Salt-stimulation of caesium accumulation in the euryhaline green microalga *Chlorella salina*: potential relevance to the development of a biological Cs-removal process

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Accumulation of Cs⁺ by *Chlorella salina* was 28-fold greater in cells incubated in the presence than in the absence of 0.5 M-NaCl. An approximate 70% removal of external Cs⁺ resulted after 15 h incubation of cells with 50 µM-CsCl and 0.5 M-NaCl. LiCl also had a stimulatory effect on Cs⁺ uptake, although mannitol did not. Cs⁺ influx increased with increasing external NaCl concentration and was maximal between 25–500 mM-NaCl at approximately 4 nmol Cs⁺ h⁻¹ (10⁶ cells)⁻¹. Little effect on Cs⁺ uptake resulted from the presence of Mg²⁺ or Ca²⁺ or from varying the external pH, and Cs⁺ was relatively non-toxic towards *C. salina*. At increasing cell densities (from 4 × 10⁵ to 1 × 10⁷ cells ml⁻¹), decreasing amounts of Cs⁺ were accumulated per cell although the rate of Cs⁺ removal from the external medium was still greatest at the higher cell densities examined. Freely suspended *C. salina* and cell-loaded alginate microbeads accumulated similar levels of Cs⁺, however, 46% of total Cs⁺ uptake was attributable to the calcium–alginate matrix in the latter case. When Cs⁺-loaded cells were subjected to hypo-osmotic shock, loss of cellular Cs⁺ occurred allowing easy Cs⁺ recovery. This loss exceeded 90% of cellular Cs⁺ when cells were washed with solutions containing ≤ 50 mM-NaCl between consecutive Cs⁺ uptake periods; these cells subsequently lost their ability to accumulate large amounts of Cs⁺. Maximal Cs⁺ uptake (approximately 85.1% removal after three 15 h incubations) occurred when cells were washed with a solution containing 500 mM-NaCl and 200 mM-KCl between incubations. The relevance of these results to the possible use of *C. salina* in a salt-dependent biological Cs-removal process is discussed.

Introduction

The contamination of aquatic ecosystems by ¹³⁷Cs, that may result from the discharge of radioactive waste effluents from nuclear reactors and nuclear fuel reprocessing plants, is of environmental concern due to the long half-life of ¹³⁷Cs (~ 30 years) and the toxic effects that may result from its accumulation into food chains (Avery *et al.*, 1992a). As a consequence, the removal of ¹³⁷Cs from waste effluents, to acceptable residual levels prior to discharge, is essential. One alternative to the use of chemical methods for the removal of other metals from solution, that has received attention in recent years, is biosorption (Gadd, 1992). Many biosorption studies have utilized denatured biomass to eliminate the toxic

effects of certain metals or to increase the biosorptive capacity of the biomass (Gadd, 1990; Avery & Tobin, 1992). However, microbial Cs⁺ accumulation is metabolism-dependent and is mediated by cellular K⁺ transport systems in most cases (Avery *et al.*, 1992a). Because of the low bioconcentration factors that are generally observed for Cs⁺ (Plato & Denovan, 1974; Avery *et al.*, 1991), an efficient microbial Cs-removal process requires an effective means of stimulating active Cs⁺ (K⁺) influx.

Among the mechanisms employed by many halo-tolerant micro-organisms to adapt to increases in external salinity is increased K⁺ accumulation (Gilmour, 1990). In *Escherichia coli*, several different K⁺ transport systems occur (Walderhaug *et al.*, 1987) although those under osmotic control are distinct from the system that mediates Cs⁺ uptake (Macaskie, 1991). However, Pick *et al.* (1986) detected a single K⁺ system in the halotolerant microalga *Dunaliella salina*, and recent work has demonstrated that a single mechanism also mediates Cs⁺ uptake in *Chlorella salina* (Avery *et al.*, 1993). It is likely

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therefore that any factor that influences K^+ uptake in these organisms will also affect Cs^+ uptake.

The purpose of this study was to assess the potential of *C. salina* for Cs^+ removal and recovery in the presence of varying external NaCl concentrations.

Methods

Organism and culture conditions. Axenic cultures of *Chlorella salina* Kufferath CCAP 211/25 were grown at 22 °C in 100 ml MN medium of composition (mg l⁻¹): MgSO₄·7H₂O, 37.5; CaCl₂·2H₂O, 18.0; NaNO₃, 750.0; K₂HPO₄, 20.0; citric acid, 3.0; FeSO₄·7H₂O, 3.0; EDTA (disodium salt), 0.5; Na₂CO₃, 20.0; H₃BO₃, 2.68; MnCl₂·4H₂O, 1.81; ZnSO₄·7H₂O, 0.222; Na₂MoO₄·2H₂O, 0.39; CuSO₄·5H₂O, 0.079; Co(NO₃)₂·6H₂O, 0.049; in 75% (v/v_{aq}) distilled water) filtered seawater (obtained from East Rock, St Andrews, Scotland, UK; filtered using Whatman no. 1 filter papers). Cultures were incubated in 250 ml Erlenmeyer flasks with rotary aeration at 150 cycles min⁻¹ and a photon fluence irradiance incident on the surface of the flasks of 120 µmol m⁻² s⁻¹, provided by white fluorescent light tubes. To examine the effect of CsCl on growth, cells from the late exponential growth phase were inoculated to an initial density of approximately 2 × 10⁵ cells ml⁻¹ (OD₆₈₀ ~ 0.1), in 100 ml MN medium containing the desired amount of CsCl, and were incubated as above. Cell numbers were determined after appropriate dilution with distilled water, using a modified Fuchs–Rosenthal haemocytometer.

Short term Cs^+ uptake experiments. Cells from the late exponential growth phase were collected by centrifugation (1200 g, 10 min), washed thoroughly with 10 mM-TAPS buffer containing 0.5 M-NaCl (unless otherwise specified), adjusted to pH 8.0 using 1 M-NaOH (unless otherwise specified), and suspended to a density of approximately 10⁶ cells ml⁻¹ in 20 ml of the same buffer. The suspension was then equilibrated for 1 h at 22 °C and a photon fluence irradiance of 120 µmol m⁻² s⁻¹. Cs^+ uptake was initiated by the addition of CsCl to the required concentration, with ¹³⁷Cs (Amersham), added as a tracer to a final activity of 0.93–7.40 kBq ml⁻¹. Where required, either NaCl, MgCl₂·6H₂O, CaCl₂·6H₂O or mannitol, at the desired concentration, was added to the cell suspension with the CsCl and ¹³⁷Cs. At intervals, 200 µl samples were removed and harvested by centrifugation (8000 g, 30 s) through a layer comprising 40% (v/v) Dow-Corning 550 silicone oil and 60% (v/v) bis-3,3,5-trimethylhexylphthalate (Fluka) in 500 µl Beckman PRO22 plastic tubes, using an Eppendorf 5412 micro-centrifuge. The bottom of each tube was cut off and placed in 5 ml Ecosint A scintillation fluid (National Diagnostics) for 24 h before measuring radioactivity using a Packard Minaxi tri-carb 4000 scintillation counter. To determine radioactivity in the external medium, 200 µl samples of supernatant were taken following centrifugation of cells (1200 g, 10 min) and analysed as described above. To assess the effect of pH on Cs^+ accumulation, cells were treated as above but washed and finally suspended in the following buffers (all 10 mM) with 0.5 M-NaCl, at the specified pH values: HEPES, pH 7; TAPS, pH 8 and 9; CHES, pH 10. The final pH was again adjusted using 1 M-NaOH. All glassware was washed with 1 M-HCl and rinsed thoroughly with distilled deionized water prior to use.

Production of alginate microbeads and preparation for Cs^+ uptake experiments. A microbead maker (obtained from Dr Gudmund Skjak-Braek, Institute of Biotechnology, University of Trondheim, Norway) was used to produce alginate beads of approximate diameter 0.5 mm. This technique relies on a high air flow, running down the outside of a needle containing alginate gel to prematurely dislodge gel droplets that form at the end of the needle. Gravity alone was sufficient to draw the gel through the needle, allowing a rate of bead formation exceeding

240 min⁻¹. Bead size was varied by adjusting the rate of air flow. A cell suspension of *C. salina*, of the appropriate cell density, was prepared as described above in 10 mM-TAPS buffer + 0.5 M-NaCl, pH 8, and mixed with an equal volume (25 ml) of 2% (w/v) sterile sodium alginate. For unloaded beads, sodium alginate was mixed with sterile buffer. The appropriate mixture was placed in the sterilized bead-maker chamber and the top was sealed. An air flow of approximately 30 ml min⁻¹ was applied to the top of the bead maker to produce microbeads of diameter 0.5 mm. As beads were formed they were dropped into a sterile solution of 0.2 M-CaCl₂, at 4 °C, and allowed to harden for 1 h. The beads were then washed thoroughly in MN medium to remove excess Ca²⁺, transferred to 250 ml Erlenmeyer flasks containing 100 ml MN medium and incubated for 24 h in the light with shaking, as described above for culture conditions. For Cs^+ uptake experiments, 10 ml of bead suspension was placed in a 10 ml measuring cylinder and, following settling of beads, the supernatant was removed by Pasteur pipette to allow addition of a further volume of bead suspension. This process was repeated until 7.5 ml packed beads were obtained. These were removed, washed and resuspended in 20 ml 10 mM-TAPS buffer + 0.5 M-NaCl, pH 8, in 100 ml conical flasks and incubated under a photon fluence irradiance of 120 µmol m⁻² s⁻¹. Cs^+ accumulation was initiated by the addition of CsCl, labelled with ¹³⁷Cs (final activity ~ 4.6 kBq ml⁻¹), to a final concentration of 50 µM. At intervals beads were separated from the suspending medium as described above for free cells. Samples (100 µl) of the resultant supernatant were added to 5 ml scintillation fluid and analysed for radioactivity as described above.

Results

*Effect of NaCl on Cs^+ accumulation by *C. salina**

Accumulation of Cs^+ after 2 h incubation in the presence of 0.5 M-NaCl was approximately 28-fold higher than for cells incubated in the absence of NaCl (Table 1). LiCl had one-half of the stimulatory effect of NaCl on Cs^+ accumulation. Mannitol, at 0.05 or 1.0 M, did not markedly stimulate Cs^+ accumulation (Table 1). The initial rate of active Cs^+ uptake (20–120 min, after cell-wall binding) by *C. salina* was determined over a range of NaCl concentrations in the presence of 50 µM-CsCl (Fig. 1). Cs^+ influx was lowest at 50 µM-NaCl [approximately

Table 1. *Effect of NaCl, LiCl and mannitol on Cs^+ accumulation by *C. salina**

Cells were harvested after 2 h incubation in 10 mM-TAPS buffer, adjusted to pH 8 using tetramethylammonium hydroxide, with 1 mM-CsCl and the specified addition. Mean values from three replicate determinations are shown ± SE.

Addition to external medium	Concn (M)	Intracellular Cs^+ [nmol (10 ⁶ cells) ⁻¹]
None	—	0.8 ± 0.4
NaCl	0.50	22.2 ± 0.3
LiCl	0.50	12.7 ± 0.4
Mannitol	1.00	1.1 ± 0.2
Mannitol	0.05	1.5 ± 0.3

Fig. 1. Effect of NaCl on the rate of Cs^+ uptake by *C. salina*. Cells were suspended to $1 \times 10^6 \text{ ml}^{-1}$ in 10 mM-TAPS buffer, adjusted to pH 8 using tetramethylammonium hydroxide, supplemented with $50 \mu\text{M}$ -CsCl and the appropriate concentration of NaCl. Rates of Cs^+ uptake ($\circ$) were determined over the first 2 h incubation. Typical results from one of three experiments are shown.

$0.3 \text{ nmol Cs}^+ \text{ h}^{-1} (10^6 \text{ cells})^{-1}$], the lowest concentration examined, and increased to a maximum level of approximately $4 \text{ nmol Cs}^+ \text{ h}^{-1} (10^6 \text{ cells})^{-1}$ between 25 and 500 mM-NaCl. The uptake rate was markedly reduced at 1 M-NaCl (Fig. 1).

The possibility of a Cs^+ - Na^+ symport in *C. salina*, which would account for the above observations, was investigated. However, although a clear stimulation of Cs^+ influx resulted from the addition of Na^+ to the external medium, any reciprocal stimulation of Na^+ influx by Cs^+ was not discernible as $^{22}\text{Na}^+$ uptake was complete within 30 s (data not shown). This was the fastest sampling time possible by the technique employed, and consequently rates of Na^+ uptake were not measured.

Influence of pH, divalent cations and cell density on Cs+ accumulation/removal by C. salina

Cs^+ accumulation by *C. salina* was similar, after 2 h incubation, over a range of external pH values from pH 7 to 10 (data not shown). A slight increase in Cs^+ accumulation was apparent at pH 8, although this was only approximately 16% greater than at pH 7.

Mg^{2+} and Ca^{2+} (supplied as chlorides) had no marked effect on Cs^+ accumulation by *C. salina* when supplied at 4 to 20-fold higher concentrations than CsCl (data not shown).

Cell density had a marked effect on Cs^+ accumulation by *C. salina* (Fig. 2a). The rate of Cs^+ uptake, on a per cell basis, decreased with increasing cell density and, after 15 h incubation, the final levels of Cs^+ accumulated

Fig. 2. Influence of cell density on Cs^+ accumulation by *C. salina*. Cells were suspended in 10 mM-TAPS buffer + 0.5 M-NaCl, pH 8, supplemented with $50 \mu\text{M}$ -CsCl. (a) Cs^+ accumulation at cell densities of 4×10^5 ($\circ$), 1×10^6 ($\bullet$), 4×10^6 ($\square$) and 1×10^7 ($\blacksquare$) ml^{-1} . (b) Data from (a) transformed to represent Cs^+ remaining in solution. Mean values from three replicate determinations are shown. SE values were smaller than the dimensions of the symbols.

Fig. 3. Effect of immobilization on Cs^+ accumulation by *C. salina*. Cells (free and immobilized) were suspended to $1 \times 10^6 \text{ ml}^{-1}$ in 10 mM-TAPS buffer + 0.5 M-NaCl, pH 8, supplemented with $50 \mu\text{M}$ -CsCl. The graph shows Cs^+ remaining in solution in the presence of free cells ($\circ$), cells immobilized in beads ($\bullet$) and cell-free beads ($\square$). Mean values from three replicate determinations are shown $\pm$ SE where these values exceeded the dimensions of the symbols.

Table 2. Uptake-efflux cycles for removal/recovery of Cs^+ by *C. salina*

Cells were suspended in 10 mM-TAPS buffer + 0.5 M-NaCl, pH 8, supplemented with the appropriate amount of CsCl. Cells were harvested after 15 h, washed once in 10 mM-TAPS buffer, adjusted to pH 8 using tetramethylammonium hydroxide and supplemented with the specified amounts of NaCl, KCl or mannitol, and then returned to the original Cs^+ -containing buffer. The washing procedure was repeated after 30 and 45 h of incubation. Mean values from three replicate determinations are shown $\pm$ SE.

CsCl concn (μM)	Addition to washing solution	Percentage removal of Cs^+ after:			Percentage recovery of accumulated Cs^+ at 15 h
		15 h	30 h	45 h	
50	0–50 mM-NaCl	71.5 $\pm$ 1.2	69.7 $\pm$ 0.7	69.5 $\pm$ 0.6	94.8 $\pm$ 1.7
50	200 mM-NaCl	71.5 $\pm$ 1.2	76.0 $\pm$ 0.0	76.0 $\pm$ 0.2	67.9 $\pm$ 0.2
50	500 mM-NaCl	71.5 $\pm$ 1.2	75.6 $\pm$ 0.0	76.3 $\pm$ 0.1	4.0 $\pm$ 0.0
50	500 mM-NaCl + 50 mM-KCl	71.5 $\pm$ 1.2	76.9 $\pm$ 0.4	77.2 $\pm$ 0.8	82.4 $\pm$ 0.0
50	500 mM-NaCl + 200 mM-KCl	71.5 $\pm$ 1.2	81.8 $\pm$ 0.2	85.1 $\pm$ 0.1	63.9 $\pm$ 0.2
50	500 mM-NaCl + 350 mM-KCl	71.5 $\pm$ 1.2	63.6 $\pm$ 0.6	61.4 $\pm$ 0.5	29.3 $\pm$ 0.3
50	500 mM-NaCl + 500 mM-KCl	71.5 $\pm$ 1.2	58.6 $\pm$ 0.9	52.3 $\pm$ 0.3	12.4 $\pm$ 0.1
50	250 mM-NaCl + 250 mM-KCl	71.5 $\pm$ 1.2	54.8 $\pm$ 1.2	51.9 $\pm$ 0.0	64.5 $\pm$ 0.7
50	200 mM-KCl	71.5 $\pm$ 1.2	45.5 $\pm$ 0.2	32.3 $\pm$ 4.9	11.3 $\pm$ 0.0
50	1 M-mannitol	71.5 $\pm$ 1.2	52.7 $\pm$ 0.8	48.2 $\pm$ 0.1	3.4 $\pm$ 0.0
1	200 mM-NaCl	79.3 $\pm$ 0.0	85.1 $\pm$ 0.2	87.8 $\pm$ 0.0	—
10	200 mM-NaCl	79.4 $\pm$ 0.1	84.9 $\pm$ 7.4	87.6 $\pm$ 0.0	—

were approximately 43.6, 29.0, 11.4 and 3.2 nmol Cs^+ (10^6 cells) $^{-1}$ at cell densities of approximately 4×10^5 , 1×10^6 , 4×10^6 and 1×10^7 cells ml^{-1} , respectively (Fig. 2a). When the data were presented as Cs^+ remaining in solution during uptake at the varying cell densities, it was evident that the initial rate of Cs^+ removal increased with increasing cell density (Fig. 2b). At the highest cell density examined (1×10^7 cells ml^{-1}), accumulation of Cs^+ was complete after approximately 7 h at which stage approximately 28 % of Cs^+ remained in solution

Removal of Cs^+ from solution by free and immobilized cells of *C. salina*

Incubation of freely suspended cells of *C. salina*, and cells immobilized in calcium-alginate beads, in the presence of 50 μM -CsCl for 15 h, resulted in an approximate 70 % removal of external Cs^+ in both cases (Fig. 3). However, approximately one-half of that was attributable to binding of Cs^+ to the calcium-alginate matrix (determined by incubation of cell-free calcium-alginate beads alone). The results indicated that *C. salina* accumulated approximately 46 % less Cs^+ when cells were present in an immobilized state than when in free suspension.

Inhibition of growth of *C. salina* by Cs^+

Growth of *C. salina* in MN medium was similar in the absence or presence of 50 μM -CsCl, with a cell doubling time during the exponential growth phase of 2.4 d and a

final cell yield after 35 d incubation of approximately 9.2×10^7 cells ml^{-1} (data not shown). However, growth of *C. salina* was inhibited at 1 mM-CsCl; the cell doubling time was increased by approximately 41.7 % and the final cell yield reduced by approximately 68.5 %, in comparison to cells incubated in the absence of CsCl.

Uptake-efflux cycles for removal/recovery of Cs^+ by *C. salina*

The efficacy of repeated incubations of *C. salina* in removal of Cs^+ from solution was investigated (Table 2). Approximately 71.5 % Cs^+ was removed after 15 h incubation of *C. salina* in buffer in the presence of 50 μM -CsCl and approximately 95 % of accumulated Cs^+ was released when cells were subsequently washed in buffers of lower osmotic strength, containing 0–50 mM-NaCl. At higher NaCl concentrations, a lower proportion of cellular Cs^+ was released and negligible Cs^+ loss resulted at 500 mM-NaCl. Re-incubation of the cells for a second 15 h period, in the original Cs^+ -containing buffer, resulted in little or no further Cs^+ accumulation. In all of the above cases, further accumulation of Cs^+ did not occur during a third incubation period (Table 2).

The inclusion of increasing amounts of KCl in washing solutions containing 500 mM-NaCl resulted in decreased levels of Cs^+ release. However, the greatest percentage removal of Cs^+ , approximately 85.1 % after three successive 15 h incubations, occurred when cells were washed in buffer containing 500 mM-NaCl and 200 mM-KCl. Washing cells in other combinations of NaCl, KCl

and mannitol resulted in lower final levels of Cs^+ removal. The percentage removal of Cs^+ was increased when cells were incubated in the presence of 1 and 10 μM - CsCl (Table 2). After incubation periods totalling 45 h, > 87% of the initial amount of Cs^+ was accumulated at these lower CsCl concentrations. Further enhancement of Cs^+ removal was achieved by using either fresh buffer or fresh cells for the second incubation period (data not shown).

Discussion

Although it is generally considered that inorganic ions, e.g. K^+ , play a role in the osmoregulation of halotolerant microalgae (Gilmour, 1990), there is some controversy as to their relative importance in osmo-adaptation compared to organic solute accumulation/synthesis (Ginzberg & Ginzberg, 1986). In the present work, Cs^+ influx in *C. salina* was markedly stimulated in the presence of NaCl indicating that, in contrast to *E. coli* (Macaskie, 1991), the K^+ transport system that mediates Cs^+ uptake by *C. salina* is under osmotic control. Rates of Cs^+ uptake at low NaCl concentrations ($\leq 50 \mu\text{M}$ - NaCl) were comparable to those observed previously for the freshwater microalga *Chlorella emersonii* in the absence of NaCl ; NaCl inhibited Cs^+ uptake by *C. emersonii* (Avery *et al.*, 1992b). The stimulatory effect of NaCl and LiCl on Cs^+ uptake by *C. salina* was not mirrored by mannitol when supplied at an equivalent osmotic strength to the salts. It was concluded that the present observations with Cs^+ were due to a specific effect of NaCl and LiCl , rather than a general osmotic effect. In certain diatoms the operation of K^+/Na^+ symports has been described (Brown & Hellebust, 1978), although any reciprocal stimulation of Na^+ uptake by Cs^+ in *C. salina* was not discernible in this study due to a very rapid accumulation of $^{22}\text{Na}^+$. It should be stressed that other mechanisms besides a symport may also explain the current observations. Ehrenfeld & Cousin (1982, 1984) have postulated that K^+/Na^+ exchange occurs in the halotolerant *Dunaliella tertiolecta* to counteract the large passive influx of Na^+ that follows hypertonic shock. However, Pick *et al.* (1986) proposed that increased K^+ uptake and Na^+ extrusion, following osmotic shock in *D. salina*, may be unrelated secondary effects resulting from an activation of the plasma membrane H^+ -ATPase.

The low level of inhibition of Cs^+ uptake by other monovalent cations in *C. salina* (Avery *et al.*, 1993), and by the divalent cations Mg^{2+} and Ca^{2+} reported here, suggests that problems frequently caused by the presence of competing ions on metal removal processes (Gadd, 1990) may not be so important in the present system. Furthermore, in contrast to the marked pH-dependence of Cs^+ accumulation by the cyanobacterium *Synecho-*

cystis PCC 6803 (Avery *et al.*, 1991), high Cs^+ uptake levels were maintained over a range of external pH values in *C. salina*.

Despite the increased removal of Cs^+ from solution at higher cell densities of *C. salina*, decreased amounts of Cs^+ were accumulated per cell. This is similar to results obtained for cobalt uptake by *C. salina* (Garnham *et al.*, 1991) and may be attributed to a decrease in the availability of exogenous solute (Meikle *et al.*, 1990) in addition to shading of incident light at high cell densities. Decreased Cs^+ accumulation was also observed in immobilized cells of *C. salina*. Immobilization has negligible influence on the photosynthesis of *C. salina* but results in a 30% decrease in the rate of respiration (Garnham *et al.*, 1992). Rb^+ and Cs^+ influx, in *Chlorella pyrenoidosa* and *C. emersonii*, respectively, are independently supported by cyclic photophosphorylation and oxidative phosphorylation (Springer-Lederer & Rosenfeld, 1968; Avery *et al.*, 1992b). It is likely that a reduction in respiration would also result in a decline in Cs^+ uptake in immobilized *C. salina*.

Hypo-osmotic shock of Cs^+ -loaded cells resulted in loss of the accumulated ion as was the case for K^+ in the halotolerant microalga *Chlorococcum submarinum* (Blackwell & Gilmour, 1991). Such treatment is known to have negligible effect on the growth or structural integrity of *C. salina* (Wong *et al.*, 1979; Garnham *et al.*, 1991). At very low salinities, the proportion of cellular Cs^+ that was released approached 100%. However, washing cells in buffer containing 500 mM- NaCl and 200 mM- KCl , between successive incubation periods, resulted in the highest final level of Cs^+ removed, approximately 85.1%. Unfortunately, the removal efficiency of Cs^+ by *C. salina* was reduced during successive incubation periods. Further experiments (data not shown) indicated that this reduced uptake was partly due to increased competition from K^+ released by cells in exchange for Cs^+ . In addition, washing procedures that allowed cells to retain the greatest capacity for further Cs^+ uptake failed to remove all cellular Cs^+ . As a consequence, subsequent accumulation of Cs^+ , to a final level equal to that obtained prior to washing, only required reduced uptake.

The maximal level of Cs^+ uptake reported here [85.1% removal from 50 μM - Cs^+ , $\sim 42.6 \text{ nmol } (10^6 \text{ cells})^{-1}$] corresponds to approximately 66 mg Cs^+ (g dry wt) $^{-1}$. This is lower than the value of 150 mg metal (g dry wt) $^{-1}$ suggested by Brierley *et al.* (1986) as being a threshold below which biological metal-removal becomes commercially non-viable, although it must be stated that environmental considerations did not take priority in this assessment. Although no improvement in Cs^+ uptake efficiency resulted from cell immobilization, advantages of this process over many other living cell systems

include its pH-independence, the low toxicity of Cs⁺ towards *C. salina* and the non-destructive treatments that may be employed for complete recovery of accumulated Cs⁺. Furthermore, despite the probable influence of external K⁺ on Cs⁺ accumulation following successive incubation periods, competition by K⁺ is low in *C. salina* (Avery *et al.*, 1993) compared to certain other micro-organisms (Avery *et al.*, 1992a), and the influence of other naturally abundant ions like Ca²⁺ and Mg²⁺ was negligible. The application of recently gained knowledge to the present system, e.g. the nutritional-regime dependence of Cs⁺ uptake in phototrophic micro-organisms (Avery *et al.*, 1992b) and possibilities arising from the selective isolation of Cs⁺-accumulating micro-organisms (Tomioka *et al.*, 1992) may prove useful in further enhancing Cs⁺-removal efficiencies by halo-tolerant micro-organisms in an applied context.

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