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Original scientific paper

Biosorption characteristics of *Spirulina* and *Chlorella* cells for the accumulation of heavy metals

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Abstract: The biosorption of heavy metals by dried *Chlorella vulgaris* and *Spirulina platensis–Spirulina maxima* cells was studied under various experimental conditions. The effects of biosorbent dosage, pH, adsorption time, temperature, initial metal concentration on the biosorption were examined. A biosorption process can be divided into two parts: the first part follows zero-order and the second part pseudo second-order kinetics. Characterization of biosorption equilibrium was evaluated employing the Langmuir and Dubinin–Radushkevich models using non-linear regression. The optimum pH range was found to be 5.0−6.0 for Pb(II) and 4.0−6.0 for Cu(II) and Cd(II) adsorption. Based on the experimental data, the maximum adsorption capacities for Pb(II), Cd(II) and Cu(II) were 144, 161 and 138 mg g^{-1} by *Chlorella* cells and 370, 201 and 165 mg g^{-1} by *Spirulina* cells. The corresponding values for activated carbon were 86, 134 and 43 mg g^{-1}, respectively.

Keywords: algae; kinetics; isotherm; Langmuir; Dubinin–Radushkevich; activated carbon.

INTRODUCTION

Heavy metal pollution is one of the most important environmental problems today.[1] Cadmium, copper and lead are heavy metals that pose serious health hazards through entry into the food chain by anthropogenic pathways.[2] Most industrial technologies for their removal are not cost effective if the concentration of the heavy metal pollution is under 100 mg L^{-1}.[3] The disadvantages of the conventional methods are long operation time or the use of other chemicals, which could introduce toxic or carcinogenic intermediates into waterbodies.

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Certain natural materials of biological origin, such as bacteria, fungi, yeast and algae possess metal-sequestering properties and therefore could be used to rapidly decrease the concentration of heavy metal ions with high efficiency. These biosorbents are ideal candidates for the treatment of high-volume and low-concentration complex wastewaters contaminated with heavy metal ions.[4,5]

Living and dead biomass of algae cells can be used to decrease environmental heavy metal pollution. Several algae were tested for their ability to adsorb heavy metals.[6–11] The use of dead biomass is favourable because it tolerates high concentrations of toxic ions, a nutrient supply unnecessary and the culture conditions are not limiting.[6] Algae are widely used for several purposes, such as food for human consumption, a source of phycocolloids, β-carotane, atraxanthine and chlorophyll.[7] The most important criteria for developing algal biosorbents to decrease harmful metal contents in soil and water are the followings: ability for rapid adsorption of huge amounts of metal ions, availability in large quantities because of worldwide cultivation and relatively cheap production.[12,13]

Spirulina and *Chlorella* cells were used by many researchers to test their ability to remove heavy metal ions from polluted water.[7,12,14,15] The use of various conditions for the adsorption resulted in different adsorption properties. Mehta and Gaur compared the different adsorption features of living and dead biomass of *Chlorella vulgaris* cells.[6] Chojnacka and co-workers examined different morphological types of *Spirulina* species to compare the binding characteristics of these algae in case of chromium, cadmium and copper.[16] Fang *et al.* described the functional groups that could play a role in the surface adsorption of copper and cadmium by *Spirulina* cells with the help of FTIR and X-ray analyses. Al-Rub studied the effect of competitive cations on copper adsorption by *Chlorella* cells.[13] However, there is a lack of comparisons with often used adsorbents, such as activated carbon, in the literature.

The present study considers the adsorption properties of two different alga strains: green microalgae *C. vulgaris* and blue–green alga (cyanobacteria) *S. platensis–S. maxima* mixture. Their properties were compared with respect to the effect of pH, temperature and biosorbent dosage on Pb(II), Cd(II) and Cu(II) biosorption, with an evaluation of the kinetics and equilibrium capacities of the biomasses. The biosorption experiments were also performed using activated carbon. The obtained results showed that these biosorption systems using algal cells represent promising alternatives for the removal of heavy metal ions from polluted aqueous environments.

EXPERIMENTAL

Materials and adsorbent

C. vulgaris and *S. platensis–S. maxima* cells were purchased (Czech Academy of Sciences) in the dried form. Activated carbon was purchased from VWR Prolabo Ltd. (USA). The heavy-metal test solutions containing Pb(II), Cd(II) and Cu(II) ions were prepared from

reagent-grade metal of hydrated $Pb(NO_3)_2$, $Cd(NO_3)_2$ and $CuCl_2$ (Fluka, Germany) in the concentration range of 25–500 mg L^{-1}.

Determination of surface charge

Dried *C. vulgaris* or *S. platensis–S. maxima* biomass (1 g L^{-1}) was suspended in 25 mL distilled water in the pH range 2–11. The required pH was regulated with 0.1 M NaOH and 0.1 M HCl solutions. The specific surface charge was determined with a Zeta Sizer (Zetasizer Nano-Z, Malvern Instruments Ltd., Malvern, UK). The values were determined after the evaluation of 25 to 50 measurements.

Effect of pH on biosorption

The pH effect was tested using single-metal solutions containing 50 mg L^{-1} Pb(II), Cd(II) and Cu(II) ion. After 24 h of incubation, samples were taken from the suspensions, and the biomass was separated from the heavy metal solutions at 10 000 rpm for 5 min. The concentration of heavy-metals in supernatants was evaluated by atomic absorption spectrometry (AAS) (Perkin–Elmer 2380) at 217 nm for Pb(II), at 228 nm for Cd(II) and at 324.8 nm for Cu(II).

Effect of adsorbent dose on biosorption

Different dosages of algal biosorbents (0.25, 0.5, 1.0 and 2.0 g L^{-1}) were used at constant pH to find the optimal concentration. Solutions with 50 mg L^{-1} initial Pb(II) ion concentration were used under batch conditions. The heavy metal content in the supernatant was measured by AAS. The efficiency of the removal was calculated using the following equation:

$$\text{Removal efficiency} = \frac{100(c_0 - c_e)}{c_0} \quad (1)$$

c_0 and c_e are the metal concentrations initially and in the equilibrium (mg L^{-1}), respectively.

Adsorption dynamics

Batch sorption studies were investigated with 50 mg L^{-1} initial heavy metal concentration. Samples were taken at desired time periods and the residual Pb(II), Cd(II) or Cu(II) concentrations were determined.

Equilibrium evaluation based on biosorption isotherms

The biomass *C. vulgaris* and *S. platensis–S. maxima* cells (1 g L^{-1}) were suspended in heavy metal solutions, which were gently agitated at room temperature. For determination of adsorption isotherms, Pb(II), Cd(II) and Cu(II) solutions were used in the concentration range of 25–500 mg L^{-1} at a biosorbent dosage of 1.0 g L^{-1}. After incubation for 24 h, samples were taken from the suspensions and the heavy metal content in the supernatant was measured by AAS. The metal uptake per gram of adsorbent, q (mg g^{-1}), was calculated using the equation:

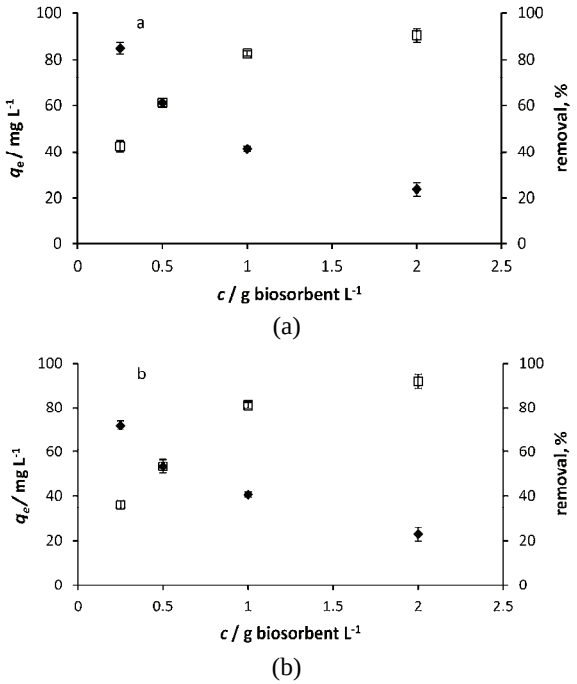
$$q = \frac{(c_0 - c_e)V}{m} \quad (2)$$

where V is the volume of the solution (L) and m is the mass of biosorbent (g). All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Effect of biosorbent dose

Biosorbent concentration is one of the most important factors affecting biosorption. In this study, it was varied between 0.25 and 2.0 g L^{-1}. The effect of the biomass loading of the adsorption of Pb(II) by *Spirulina* and *Chlorella* cells is shown in Fig. 1, from which it could be seen that the value of biomass concentration influenced the uptake capacity. The uptake capacity increased[17] with decreasing dosage of the biosorbent, which may be due to interference between the binding sites on the biosorbent. A similar relationship was reported in the literature.[7,12,13] Such an adsorption behaviour could be described by the Kroeker relation. With increasing weight of biosorbent in the solution of defined volume and concentration, the specified amount adsorbed by the adsorbent decreased. By both organisms, a dosage of 1.0 g L^{-1} resulted in an efficiency of more than 80 %. On increasing the biosorbent concentration further (2.0 mg L^{-1}), the adsorbed amount increased by only 10 %. In the further experiments, an alga concentration of 1.0 g L^{-1} was used as the optimum for biosorption.



(a)

(b)

Fig. 1. Adsorption capacities of: a) *Spirulina* and b) *Chlorella* cells, in dependence on the biomass concentration at concentration of 50 mg lead ions. □ represents the removal in percent, and ◆ is the adsorption capacity.

Effect of pH on biosorption

The pH is an important factor in biosorption processes. Based on FTIR spectra analysis, the most dominant role in the binding play the carboxyl groups

from proteins and carboxylated polysaccharides.[18] These groups have the highest affinity for metal ions because they are deprotonated in the pH range of the highest adsorption. Other groups, such as phosphate, hydroxyl and amino groups, present in the surface area of alga cells can play only a minor role; their pK_a values allow deprotonation only at higher pH values.[16] The zeta potential determination of alga cell surfaces shows that they are negatively charged above pH 3 by both species (Fig. 2). Parallel to these findings, the adsorption profile by *Spirulina* and *Chlorella* cells are shown in Fig. 3 for different pH values. In the cases of Pb(II), Cd(II) and Cu(II) biosorption on *Chlorella* cells and for Pb(II) and Cu(II) adsorption on *Spirulina* cells, the uptake capacities were the lowest at pH 3 and remained nearby constant between pH 4−6. The adsorption of Cd(II) on *Spirulina* cells was not affected by the pH value. Above pH 6, Cu(II) begins to precipitate, which made the adsorption more difficult.

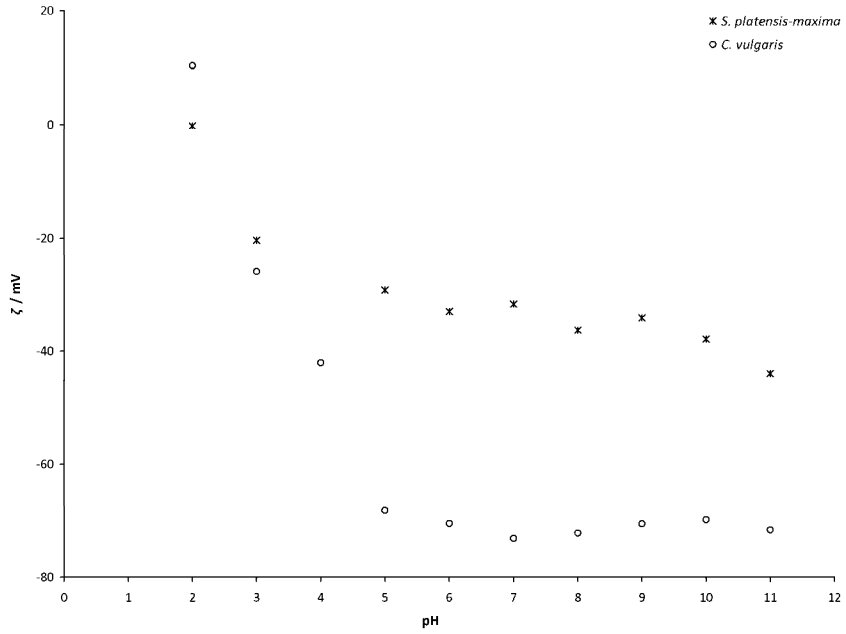


Fig. 2. Zeta potential values of *S. platensis*−*S. maxima* and *C. vulgaris* cells in the pH range of 2−11 for a biomass concentration of 1 g L^{-1} at 295 K.

The experimental results indicated that the biosorption process was effective in the pH range 5–6 for Pb(II) and pH 4−6 for Cd(II) and Cu(II) adsorption and thus no pH adjustment was necessary, because the initial pH values of all the metal solutions were within these ranges.

Effect of time on the biosorption

The adsorption of the heavy metal ions on the active sites of several types of biomass is generally considered very rapid.[2] This process may be independent of the initial metal concentration and the first period of the adsorption could be described with zero order adsorption kinetics. The practical aspect of the rapid kinetics is that a small reactor can ensure the cost-effective use.[14] Based on the morphology of the examined organisms, the heavy metal ions adsorb to the sheath

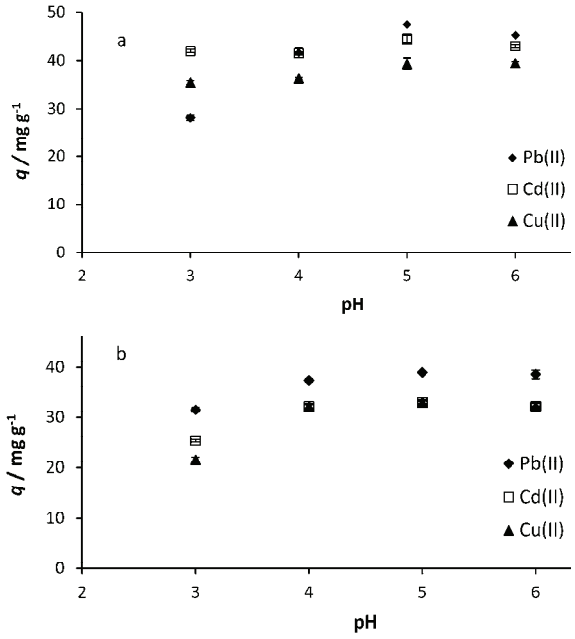


Fig. 3. Effect of pH on lead, cadmium and copper ions biosorption by: a) *Spirulina* and b) *Chlorella*, at 50 mg L^{-1} of heavy metal concentration and 1.0 g L^{-1} of algal cell concentration.

of the cells, very likely mainly by electrostatic attraction and complexation.[12] For the second phase of the adsorption, the chemisorption mechanism seems to be the most important force. The rate limiting step is the biosorption of positively charged heavy metal ions onto the cell surface.[19] Based on this finding, pseudo-second order kinetics were used to evaluate the kinetic parameters, because it takes into account the interaction between adsorbent and adsorbate.[2,6,15] The correlation coefficients for the second-order kinetic model were close to 1.0 for all cases. Other mechanisms examined in the kinetic evaluation were not so significant and hence the roles of physical adsorption and intraparticle diffusion were limited.[13,16] The zero order kinetic constants (k) and the pseudo-second order kinetic constants ($k_{2,ad}$), together with the calculated ($q_{eq,cal}$) and experimentally determined ($q_{eq,exp}$) adsorbed amounts of lead, cadmium and copper ions are presented in Table I.

After the first period of 8–10 min of adsorption (zero order kinetics), the equilibrium was established in 20–25 min (pseudo-second order kinetics) for each observed heavy metal ion, and no more heavy metal ions were adsorbed after this time. The data presented in Fig. 4 show that 70–90 % of the soluble ions were removed from the system. This adsorption profile may be characteristic for monolayer coverage. Similar findings were reported by Areco *et al*. for the adsorption of different heavy metals by *Avena fatua*.[20] Aksu reported the kinetic and thermodynamic parameters of Ni(II) adsorption by *C. vulgaris*.[14]

TABLE I. Constants for zero-order and pseudo second-order adsorption kinetic for the adsorption of lead, cadmium and copper ions by *Spirulina* and *Chlorella* cells

Biosorbent	Heavy metal	Zero-order kinetics	Pseudo-second order kinetics			
		k mg L^{-1} min^{-1}	$k_{2,ad}\times10^2$ g mg^{-1} min^{-1}	$q_{eq,cal}$ mg g^{-1}	R^2	$q_{eq,exp}$ mg g^{-1}
S. plat.– S. max.	Pb(II)	8.55	29	41.3	0.99	41.2
	Cd(II)	8.84	2.2	47.5	0.98	47.8
	Cu(II)	7.00	3.9	37.7	0.99	37.4
C. vulgaris	Pb(II)	7.57	5	40.3	0.99	40.6
	Cd(II)	6.48	52	32.7	0.99	32.4
	Cu(II)	5.9	8	31.3	0.99	31.2

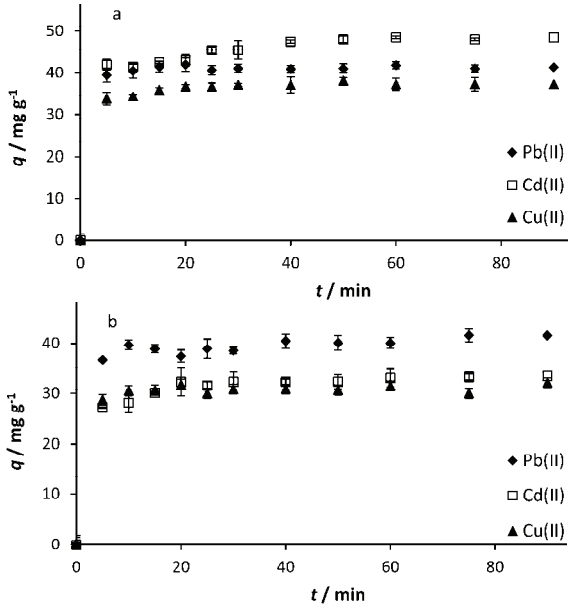


Fig. 4. A plot of the adsorbed amount *vs*. time for: a) *Spirulina* and b) *Chlorella* cells, at 50 mg L^{-1} initial lead, cadmium and copper ions concentrations.

Equilibrium studies of biosorption

The adsorption equilibrium relationship at a given temperature is referred to as an adsorption isotherm. Equations widely used to describe adsorption iso-

therms are the Langmuir, Freundlich, Dubinin–Radushkevich two-parameter models and Redlich–Peterson and Sips three-parameter models.[2,15,19,21,22] They describe the relationship between solute concentration remaining in the solution and the amount of solute adsorbed per unit mass of adsorbent. In this study, the Langmuir isotherm model was used because it is based on monolayer coverage over the cell wall, which could be inferred from the adsorption rate observations. It assumes identical surface sites that can accommodate one adsorbed particle. A number of studies demonstrated that despite these simplifications of the adsorption process, the Langmuir isotherm[2,15,16,20] could be used to interpret the biosorption data.[2] The model is given by Eq. (3):

$$q_e = \frac{q_{max} b c_e}{1 + b c_e} \quad (3)$$

where b is the adsorption equilibrium constant including the affinity of the binding sites (L mg^{-1}), c_e and q_e are unadsorbed metal ions in solution and adsorbed metal ions on the biosorbent at equilibrium, respectively, q_{max} is the maximum amount of metal ion per unit weight of adsorbent necessary to form a complex monolayer on the surface of the adsorbent (mg g^{-1}). The experimental values compared with the calculated data are presented in Table II. The highest b values (0.089 and 0.17) are obtained for Pb(II) adsorption for *Spirulina* and *Chlorella* cells, these values indicate steep initial slope and high affinity. Lower b values were calculated for Cd(II) and Cu(II) adsorption. According to this, the fitted curves have less steep initial slope.

TABLE II. The Langmuir and Dubinin–Radushkevich isotherm constants for lead, cadmium and copper ions adsorption by *Spirulina* and *Chlorella* cells at 295 K

Biosorbent	Heavy metal	Langmuir isotherm				Dubinin–Radushkevich isotherm		
		Q_{max} mg g^{-1}	B L mg^{-1}	R^2	$Q_D \times 10^3$ mol g^{-1}	E kJ mol^{-1}	R^2	Q_{exp} mg g^{-1} (mmol)
S. plat.– S. max.	Pb(II)	413	0.089	0.97	4.9	13.4	0.86	370 (1.8)
	Cd(II)	298	0.019	0.99	9.2	9.5	0.97	201 (1.8)
	Cu(II)	262	0.0039	0.99	9.6	8	0.99	165 (2.6)
C. vulgaris	Pb(II)	151	0.17	0.97	1.5	15.1	0.85	144 (0.7)
	Cd(II)	280	0.0085	0.99	8.7	8.5	0.95	161 (1.4)
	Cu(II)	233	0.0044	0.98	8.5	8	0.95	138 (2.2)

The parameters of this model were compared with the values calculated from the Dubinin–Radushkevich model,[21,22] which can describe the porous structure of a sorbent. It is more universal than the Langmuir isotherm model as it assumes a heterogeneous surface and not identical binding sites. The advantage of this model is that the mean energy of sorption can be determined and the magnitude of this value could be used to estimate the type of the sorption reaction. E values between 8.0−16 kJ mol^{-1} are in the range of an ion-exchange mechanism.[21,22]

This isotherm is quite often used to describe adsorption equilibrium.[21,22] The isotherm is given by Eqs. (4)–(6):

$$Q_0 = Q_D \exp\left(B_D \varepsilon_D^2\right) \tag{4}$$

$$\varepsilon_D = RT \ln\left(1 + \frac{1}{c_e}\right) \tag{5}$$

where Q_D characterizes the monolayer saturation capacity (mol g^{-1}), B_D is the Dubinin–Radushkevich model constant (mol^2 kJ^{-2}) and ε_D is the Polanyi potential. The mean adsorption energy can be calculated as follows:

$$E = \frac{1}{\sqrt{2B_D}} \tag{6}$$

The calculated E values are given in Table II, which shows that the values of E were 13.4 and 15.1 kJ mol^{-1} for Pb(II), 9.5 and 8.5 kJ mol^{-1} for Cd(II) and 8.0 kJ mol^{-1} for Cu(II) adsorption by *Spirulina* and *Chlorella* cells, respectively. The obtained E values were within the range of those denoting an ion-exchange mechanism was operative. The q_{max} values calculated from both models were much higher than the experimental values, which could indicate that the highest adsorbed amount was not reached. The fitted curves for the Langmuir and Dubinin–Radushkevich isotherms are shown in Figs. 5 and 6, respectively, for adsorption by *Spirulina* and *Chlorella* cells.

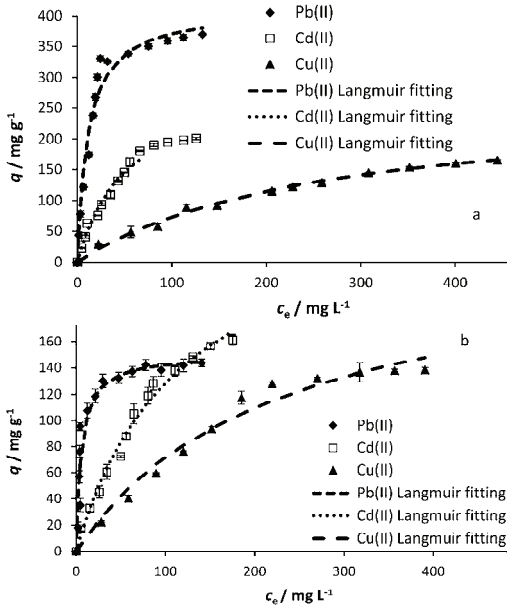


Fig. 5. Adsorption isotherms and Langmuir fitting of the adsorption of lead, cadmium and copper ions by: a) *Spirulina* and b) *Chlorella* cells, at 295 K.

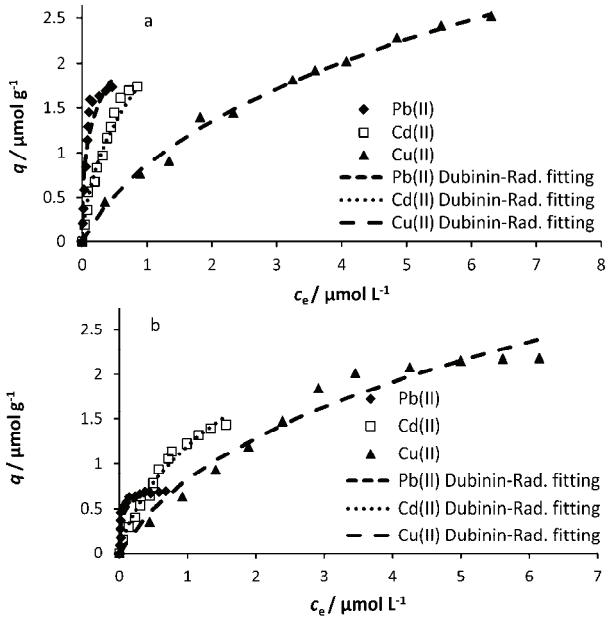


Fig. 6. Adsorption isotherms and Dubinin–Radushkevich fitting of the adsorption of lead, cadmium and copper ions by: a) *Spirulina* and b) *Chlorella* cells, at 295 K.

Effect of temperature

In most cases, the effect of temperature in the range of 295–313 K was negligible. The adsorptions did not change or showed only a slight increase with decreasing temperature. This finding was in good agreement with previously reported data.[23,24] A biosorption process may be slightly exothermic due to physical adsorption, however the contribution of physical adsorption in the overall process was maximum 4 %.[16] It could be assumed based on the obtained results that the heavy metal binding was mainly chemisorption based on ion-exchange. As an example, the isotherm for the adsorption of lead ions by *Chlorella* cells can be seen in Fig. 7.

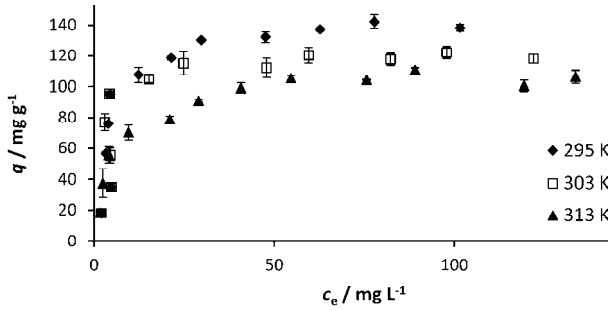


Fig. 7. Adsorption isotherms of lead ion adsorption by *Chlorella* cells at 295, 303 and 313 K.

Comparison with activated carbon

The experimental values for Pb(II), Cd(II) and Cu(II) adsorption on activated carbon were 86, 134 and 43 mg g^{-1}, respectively. The green alga *Chlorella* removed about 70 % more Pb(II) and 20 % more Cd(II) than activated carbon, and 4 times more Cu(II). The *Spirulina* strains were excellent biosorbents; they adsorb about 50 % more Cd(II) and 4 times more Pb(II) and Cu(II) than activated carbon. The adsorbed amounts of heavy metals by *Chlorella* cells, *Spirulina* cells and activated carbon are summarized in Table III. There is only little information about the comparison of biosorbents for heavy metal ions and activated carbon.[1,23]

TABLE III. Amounts of heavy metals adsorbed by *Chlorella*, *Spirulina* cells and activated carbon

Sorbent	Heavy metal							
	Pb(II)		Cd(II)		Cu(II)		Zn(II)	
	mg	mmol	mg	mmol	mg	mmol	mg	mmol
S. plat.–S. max.	370	1.8	201	1.8	165	2.6	128	2.0
C. vulgaris	144	0.7	161	1.4	138	2.2	127	1.9
Activated carbon	86	0.4	134	1.2	43	0.7	27	0.4

CONCLUSIONS

The biosorption of lead, cadmium and copper ions by dried *C. vulgaris* and *S. platensis–S. maxima* cells were studied using the batch technique. The adsorption was effective over a wide range of algal concentrations. The biosorption system containing 1.0 g L^{-1} adsorbent was optimal, and at this composition about 80 % of the heavy metal ions could be removed. An optimum pH range (pH 4–6) was determined for the biosorption process, which confirms the applicability of this system for wastewater treatment both at low and high heavy metal concentrations. In case of each metal by both organisms, the uptake capacity was the lowest at pH 3, and remained nearly constant between pH 4–6. Equilibrium was reached in 20–25 min for each heavy metal ion, and after this time, no more heavy metal ions were adsorbed. 70–90 % of the soluble ions was removed from the system. The biosorption process could be divided into two steps: the first part follows zero-order, the second part pseudo second-order kinetics. The equilibrium evaluation was described with adsorption models given by Langmuir and Dubinin–Radushkevich, and both models exhibited good fitting to the experimental values. The adsorption order was Pb < Cd < Cu for *Chlorella*, and Pb ≈ Cd < Cu for *Spirulina*. *Chlorella* and *Spirulina* algae biomass should be considered for use to decontaminate water from heavy metals.

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ИЗВОД

БИОСОРПЦИОНЕ КАРАКТЕРИСТИКЕ ЋЕЛИЈА *Spirulina* И *Chlorella* ПРИ АКУМУЛИРАЊУ ТЕШКИХ МЕТАЛА

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Биосорпција тешких метала осушеним ћелијама *Chlorella vulgaris* и *Spirulina platensis–Spirulina maxima* изучавана је у разним експерименталним условима. Испитани су утицаји дозе биосорбента, pH, времена адсорпције, температуре и почетне концентрације метала на биосорпцију. Процес биосорпције се може поделити на два дела: први део се одвија по кинетичком закону за реакцију нултог реда, а други део по кинетичком закону за реакцију псеудо-другог реда. Карактеристике биосорпционе равнотеже су одређене моделима Ленгмира и Дубињин–Радушкевича, коришћењем нелинеарне регресије. Нађено је да је оптимални опсег pH 5,0 до 6,0 за адсорпцију Pb(II) и 4,0 до 6,0 за Cu(II) и Cd(II). Максимални капацитети адсорпције за Pb(II), Cd(II) и Cu(II) су били 144, 161 и 138 mg g^{-1} на ћелијама *Chlorella*, а 370, 201 и 165 на ћелијама *Spirulina*, према експерименталним резултатима. Те вредности за активни угаљ су биле 86, 134 и 43 mg g^{-1}, редом.

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