Isotherm studies for the determination of Cd(II) ions removal capacity in living biomass of a microalga with high tolerance to cadmium toxicity.

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Abstract

The biosorption characteristics of Cd(II) ions using the living biomass of the marine diatom *Phaeodactylum tricornutum* were investigated. This microalga is a highly tolerant species to cadmium toxicity, for this reason is interesting to know its potential for use in the removal of Cd(II) ions. The use of living biomass offers better possibilities than dead biomass since cadmium can also be bioaccumulated inside the cells. For this purpose, tolerant species are necessary. *P. tricornutum* is within this category and in the present manuscript is demonstrated that this microalga has a very good potential for bioremediation of Cd(II) ions. Cadmium removed by the cells was divided into three fractions: total, intracellular and bioadsorbed. The experiments were conducted for 96 h in a concentration range of 1-100 mg Cd(II)/L. Each fraction was characterized every 24 h by sorption isotherms. The experimental isotherm data were analysed using the Langmuir, Freundlinch, Dubinin-Radushkevich and Temkin equations. The biosorption was well described by Langmuir isotherm followed by Freundlich. The worst model was Temkin. The biosorption capacity of this microalga for Cd(II) ions was found to be 67.140 ± 3.247 mg/g after 96 h with approximately 40% of this capacity in the intracellular fraction. The bioconcentration factor determined was 2204.7 after 96 h and with an initial Cd(II) concentration of 1 mg/L.

Keywords: cadmium, biosorption isotherm, bioaccumulation, Phaeodactylum tricornutum, living biomass

1. Introduction

Heavy metal pollution remains one of the major environmental problems. Since these elements persist without degradation indefinitely, they can accumulate throughout the food chain with adverse health effects in humans. Thus, cadmium is a common pollutant introduced into aquatic ecosystems through discharges from many industries. This heavy metal is considered a non-essential and non-beneficial element to organisms, causing toxicity (Hasanuzzaman and Fujita 2013; Maret and Moulis 2013). For this reason is mandatory that the cadmium levels in the water bodies must be reduced to allowable concentrations. In this way, the development of systems for the removal of this metal from aqueous solutions is important for the protection of the environment and human health.

Although there are different methods for the removal of metals, biosorption technology has gained important credibility during recent years because of its eco-friendly nature, cost-effectiveness, no production of toxic secondary products and excellent performance. These properties lead to biological remediation techniques are preferred to physical or chemical technologies. These procedures involve the use of a biological material as metal sorbent. Different biological materials can be used for the effective removal and recovery of metals (Dixit and Singh 2013; Karnika et al. 2007; Sekabira et al. 2011; Volesky 2001); nevertheless, over the last few years, microalgal biomass has become an important and attractive material for biosorbents. The potential of many microalgae and cyanobacteria in the removal of heavy metal ions from aqueous effluents has been well demonstrated (Monteiro et al. 2012; Rajamani et al. 2007; Torres et al. 2013). This biomass has high metal binding capacities due to the composition of the cellular surface of the microalgae, which provides numerous metal binding sites (bioadsorption). In addition, microalgae can take up metal ions into the cell and accumulate them in large quantities (bioaccumulation) (Bayramoĝlu et al. 2006; Inthorn et al. 2002; Pérez-Rama et al. 2010; Volesky 1990). These properties give microalgae a great advantage as metal bioconcentrators in view of their use in biological water remediation techniques.

The use of living biomass has the ability to remove metals by these two processes, bioadsorption and bioaccumulation. At present, most of the studies on heavy metal biosorption focused on dead biomass using different physicochemical pre-

treatments (Michalak et al. 2013). Although, the use of dead biomass eliminates the problems of toxicity and proper maintenance, metals are only removed by bioadsorption. In addition to the removal by these two processes, the use of live microalgae has other advantages over dead biomass such as the possibility of improvement through genetic or metabolic engineering approaches. An ideal species of microalga that can be used as living biomass should have a number of requirements; survive in presence of high metal concentrations and bioaccumulate high concentrations. If the microalga can grow in presence of metal concentrations considered toxic, it can produce new biomass to remove a higher amount of this metal. For these reasons, the metal tolerance in microalgal cells is a key aspect in the use of certain microalgae as living biomass for the removal of metals.

Phaeodactylum tricornutum is a marine microalga with a high tolerance to cadmium toxicity (Torres et al. 1995). Furthermore, previous studies showed that this microalga is able to remove this metal by both biosorption and bioaccumulation (Torres et al. 1998). Due to these features, it would be very interesting to characterize the living biomass of this microalga through studies of sorption isotherms. The isotherm equations allow obtaining parameters that refer to the properties of the biomaterial and its affinity for the sorbate at a fixed temperature and pH. Thus, the main objective of the present study is to investigate, using the sorption isotherm models (Langmuir, Freundlich, Dubinin-Radushkevich and Temkin), the cadmium biosorption capacity of the marine microalga *P. tricornutum*. For a better characterization, three fractions were studied, total cadmium removed, cadmium removed intracellularly and cadmium removed by bioadsorption. Efficiency of removal, maximum sorption capacity, binding constants and bioconcentration factor were obtained; also, the importance of the intracellular removal in a high tolerance species is discussed.

2. Experimental

2.1. Microalga, reagents and equipment

The microorganism used in the present study was the marine microalga *Phaeodactylum tricornutum* Bohlin (*Bacillariophyceae*).

Analytical grade chemicals (Sigma-Aldrich®, St. Louis, MO, USA) and double deionised water (Milli-Q® Millipore, 18.2 MΩ/cm resistivity) were used for all

solutions. The seawater used for the cultures was natural organic-free seawater passed through a Millipore filter of pore size 0.45 µm, through a charcoal column to eliminate organic quelating substances and without addition of growth medium. Finally, the seawater was sterilised at 121ºC for 20 min. All assays were carried out in this unenriched seawater with a salinity of 35 ‰ and pH = 8.2.

A stock solution of cadmium was prepared by dissolving $CdCl_2$ in Milli-Q water to give a final concentration of 10 g/L of Cd(II).

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, Germany).

2.2. Batch biosorption experiments

P. tricornutum cells were culture in glass bottles (PYREX) for 96 h containing 500 mL of seawater. The bottles were previously rinsed with 10% nitric acid for 24 h and several times with Milli-Q water. In order to study the biosorption potential of *P. tricornutum* for cadmium, different concentrations of this metal were used. Inside the bottles, the seawater with an appropriate volume of the cadmium stock solution to obtain the cadmium(II) concentrations of 1, 5, 10, 25, 50, 75 and 100 mg/L were placed. Finally, the microalga (at the middle of the logarithmic phase) was added to obtain an initial density of $25x10^4 \pm 2x10^4$ cells/mL equivalent to 0.019 ± 0.002 mg/mL of dry biomass. Cultures were maintained photoautotrophically at a constant temperature of 18 ± 2ºC and under illumination of 68 µE/(m^2s), with a dark:light cycle of 12:12 h. Cultures were gently shaken every 24 h to ensure homogeneous exposure to metal.

Samples from each culture were taken every 24 h of exposure. The experiments were repeated three times and the average results ± S.D. are presented in the present work. A control cultures without cadmium was also included to study the toxic effect of cadmium.

2.3. Dry weight determination

The dry weight of the cultures was obtained by filtration (Zhu and Lee 1997). 0.45 µm acetate membrane filters (Millipore, Billerica, USA) were previously dried and weighed. A known volume from each culture was filtered through these filters. The

filters with the samples were washed three times with 0.5 M ammonium formate for the elimination of salts absorbed on the cell surface and present in the intracellular water. The filters were then dried in an oven at 90ºC for 24 h until constant weight. Finally, the filters were cooled in a desiccator for 30 min before being weighed again on the same balance. The dry weight of the sample was calculated by the difference between the two weights of the same filter.

2.4. Determination of cadmium removed by the cells

Three fractions of cadmium removed by *P. tricornutum* cells were obtained for a better characterization of this process: total removed cadmium, intracellular cadmium and bioadsorbed cadmium.

Total cadmium removed by the cells was determined by filtration of an aliquot from each culture of *P. tricornutum* through two superimposed 0.45 µm MF-Millipore filter. Each filter was separately digested for 24 h with 1 mL of 15 M HNO_3 and 0.5 mL of 72% (w/w) $HClO_4$. Cadmium was measured in the solution of both filters and the lower filter was used as a blank.

Intracellular cadmium was determined centrifuging an aliquot from each culture at 4000 x g for 5 min and the pellet resuspended in the same volume of natural seawater containing 0.02 M EDTA for 20 min. EDTA was used in experiments of desorption of metals since it has the ability to complex heavy metals and put them back into the solution (Lezcano et al. 2011; Olguín and Sánchez-Galván 2012). Afterwards, the cells were centrifuged and washed twice with natural seawater. This washing with EDTA removed cadmium adsorbed on the cell surface and allowed to determine the intracellular cadmium. The final pellet obtained was digested as in total cadmium determination.

Finally, cadmium bioadsorbed onto the cell surface was calculated by subtracting the intracellular cadmium from the total removed cadmium.

2.5. Determination of biosorption isotherms

Four sorption isotherms were considered to study the characteristics of the living biomass of *P. tricornutum* in the removed of cadmium. The Langmuir (Langmuir 1918) (1), Freundlich (Freundlich 1906) (2), Temkin (Temkin and Pyzhev 1940) (3) and

Dubinin-Radushkevich (Dubinin and Radushkevich 1947) (4) equations were used in the present study and they were expressed respectively by:

$$q_e = \left(q_{max} b C_e\right) \Big/ (1 + bC_e) \quad (1)$$

$$q_e = K_F C_e^{1/n} \quad (2)$$

$$q_e = (RT/b_T)\, Ln(A_T C_e) \quad (3)$$

$$q_e = q_{max} e^{-B_D \varepsilon^2} \quad (4)$$

where q_e (mg/g) is the amount of cadmium sorbed at equilibrium per unit mass of *P. tricornutum*, C_e (mg/L) is the cadmium concentration in solution at equilibrium, q_{max} (mg/g) is the maximum sorption capacity or theoretical isotherm saturation capacity, b (L/mg) is the constant related to the affinity for the biomaterial, K_F is an indicator of the sorption capacity and n is of the intensity, A_T is the Temkin isotherm equilibrium binding constant (L/g), corresponding to the maximum binding energy, b_T is a constant related to the heat of sorption (kJ/mol), R is the gas constant (8.314 J/mol K), T is the absolute temperature, B_D is related to the free energy sorption per mole of the sorbate and ε is the Polanyi potential which is related to the equilibrium concentration as follows:

$$\varepsilon = RTLn\left(1 + 1/C_e\right) \quad (5)$$

The apparent energy (E_D, KJ/mol) of sorption from Dubinin-Radushkevich isotherm model can be computed using the equation 6:

$$E_D = 1/\sqrt[2]{2B_D} \quad (6)$$

According to Hall et al. (1966), the essential features of the Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter, R_L, which is defined by the following relationship:

$$R_L = 1/(1 + bC_i) \quad (7)$$

where C_i is the initial cadmium concentration (mg/L) and b is the Langmuir constant (L/mg).

These isotherms and their parameters were calculated for the free fractions of cadmium removed (total, intracellular and bioadsorbed) every 24 h of culture for 96 h.

2.6. Bioconcentration factor

The bioconcentration factor (BCF) was applied to discuss the metal accumulation. This factor was calculated for the fraction that corresponds to the total cadmium removed by this microalga. It was calculated as follows:

$$BCF = \frac{mg\ cadmium\ removed/kg\ dry\ weight}{residual\ mg\ cadmium\ in\ solution/L\ solution} \quad (8)$$

2.7. Statistical analysis

All data represent the mean of three independent experiments. Sorption data were fitted to the isotherms (eqs. (1), (2), (3) and (4)) using nonlinear regression analysis. All statistical analysis and plots were performed using Sigmaplot for Windows version 12.5 (Systat Software, Inc.). Experimental data among treatments were compared with one-way ANOVA using SPSS version 21.0 (SPSS Ibérica, Spain). Tukey's multiple comparisons test was carried out to distinguish significant differences. The significant differences was set among treatments at $p<0.05$.

3. Results and Discussion

The use of living biomass in the removed of metals involves having species that tolerate high concentrations of a metal by some intracellular mechanism that also can retain metal ions. In this way, a significant fraction of removed cadmium lies in the intracellular compartment, increasing the amount of cadmium removed when compared to dead biomass in which the removal process is performed only by bioadsorption. Microalgae, whose tolerance mechanisms prevent the incorporation of metal or have systems for its expulsion (Folgar et al. 2009) will be unsuitable for use in this process. These microalgae, although metal-tolerant, the amount of metal removed is virtually comparable to the removed as dead biomass. *Phaeodactylum tricornutum* belongs to the first category. It is a marine microalga that is characterized by a high tolerance to cadmium toxicity with an EC_{50} of 22.39 mg Cd(II)/L after 96 h of exposure (Torres et al. 1995). Its tolerance is because its response to the toxicity of this element is the intracellular synthesis of complexing cadmium compounds, phytochelatins (Torres et al.

1997). These properties make this microalga in a very interesting microorganism to study its ability to remove cadmium.

3.1. Effect of cadmium on the growth and the final biomass production of P. tricornutum

Figure 1 shows the growth curves obtained with the different cadmium treatments. The toxic effect of this element increased as its initial concentration increased in the cultures. With the culture conditions used in the experiments, the control cultures reached the stationary phase at 96 h. Obviously, after this time of culture, the yielded biomass (dry weight) decreased as cadmium increased. ANOVA and Tukey's test showed that the differences were significant, with the exception of the control and the concentration of 1 mg/L, which did not show significant differences. No cell growth was observed in the cultures with the higher cadmium concentrations assayed (75 and 100 mg/L), which also did not show significant differences. *P. tricornutum* can tolerate cadmium well since biomass is still produced, even in the cultures with cadmium concentrations of 25 or even 50 mg/L; moreover, the cultures with 1 mg Cd(II)/L and controls had no differences.

3.2. Analysis of biosorption isotherms

Four sorption models were considered to identify the properties that best describe the cadmium biosorption by living cells of *P. tricornutum*, providing information on the biosorption potential of this material by means of easily interpretable constants. The Langmuir and Freundlich models are well established and usually applied to study the biosorption processes. Dubinin-Radushkevich is generally applied to express the sorption mechanism with a Gaussian energy distribution onto a heterogeneous surface and allows to estimate the apparent energy of sorption. Temkin (with its application, rather limited to liquid-solid adsorption equilibrium) was also used to estimate the heat of sorption process. The validity of the models was assessed by the correlation coefficient R^2. Table 1 shows the parameters obtained ± S.E. for each model and for each fraction of cadmium removed by *P. tricornutum* throughout time of exposure.

3.2.1. The Langmuir isotherm

The sorption data were analysed according to the Langmuir isotherm equation (eq. 1). Figure 2 shows the Langmuir isotherm plots and Table 1 shows the parameters obtained and the coefficients of correlation for the three fractions analysed. In all cases, these isotherms were found to be well fitted to the experimental data over the whole cadmium concentration range assayed and for all days analysed with very high coefficients of determination. The R^2 values suggest that this isotherm provides a very good model to describe the process of cadmium removal by the living biomass of *P. tricornutum*.

3.2.1.1. Total cadmium removed

The Langmuir isotherm allowed to calculate the maximum cadmium removal capacity, q_{max}. This capacity increased throughout the days of culture, reaching its maximum value after 72 h with 67.140 ± 3.247 mg Cd/g. When it is compared with other biosorbents, *P. tricornutum* offers excellent possibilities for use in the removal of cadmium. Thus, another microalga, *T. suecica*, had a maximum biosorption capacity of 40.22 mg Cd/g after 72 h and also using living biomass (Pérez-Rama et al. 2010). The biomass of *Pleurotus platypus* had a capacity of 34.96 mg/g (Vimala and Das 2009), *Chlorella vulgaris* of 62.3 mg/g at pH 4.0 (Aksu 2001) or *Ceramium virgatum* of 39.7 mg/g (Sarı and Tuzen 2008). These data indicate that *P. tricornutum* has considerable potential for the removal of cadmium ions from aqueous solutions.

Although adsorption experiments with dead materials typically used relatively short contact times to reach the equilibrium and a rapid sorption is among desirable parameters, with the use of live biomass the times can be extended without excessive loss of efficiency, because the removed amount increases with time due to the generation of new biomass or higher uptake into cells. In fact, the maximum amount of cadmium removed by *P. tricornutum* doubled from 24 to 72 h.

Various factors influence the biosorption capacity of metals such as temperature, pH or media components. In the present study and in order to maintain the cells alive and metabolically active, the culture conditions were with seawater and pH = 8.2. However, these conditions could be considered suboptimal for a process with dead biomass. Most studies showed that the maximum capacity is achieved at pH in the range of 5-6 (Sarı and Tuzen 2008; Tangaromsuk et al. 2002) with a decrease in the efficiency at higher pH due to the formation of anionic hydroxide complexes. Furthermore,

experiments were carried out in natural seawater, which is a more complex system (although more real) that perform experiments in deionized water. Ahmady-Asbchin et al. (2013) found that the biosorption of other metals (nickel and cooper) on *Sargassum angustifolium* was lower in tap water than in deionized water. For these reasons, the distribution of cadmium species at the beginning of the experiments and using a standard seawater composition (Millero et al. 2008) were predicted using the Visual MINTEQ software (Version 3.1) for Windows (Gustafsson 2013). Data obtained with this program (using the highest cadmium concentration) shows that eight main cadmium species can exist under these conditions (Fig. 3) with approximately 50 and 45% of total species present as $CdCl^+$ and $CdCl_2$ (aq) respectively, both undersaturation. Therefore, it can be concluded that Cd-Cl complexes are the most abundant chemical form of dissolved cadmium in the conditions used in the present experiments. These complexes are less strongly sorbed than free Cd^{2+} ion, remaining in the solution and thereby reducing the amount of total cadmium removed. Despite this, the cadmium removal capacity remained high in this microalga; thus, the maximum adsorption capacity of *S. obliquus* CNW-N was higher than that of *P. tricornutum* with 68.6 mg/g after 15 min (Chen et al. 2012). However, it should be taken into account that the experiments were performed in deionized water.

3.2.1.2. Bioadsorbed cadmium

The maximum removal capacity obtained for this fraction and deduced from the Langmuir model was 43.670 ± 4.064 mg Cd/g after 72 h of culture. This capacity also increased throughout the days of culture. In all cases, this capacity was always less than the total maximum obtained. This is because the intracellular fraction contributes to add more cadmium in the removal capacity. If this fraction was not considered, the amount of bioadsorbed cadmium would be equivalent to the amount removed in an experiment with dead biomass.

3.2.1.3 Intracellular cadmium

Although the application of adsorption isotherms on surfaces to the incorporation of the metal into the cell may be debatable, the data indicated good fits. This fact allowed obtaining parameters that can serve as a reference to characterize and quantify the process. With this in mind, the removal capacity in this fraction increased as the culture time increased. The maximum removal capacity reached in this fraction,

calculated by Langmuir isotherm, was 27.233 ± 1.212 mg Cd/g after 72 h of culture (Table 1). This fraction is important, reaching more than 90% of the total cadmium removed at the lowest concentration of the metal. This percentage decreased throughout time of culture and with the increase of the cadmium concentration, become more dominant the bioadsorbed fraction. However, although this percentage decreased, the amount of cadmium removed in the intracellular fraction progressively increased during the culture time. Finally, in *P. tricornutum*, after 96 h of culture, about 45% of removed cadmium remained intracellularly. It is proved with *P. tricornutum* that the bioaccumulation of this metal depends on the lability of the cadmium species in the medium (Yan et al. 2011). The non-addition of culture medium probably favoured a higher bioaccumulation of this metal in the cells. Moreover, a recent study with *Zea mays* and *Brassica juncea* showed that Cl^- enhanced cadmium uptake by these plants (Lopez-Chuken et al. 2010). The Visual MINTEQ software showed a predominance of chlorine complexes in the solution used in the experiments.

One response to the cadmium toxicity in this species is the biosynthesis of phytochelatins, peptides rich in cysteine. In fact, the thiol content of this microalga increases when it is exposed to cadmium (Torres et al. 1997). Phytochelatins form complexes with cadmium, via thiol groups of the cysteines, reducing its toxicity. The more efficient this mechanism, more cadmium could be stored and therefore, the amount of bioaccumulated cadmium will be higher. In fact, *P. tricornutum* synthesizes long-chain phytochelatins, which are more efficient in binding cadmium (Torres et al. 1997). Therefore, this fraction contributes significantly to increasing the amount of cadmium removed. Other examples also show this feature; thus, the immobilized live *Oscillatoria* sp. H1 also showed a higher biosorption capacity than the dry biomass and heat-inactivated *Oscillatoria* sp. H1 (Katırcıoğlu et al. 2008). Live *Spirulina* sp. was found to be more potent for bioremediation than dead *Spirulina* (Doshi et al. 2007).

The separation factor values (R_L) were calculated for the entire concentration range by means of equation (7). These values were plotted against the initial cadmium concentrations (fig. 4) for the three fractions and for each time of exposure. This figure shows that $0<R_L<1$, indicating a favourable sorption in all cadmium concentrations assayed and for the three fractions. Note that throughout the time of exposure, the intracellular fraction showed the lowest values of the separation factor, suggesting a more favourable sorption.

3.2.2. Freundlich isotherm

Figure 5 shows the plots of this isotherm for the three fractions analysed every 24 h. The Freundlich constants obtained for each fraction are shown in Table 1. Freundlich model also described well the sorption of cadmium by living cells of *P. tricornutum*. However, the correlation coefficients were slightly lower than those of the Langmuir model (Table 1). This isotherm was applied in order to determine the sorption intensity of the sorbent for the sorbate. In this way, Freundlich equation describes sorption on a highly heterogeneous surface and for a good sorbent, $0.2<1/n<0.8$, a smaller value of $1/n$ indicates better sorption and formation of rather strong bond between the sorbate and sorbent. The values of $1/n$ obtained with *P. tricornutum* were always below 1 which indicates good and favourable sorption properties. Furthermore, the lowest values were always in the intracellular fraction, indicating a more strength of sorption. *P. tricornutum* accumulates cadmium intracellularly in an inert form, bounded to phytochelatins, which makes its retention stronger. The K_F values (a relative measure of sorption capacity) obtained can be considered slightly low, when compared with literature data (Dönmez et al. 1999), but must be taken into account that the experiments were conducted under experimental conditions different to those commonly used in experiments with dead biomass, a more complex medium and slightly alkaline pH.

3.2.3. Dubinin-Radushkevich isotherm

Although the Langmuir and Freundlich isotherm models are widely used, they do not give information on the adsorption mechanism. Therefore, the equilibrium data were tested with the Dubinin-Radushkevich model. This isotherm model predicts the nature of the sorbate sorption onto the sorbent and it is used to calculate the mean free energy of sorption. The empirical equation of this model is represented in the equation 3 and the plots obtained are shown in the figure 6. The R^2 values show in Table 1 indicates that Dubinin-Radushkevich model described worse the relationship between the amount of cadmium removed and the living biomass of *P. tricornutum* across the concentration range studied. Despite this, the sorption energy values calculated by means of this isotherm were in the range 0.055-0.16 kJ/mol, values below those usually obtained in the removal of heavy metals (Abdel-Aty et al. 2013). When the value of E_D is less than 8 KJ/mol, the sorption process is said to be dominated by physisorption. If E_D is between 8 and 16 KJ/mol, the process is dominated by a chemical ion exchange

mechanism and a value of E_D higher than 16 KJ/mol, the process is dominated by chemical particle diffusion (Bering et al. 1972). The values obtained indicated that the sorption process was dominated by physisorption. However, slightly higher values were obtained in the intracellular fraction.

3.2.4. Temkin isotherm

The isotherm constants and correlation coefficients are presented in Table 1. The application of this isotherm is shown in Figure 7. Temkin model showed the worst correlation coefficients. The variation of sorption energy was positive, indicates that the process was exothermic. As in Dubinin-Radushkevich, the value obtained indicated physisorption.

3.3.Bioconcentration factor for cadmium

To evaluate the ability of *P. tricornutum* to concentrate cadmium from the external solutions, the bioconcentration factor (BCF) was calculated. This factor provides an index of the ability of the microalga to concentrate the metal with respect to the metal concentration in the surrounding medium. If BCF was over one, metal was bioconcentrate. Figure 8 shows the BCFs obtained for the different cadmium concentrations, for each time of exposure and calculated by means of equation 8. The BCFs were always higher than 1. The highest BCF was obtained in cultures exposed to the lowest cadmium concentration assayed (1 mg/L) and after 96 h. As cadmium increased in the medium, the BCF decreased and reached the minimum value in the cadmium concentration of 100 mg/L. Two reasons can explain this result; the first is that at low cadmium concentrations, the ratio of binding sites to the amount of cadmium in the medium is high, and thus, a complete sorption can occur. When the cadmium concentration increases, the binding sites are rapidly saturated preventing the binding of more cadmium. The second reason is related with the toxicity of this metal, as cadmium increased in the medium, its toxic effect also increased, reducing the amount of cadmium removed intracellularly. Despite this toxic effect, the BCF obtained with the living biomass of *P. tricornutum* was 2204.7 with a cadmium concentration of 1 mg/L and even a BCF of 419.3 was achieved in the cadmium concentration of 100 mg/L. This result highlights the importance of metal-tolerant living cells that can incorporate it into the cellular interior. In addition, this calculated bioconcentration factor was >1000, suggesting that *P. tricornutum* biomass was a very good cadmium accumulator. In

general, microalgae are considered good accumulator of metals (Inthorn et al. 2002; Sekabira et al. 2011) with a high potential for application in bioremediation issues.

4. Conclusion

The present study focused on the biosorption of Cd(II) ions by living biomass of the marine diatom *P. tricornutum*. The obtained results showed that this biomass could be used as an efficient sorbent material for the removal of this metal from aqueous solutions. The Langmuir isotherm was demonstrated to provide the best correlation coefficient and the worst was Temkin. The maximum sorption capacity, obtained with the Langmuir isotherm, was 67.140 ± 3.247 mg/g after 72 h with approximately 40% of this capacity in the intracellular fraction, which is indicative of the importance of this fraction and therefore of the use of living biomass. The bioconcentration factor reached was 2204.7 in the cadmium concentration of 1 mg/L, after 96 h of exposure.

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Figure captions

Figure 1.

Effect of cadmium concentrations on the growth of *P. tricornutum* for 96 h of exposure. Values represent mean ± S.D. Different letters indicate significant differences at the 5% level according to the Tukey's test.

Figure 2.

The Langmuir isotherm plots of total, intracellular and bioadsorbed cadmium in living cell of *P. tricornutum* every 24 h of exposure to the metal.

Figure 3.

Cadmium speciation at the beginning of the experiments in standard seawater with pH of 8.2 (at 18ºC and 100 mg Cd(II)/L). Chemical speciation modelling was performed using the software Visual MINTEQ 3.1.

Figure 4.

Separation factor for Cd(II) ions as a function of the initial metal concentrations for the three fractions of cadmium removed by *P. tricornutum*.

Figure 5.

The Freundlich isotherm plots of total, intracellular and bioadsorbed cadmium in living cells of *P. tricornutum* every 24 h of exposure to the metal.

Figure 6.

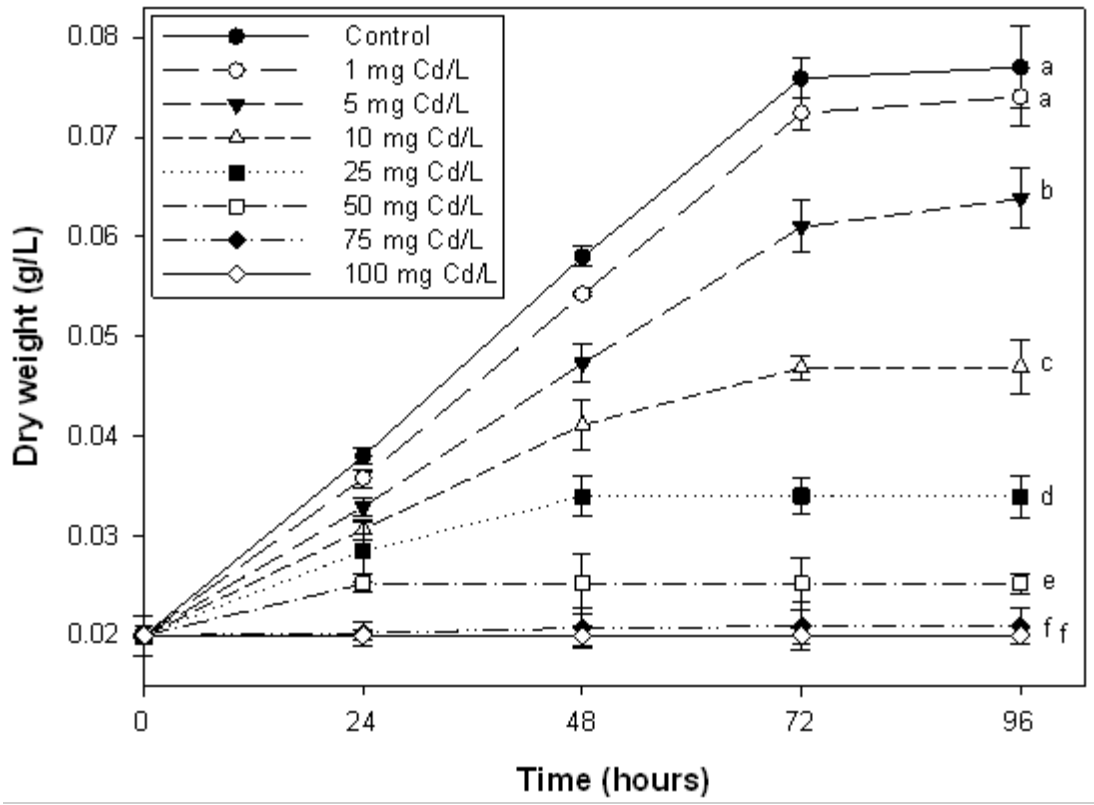
The Dubinin-Radushkevich isotherm plots of total, intracellular and bioadsorbed cadmium in living cells of *P. tricornutum* every 24 h of exposure to the metal.

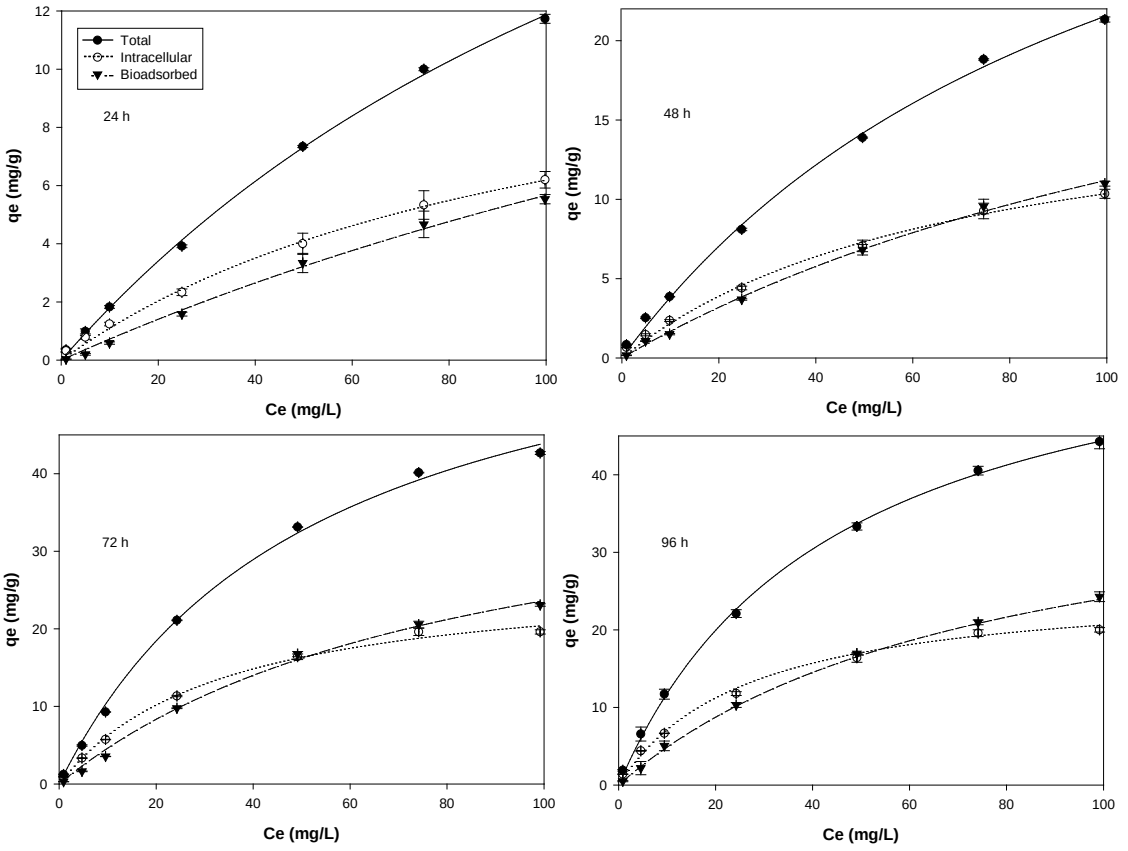
Figure 7.

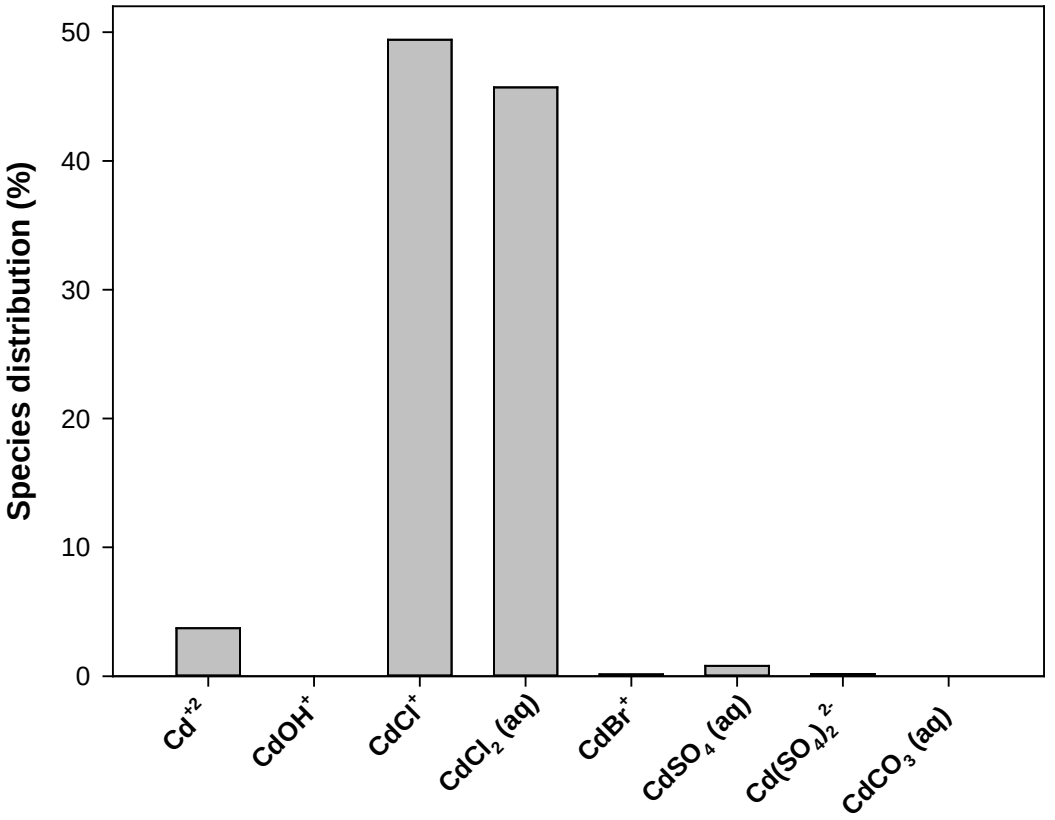
The Temkin isotherm plots of total, intracellular and bioadsorbed cadmium in living cells of *P. tricornutum* every 24 h of exposure to the metal.

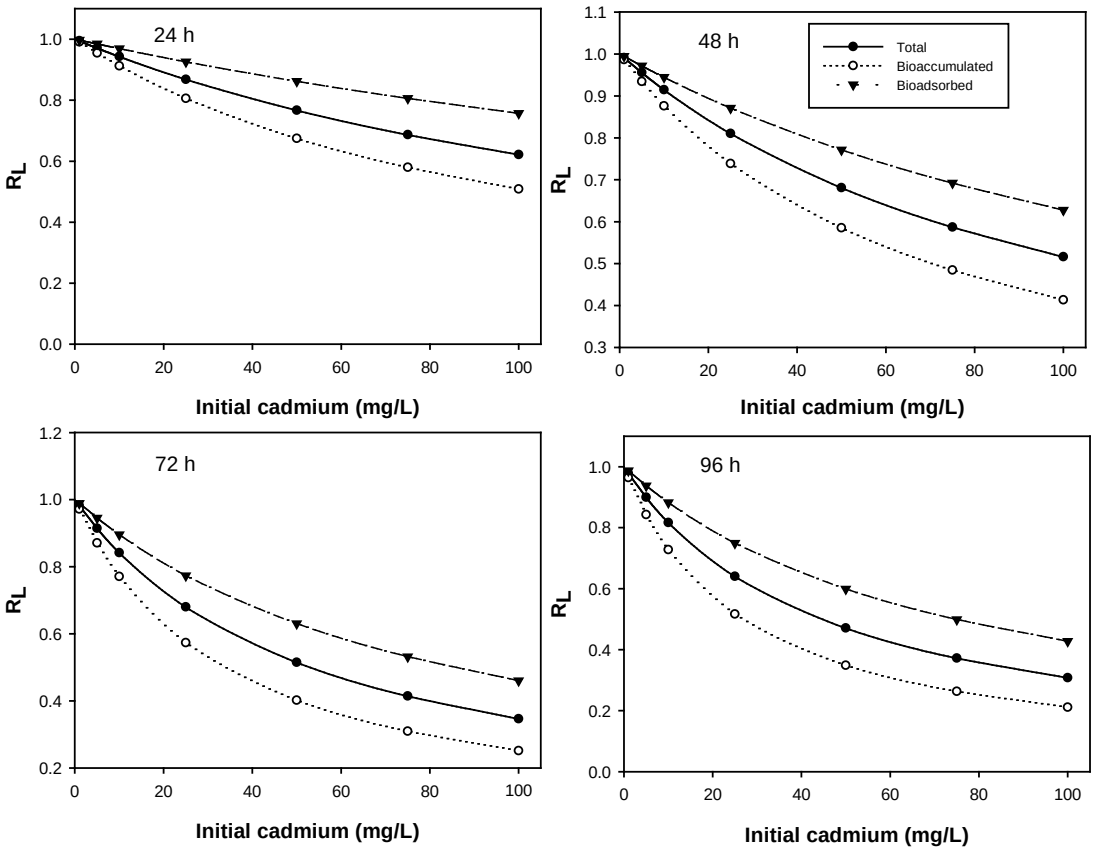
Figure 8.

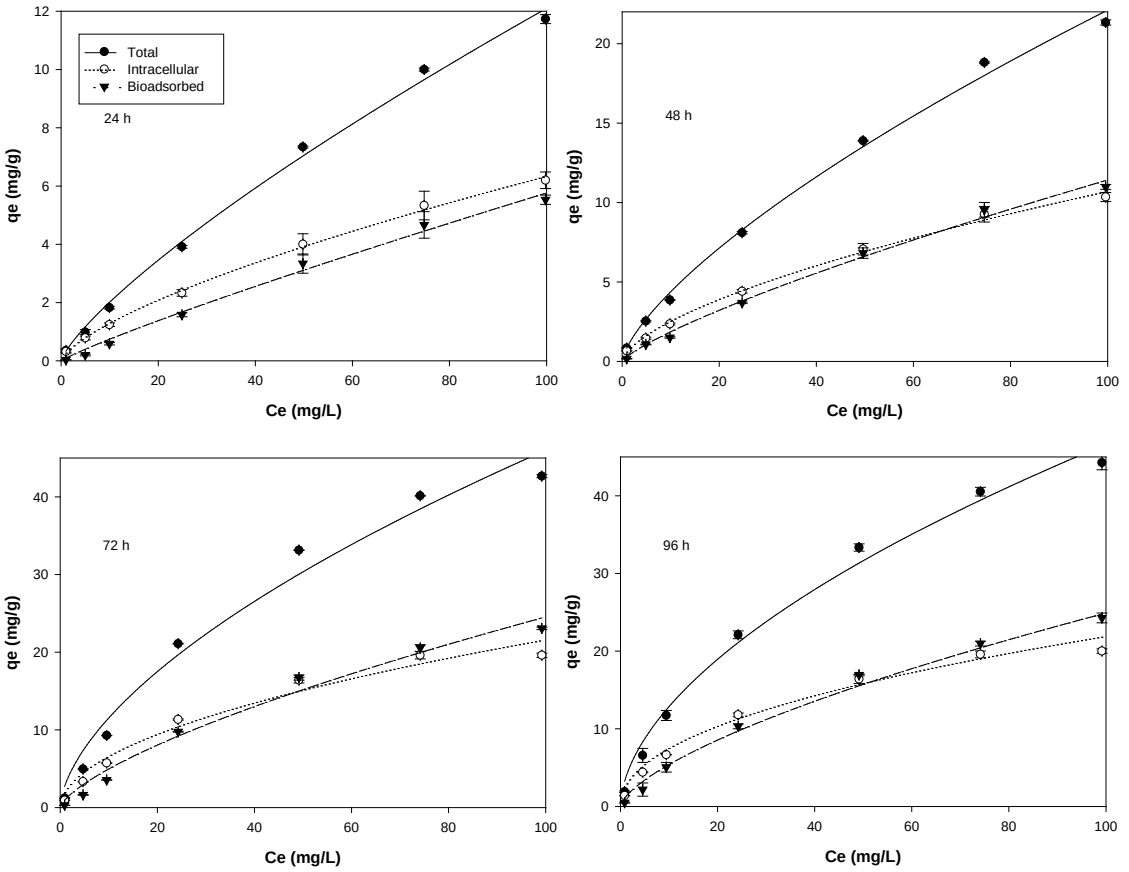
Mean bioconcentration factor (BCF) values obtained for the living biomass of *P. tricornutum* exposed to different cadmium concentrations and for each time of exposure.

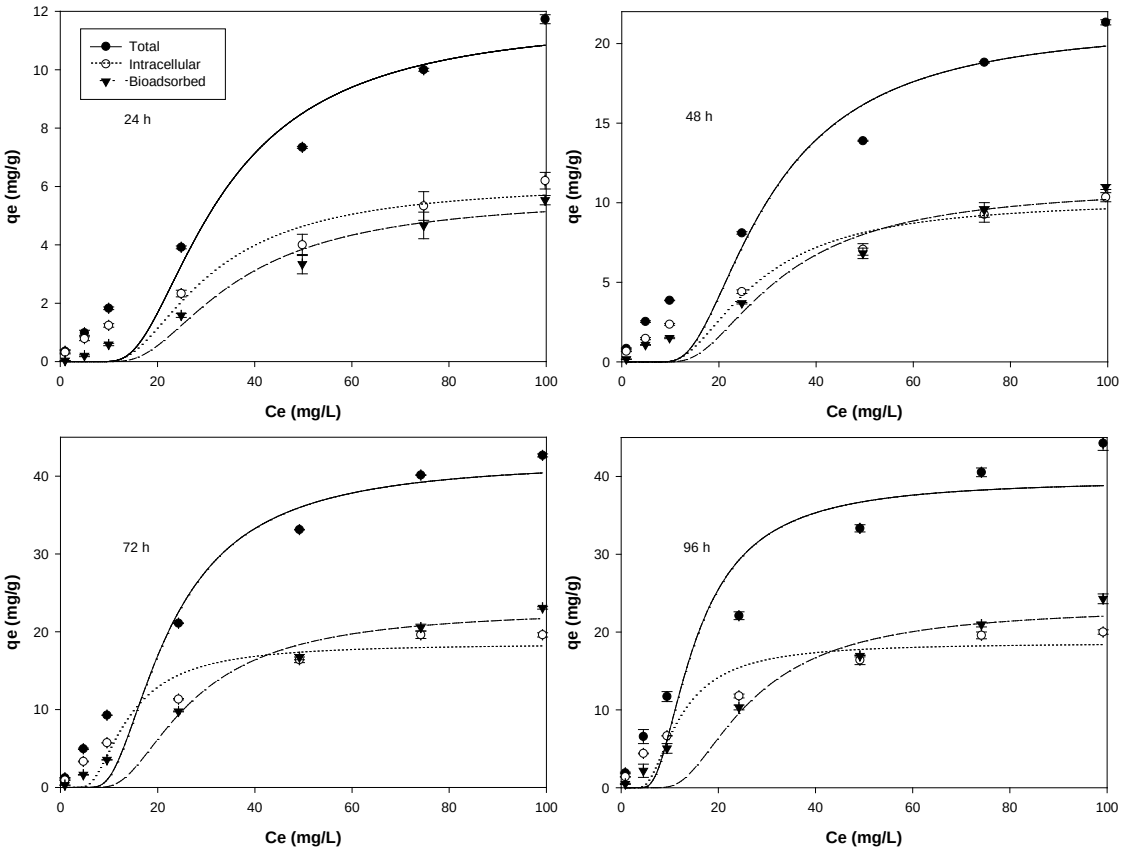

Control
1 mg Cd/L
5 mg Cd/L
10 mg Cd/L
25 mg Cd/L
50 mg Cd/L
75 mg Cd/L
100 mg Cd/L
Dry weight (g/L)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0
24
48
72
96
Time (hours)
a
a
b
c
d
e
f f

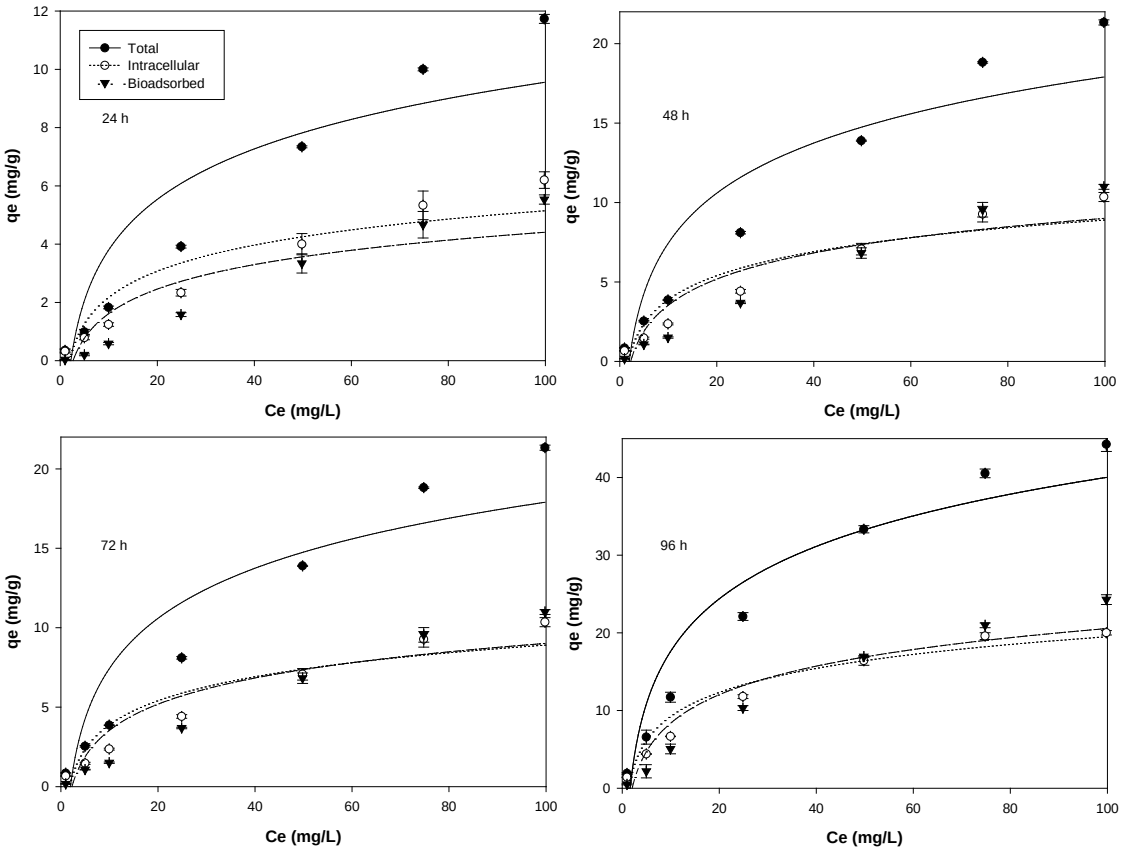

Total
Intracellular
Bioadsorbed
24 h
48 h
72 h
96 h
qe (mg/g)
Ce (mg/L)


Species distribution (%)
50
40
30
20
10
0
Cd+2
CdOH+
CdCl+
CdCl2 (aq)
CdBr+
CdSO4 (aq)
Cd(SO4)2 2-
CdCO3 (aq)


24 h
48 h
72 h
96 h
Total
Bioaccumulated
Bioadsorbed
R_L
Initial cadmium (mg/L)


Total
Intracellular
Bioadsorbed
24 h
48 h
72 h
96 h
qe (mg/g)
Ce (mg/L)


Total
Intracellular
Bioadsorbed
24 h
48 h
72 h
96 h
qe (mg/g)
Ce (mg/L)


Total
Intracellular
Bioadsorbed
24 h
48 h
72 h
96 h
qe (mg/g)
Ce (mg/L)

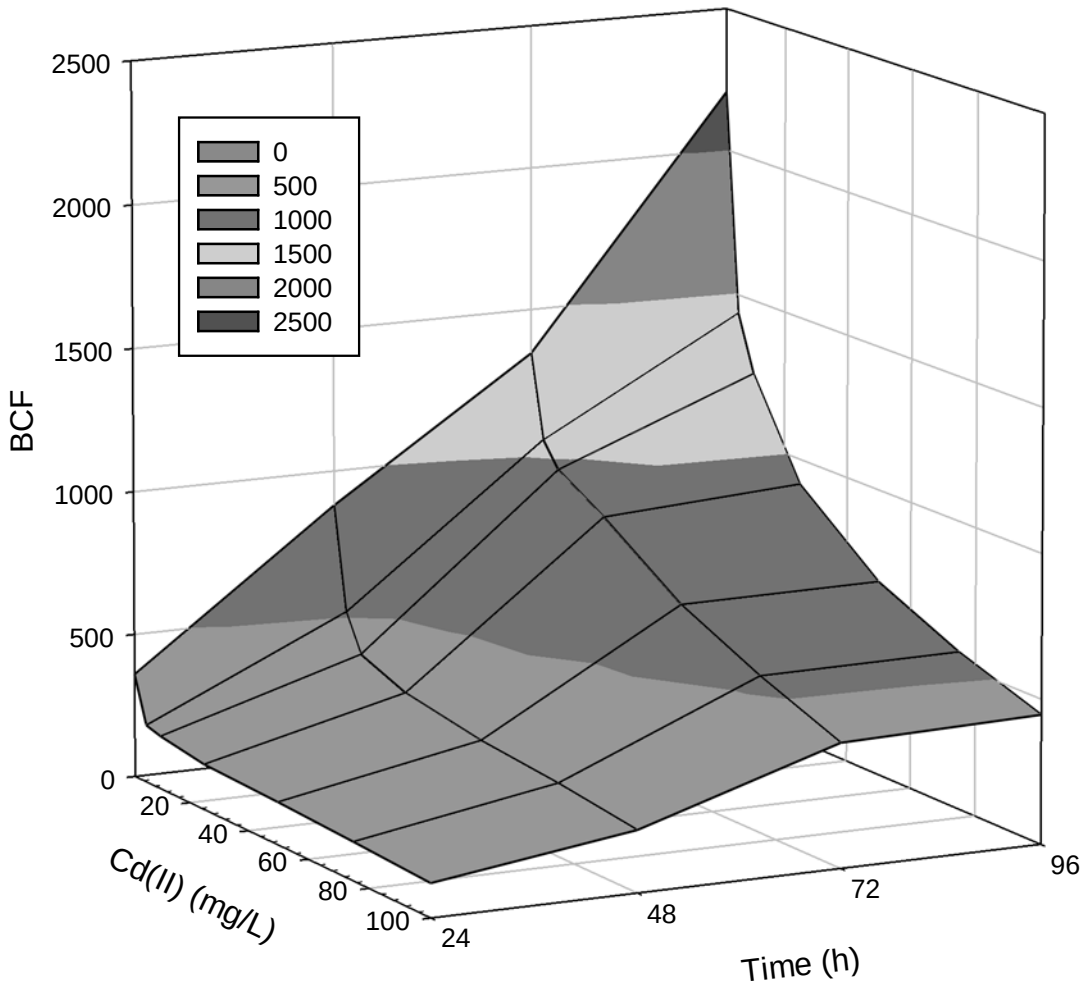

2500
2000
1500
1000
500
0
BCF
0
500
1000
1500
2000
2500
0
20
40
60
80
100
Cd(II) (mg/L)
24
48
72
96
Time (h)

Table 1. Summary of isotherm model parameters ± S.E. for the three analysed fractions of Cd(II) ions removed by *P. tricornutum* cells.

Isotherm/Parameters		Total time (h)				Intracellular time (h)				Bioadsorbed time (h)			
		24	48	72	96	24	48	72	96	24	48	72	96
Langmuir	q_{max} (mg/g)	31.331 ± 2.604	44.533 ± 3.784	67.140 ± 3.247	64.101 ± 1.696	12.585 ± 1.308	17.658 ± 1.496	27.233 ± 1.212	26.030 ± 1.261	23.277 ± 7.100	30.060 ± 3.667	43.670 ± 4.064	41.875 ± 2.054
	b (L/mg)	0.006± 0.001	0.009 ± 0.001	0.019 ± 0.002	0.023 ± 0.001	0.01 ± 0.001	0.014 ± 0.002	0.03 ± 0.004	0.037 ± 0.005	0.003 ± 0.001	0.006 ± 0.001	0.012 ± 0.002	0.014 ± 0.001
	R^2	0.999	0.998	0.998	0.999	0.998	0.997	0.996	0.997	0.997	0.998	0.997	1.000
Freundlich	K_F (mg/g)/(mg/L)$^{1/n}$	0.336 ± 0.051	0.870 ± 0.122	2.895 ± 0.770	3.540 ± 0.574	0.265 ± 0.025	0.600 ± 0.067	2.010 ± 0.511	2.453 ± 0.463	0.097 ± 0.027	0.307 ± 0.059	1.007 ± 0.305	1.227± 0.216
	$1/n$	0.779 ± 0.036	0.703 ± 0.033	0.601 ± 0.063	0.560 ± 0.039	0.689 ± 0.022	0.626 ± 0.027	0.515 ± 0.061	0.473 ± 0.044	0.887 ± 0.063	0.786 ± 0.044	0.693 ± 0.071	0.655 ± 0.043
	R^2	0.997	0.996	0.981	0.992	0.998	0.997	0.973	0.984	0.994	0.995	0.985	0.994
Dubinin-Radushkevich	q_{max} (mg/g)	11.761 ± 1.072	21.251 ± 2.105	42.006 ± 3.569	39.565 ± 3.689	6.118 ± 0.663	10.181 ± 1.136	18.390 ± 1.445	18.411 ± 1.675	5.665 ± 0.419	11.074 ± 0.977	22.930 ± 1.598	23.369 ± 2.223
	B_D(mol^2/J^2)	1.4E-04 ± 4.20E-05	1.2E-04 ± 4.1E-05	6.5E-05 ± 2.5E-05	3.2E-05 ± 1.4E-05	1.2E-04 ± 4.5E-05	9.7E-05 ± 4.1E-05	2.5E-05 ± 9.5E-06	1.9E-05 ± 8.3E-06	1.7E-04 ± 3.9E-05	1.4E-04 ± 4.0E-05	9.4E-05 ± 2.5E-05	9.0E-05 ± 3.4E-05
	E (KJ/mol)	0.060 ± 0.009	0.065 ± 0.011	0.088 ± 0.017	0.126 ± 0.028	0.065 ± 0.012	0.072 ± 0.015	0.141 ± 0.027	0.160 ± 0.034	0.055 ± 0.006	0.060 ± 0.009	0.073 ± 0.01	0.074 ± 0.014
	R^2	0.970	0.965	0.970	0.925	0.960	0.958	0.942	0.921	0.978	0.970	0.980	0.961
Temkin	b_T (kJ/mol)	0.969 ± 0.198	0.536 ± 0.100	0.251 ± 0.037	0.257 ± 0.034	1.883 ± 0.357	1.124 ± 0.193	0.544 ± 0.067	0.564 ± 0.061	1.998 ± 0.439	1.027 ± 0.206	0.466 ± 0.079	0.471 ± 0.075
	A_T (L/mg)	0.459 ± 0.242	0.528 ± 0.258	0.574 ± 0.215	0.689 ± 0.237	0.549 ± 0.283	0.621 ± 0.294	0.708 ± 0.237	0.890 ± 0.279	0.381± 0.203	0.455 ± 0.228	0.480 ± 0.198	0.557 ± 0.208
	R^2	0.827	0.848	0.900	0.911	0.848	0.868	0.927	0.940	0.805	0.829	0.870	0.881