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The Chemical Engineering Journal 65 (1997) 13–19

CHEMICAL ENGINEERING JOURNAL

Cell surface area as a major parameter in the uptake of cadmium by unicellular green microalgae

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Received 12 September 1995; accepted 10 January 1996

Abstract

The mathematical model previously developed in the Department of Chemical Engineering, Monash University for the metal uptake by living algal cells has been revised to take into account the actual surface area through which transfer occurs. Cadmium uptake data obtained using the three algal species *Chlorella vulgaris, Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* were able to be fitted satisfactorily by the revised model.

Keywords: Microalgae; Metal ion; Cadmium; *Chlorella vulgaris*; *Chlorella pyrenoidosa*; *Chlamydomonas reinhardtii*

1. Introduction

In a previous article [1], the authors have indicated the importance of algae as part of the food chain and their role in the bioaccumulation of toxic metals. They have shown that it is possible to describe mathematically the uptake of cadmium ions by the fresh water green microalgae, *Chlorella vulgaris*, *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* in terms of an initial passive surface adsorption followed by an active transport of metal ions into the cell cytoplasm. These studies follow the work of Khummongkol et al. [2] and Ting et al. [3] also conducted in this department.

The model that has been developed can be expressed in terms of four key equations.

$$m = KC_1 \quad \text{(surface adsorption)} \tag{1}$$

$$m + X(C_1 + C_2) = A \quad \text{(metal mass balance)} \tag{2}$$

$$\frac{d(XC_2)}{dt} = \frac{XR_1}{K+X}\left[A - C_2(X + R_2(K+X))\right] \tag{3}$$

(membrane transport)

together with a growth expression

$$dX/dt = L$$

or similar. (The meanings of the symbols are given in the Nomenclature, at the end of the article.)

The model parameters K, R_1, R_2 and L are estimated from experimental data. A numerical solution is obtained using the differential equation software Matlab to solve the membrane transport equation (Eq. (3)), and the surface adsorption equation (Eq. (1)) together with the growth equation (Eq. (4)).

2. Materials and method

Organisms, culture techniques, cell dry weight measurement and metal analysis methods are the same as have been described previously [1].

2.2. Size distribution analysis of algal cells

The size distributions of the algal cells were determined by using a Malver laser diffraction particle sizer (Malvern Master Sizer/E). A sample of algal culture (contained in a small glass cell and continuously stirred by a magnetic stirrer to maintain uniform cell suspension) was placed in the path of the He–Ne laser beam and the beam scatter measured. The instrument software uses the Fraunhofer diffraction to provide information on the size distribution of the solids in the sample, together with the calculated volume mean diameter (VMD) and the Sauter mean diameter (SMD).These diameters are defined as follows.

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PII S1385-8947(96)03091-4

Table 1
Results of long term uptake studies of cadmium by *Chlorella vulgaris*

Alga *Chlorella vulgaris*	Sauter mean diameter (μm)	Specific surface area $\times 10^{-2}$ ($m^2\ mg^{-1}$ cells)	Final total cadmium uptake	
			$\times 10^{-5}$ (mMol mg^{-1} cells)	$\times 10^{-3}$ (mMol m^{-2} cells)
Culture 1	2.76	2.20	3.40	1.71
Culture 2	4.40	1.30	2.29	1.70
Culture 3	6.13	0.98	1.64	1.74

$$\mathrm{VMD} = \frac{\sum_{i=1}^{n} V(di)d}{\sum_{i=1}^{n} V(di)} \quad (5)$$

$$\mathrm{SMD} = \frac{\sum_{i=1}^{n} V(di)}{\sum_{i=1}^{n} V(di)/di} \quad (6)$$

The instrument software also calculates the specific surface area (SSA) of a unit volume of particles from:

$$\mathrm{SSA} = (\text{total area})/(\text{total volume}) = \frac{\sum(Vi/di)}{\sum(Vi)} \quad (7)$$

$$\mathrm{SSA} = 6/\mathrm{SMD}$$

As with the statistical parameters, the specific surface area is calculated from the derived diameters.

3. Experimental results and discussion

3.1. Long term uptake of cadmium on C. vulgaris at different initial cell size distributions

Different cultures of the same algal species were found to have different initial cell size distributions and there were also differences in the cell size distributions from species to species. In order to investigate the effect of cell size distribution on the uptake and the values for the model parameters, a set of long term experiments was undertaken using the same experimental conditions reported previously [1] with three cultures of *C. vulgaris* each having different cell sizes. The Sauter mean diameters of the cells were 2.76 μm, 4.36 μm and 6.13 μm respectively. The cultures were spiked with cadmium ions (in the form of cadmium nitrate solution) to give an initial cadmium concentration of 1.78×10^{-2} mMol l^{-1} (2 ppm). A summary of the results for the long term uptake of cadmium is given in Table 1 and the estimated model parameters for the three different initial cell size distributions are given in Table 2.

The results obtained for any single individual algal size can be described satisfactorily by the model developed previously [3], but the final uptake per unit mass of the cells is different for each cell size even when the same algal species is being studied. This result is not of much value in any design calculation.

Surfaces of the same algal species absorbing the same metal ion under the same conditions show different final uptakes per unit mass but essentially the same final uptake per unit area of the cells (Table 1). This suggests that when comparing uptake results from different algal species, or from the same species but with different cell sizes, uptake data should be expressed in terms of the surface area and not in terms of a unit mass of dry cells (even though this latter figure is far easier to obtain experimentally). It has been shown that there are long time changes in the mean cell size distributions when there is metal uptake, and so this too must be considered. The mathematical model needs to be refined to take into account the surface area of the cells before it can be considered to be a useful tool for design purposes.

4. Modified mathematical model

4.1. Surface adsorption isotherm

Since the first stage in any active uptake is highly dependent on the surfaces provided by the algal cells, the specific

Table 2
Estimated model parameters based on the long term uptake of cadmium on *Chlorella vulgaris* at different initial cell sizes

Alga *Chlorella vulgaris*	Initial cell concentration X_0 ($mg\ l^{-1}$)	Adsorption constant K ($mg\ l^{-1}$)	Linear rate of growth L ($mg\ l\ h^{-1}$)	Carrier rate constant R_1 (h^{-1})	Ratio of rate constants R_2 (dimensionless)
Culture 1	220	300	6.3	0.308	0.73
Culture 2	180	555	7.3	0.123	1.30
Culture 3	220	740	6.0	0.052	30.80

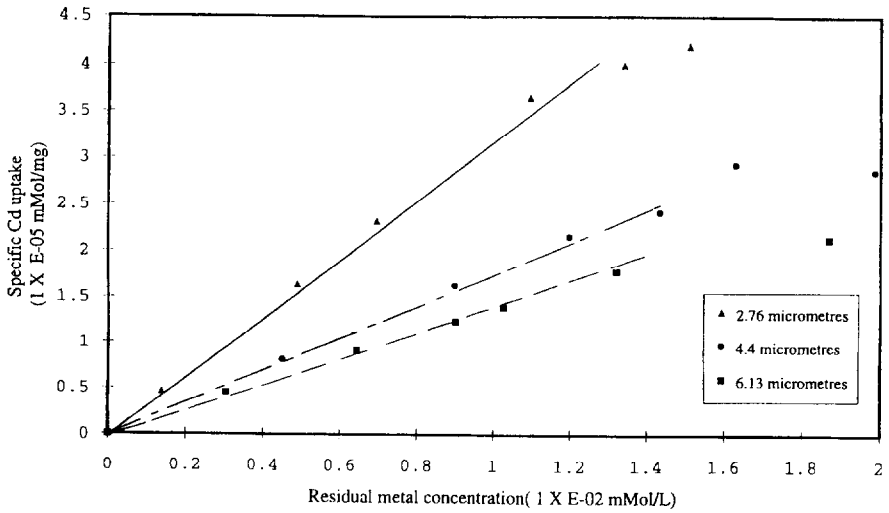

Fig. 1. Adsorption isotherms of *C. vulgaris* used in the initial cadmium uptake study at different mean cell sizes of 2.76, 4.40, and 6.13 μm diameter.

surface area must be an important parameter in any adsorption equation. It was shown previously that the value of the adsorption constant K is inversely proportional to the specific surface area of the cells. The results for the adsorption constants for different algal species and also for *C. vulgaris* with different mean cell sizes indicate that smaller cell sizes provide a larger surface areas and lower values of adsorption constants based on a unit mass of cells and *vice versa*. Both Ting and coworkers [3] and Khummongkol and his coworkers [2] mentioned this fact but apparently missed its real significance. One may write:

$$K_s = KS_p \tag{8}$$

and Eq. (1) becomes:

$$m = K_s C_{1s} \tag{9}$$

and

$$C_{1s} = C_1 / S_p \tag{10}$$

Eq. (8) suggests that for any single algal species one should obtain a fixed value for the isothermal surface adsorption constant K_s independent of the algal cell size and its specific surface area. The specific surface uptake of metal ions on the cell (C_{1s}) can be determined using Eq. (10) for any given culture.

4.2. Membrane transport equation

The membrane transport equation takes into account the intracellular transfer. This equation was shown previously to describe the intracellular uptake, C_2 for the different size distributions with reasonable accuracy. The same differential equation (Eq. (3)) can be used for the prediction of the intracellular uptake as was used *per unit mass of the cells*. The values of the model parameters K, R_1 R_2 and L can be estimated as before using the mathematics software Matlab.

4.3. Final total metal uptake per unit surface area

The evaluation of the total metal uptake per unit surface area is shown below:

$$\text{Total metal uptake} = C_{1s} + \frac{C_2}{S_p}$$

the parameters having been previously defined. From a theoretical point of view, the final metal uptake per unit surface area is expected to be constant for each algal species independent of the initial cell size under fixed environmental conditions and this was found experimentally to be correct.

4.4. Experimental study using C. vulgaris

The data obtained with the three algal species *C. vulgaris*, *C. pyrenoidosa* and *C. reinhardtii* reported previously [1] and also the data obtained from uptake experiments conducted using the three different mean cell size cultures of *C. vulgaris* have been recalculated in terms of the revised surface adsorption transport model to assess the general applicability of the modified model. The results follow.

4.5. Surface adsorption isotherms

The adsorption isotherms for *C. vulgaris* with three different initial cell sizes are shown in Fig. 1, which gives the residual metal concentrations as a function of the specific cadmium uptake. The data fall on straight lines, one for each cell size. However when the data are re-evaluated to give the uptake in terms of unit surface area (C_{1s}) and again plotted as a function of cadmium remaining in solution, a single straight line is obtained as predicted by Eq. (9). These results are shown as Fig. 2.

It is clear that at low concentrations of cadmium (up to about 1.5×10^{-2} mMol Cd l^{-1}) a linear relationship exists between the surface cadmium concentration per unit area and the residual cadmium concentration in solution. The surface

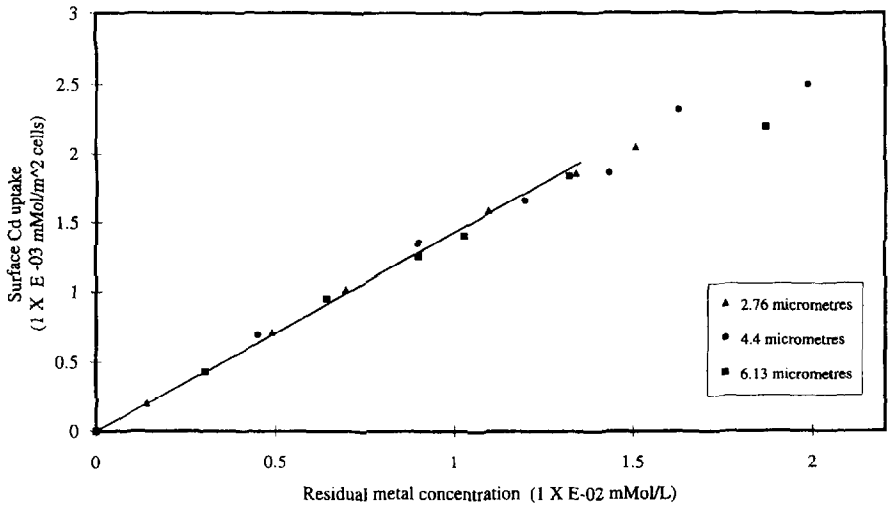

Fig. 2. Surface adsorption isotherm for *C. vulgaris* at different mean cell sizes of 2.76, 4.40, and 6.13 μm diameter.

adsorption constant K_s can be determined from the gradient of this line and has a value of 7.09 $m^2 l^{-1}$. It is constant for the *C. vulgaris* strain used in this study. Table 3 gives a summary of the results obtained for the adsorption isotherms of *C. vulgaris* with these different mean cell sizes.

4.6. Long term uptake

The experimental values for the cadmium specific surface uptake, (C_{1s}) for *C. vulgaris* and the data calculated using the sorption model were evaluated and are shown as Figs. 3–5. The experimental data are shown as individual data points, the model predictions as full lines. The experimental values and the model predictions are in good agreement.

The results obtained from the long term uptake studies were re-evaluated in terms of the surface area and the total uptake per unit surface of the algal cells calculated. The results are shown as Fig. 6 where it is seen that the total uptake approaches a single value for all three cultures of *C. vulgaris*.

4.7. Experimental study on C. pyrenoidosa and C. reinhardtii

The experimental data on the surface uptake of the other two algal species, *C. pyrenoidosa* and *C. reinhardtii* reported previously were also re-evaluated in terms of the surface area rather than the mass and are shown as Figs. 7 and 8. Again the model predictions and the experimental data are in good agreement. A summary of the results for the long term uptake of cadmium on the three algal species used in this study is given in Table 3.

Table 3
Summary of results on long term uptake of the algal cells

Algal species	Initial cell concentration X_0 ($mg\ l^{-1}$)	Sauter mean diameter (μm)	Specific surface area S_p $\times 10^{-2}$ ($mMol\ mg^{-1}$) cells)	Adsorption constant K ($mg\ l^{-1}$)	Surface adsorption constant K_s ($m^2\ l$)	Final total uptake $C_{1s}+C_2/S_p$ $\times 10^{-3}$ ($mMol\ m^{-2}$ surface)
Chlorella vulgaris (culture 1)	220	2.76	2.3	300	7.05	1.71
Chlorella pyrenoidosa	190	2.81	2.1	215	4.5	1.10
Chlorella vulgaris (culture 2)	180	4.40	1.3	555	7.2	1.70
Chlamydomonas reinhardtii	230	6.02	0.99	3025	29.9	2.41
Chlorella vulgaris (culture 3)	220	6.13	0.97	740	7.23	1.74

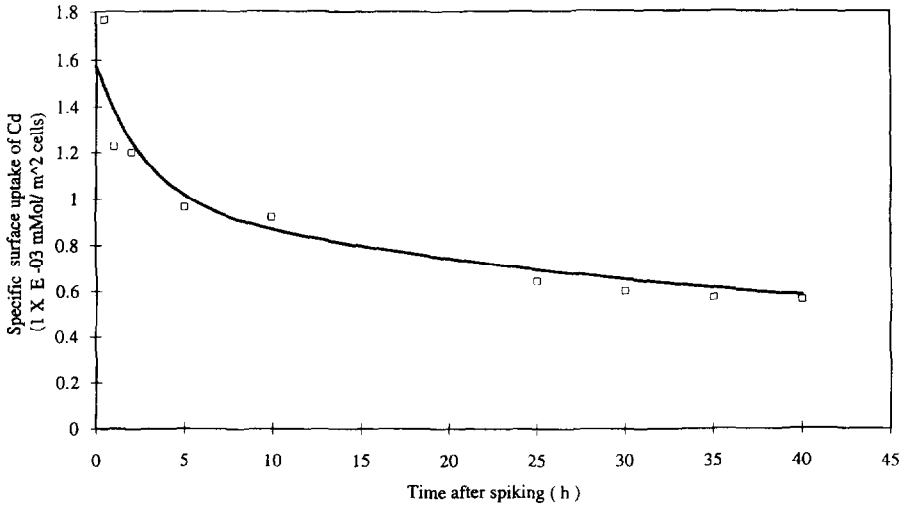

Fig. 3. Surface uptake of cadmium on cells (C_{1s}) during the long term uptakeby *C. vulgaris* culture 1 (2.76 μm diameter).

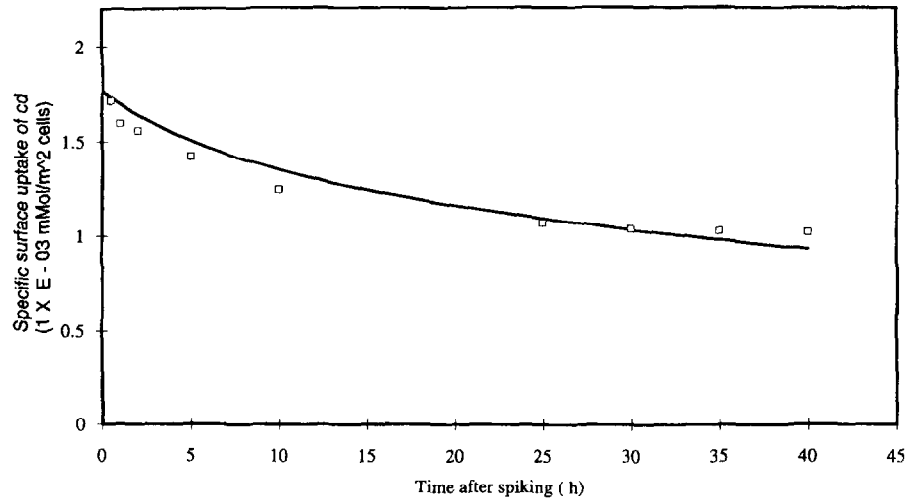

Fig. 4. Surface uptake of cadmium on cells (C_{1s}) during the long term uptake by *C. vulgaris*, culture 2 (4.40 μm diameter).

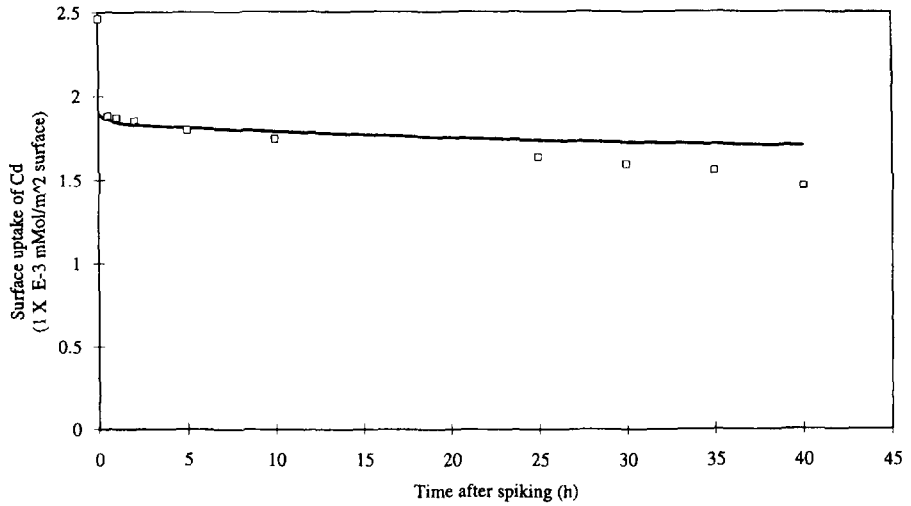

Fig. 5. Surface uptake of cadmium on cells (C_{1s}) during the long term uptakeby *C. vulgaris* culture 3 (6.13 μm diameter).

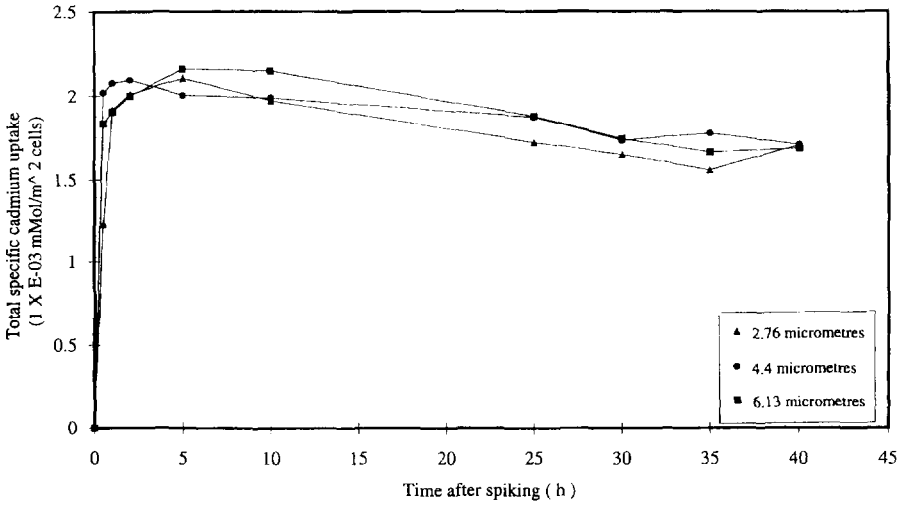

Fig. 6. Total cadmium uptake per unit surface area ($C_{1s} + C_2/S_p$) for *C. vulgaris* for cultures with different mean cell diameters of 2.76, 4.40, and 6.13 μm.

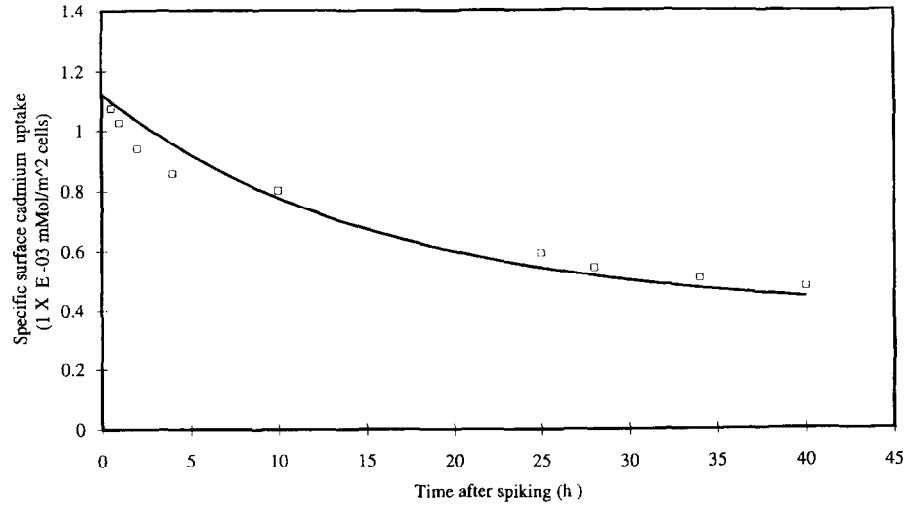

Fig. 7. Surface uptake of cadmium on cells (C_{1s}) during the long term uptakeby *C. pyrenoidosa* (mean cell size 2.81 μm diameter).

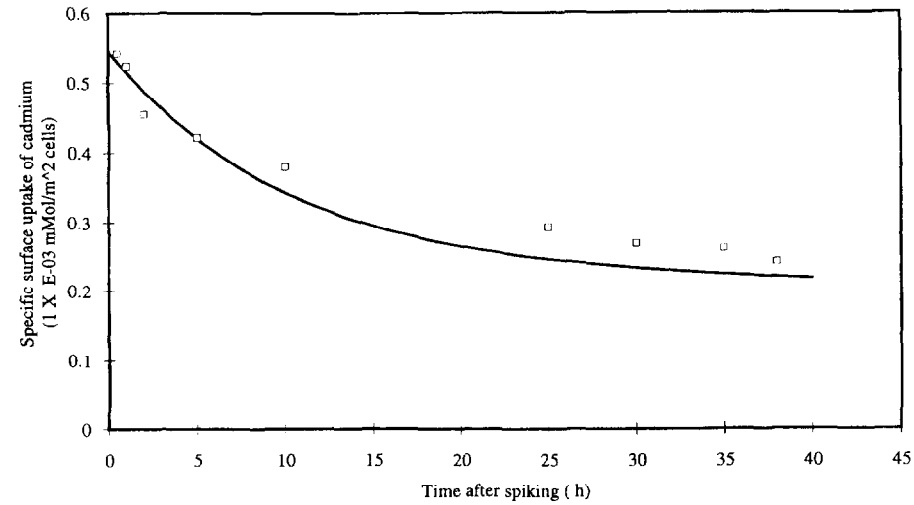

Fig. 8. Surface uptake of cadmium on cells (C_{1s}) during the long term uptakeby *C. reinhardtii* (mean cell size 6.02 μm diameter).

From Table 3, it is clear that even with cells of the same approximate size but of different species the uptake kinetics are quite different. It is also clear that the alga *C. reinhardtii* has a higher total uptake of cadmium per unit area of cell surface and *C. pyrenoidosa* a lower total uptake than *C. vulgaris*.

When just the initial surface uptakes are considered, *C. vulgaris* has the highest surface uptake for cadmium while *C. reinhardtii* has the lowest. Thus quite eroneous conclusions can be drawn if only short term data are considered.

5. Conclusions

1. For a single algal species with different initial mean cell sizes, the value for the surface adsorption constant (K_s) is constant whereas the values of the adsorption constants (K) based on a unit cell mass vary depending on the algal cell sizes.
2. The final total uptake per unit surface area of the cells for a single algal species is constant under fixed environmental conditions.
3. As a revised model, the Surface Adsorption Transport Model has been successfully used to describe the uptake of cadmium ions by three algal species *C. vulgaris*, *C. pyrenoidosa* and *C. reinhardtii*.
4. The results in terms of the total surface uptake can be used directly to compare the ability of different algal species and strains to absorb metal ions from solution.
5. Under the experimental conditions used in this study, the alga *C. reinhardtii* showed the highest total and intracellular uptake per unit surface area for cadmium out of the three algal species studied. *C. vulgaris* showed the highest surface uptake per unit area suggesting that this species has a larger number of active surface sites and a higher affinity for the uptake of cadmium than the other species tested.

Nomenclature

A	total metal concentration in system (mMol l^{-1})
C_1	metal concentration on the cell wall (mMol mg^{-1})
C_{1s}	specific surface metal uptake on cell (mMol m^{-2} cell surface area)
C_2	metal ion concentration within the cell (mMol mg^{-1})
d_i	the mean diameter of size band i (m)
K	adsorption constant (mg l^{-1})
K_s	surface adsorption constant (m^2 l^{-1})
L	cell productivity (mg l^{-1} h^{-1})
m	metal ion concentration in the solution (mMol l^{-1})
R_1	carrier rate constant (h^{-1})
R_2	ratio of rate constants
S_p	specific surface area of the cells (m^2 mg^{-1} of dried cells)
t	time (h)
V_i	volume in size band i
$V(d_i)$	volume frequency of cell i
X	cell dry weight (mg l^{-1})

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