

Sorption isotherm studies of Cd(II) ions using living cells of the marine microalga *Tetraselmis suecica* (Kylin) Butch

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Abstract

The present work reports the use of living cells of the marine microalga *Tetraselmis suecica* for the biosorption of cadmium ions. For a better understanding of the biosorption characteristics, three fractions of removed cadmium (total, bioadsorbed and intracellular) were measured in the cells after 24 and 72 h of exposure to different initial cadmium concentrations (0.6–45 mg L⁻¹). Both the Langmuir and Freundlich models were suitable for describing the sorption of cadmium ions by this microalga. The maximum sorption capacity was estimated to be 40.22 mg Cd g⁻¹ after 72 h using the Langmuir sorption model. In the lower cadmium concentrations, metal removed intracellularly was higher than that removed on the microalgal cell surface. Therefore, the intracellular fraction contributed more to the total removed cadmium than the fraction bioadsorbed to the cellular surface. The results showed that the cadmium removal capacity using living biomass could be much more effective than with non-living biomass due to the intracellular bioaccumulation. According to the microorganism selected and its tolerance to the toxic effect of the metal, the cadmium content in the intracellular fraction can become very significant, just like it happened with *Tetraselmis*.

Keywords

Cadmium; Marine microalga; *Tetraselmis suecica*; Biosorption; Living cells

1. Introduction

The mobilization of heavy metals in the environment because of industrial activities is of serious concern due to the toxicity of these elements in humans and other organisms. Metal contamination is specially concentrated in coastal areas, which are sometimes polluted by several metals (Furness and Rainbow, 1990). This pollution represents a significant

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environmental problem because metals are non-biodegradable and they are bioaccumulated throughout the food chain. Among toxic metals, cadmium is one of these elements responsible for polluting the ecosystems and with the greatest potential hazard to humans and the environment. Cadmium is not essential for the living beings and it causes various deleterious effects on them (Bertina and Averbek, 2006), being considered a priority pollutant. This metal is widely used in industrial processes (electroplating, batteries, paints, etc.) and many effluents contain high concentrations of this element. As a result of this, in recent years, there is an increase in the demand of processes for cadmium ions removal from polluted environments.

Conventional treatment methods for the removal of metal ions, including chemical precipitation, ion exchange and adsorption, electrochemical deposition, reverse osmosis, evaporation, etc., do not seem to be economically feasible and they have disadvantages and limitations concerning their use. These techniques are often ineffective or expensive when the concentrations of the metal ions are low, in the order of 1–100 mg L⁻¹. In addition, all these methods have high reagent and energy requirements. New technologies are required that can reduce heavy metal concentrations to environmentally acceptable levels at affordable costs. Biosorption has recently attracted growing interest. The use of biological materials as biosorbents for metals is an effective and economical alternative to other treatments (Volesky, 2001). Moreover, this methodology offers an eco-friendly approach for bioremediation of metal contaminated environments.

Different biological materials can be used for the effective removal and recovery of metals; nevertheless, the use of microbial biomass offers a potential alternative, which is in continuous development and improvement. In this way, biosorption of metals has been reported in bacteria, yeast, fungi, cyanobacteria and microalgae. Among these microorganisms, microalgal and cyanobacterial biomass have showed a high capacity to remove metals both as free (Çetinkaya Dönmez et al., 1999 and Radway et al., 2001) and immobilized (Shashirekha et al., 2008) biomass; in case of cadmium, even better than the biomass of other microorganisms or other biosorbents (Aksu, 2001, Bayramoğlu et al., 2006, Doshi et al., 2007, Tangaromsuk et al., 2002 and Torres et al., 1998). In spite of this, there are few studies about the use of microalgal biomass to remove cadmium, and in particular about the kinetic and isotherm characteristics of this process.

When the microbial biomass is used for this purpose, it is interesting to consider the possibility of using living microorganisms. The ability of living microorganisms to remove metals is evaluated in few studies (Doshi et al., 2007, Folgar et al., 2009, Pérez-Rama et al., 2002, Torres et al., 1998, Watanabe et al., 2003 and Yilmaz and Ensari, 2005). In this case, biosorption involves a combination of active and passive mechanisms. The first step is a passive uptake, rapid and reversible binding to the cellular surfaces. The second one is an active uptake, related to metabolic activity, and that it allows the intracellular bioaccumulation (Hu et al., 1996 and Kwan and Smith, 1991). Precisely, the use of living biomass allows the additional use of this second step to bioaccumulate more amount of metal inside the cell, therefore benefiting the removal of these elements. Nevertheless, the use of non-living biomass

would eliminate the problem of metal toxicity on the cells. For this reason, in order to achieve that this process could be significant, it is necessary that the used cells are so tolerant, as it is possible, to the toxic action of the metal. Under these conditions, the energy metabolism of the cells would remain active. In addition, it would be desirable that the intracellular tolerance mechanisms are suitable. These mechanisms would allow supporting a high uptake capacity in order to enhance the biosorption process.

The objective of this study is to investigate, using the sorption isotherm models, the cadmium biosorption capacity and its characteristics by living cells of the marine microalga *Tetraselmis suecica*, whose cells have a high tolerance to this metal (Pérez-Rama et al., 2006). Previous studies have showed that this microalga could be an effective system for cadmium removal (Pérez-Rama et al., 2002). The importance of the intracellular removal is discussed and demonstrated in this work.

2. Experimental procedures

2.1. Microalga, reagents and equipment

The microorganism employed in the present study was the marine microalga *T. suecica* (Kyllin) Butch.

Analytical grade chemicals and double deionised water (Milli-Q Millipore, 18.2 MΩ cm⁻¹ resistivity) were used for all solutions.

The seawater used to carry out the cultures was natural enriched seawater. Natural seawater was passed through a 0.45 µm-pore Millipore filter and a charcoal column (Activated Charcoal Norit®, Sigma–Aldrich) to eliminate organic chelating substances and subsequently sterilized at 121 °C for 20 min. The assays were carried out in this natural seawater with the addition of inorganic nutrients (Fábregas et al., 1986) but without Ethylenediaminetetraacetic acid (EDTA) and Tris. The salinity was 35‰ and the initial pH of the culture was 7.8.

A stock solution of cadmium was prepared by dilution of CdCl₂ in Milli-Q water to a final cadmium concentration of 10 g L⁻¹.

Cadmium concentrations in the solutions were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a VG Elemental Plasma Quad 2 ICP-MS System (VG Elemental, 63069 Offenbach, Germany).

2.2. *T. suecica* culture conditions

T. suecica cells were cultured for 72 h in the natural enriched seawater media containing several concentrations of CdCl₂. For the experiments, appropriate volumes of the cadmium stock solution were added to the enriched seawater to obtain cadmium concentrations of 0.6, 3, 6, 15, 30 and 45 mg L⁻¹.

Cultures were carried out in 250 mL glass bottles (PYREX). The bottles were previously rinsed with 10% (v/v) nitric acid and several times with Milli-Q water. Inside these bottles, the enriched

seawater and the volume of the cadmium stock solution were placed with the *T. suecica* cells (at the middle of the logarithmic phase) at an initial density of $25 \pm 0.4 \times 10^4$ cells mL⁻¹, equivalent to 0.046 ± 0.002 mg of dry biomass mL⁻¹. Cultures were maintained at 18 ± 1 °C under a light intensity of $68 \mu\text{E m}^{-2} \text{s}^{-1}$ using cool fluorescent light with 12:12 h of light–dark cycle and they were agitated by aeration with sterile air, at flow rate of 2 L min⁻¹. Samples of the experiments were taken after 24 and 72 h of exposure. The biosorption experiments were repeated three times and the average results are presented in this work.

2.3. Determination of cadmium removed by the cells

Three fractions of removed cadmium were obtained for a better understanding of this process: total removed cadmium, intracellular cadmium and bioadsorbed cadmium.

Total cadmium in the cells was determined by filtration of 15 mL aliquots from each culture of *T. suecica*. Each aliquot was filtered through two superposed 1.2 μm MF-Millipore filters. Filters were separately digested for 24 h with 1 mL of 15 M HNO₃ and 0.5 mL of 72% (w/w) HClO₄. Cadmium was measured in both filters and the lower filter was used as blank.

Intracellular cadmium was measured in the following way. A 25 mL aliquot from each microalgal culture was centrifuged at $4000 \times g$ for 5 min, the pellet was resuspended for 20 min in 25 mL of a solution containing 0.02 M EDTA dissolved in natural seawater. Afterwards, the cells were centrifuged and washed twice with natural seawater. The EDTA washing removed cadmium adsorbed on the cell surface, thereby allowing only intracellular cadmium to be measured. The washed pellet was digested as in total cadmium determination.

Cadmium bioadsorbed onto the cell surface was determined by subtracting the intracellular cadmium concentration from the total removed cadmium.

The cadmium removal percentage (%) was calculated based on cadmium added initially to each culture.

2.4. Cadmium measurement

Digested samples were brought to a final volume of 5 mL with Milli-Q water. Finally, cadmium ions present in the samples were measured by ICP-MS. The limit of detection (LOD) was 0.25 $\mu\text{g L}^{-1}$.

2.5. Biosorption isotherms

Two sorption isotherms were considered to identify the isotherm that better describes the cadmium biosorption by the living biomass of *T. suecica*. The linearized forms of the Langmuir and Freundlich equations were used for analysis and they are given as:

equation(1)

$$1/q_e = 1/q_{\max} + 1/(q_{\max} b C_e)$$

equation(2)

$$\ln q_e = \ln K_F + 1/n \ln C_e$$

where q_e (mg g⁻¹) is the equilibrium metal uptake, C_e (mg L⁻¹) is the concentration of metal in solution at equilibrium, q_{max} (mg g⁻¹) is the maximum uptake by the biomass and b , K_F and n are the constants corresponding to the respective isotherms. b (L mg⁻¹) is the constant related to the affinity for the material, K_F and n are the indicators of the sorption capacity and sorption intensity, respectively.

Isotherms were calculated for the three fractions of cadmium removed (total, intracellular and bioadsorbed) after 24 and 72 h of exposure.

2.6. Statistical analysis

All data represent the mean of three independent experiments. Standard deviation and error bars are indicated whenever necessary. Sorption data were fitted to Langmuir and Freundlich isotherm models (Eqs.(1) and (2)) using linear regression analysis. All statistical analysis and plots were performed using SigmaPlot for Windows version 10 software (Systat Software, Inc).

3. Results and discussion

T. suecica is a marine microalga species with a high tolerance to cadmium (Pérez-Rama et al., 2006), for this reason is interesting to study the properties of cadmium biosorption that this microalga has when it is used as living biomass. Living biomass has the property by means of which, cadmium can be removed not only by bioadsorption but also by bioaccumulation inside the cell. This additional fraction enhances the ability to remove a higher amount of metal. Because of this, cells with high tolerance to cadmium are preferred to achieve better metal bioaccumulation possibilities, especially, as the metal concentration increases in the medium.

Previous studies have shown that *T. suecica* has a median effective concentration (EC50) of 7.9 ± 1 mg L⁻¹ of cadmium after six days of exposure and it was able to survive in a medium containing a cadmium concentration as high as 15 mg L⁻¹ (Pérez-Rama et al., 2002).

3.1. Effect of the initial concentration of cadmium

The initial concentration of the metal in the medium influenced its uptake by the cells. In this way, the effect of the initial concentration of cadmium on the percentage of metal removed in the three fractions of *T. suecica* cells after 24 and 72 h of exposure is shown in Fig. 1 (a and b). After 24 h, the percentage of total removed cadmium descended as the metal concentration added to the medium increased. In all the cadmium concentrations, the percentage of cadmium removed by means of bioadsorption was higher than that removed in the intracellular fraction. This difference increased as the cadmium concentration increased in the medium. The maximum percentage of removed cadmium was obtained in the lowest concentration of this metal. As cadmium concentration increased, the efficiency progressively decreased. In all the

tested concentrations, the percentage of cadmium removed intracellularly was high, being always greater than 14% (Table 1).

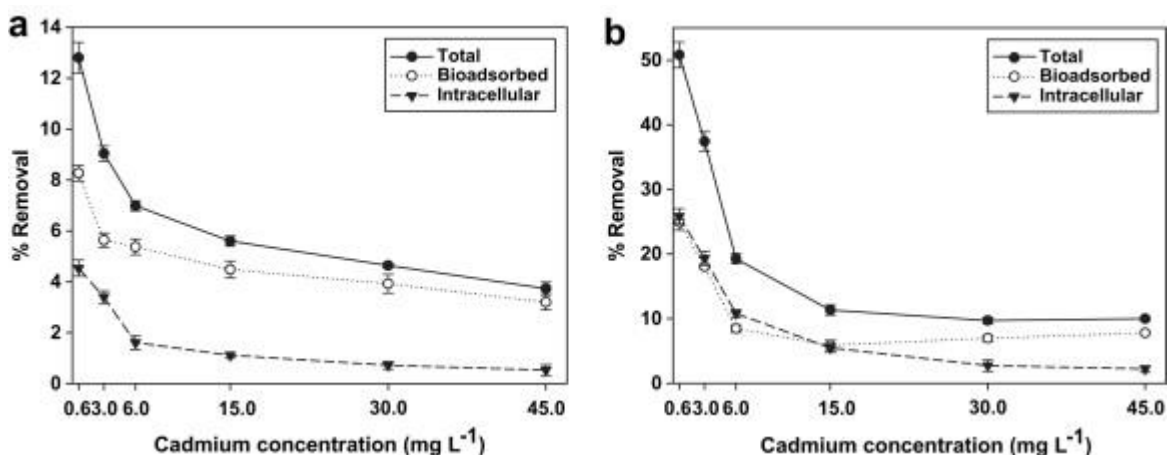


Fig. 1.

Percentage of cadmium removed by living cells of the marine microalga *Tetraselmis suecica* after 24 h (a) and 72 h (b) in relation to different cadmium concentrations in the medium.

Table 1.

Relative percentages of cadmium removed in the bioadsorbed and intracellular fractions measured in the *Tetraselmis suecica* cells.

Initial Cd concentrations (mg L ⁻¹)	24 h		72 h	
	Bioadsorbed	Intracellular	Bioadsorbed	Intracellular
0.6	64.58 ± 2.1	35.42 ± 1.9	49.15 ± 2.6	50.85 ± 2.3
3	62.35 ± 1.1	37.65 ± 2.2	48.29 ± 1.3	51.71 ± 1.1
6	76.92 ± 1.9	23.08 ± 0.8	43.96 ± 2.2	56.04 ± 1.4
15	80.09 ± 3.1	19.91 ± 1.5	51.67 ± 3.1	48.33 ± 2.4
30	84.48 ± 2.9	15.52 ± 1.1	71.82 ± 1.1	28.18 ± 0.9
45	85.71 ± 4.2	14.29 ± 0.9	77.78 ± 4.1	22.22 ± 0.7

Data are means ± S.D. (n = 3).

On the other hand, after 72 h of exposure, the percentage of removed cadmium was higher than that removed after 24 h in all the concentrations assayed (Fig. 1b). A significant fact was that, in the lower cadmium concentrations (0.6, 3 and 6 mg L⁻¹), the intracellular fraction presented the highest percentage of removed cadmium in relation to the bioadsorbed fraction. However, the percentage of intracellular cadmium decreased from the concentration of 6 mg L⁻¹, remaining below the bioadsorbed (Table 1). It is important to point out that precisely from this concentration, which coincided with the EC50 value, when the percentage of intracellular cadmium descended. This may be attributed to the toxic effect of cadmium. With the increase of cadmium concentration in the medium, the toxic effect of this metal also increased; this reduced the energy metabolism and therefore, the active uptake of the metal to the cellular interior declined. Due to the fact that the metal incorporation to the cellular interior is a passive initial process followed by another active, the reduction of the last one supposed a lesser accumulation of the metal.

In addition, as seen from Table 1, the percentage of intracellular cadmium was always higher at 72 h than at 24 h in all the concentrations tested, being its value higher than 22%. These values demonstrate, once again, the importance of the intracellular removal.

3.2. Analysis of biosorption isotherms

Two sorption models were considered to identify the properties that describe in a better way the biosorption of cadmium ions by living cells of *T. suecica*. The Freundlich and Langmuir models are well known and they were used in this study.

With the data obtained in the experiments, it is observed that it was possible the application of the two isotherms to the three fractions of cadmium measured in this microalga. All isotherm models fit very well and they gave linear plots. In view of the values of linear regression coefficients (R^2) given in Table 2, it was noted that the Langmuir isotherm model exhibited a better fit to the sorption data than the Freundlich isotherm model.

Table 2.

Isotherm model constants for the cadmium removal by living biomass of *T. suecica*.

		Langmuir			Freundlich		
		q_{\max} (mg g ⁻¹)	b (L mg ⁻¹)	R^2	K_F	1/n	R^2
24 h	Total	30.128	2.579	0.994	0.362	0.782	0.997
	Bioadsorbed	26.752	1.557	0.992	0.431	0.765	0.994
	Intracellular	5.170	0.886	0.997	1.697	0.558	0.970
72 h	Total	40.222	5.683	0.993	0.183	0.820	0.995
	Bioadsorbed	29.164	1.808	0.994	0.732	0.998	0.981
	Intracellular	23.876	1.925	0.999	0.485	0.716	0.972

3.2.1. Langmuir isotherm

The Langmuir isotherm was used for the estimation of maximum biosorption capacity. Fig. 2 (a and b) shows the plots of this isotherm for the three fractions measured after 24 and 72 h of exposure, respectively. The Langmuir isotherm provides good information about the cadmium uptake by *T. suecica* because the coefficients of determination (R^2) generated were 0.994, 0.992 and 0.997 for total, bioadsorbed and intracellular cadmium respectively, after 24 h. Moreover, in the case of 72 h, the obtained coefficients were also high, 0.993, 0.994 and 0.999, respectively (Table 2). These data suggested that the process of biosorption of cadmium ions by biomass of *T. suecica* followed the Langmuir model.

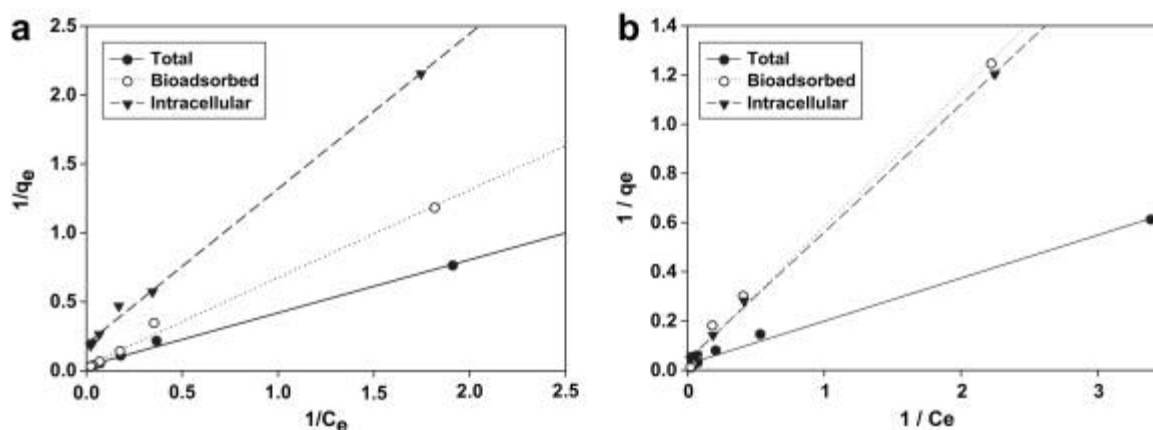


Fig. 2.

The linearized Langmuir isotherms of total, bioadsorbed and intracellular cadmium in living cells of *T. suecica* after 24 h (a) and 72 h (b) of exposure to the metal.

3.2.1.1. Total cadmium removed

The maximum biosorption capacity (total removed cadmium) derived from the linear regression equation of this model was 30.13 mg g⁻¹ after 24 h. This capacity increased to 40.22 mg g⁻¹ after 72 h. When it is compared to other biosorbents, *T. suecica* had similar properties to yeast cells *Saccharomyces cerevisiae* and *Kluyveromyces fragilis*, which had a maximum biosorption capacity of 35 and 40 mg g⁻¹ respectively (Hadi et al., 2003). This cadmium removed by the microalga was higher than that removed by whole mycelia of *Aspergillus niger*, *Rhizopus oryzae*, and *Mucor rouxii* with a metal binding capacity of 7.1, 31.6 and 27.1 mg g⁻¹ respectively (Baik et al., 2002). Nevertheless, this maximum capacity was lower than that of other microalgae, for example, *Chlorella vulgaris* had a maximum capacity of 62.3 mg g⁻¹ at pH 4.0 (Aksu, 2001).

Matsunaga et al. (1999) performed screening of marine microalga for cadmium removal. Twenty-four strains were tested and after 12 d of incubation, the marine microalga *Chlorella* sp. NKG16014 showed the highest cadmium removal capacity. In this case, the maximum cadmium sorption was 37 mg Cd g⁻¹ using dry cells.

Another microalga, which also presented high tolerance to cadmium, was *Dunaliella salina*. It was capable of removing a maximum amount of cadmium of 52 mg g⁻¹ after 24 h and under the same culture conditions than *T. suecica* (Folgar et al., 2009). Finally, living cells of the cyanobacterium *Spirulina* sp. reached a value of 625 mg g⁻¹ at pH 6.0 (Doshi et al., 2007).

Earlier studies on heavy metal biosorption have shown that pH is an important parameter affecting the biosorption process. The most studies showed that the maximum biosorption capacity is achieved at pH in the range 5.0–6.0 (Aksu, 2001, Bayramoğlu et al., 2006 and Tangaromsuk et al., 2002), as pH increases the biosorption capacity decreases. In the present study, the pH was initially 7.8 with the purpose of keeping the cells with their metabolism active

and therefore that the cells were alive. At pHs upper than 6.0, the complexation of cadmium ions by hydroxyl groups would prevent the metal sorption, reducing the maximum capacity. It is also necessary to bear in mind that this removal capacity was measured in the presence of the components of a culture medium with seawater, in which, cadmium co-exist with large amounts of sodium and other cations with the same valence (mainly calcium and magnesium). This situation is more realistic than the simple fact of exposing the biomass to a metallic solution in deionised water. The influences of major nutrients additions on metal sorption in the marine microalga *D. salina* were examined in (Shun-Xing et al., 2007), showing differences in the uptake of metals under different nutrient regimes.

As seen from Table 2, the values of b obtained were high. A large value of b also implied strong bonding of cadmium to the *T. suecica* cells.

3.2.1.2. Bioadsorbed cadmium

Regarding to the cadmium that was bioadsorbed by the living biomass of *T. suecica* after 24 h of culture, the maximum removal capacity in this fraction that is deduced from Langmuir was 26.75 mg g⁻¹. This capacity was 29.16 mg g⁻¹ after 72 h. As it is observed, this capacity was below the maximum value that this microalga was capable of removing. The explanation of this fact is that, to this cadmium removed by means of bioadsorption, it is necessary to add the amount removed in the intracellular fraction. This mechanism increased the total amount of cadmium ions removed from the medium. If only this fraction is considered, it might be comparable to a situation in which non-living biomass is used, in which cadmium is only bioadsorbed.

3.2.1.3. Intracellular cadmium

The maximum cadmium removal capacity in the intracellular compartment calculated through the linearized form of the Langmuir isotherm was only 5.17 mg g⁻¹ after 24 h. Nevertheless, this capacity increased significantly after 72 h, reaching a value of 23.88 mg g⁻¹ (Table 2). Cadmium entering the cytoplasm may be captured by phytochelatins (PCs), thiol-rich compounds, whose synthesis is induced in presence of external cadmium. Within the cytosol, PCs form complexes with cadmium that decrease its toxicity. Precisely, in case of *T. suecica* was after 72 h when the highest phytochelatin accumulation took place in the tested concentrations (Pérez-Rama et al., 2006). Nassiri et al. (1997) demonstrated that cadmium was stored specifically in the osmiophilic vesicles of *T. suecica*, probably bounded to these molecules. This tolerance mechanism would explain this increase in the capacity of cadmium removed by means of bioaccumulation, constituting an important fraction of the removed cadmium.

Cadmium removal by *Chlorella* sp. NKG16014 was also evaluated by measuring the amount of cell adsorption and intracellular bioaccumulation (Matsunaga et al., 1999). After 12 d of

incubation, 67% of the removed cadmium was accumulated intracellularly and only a 25% was adsorbed on the microalgal cell surface. Therefore, in this microalga, the cadmium uptake by the living cells was also enhanced by intracellular accumulation. The same happened with *T. suecica*, in which, only after 72 h, between 50 and 60% of the removed cadmium remained intracellularly. Moreover, in case of this microalga, the increase in the amount of cadmium removed intracellularly was higher than in case of other microalgae; for example, in *D. salina* the amount of cadmium removed intracellularly was lower than 40% (Folgar et al., 2009).

3.2.2. Freundlich isotherm

Freundlich model effectively described the sorption of cadmium by living cells of *T. suecica*. In this way, the Freundlich isotherm model was used to estimate the biosorption intensity of *T. suecica* cells towards cadmium. Application of this isotherm is shown in Fig. 3 (a and b). The Freundlich sorption constants (K_F and $1/n$) evaluated from the isotherms with the correlation coefficients are shown in Table 2. As seen in table, regression correlation coefficients for all the measured fractions and for the two exposure times were very high. These values indicated that the sorption isotherm followed the Freundlich equation satisfactorily within the range of cadmium concentrations assayed.

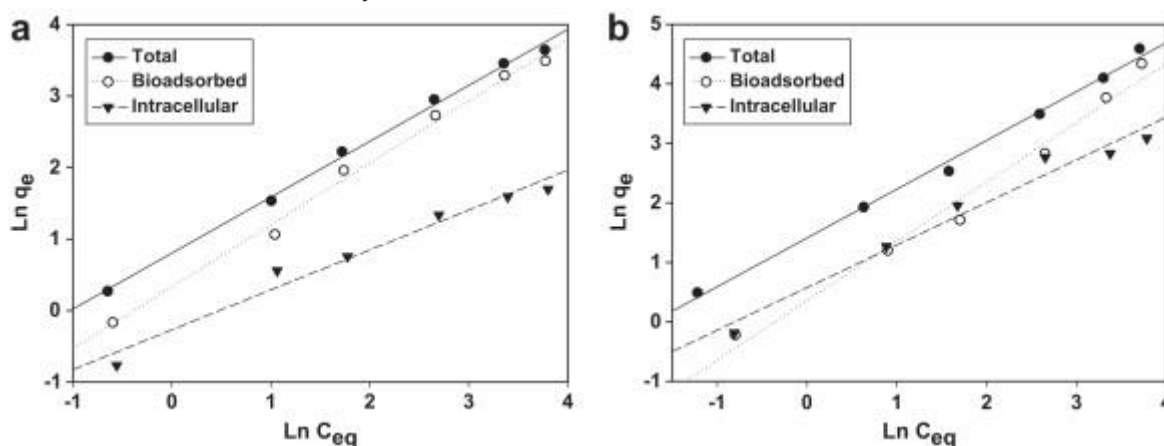


Fig. 3.

The linearized Freundlich isotherms of total, bioadsorbed and intracellular cadmium in living cells of *T. suecica* after 24 h (a) and 72 h (b) of exposure to the metal.

Values of n over one suppose a beneficial sorption, indicating that cadmium ions are favourably sorbed by *T. suecica* cells. At both 24 and 72 h, the value of n obtained for the intracellular cadmium was higher than the obtained for the bioadsorbed fraction. The values of $1/n$ lower than unity are an indication that a significant sorption takes place at low concentration, but the increase in the amount sorbed with concentration becomes less significant at higher concentrations (Teng and Hsieh, 1998). In case of the living biomass of *T. suecica*, the obtained data showed the minor value of $1/n$ for the fraction removed intracellularly, which

indicated that the intracellular removal was higher at low metal concentrations in the medium than at high ones, being this due to the toxic effect of the metal.

The K_F value is an indication of the biosorption capacity. The values of K_F obtained using these isotherms were low for the three fractions, when compared with typical values in the literature. It is common to find higher values, or even much higher, than one. This low sorption capacity could be due to the composition of the culture medium, whose components interfered in the metal biosorption capacity.

4. Conclusion

The aim of this work was to find the cadmium removal characteristics of living biomass of the marine microalga *T. suecica*. Three fractions of cadmium in the cells were measured (total, bioadsorbed and intracellular) after 24 and 72 h, and Langmuir and Freundlich models were applied successfully to describe the process. The obtained results showed that the sorption data fitted very well to both models for the three measured fractions. Based on the Langmuir model, living cells of this microalga could remove cadmium at a maximum capacity of 40.22 mg Cd g⁻¹ after 72 h of exposure. In the lower cadmium concentrations assayed, cadmium removed intracellularly was higher than that adsorbed on the microalgal cell surface, contributing significantly to the total cadmium removal. Living cells of this microalga are more suitable than non-living biomass to remove a higher amount of cadmium. This is because the metal that has been bioaccumulated inside the cells contributed to this removal significantly, being even higher than the cadmium removed by biosorption to the external surface of the cells. This happened while the toxic effect of the metal was not high enough as to prevent the intracellular bioaccumulation.

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References

- Z. Aksu
Equilibrium and kinetic modelling of cadmium(II) biosorption by *C. vulgaris* in a batch system: effect of temperature
Sep. Purif. Technol., 21 (2001), pp. 285–294
- W.Y. Baik, J.H. Bae, K.M. Cho, W. Hartmeier
Biosorption of heavy metals using whole mold mycelia and parts thereof
Bioresour. Technol., 81 (2002), pp. 167–170
- G. Bayramoğlu, I. Tüzün, G. Çelik, M. Yılmaz, M.Y. Arica

Biosorption of mercury(II), cadmium(II) and lead(II) ions from aqueous system by microalgae *Chlamydomonas reinhardtii* immobilized in alginate beads
Int. J. Miner. Process, 81 (2006), pp. 35–43

G. Bertina, D. Averbeck

Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review)

Biochimie, 88 (2006), pp. 1549–1559

Çetinkaya Dönmez et al., 1999

G. Çetinkaya Dönmez, Z. Aksu, A. Öztürk, T. Kutsal

A comparative study on heavy metal biosorption characteristics of some algae

Process Biochem., 34 (1999), pp. 885–892

H. Doshi, A. Ray, I.L. Kothari

Biosorption of cadmium by live and dead *Spirulina*: IR spectroscopic, kinetics and SEM studies

Curr. Microbiol., 54 (2007), pp. 213–218

J. Fábregas, C. Herrero, B. Cabezas, J. Abalde

Biomass production and biochemical composition in mass cultures of the marine microalga *Isochrysis galbana* Parke at varying nutrient concentrations

Aquaculture, 53 (1986), pp. 101–113

S. Folgar, E. Torres, M. Pérez-Rama, A. Cid, C. Herrero, J. Abalde

Dunaliella salina as a marine microalga highly tolerant to but a poor remover of cadmium

J. Hazard. Mater., 165 (2009), pp. 486–493

R.W. Furness, P.S. Rainbow

Heavy Metals in the Marine Environment

CRC Press, Inc, Boca Raton, Florida (1990)

B. Hadi, A. Margaritis, F. Berruti, M. Bergougnou

Kinetics and equilibrium of cadmium biosorption by yeast cells *S. cerevisiae* and *K. fragilis*

Int. J. Chem. Reactor Eng., 1 (2003), pp. 1–18

S. Hu, C.H. Tang, M. Wu

Cadmium accumulation by several seaweeds

Sci. Total Environ., 187 (1996), pp. 65–71

K.H.M. Kwan, S. Smith

Some aspects of the kinetics of cadmium and thallium uptake by fronds of *Lemna minor* L

New Phytol., 117 (1991), pp. 91–102

T. Matsunaga, H. Takeyama, T. Nakao, A. Yamazawa

Screening of marine microalgae for bioremediation of cadmium-polluted seawater
J. Biotechnol., 70 (1999), pp. 33–38

Y. Nassiri, J. Wéry, J.L. Mansot, T. Ginsburger-Vogel
Cadmium bioaccumulation in *Tetraselmis suecica*: an electron energy loss spectroscopy (EELS) study
Arch. Environ. Contam. Toxicol., 33 (1997), pp. 156–191

M. Pérez-Rama, J. Abalde, C. Herrero, E. Torres
Cadmium removal by living cells of the marine microalga *Tetraselmis suecica*
Bioresour. Technol., 84 (2002), pp. 265–270

M. Pérez-Rama, E. Torres, J. Abalde
Composition and production of thiol constituents induced by cadmium in the marine microalga *Tetraselmis suecica*
Environ. Toxicol. Chem., 25 (2006), pp. 128–136

J.C. Radway, E.W. Wilde, M.J. Whitaker, J.C. Weissman
Screening of algal strains for metal removal capabilities
J. Appl. Phycol., 13 (2001), pp. 451–455

V. Shashirekha, M.R. Sridharan, S. Mahadeswara
Biosorption of trivalent chromium by free and immobilized blue green algae: kinetics and equilibrium studies
J. Environ. Sci. Health A, 43 (2008), pp. 390–401

L. Shun-Xing, H. Hua-Sheng, Z. Feng-Ying, D. Nan-Sheng, L. Fang
Influence of nitrate on metal sorption and bioaccumulation in marine phytoplankton, *Dunaliella salina*
Environ. Toxicol., 22 (2007), pp. 582–586

J. Tangaromsuk, P. Pokethitiyook, M. Kruatrachue, E.S. Upatham
Cadmium biosorption by *Sphingomonas paucimobilis* biomass
Bioresour. Technol., 85 (2002), pp. 103–105

H. Teng, C. Hsieh
Influence of surface characteristics on liquid-phase adsorption of phenol by activated carbons prepared from bituminous coal
Ind. Eng. Chem. Res., 37 (1998), pp. 3618–3624

E. Torres, A. Cid, C. Herrero, J. Abalde
Removal of cadmium ions by the marine diatom *Phaeodactylum tricornutum* Bohlin accumulation and long-term kinetics of uptake
Bioresour. Technol., 63 (1998), pp. 213–220

B. Volesky

Detoxification of metal-bearing effluents: biosorption for the next century
Hydrometallurgy, 59 (2001), pp. 203–216

M. Watanabe, K. Kawahara, K. Sasaki, N. Noparatnaraporn
Biosorption of cadmium ions using a photosynthetic bacterium, *Rhodobacter sphaeroides* S and a marine photosynthetic bacterium, *Rhodovulum* sp. and their biosorption kinetics
J. Biosci. Bioeng., 95 (2003), pp. 374–378

E.I. Yilmaz, N.Y. Ensari
Cadmium biosorption by *Bacillus circulans* strain EB1
World J. Microbiol. Biotechnol., 21 (2005), pp. 777–779

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