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Uptake of Cadmium by *Pseudokirchneriella supcapitata*

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ABSTRACT

In this work the microalgae Pseudokirchneriella supcapitata *was used for the removal of the cadmium in liquids. The accumulations of metal ions by the alga occur in two stages: a very fast absorption (passive adsorption) proceeded by a slower absorption (activate absorption). A mathematical model based on the surface absorption and on the transport into the interior of the cellular membrane was developed. The simulation model kinetic parameters were experimentally obtained. Through the results observed, the mathematical model was shown to be suitable when compared to the experimental results, confirming the validation of the mathematical model.*

Key words: Cadmium, microalgae, heavy metals, mathematical model

INTRODUCTION

Some heavy metals cause strong impact on the stability of ecosystems and cause adverse effects in human beings even in amounts of the order of milligrams or micrograms. These metals must be removed from the environment in order to avoid habitat degradation and pollution (Steinkellner et at, 1998). Cadmium accumulates in human tissue in function of its affinity by functional bonding agents such as sulfydryl, hydroxyl, carboxylic and phosphoryl groups. Besides, it competes with the essential elements and may cause inhibition of important enzymatic functions (Baes, 1976; Castro, 2000). Many studies have been based on the effects of the cadmium on: enzymatic activities, phosphorization oxidative, permeability and integrity of the cellular membrane and synthesis of proteins. (Hughes and Poole, 1989; Trevors et al., 1986).

The main cadmium applications are found in the batteries production (Ni-Cd), in the manufacturing of pigments for ceramic materials, in the PVC (polyvinyl chloride) stabilization agents and in galvanoplastics processes. It is vital to limit the concentration of these metals in the sources of human consumption. According to our legislation, the content of these elements in waters aimed at the domestic supply should not exceed 20 μgL^{-1} and in the case of the cadmium; the upper limit is of $1\mu gL^{-1}$. (Castro, 2000).

Generally, the treatment of metal-contaminated effluents includes physical-chemical processes such as flocculation and/or precipitation, electrolysis, crystallization and adsorption. However, these processes are expensive and produce new residuals, thus changing the type of problem. The search for cheaper and definitive solutions led to the development of technologies based on the use of microorganisms as heavy metals removing agents (Ting et al, 1989).

Microorganisms have been commonly reported to be capable of sequestering heavy metals and concentrating them up to several thousand times

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(or more) over them. The sequestering capacity as well as accumulation mechanisms can vary thoroughly in agreement with the microbial species. External factors as pH, temperature, absence or presence of nutrients and other metals also influence in the mechanism actuate and, consequently, in the efficiency and accumulation selectivity (Crist et al., 1988; Nakagima and Sakaguchi, 1986).

In this work, the uptake of cadmium by the microalgae *Pseudokirchneriella supcapitata* was evaluated.

MATERIALS AND METHODS

Organism

The microorganism used in the present work was the unicellular microalgae *Pseudokirchneriella supcapitata*, obtained from samples collected in the Ecotoxicology Laboratory - Universidade Federal da Bahia (UFBA).

Growth Medium

The growth medium was prepared with deionized water and sterilized in autoclave at pressure of 1.5 kgf/cm² and temperature of 127°C. Soon after, 100 mL of *P. supcapitata* was added in 1 L. Table 1 presents the culture medium composition.

Experimental Cultures

The second group of experiments was conducted using the same culture conditions mentioned above. After 60 h of inoculation, the cadmium was added at an initial concentration of $2x10^{-5}$ $molL^{-1}$. Soon after, samples were collected at several time intervals.The third group of experiments was performed in order to evaluate the cadmium effect on the algae growth. The cadmium was added along with the inoculum in order to observe the algae behavior. Initial concentration of Cd was $2x10^{-5}$ $molL^{-1}$.

Table 1 - Composition of growth medium

Components	**Concentration ($molL^{-1}$)**
Calcium Nitrate Tetrahydrate	0.17
Potassium Nitrate	0.99
Magnesium Sulphate	0.12
Potassium Phosphate	0.23
Copper Sulphate Pentahydrate	$0.12x10^{-3}$
Ammonium Molybdate Tetrahydrate	$0.05\ x10^{-3}$
Zinc Sulphate	$0.02\ x10^{-3}$
Manganese Nitrate	$0.24\ x10^{-3}$
Monohydrate Citric Acid	$0.46\ x10^{-3}$
Boric Acid	$0.97\ x10^{-3}$
Ferric Chloride	$6.04\ x10^{-3}$
Ferrous Sulphate Heptahydrate	$2.24\ x10^{-3}$
Hydrated Ferric Citrate	$4.85\ x10^{-3}$
Sodium Bicarbonate	$0.18\ x10^{-3}$

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Analysis of the Metal

The analysis of the residual metal concentrations in the solution and in the membrane was done by Atomic Absorption Spectroscopy (Varian).

Mathematical Modeling

The phenomenon of metal uptake by the microorganisms is quite complex: the actual attachment of the metal ions on the cellular surface may include the physical adsorption, ion exchange and chemisorption. In this work, the term adsorption is used to describe any of these possible modes of interactions between the metal ion and the surface of the cells without distinction. The uptake of metal ions by microorganisms occurs in two stages:

1st) Rapid uptake (passive uptake)
2nd) Slower uptake (active uptake)

During the passive uptake, the metal ions are adsorb onto surface of the cells within a few seconds or minutes. In the second uptake stage, the metal ions are transported across the cell membrane and into the cytoplasm. The following suppositions are made in the modeling of the uptake process:

(i) The metal in solution at the cell surface, m (mmol metal/L), is in equilibrium with the metal adsorbed on the cell, C_1 (mmol metal/mg cell dry wt). A linear relationship between these two is assumed:

$$m = KC_1 \tag{1}$$

where K = adsorption constant (mg cell dry wt/L)

(ii) The metal ion adsorbed on the cell surface traverses the cell membrane through the agency of carrier molecules present on both sides of the membrane. The carrier mediated mechanism is analogous to an enzyme-substrate coupling scheme:

$$C_1 + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CE \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C_2 + E \tag{2}$$

where: C_1 = extracellular metal concentration (mmol/L)
C_2 = intracellular metal concentration (mmol/L)
E = carrier concentration (mmol/L)
CE = metal-carrier complex concentration (mmol/L)
k_1, k_{-2} = chemical reaction rate constants (mg/mmol.h)
k_{-1}, k_2 = chemical reaction rate constants (h^{-1})

(iii) The diffusion of the metal-carrier complex CE through the cell membrane is very rapid and the concentrations of E and CE are small compared with C_1 and C_2, the metal ion concentrations outside and inside the cells, respectively.

iv) Pseudo-steady state is assumed; the is no net accumulation of the metal-carrier complex and d(CE)/dt = 0.

(v) On a unit volume basis, the carrier content of each cell is essentially constant, and so the total carrier concentration in the system, E_t, is given by

$$E_t = [E]+[CE] \tag{3}$$

(vi) One mole of carrier reacts with 1 mol metal ion to produce 1 mol metal-carrier complex.

From assumption (iv), the reaction velocity v for the formation and decomposition of the metal-carrier complex is given by:

$$\frac{d[CE]}{dt} = k_1[C_1][E] - k_{-1}[CE] = 0 \tag{4}$$

$$v = \frac{d[C_2]}{dt} = k_2[CE] - k_{-2}[C_2][E] \tag{5}$$

Using equations (3) - (5), we obtain:

$$v = E_t \frac{Z_1[C_1] - Z_2[C_2]}{1 + Z_3[C_1] + Z_4[C_2]} \tag{6}$$

and

$$CE = E_t \frac{Z_3[C_1] - Z_4[C_2]}{1 + Z_3[C_1] + Z_4[C_2]} \tag{7}$$

where:

$$Z_1 = \frac{k_1 k_2}{k_{-1} + k_2}$$

$$Z_2 = \frac{k_{-1} k_{-2}}{k_{-1} + k_2}$$

$$Z_3 = \frac{k_1}{k_{-1} + k_2}$$

$$Z_4 = \frac{k_{-2}}{k_{-1} + k_2}$$

From assumption (iii), [CE] is small, so from equation (6), $1 + Z_3[C_1] + Z_4[C_2] \approx 1$. Hence equation (6) approximates to

$$v = E_1\{Z_1[C_1] - Z_2[C_2]\} \tag{8}$$

Replacing E_t by the product xp , where x is cell dry weight (mg/L) and p is the carrier content of the cells (mmol/mg),

$$v = xp\{Z_1[C_1] - Z_2[C_2]\} \tag{9}$$

By defining R_1, the carrier rate constant, as $p(Z_1)$ and R_2, the ratio of rate constants, as Z_2/Z_1 and writing v as $d(x[C_2])/dt$, one obtains

$$\frac{d(x[C_2])}{dt} = xR_1\{[C_1] - R_2[C_2]\} \qquad (10)$$

The metal mass balance gives

$$A = m + x\{[C_1] + [C_2]\} \qquad (11)$$

where A is the total metal concentrations in a unit volume of system and m is the concentration of the metal ion in solution.

Substituting (1) and (11) into equation (10), one obtains:

$$\frac{d(x[C_2])}{dt} = \frac{xR_1}{K + x} \cdot \{A - [C_2](x + R_2(K + x))\} \qquad (12)$$

This final expression provides a description of the model from which an analytical solution may be obtained.

The cells growth (x) for the equation (12), can followed three models

I.. Uptake by viable cells without growth, $x = x_o$

II. Uptake by cells with linear growth

$$\frac{dx}{dt} = L \qquad (13)$$

where L is the linear growth rate (mg dry wt/Lh).

III. Uptake by cells with exponential growth,

$$\frac{dx}{dt} = \mu x \qquad (14)$$

where μ is the exponential growth rate (h^{-1}).

Estimation of model parameters K_1 $R_1$and R_2

At the initial stage of the metal uptake, only surface adsorption is significant so that $[C_2]$ my be assumed to be zero in equation (11). At time t = 0, $x = x_0$. Hence, with equation (1), one obtains

$$K = \frac{m}{A - m} x_0 \qquad (15)$$

An estimate of the adsorption constant K can therefore be made using results obtained at the beginning of the experiments.

From equations (1) and (11), the intracellular metal concentration $x[C_2]$ my be writen as

$$x[C_2] = \frac{KA - m(K + x)}{K} \qquad (16)$$

Substituing equations (1) and (16) into equation (10), yelds

$$\frac{d\{(KA - m(K + x))/K\}}{dt} =$$

$$\frac{xmR_1}{K} - \frac{KA - m(K + x)}{K} R_1 R_2 \qquad (17)$$

By defining two variables α and β as

$$\alpha = \frac{KA - m(K + x)}{K} \qquad \beta = \frac{xm}{K}$$

equation (17) becomes

$$\frac{d\alpha}{dt} = \beta R_1 - \alpha R_1 R_2 \qquad (18)$$

From experimental data, α was able to be expressed as a second degree polynomial function of t, and so dα/dt expressed as a function of t. Likewise, β was able to be calculated using values of x and m at different times. By substituting dα/dt, α and β evaluated at different times into equation (18), the carrier rate constant R_1 and the ratio of rate constants R_2 were estimated using a linear least-square analyses routine (MATLAB).

Having obtained an estimate of the three constants (K, R_1 e R_2) from experimental data, equation (12) along with the growth model that in this case presented a exponential behavior, equation (14) was able to be solved by numerical means using Runge Kutta method.

RESULTS AND DISCUSSION

The results of the experiments showed that the metal ion concentration was not lost during culture, sampling and analysis. This showed that decrease in the metal residual concentration on algae-metal experiments could be attributed to the removal by the algae cells.

Cell growth for the first and second groups of experiments

At 25°C and under constant light, the culture grew normally. Growth experimental data were tested using linear and exponential models. The most suitable model for *P. subcapitata* was the exponential growth model.

The exponential model follows the equation (14), $\frac{dx}{dt} = \mu x$, which analytical integration of the equation whit the initial conditions is presented as follows

$$x = x_0 e^{\mu \cdot t} \qquad (19)$$

where, μ is the specific growth rate considered as constant during experiments. The model parameters were found from experimental data using the approximation of the least-square, thus obtaining the following equation:

$$x = 16{,}959 e^{0{,}00685 \cdot t} \qquad (20)$$

The slope comparing the model, equation (20) to the experimental values is presented in Fig. 2, first group; one observes that the exponential model represents satisfactorily the *P. subcapitata* growth behavior.

The second experiment was conducted with the objective of evaluating the removal of cadmium after 60 h incubation and also to observe the cellular growth with the addition of cadmium to the culture medium. After 60 h of algae development, cadmium as cadmium nitrate was added to the culture medium (final concentration $2x10^{-2}$ mmolL^{-1}). The cellular growth suffered no alterations in the second group of experiments.

Estimative of parameters K, R_1 e R_2 for the second group of experiments

The uptake of cadmium from the culture medium is analyzed for the calculation of the constants K, R_1 and R_2. The estimative of the adsorption constant K followed the procedure of the anterior section,

using the equation (15), $K = \frac{m}{A - m} x_0$.

Where m = 0.01891 mmolL^{-1} (metal concentration in the solution at the first moments, in other words, t = 0.25 h), A = 2 x 10^{-2} mmolL^{-1} and x_0 = 16.15 mgL^{-1} (cellular concentration at the first moments, t = 0.25 h). Therefore, the value of the adsorption constant is K = 280.18 mgL^{-1}. The estimative of R_1 and R_2 was given as follows: Experimental data from 0.25 to 72 hours were initially used, period in which the adsorption into the inner of the cell was intense; after this period, although adsorption at the cell wall still occurs, the adsorption into the inner of the cell is almost null.

Using equation (18), $\frac{d\alpha}{dt} = \beta R_1 - \alpha R_1 R_2$, where by definition

$$\alpha = \frac{KA - m(K + x)}{K} \quad \text{and} \quad \beta = \frac{xm}{K}$$

The values of α and β were calculated from the adsorption constant K, the total cadmium concentration A and from the values of m and x that vary with time during the experiments. The variable α was adjusted to a second degree polynomial in function of time.

An equations system similar to equation (18) was constructed. The system was solved using the MATLAB software with optimization through the use of the least-square method. Fig. 1 shows the uptake of cadmium with initial concentration of $2x10^{-2}$ mmolL^{-1}, under standard conditions. The metal ion concentration dropped 5.5% at the 15 initial minutes. A slow uptake of cadmium was observed later. The model proposed in this work is in agreement with the instant decrease observed in the metal residual concentration in the solution caused by the metal ion adsorption at the algae cell walls. A slow increase in the metal adsorption was also observed. As observed from the Figure, the experimental results approximated to the simulated model.

Cadmium specific adsorption (mmol Cd / mg dry cell) is also presented in Fig. 1. The model became again consistent, once an increase on the metal ions in the cell was observed. The increase on the metal ion intracellular concentration was due to the metal ions membrane transportation; this effect decreased with time due to the cell continuous growth and the reduction in the metal ions concentration in the solution.

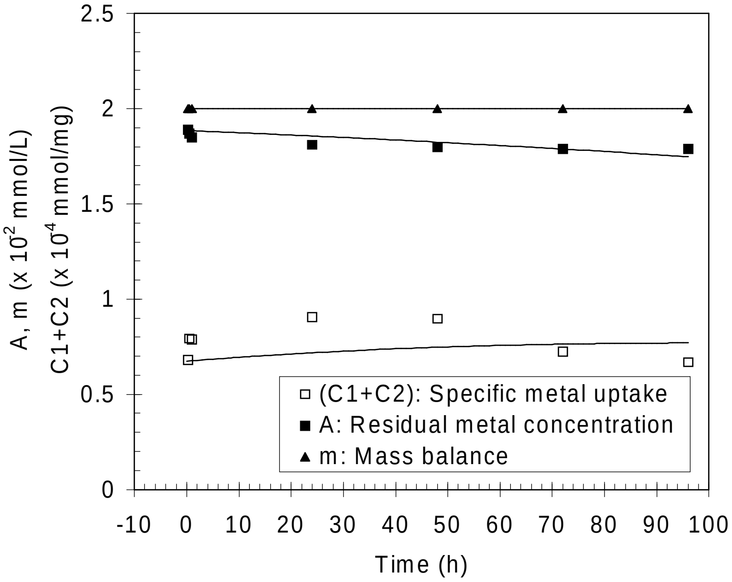

Figure 1 - Uptake of cadmium at 25°C with illumination. The model results are shown as solid lines.

Cellular growth for the third group of experiments

The exponential model presented a better correlation if compared to the linear model, also presenting a growth behavior similar to the first and second groups of tests. The exponential growth equation, according to procedure previously described, is presented below:

$$x = 12{,}372e^{0,00732 \cdot t} \qquad (21)$$

Fig. 2 shows the graph comparing the exponential model to experimental data. Addition of cadmium at the beginning of the experiment inhibited the algae growth that developed with a slower velocity.

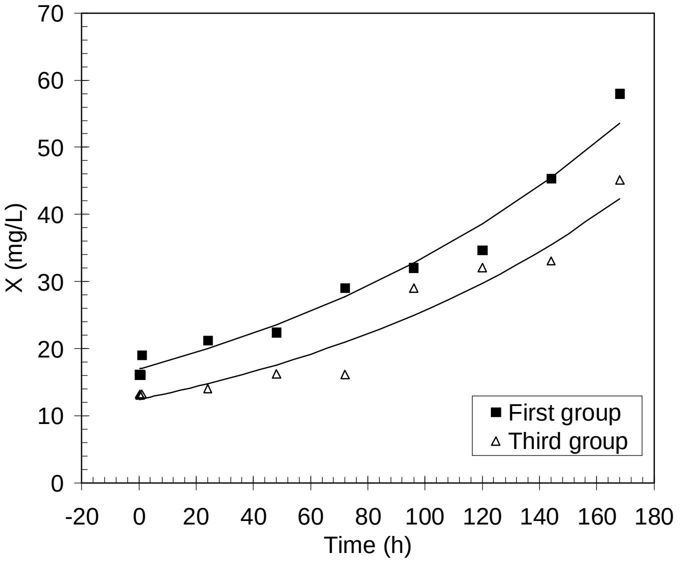

Figure 2 - Algae growth slopes without addition of cadmium (first group) and with addition of cadmium since the beginning of the inoculation (third group).

Fig. 3 shows the graph of the cadmium adsorption when it was added at the beginning of the cellular growth. A higher absorption of cadmium ions occurred at the beginning of the experiment followed by a slow absorption.

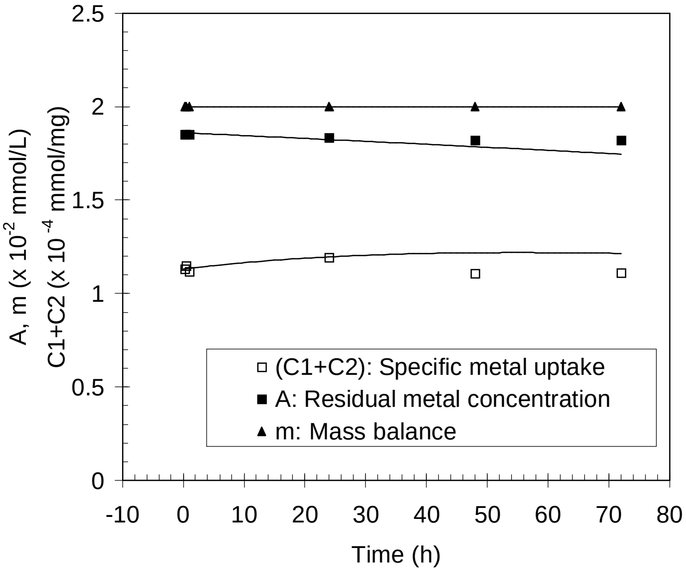

Figure 3 - Uptake of cadmium at 25°C with illumination. The model results are shown as solid lines.

Table 2 shows the values of the constants related to both the groups.

Table 2 - Model parameters

Experiments	Medium contitions		K	R_1	R_2
	°C	pH	(mg/L)	(h^{-1})	(ad.l)
2nd group	25	7.0	280.18	0.004	1.33177
3rd group	25	7.0	164.6	0.0045	4.4258

CONCLUSIONS

The mathematical model developed describes the uptake of cadmium metal ions by *P. subcapitata*. The model is described by two removal mechanisms: surface adsorption and transportation through the membrane. It was observed that a growth inhibition occurred when cadmium was added along with the inoculum in the 3rd group. The surface adsorption (passive adsorption) involved no metabolic process and did not either requires any energy from the cells. The transportation through the membrane (activate transportation) depends on the metabolic process, light intensity (in order to perform photosynthesis), and temperature. In this work, the temperature was maintained as constant at approximately 25°C and pH = 7.0.

The effect of a variation under medium conditions reflected on the changes of the model parameters K, R_1 and R_2. It was observed that changes on the model parameters occurred with the addition of cadmium with the growth and addition after 6-hours growth.

The adsorption constant K is a property characteristic of the cell surface and it is expected to be dependent on the different medium conditions, particularly composition and pH. The constant of the rate R_1 and the constant of the transporter R_2 are medium-dependent. The model validity was confirmed for the system, once *P.*

subcapitata accumulated cadmium ions from the solution.

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RESUMO

Neste trabalho utilizou-se a microalga *Pseudokirchneriella supcapitata* para a remoção do cádmio em líquidos. A metodologia consistiu de três conjuntos de experimentos: o primeiro conjunto teve como objetivo a avaliação do crescimento da alga, o segundo foi à avaliação da remoção de cádmio e o terceiro a avaliação do crescimento da alga sendo adicionado o cádmio junto com o inóculo no tempo igual a zero, também foi avaliada a remoção de cádmio neste terceiro experimento. O acúmulo de íons metálicos pela alga ocorre em duas etapas: uma absorção muito rápida (adsorção passiva) seguida por uma absorção mais lenta (absorção ativa) Desenvolveu-se um modelo matemático baseado na absorção da superfície e no transporte para o interior da membrana celular. A obtenção dos parâmetros cinéticos do modelo de simulação foi obtida experimentalmente. Pelos resultados observados o modelo matemático proposto mostrou-se adequado quando comparado aos resultados experimentais, confirmando a validação do modelo matemático proposto.

REFERENCES

Assis, J. C. (1998), Água sob medida. *Agroanalysis*, **18,** 83-88.

Baes, C. F. et al. (1979), The Hidrollysis of Cations, New York: John W. and Sons. pp. 4, 6, 15 e 24.

Castro, M. T. P. (2000), Tese de Doutorado Unicamp. pp.33-39.

Crist, R. H. et al. (1988), Interactions of metals and prótons with algae, *Env. Sc. Technology.*, USA, **22**, 755-760.

Gadd, G. M. (1988), Acumulation of metals by microorganisms and algae, *Biotechonology -A comprehensive treatise*, Winheim, **6b**, 401-433.

Hughes, M. N. and Poole, R. K. (1989), Metals and Microorganisms. Londres: Chapman and Hall Ltd.

Nakajima, A. and Sakagushi, T. (1986), Selective accumulation of heavy metals by microorganisms, *Appl. Biotechonol.*, Berlim, **24**, 59-64.

Steinkellner H. et al. (1998), Genotoxic effects of heavy metals: comparative investigation with plant bioassays. *Environ Mol Mutagen*, 31-91.

Ting, Y. P. et al. (1989). Uptake of cadmium and zinc by algae *Chlorella vulgaris*, *Biotechnol., Bioeng.* , New York, **34**, 990-999.

Trevors, J. T. et al. (1986), Cadmium transport resistente and toxicity in bacteria, algae and fungi. *Microbiol.*, Otawa, **32**, 447-467.

Casiraghi, M. P. (2003), Remoção de Cádmio em Líquidos pela Microalga *Pseudokirchneriella supcapitata*, Dissertação (Mestrado), Universidade Federal da Bahia, Brazil.

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