alginates in the cell wall could play an important role in Co^{2+} binding. There are wide variations in cell wall composition among eukaryotic algae, the only wall component common to all being cellulose, poly(β-1:4-glucopyranose) (Bold et al. 1980). This is reflected in variations in binding of metals by different algal species. It seems likely that the binding of cobalt by *C. salina* may have a similar mechanism to that described by Crist et al. (1981), although it should be pointed out that the cell wall structure of *C. salina* has not been completely elucidated and it is therefore not possible to determine the exact mechanism involved.

The influence of competing cations was greatly enhanced in the presence of 0.5 M NaCl, explained by the greater competition for binding sites due to the additional Na^+. The competing effect of Na^+ was further illustrated by increased cobalt biosorption by cells of *C. salina* previously grown in the presence of 0.5 M NaCl but then washed in distilled water to remove Na^+ bound to the cell wall. Of all the potentially competing cations, Na^+ should be considered most important when considering cobalt biosorption by *C. salina* in the natural environment. In estuarine habitats *C. salina* could be subject to changes in Na^+ concentration ranging from almost zero to approximately 0.5 M, which could affect amounts of cobalt taken up. It should be noted that, apart from the Na^+ and K^+, mono- and divalent cations used were at a relatively low concentration of 25 μM; at higher concentrations some may have effects on cobalt biosorption, e.g. Ca^{2+} and Mg^{2+}. These occur at concentrations of approximately 15 mM and 29 mM in the marine environment and have been shown to interfere with heavy metal uptake in a variety of organisms (Wong 1980; Gadd 1988; Reed and Gadd 1990). It is also noteworthy that Na^+ and K^+ did not affect cobalt biosorption at a concentration of 25 μM. Ca^{2+} and Mg^{2+} are particularly important in aquatic habitats since they can vary markedly depending on the degree of water hardness (Wong 1980). It is possible that Mg^{2+}, Ca^{2+} or other ions caused the slight decrease in cobalt binding in MN media.

Although pH is relatively stable in the marine environment, it can vary from 6–8 in estuaries, depending on dissolved CO_2 and the pH of waste being released into an estuary (Campbell 1983). Cobalt biosorption in *C. salina* was shown to be dependent on pH, with increasing pH increasing biosorption. This effect has also been reported in *Chlorella vulgaris* with a variety of metals including cobalt (Darnall et al. 1986). Greene and Darnall (1990) suggest that in microalgae active metal binding sites (such as carboxyl and amino groups) can also bind protons and these may therefore compete with cobalt for binding sites in *C. salina*. Increased binding with increased external pH has been noted for several metals and a variety of different microorganisms (Gadd 1988). In some cases, hydroxide formation occurs which is more evident at higher pH values, and this favours biosorption. Furthermore, and particularly in the presence of high NaCl concentrations, some metals form anionic chloride species, e.g. $CdCl_3^-$ and biosorption may be affected (Gadd 1988). However, Clark (1989) suggests that cobalt probably exists as Co^{2+} or $CoCl^+$ in ocean waters.

A significant proportion of cobalt taken up by *C. salina* was readily washed off the cells (desorbed) by simple chemical treatments. Desorption was increased by a decrease in pH or an increase in external Na^+. This is explained by protons and Na^+ replacing cobalt bound to the cell wall in an ion-exchange mechanism as described above (Greene and Darnall 1990). It is possible that environmental conditions, such as tidal changes in an estuary, could free a percentage of metal previously bound. The occurrence of dense algal blooms, which can be encouraged by nutrients in sewage, is common in the natural environment (Mellanby 1980). The effect of cell density is therefore of great interest but is an area often ignored by other workers. Here it was found that, although increasing cell density increased the total amount of cobalt removed from solution, it decreased

the specific amount of cobalt taken up by the cells. Meikle et al. (1990) explain similar observations with yeast cells by describing how high cell densities can limit the availability of exogenous solutes for transport, thus effecting the rate of transport and also external and internal solute concentrations. In addition, at high densities there may be electrostatic interactions between binding sites on cells, thus affecting the binding of Co^{2+} and other metals (Gadd 1988).

After microalgae have accumulated metals they may sink, transporting metals to deeper waters and sediments, and/or be ingested by herbivores in surface waters, resulting in assimilation of the metal into the food chain (Fisher et al. 1983). This work has shown that marine microalgae, like *C. salina*, could be an important component in the assimilation of cobalt/^{60}Co into estuarine food chains since it can passively bind significant amounts of cobalt. Such biosorption of cobalt was dependent upon cell density, pH, salinity and competing cations which has obvious implications in respect of the estuarine environment. In addition, it was shown that cobalt biosorption was partly reversible and dependent again on external pH and salinity, which is of possible relevance to the estuarine environment where large fluctuations in salinity occur in response to tidal movements and rainfall. Finally, it should be noted that equally significant amounts of cobalt can be accumulated by an active process in *C. salina* over longer time periods. In order to assess fully the possible importance of *C. salina* in the accumulation of cobalt form marine/estuarine environments, this phase should be further studied in detail.

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