Chemical Engineering Journal 259 (2015) 806–813

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Biosorption of Cu^{2+} and Ni^{2+} by *Arthrospira platensis* with different biochemical compositions

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HIGHLIGHTS

- Different biomass compositions of *A. platensis* used for Cu^{2+} and Ni^{2+} biosorption.
- Dry carbohydrate-enriched biomass exhibited higher biosorption capacities.
- In contrast, living carbohydrate-enriched biomass had lower biosorption capacities.

ARTICLE INFO

Article history:
Received 3 April 2014
Received in revised form 9 August 2014
Accepted 13 August 2014
Available online 20 August 2014

Keywords:
Arthrospira platensis
Biomass composition
Biosorption
Carbohydrates
Heavy metals

ABSTRACT

This study is focused on copper and nickel biosorption onto *Arthrospira platensis* biomass of different biochemical compositions. Four types of *A. platensis* were employed, namely: (1) typical dry biomass (TDB), (2) carbohydrate-enriched dry biomass (CDB), (3) typical living biomass (TLB), and (4) carbohydrate-enriched living biomass (CLB). The CDB was produced using a cultivation mode where phosphorus was the limiting nutrient. The biosorption of both metals investigated was shown to be very fast. Most of the metal sorption capacity of the biomass was filled within 15–30 min, and equilibrium was achieved within 30–60 min. The cultivation conditions (nutrient repletion or depletion) did not affect the pattern of copper and nickel biosorption kinetics. The capacity for copper ions biosorption was significantly positively affected by the accumulation of carbohydrates in the dry biomass, but was negatively affected by the accumulation of carbohydrates in the living biomass. For nickel ions, the alteration of biomass had a little but positive effect on the dry biomass, and a greater negative effect (about 30% lower biosorption capacity) on the living biomass. Living biomass exhibited a higher biosorption capacity than dry biomass, for both metals. The biosorption of copper and nickel onto *A. platensis* biomass occurred mainly due to the mechanisms of ion exchange and complexation, and less to physical adsorption.

1. Introduction

Industrial processes, such as fertilizer production, battery manufacturing, metallurgy, electronics, tanneries, etc. generate wastewater contaminated with heavy metals, which may lead to environmental problems that should be unavoidably addressed. Among the most frequently contained heavy metals in industrial wastewater are copper and nickel. Copper is known to cause irritation, headaches, stomachache and diarrhea, while nickel is known to cause-chronic bronchitis and lungs cancer among others health problems [1–3]. Various physicochemical or biological technologies are currently available for the treatment of wastewaters contaminated by heavy metals. However, each of these technologies has its disadvantages, such as high costs, low efficiency, and production of undesirable secondary sludge [2]. Dry or living biomass is considered to be a promising cost-effective way for the sorption of heavy metals, especially for wastewater with relative low metal concentrations (below 100 mg/L) [4–6].

Among the various biomasses available, microalgae and cyanobacteria have gained interest as potential biosorbents, due to their relative ease of production using wastewater as the cultivation

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http://dx.doi.org/10.1016/j.cej.2014.08.037
1385-8947/

Nomenclature

b	Langmuir isotherm constant (L/mg)	n	Freundlich adsorption intensity (−)
C_{eq}	metal concentration in the aqueous phase at equilibrium (mg/L)	q_{eq}	amount of sorbed metal per unit weight of biomass at equilibrium (mg/g)
C_o	initial metal concentration in the aqueous phase (mg/L)	$q_{eq,calc}$	calculated value of q_{eq}
C_s	biosorbent concentration (g/L)	$q_{eq,exp}$	experimental value of q_{eq}
E	mean free energy of sorption (J/mol)	q_{max}	theoretical maximum biosorption capacity (mg/g)
I	intra-particle diffusion intercept (mg/g)	q_s	theoretical isotherm saturation capacity (mol/g)
k_1	first-order rate constant (1/min)	q_t	amount of sorbed metal per unit weight of biomass at time t (mg/g)
k_2	second-order rate constant (g/mg min)	R	the gas constant (J/mol K)
K_{DR}	Dubinin–Radushkevich isotherm constant (mol^2/kJ^2)	R^2	coefficient of determination (−)
K_F	Freundlich isotherm constant ($(mg/g)(L/g)^n$)	R_L	Langmuir dimensionless separation factor (−)
k_I	intra-particle diffusion rate constant ($mg/g\ min^{1/2}$)	ε	Dubinin–Radushkevich isotherm constant (mol/kJ)
k_{p1}	pseudo first-order rate constant (1/min)	χ^2	Chi-square statistic
k_{p2}	pseudo-second order rate constant (g/mg min)		

medium, their relative high sorption capacities, and their strong metal ion sorption selectivity [7–10]. Metal sorption is a complex process, which is affected by several parameters, such as the solution pH, temperature, and ionic strength. Besides these parameters, the functional groups of the biomass surface (cell wall), such as carboxyl, amide/amine, hydroxide, phosphate groups, etc., which are related to biomass macro-molecules like carbohydrates, proteins and lipids, significantly affect the metal sorption capability [11]. However, the relative content of these macro-molecules is significantly affected by the conditions under which microalgae or cyanobacteria are grown [12]. It should be noted that stress grow conditions trigger the accumulation of lipids, carbohydrates, and other secondary metabolites in the biomass [13]. Nevertheless, there is little known about the effect of the biomass composition alteration, due to stress conditions, on metal ion biosorption capacity [9]. To the best of our knowledge, no previous published study has explored the copper or nickel biosorption capacity of different biomass composition types of microalgae or cyanobacteria. The aim of this study was to investigate the copper and nickel biosorption capacity of dry and living carbohydrate rich *Arthrospira platensis*, produced by a phosphorus limitation process.

2. Materials and methods

2.1. Microorganism and cultivation conditions

The cyanobacterium *A. platensis* (SAG 21.99) used in this study was cultivated in Zarrouk medium within 10 L plastic cubical photobioreactors (PBR), which were kept at 30 ± 2 °C in semi-continuous cultivation mode with a dilution rate of 0.1 1/d. The illumination was performed in two of the four sides of the PBR (12 klx on the one and 15 klx on the other side). Agitation of the broth was achieved with aeration using filtered air provided by an air pump. The biomass composition alteration of *A. platensis* was achieved by cultivating *A. platensis* under phosphorus limitation conditions [14]. Two types of biomass composition were used (for both dry and living biomass): (1) conventional biomass composition (typical type) consisting of 45–55% proteins, 10–20% carbohydrates, and 5–7% lipids, and (2) carbohydrate-enriched biomass (P-limited type) composition consisting of 25–28% proteins, 50–60% carbohydrates, and 4–6% lipids [14].

2.2. Preparation of A. platensis for biosorption

The *A. platensis* biomass was harvested by filtration and was rinsed with deionized (DI) water. To ensure the removal of the cultivation medium salts, the biomass was additionally washed twice by resuspension in DI water, followed by separation with centrifugation (5000 rpm for 5 min). The washed *A. platensis* biomass was dried overnight in an oven at 80 °C. Subsequently, the dried biomass was milled (IKA Labortechnik, A10), sieved through a metal sieve (300 μm pores), and stored in plastic containers inside an exsiccator containing silica gel to prevent moisture absorption by the biomass.

2.3. Experimental procedures

The present study consisted of three experimental series. In the first series, the biosorption kinetics were investigated with a biomass concentration of 0.5 g/L, and a metal concentration of 100 mg/L. Samples were collected at 1, 5, 15, 30, 60 and 120 min, and subjected to metal concentration determination. The experiments with living biomass were conducted over a time period of 48 h (data are not shown but discussed). In the second experimental series, five different metal concentrations (12.5, 25, 50, 100, and 200 mg/L) were used for the construction of appropriate equilibrium isotherms. In the case of dry biomass, the contact time was 90 min, while in case of the living biomass the contact time was extended to 48 h in order to allow for possible metal bioaccumulation. In the third experimental series, four different biomass (biosorbent) concentrations (0.125, 0.25, 0.5 and 1 g/L) were used, so that the effect of biosorbent dosage on biosorption capacity could be investigated. In addition, for the investigation of the possible biosorption mechanisms involved, the following experiments procedures were followed: (1) the cations Mg^{2+}, Na^+, and K^+ were determined at the end of each biosorption experiment, (2) metals were desorbed with the use of NH_4NO_3, $CaCl_2$ and Na_2-EDTA solutions, and (3) the various biosorption capacities were determined by the methylene blue method.

2.4. Biosorption experiments

The biosorption experiments were performed in a batch mode by placing 25 mL solution in a 50 mL polyethylene centrifuge tube, which was agitated with a horizontal agitation plate at 100 rpm. Heavy metal solutions were prepared with Cu^{2+} and Ni^{2+} stock solutions (1 g/L) by dissolving appropriate amounts of $CuSO_4$ and $NiSO_4{\cdot}6H_2O$ (analytical grade, Merck), respectively, in DI water.

The initial solution pH, in all three experimental series, was adjusted to 5 and 6 for Cu^{2+} and Ni^{2+}, respectively, in order to avoid metal precipitation. However, it should be noted that these pH values are very low and could have a negatively impact on the living

Table 1
List of the mathematical models used in the study. For more details see the Supplemental material.

Mathematical model	Equation	
Kinetics models		
First-order model	$\ln q_t = \ln q_{eq} - k_1 t$	(2)
Pseudo first-order model	$\ln(q_{eq} - q_t) = \ln q_{eq} - \left(\frac{k_{p1}}{2.303} t\right)$	(3)
Second-order model	$\frac{1}{q_t} = \frac{1}{q_{eq}} + k_2 t$	(4)
Pseudo second-order	$\frac{t}{q_t} = \frac{1}{k_{p2} q_{eq}^2} + \frac{t}{q_{eq}}$	(5)
Intra-particle diffusion model	$q_t = k_I \sqrt{t} + I$	(6)
Equilibrium isotherm models		
Langmuir isotherm	$q_{eq} = \frac{q_{max} b C_{eq}}{1 + b C_{eq}}$	(7)
Freundlich isotherm	$q_{eq} = K_F \sqrt[n]{C_{eq}}$	(8)
Dubinin–Radushkevich (DR) isotherm	$q_{eq} = q_s e^{-K_{DR} \varepsilon^2}$	(9)
Goodness of the fit		
Composite fractional error function (CFEF)	$CFEF = \sum_{n=1}^{n} \left[\frac{(q_{eq,exp} - q_{eq,calc})^2}{q_{eq,exp}}\right]$	(10)
Square statistic (χ^2)	$\chi^2 = \sum_{n=1}^{n} \left[\frac{(q_{eq,exp} - q_{eq,calc})^2}{q_{eq,calc}}\right]$	(11)

cells of *A. platensis*. By macro- but as well by microscopical examination it was observed that the cells of both types of the living *A. platensis* were intact and no cell lysis or other defacement was observed up to 72 h of retention on a solution with pH 5 and 6 containing $MgSO_4$ equimolar to the metal salts used (100 mg/L of metals). The solution pH was adjusted using 0.1 M HNO_3 and/or 0.01 M NaOH. The experiments were performed by combining pH adjusted biomass stock solutions (1 g/L in DI water) with heavy metal stock solutions at a ratio of 1:1.

The amount of a metal sorbed onto *A. platensis* at equilibrium, q_{eq} (mg/g), was calculated with the following equation:

$$q_{eq} = \frac{C_o - C_{eq}}{C_s} \quad (1)$$

where C_o, C_{eq}, and C_s are the initial metal concentration, the metal concentration at equilibrium, and the sorbent (biomass) concentration in the solution, respectively. All experiments were conducted in an air-conditioned room with temperature in the range 26–28 °C.

2.5. Analytical methods

All Cu^{2+}, Ni^{2+}, and Mg^{2+} ion concentrations were determined with an atomic absorption spectrophotometer (Varian, SpectrAA 200). For the determination of the metal concentration in each solution, 500 μL of sample was withdrawn at the preselected time, *t*, and was placed in an eppendorf type centrifuge tube (1.5 mL). Then, 1 mL of DI water was added to the centrifuge tube, and the diluted sample was centrifuged for 2 min at 10,000 rpm. Subsequently, the supernatant was collected, diluted with appropriate DI water, and analyzed. The K^+ and Na^+ ion concentrations were determined with a flame photometer (Sherwood Scientific, model 400), followed by separation of the biomass from the solution by centrifugation at 5000 rpm for 5 min.

In order to calculate the physical adsorption capacity of the biosorbents, the methylene blue method was performed as suggested by Chojnacka et al. [15]. Briefly, in a 5 mL solution sample containing 0.5 g/L biosorbent, 15 μL of 11.1 g/L methylene blue aqueous solution was added. The samples were left for 30 min at room temperature. Then, the samples were centrifuged (2 min at 10,000 rpm), and the supernatant was used to determine the amount of methylene blue adsorbed onto the biomass by spectrophotometry at 665 nm.

The possible biosorption mechanisms were investigated by a series of desorption experiments. These experiments were performed over a 90 min period, with 0.5 g/L sorbent, which was saturated with metals in 1 M NH_4NO_3 and 0.1 M $CaCl_2$ for investigation of possible ion-exchange, and 0.1 M Na_2-EDTA for investigation of possible complexation. The concentration of the living biomass of *A. platensis* was determined by spectrophotometry at 560 nm [16]. All experiments were performed in triplicates and the average values were recorded.

3. Results and discussion

3.1. Biosorption kinetics

The biosorption kinetic experimental data were fitted with five different models [17–19] which are listed in Table 1. The kinetic biosorption experimental data suggested that the biosorption of both of the metals used in this study was very fast. Most of the metal sorption capacity of the biomass was filled within 15–30 min, while saturation (equilibrium) was reached within 30–60 min for all biomass types used (see Fig. 1). Biosorption kinetics with living biomass exhibited some fluctuations, at times up to 48 h (data not shown) which are probably attributed to the bioaccumulation of metals, i.e., their uptake and/or excretion by metabolically driven (energetically) process [20]. Bioaccumulation is often related to the activation of a defense mechanism by which microorganisms handle metabolically the presence of a toxic metal [8]. However, biosorption process is not a metabolically driven process and therefore it displays generally fast metal uptake [7,15].

The kinetic biosorption data for all cases shown in Fig. 1 follow a similar pattern which suggests that the cultivation conditions (nutrient repletion or depletion) do not affect significantly the pattern of biosorption kinetics. In a relatively similar study, Chojnacka et al. [15] reported that three different metabolic modes of growth (photoautotrophy, heterotrophy, and mixotrophy) of *Spirulina* sp.

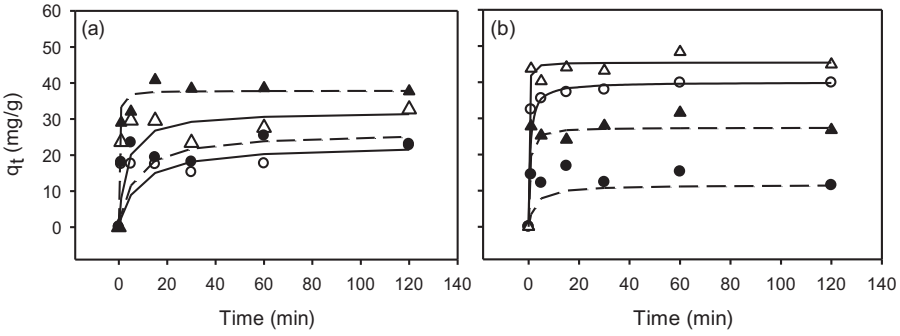

Fig. 1. Experimental data for (a) Cu^{2+} and (b) Ni^{2+} biosorption onto four different types of *A. platensis* biomass. The curves represent the best-fitted pseudo second-order kinetic model. (Here, ○: TDB, ●: CDB, △: TLB, and ▲: CLB, solid curves: typical biomass, and dashed curves: carbohydrate-enriched biomass.)

Table 2
Kinetic model parameters for Cu^{2+} and Ni^{2+} biosorption onto *A. platensis*.[a]

		First-order	Pseudo first-order	Second-order	Pseudo second-order				Intra-particle diffusion model			
		R^2	R^2	R^2	R^2	$q_{e,exp}$ (mg/g)	q_{calc} (mg/g)	k_{p2} (1/min)	CFEF	R^2	k_I (mg/g/min$^{1/2}$)	I (mg/g)	CFEF
Cu^{2+}	TL	0.247	0.656	0.292	0.986	32.15	31.27	0.0104	15.45 (13.61)	0.289	0.534	24.96	1.81 (0.76)
	CL	0.682	0.231	0.226	0.999	37.88	37.84	0.1936	1.38 (1.35)	0.382	0.763	32.4	1.74 (1.43)
	TD	0.329	0.769	0.534	0.974	22.73	21.93	0.0101	14.03 (12.40)	0.419	0.435	15.74	1.03 (0.14)
	CD	0.352	0.136	0.257	0.991	23.53	23.24	0.028	7.12 (5.87)	0.257	0.432	18.97	1.68 (0.73)
Ni^{2+}	TL	0.028	0.798	0.273	0.998	45.45	45.42	0.2547	0.88 (0.59)	0.337	0.411	41.95	0.51 (0.22)
	CL	0.071	0.066	0.091	0.994	27.47	27.38	0.0908	3.32 (2.71)	0.117	0.241	25.98	1.05 (0.48)
	TD	0.978	0.869	0.569	0.999	40.65	40.43	0.0385	2.12 (2.07)	0.822	0.701	33.45	0.2 (0.13)
	CD	0.055	0.097	0.192	0.991	11.76	11.53	0.0211	8.86 (7.69)	0.098	0.178	14.67	4.1 (0.84)

[a] Values in brackets correspond to the CFEF estimates for the first 15 min.

did not affect the pattern of biosorption kinetics of copper, chromium, and cadmium.

The linear regression analysis of the kinetic biosorption data showed that the model, which fits best the experimental data, is the pseudo second-order model ($R^2 > 0.976$), while the first-order, pseudo first-order, and second-order models yielded relatively low R^2 values (see Table 2). This result is in agreement with previous findings reported in the literature for typical dry [17,21] and typical living biomass [22], but no published work which investigated the biosorption kinetics of carbohydrate-enriched biomass exists.

The pseudo second-order model assumes that two (serial or parallel) reactions are taking place, a fast first one and a slower second one [19]. For most of the cases investigated here, the calculated CFEF values were relative high (see Table 2). It is worthy to note that a significant fraction of the calculated CFEF values is due to the discrepancies between model and experimental data during the first 15 min of the biosorption process (see bracketed values in Table 2). Thus, although the pseudo second-order model describes well the overall biosorption kinetics (based on the high R^2 values), it underestimates the early time data (first 15 min).

Fig. 2 shows the behavior of the intra-particle diffusion model. In the most of the cases considered here, three distinct linear time-fractions exist. The presence of multi-linear plot sections in conjunction with $I \neq 0$, suggest that the biosorption process was not limited by intra-particle diffusion, and that additional sorption processes and external mass transfer could be involved [19,23,24].

For Cu^{2+}, the highest pseudo second-order kinetic constant value of k_{p2} = 0.194 g/(mg min) was obtained with the CLB, while for Ni^{2+} the highest constant value of k_{p2} = 0.255 g/(mg min) was obtained with the TLB. Note that these k_2 values were about one order of magnitude higher than the corresponding values obtained for all other types of biomass employed in this study, and reflect the steep slope of the curve during the first 15 min of the biosorption kinetics.

3.2. Biosorption equilibrium isotherms

The parameter values of the various isotherm models (see Table 1) employed in this study are listed in Table 3. Clearly, in most of the cases considered here, the Freundlich isotherm fitted best the copper, while the Langmuir isotherm fitted best the nickel biosorption linearized data (see Fig. 3). However, the calculated CFEF values suggest that the Langmuir model represents better the dry biomass case, while the Freundlich model represents better the living biomass case. This observation could be explained on the basis of the theoretical assumptions of each model. The Freundlich model assumes multilayer sorption, and that the biosorbents are heterogeneous without a uniform distribution of affinities [25,26], which may be a better representation of living biosorption due to the presence of possible bioaccumulation. Whereas, the Langmuir model assumes monolayer sorption with finite number of sorption sites, without lateral interaction and steric hindrances [25], a description which could be more suitable to dry biomass due to the absence of the bioaccumulation process. These findings agree with those of Mehta and Gaur [27] who studied the sorption of copper and nickel onto living biomass of *Chlorella vulgaris*. Contrarily, Doshi et al. [22], reported that the biosorption of copper, nickel and chromium onto living *A. platensis* biomass can be described by Langmuir isotherms.

The estimated values of the Langmuir constant b, and the R_L parameter confirm that the affinity of living cells for copper was higher than that of dried cells, while in case of nickel there are no considerable differences. The estimated values of the Freundlich constant n were $n > 1$ for all biomass types considered here, indicating a favorable sorption for both metals. Note that the constant

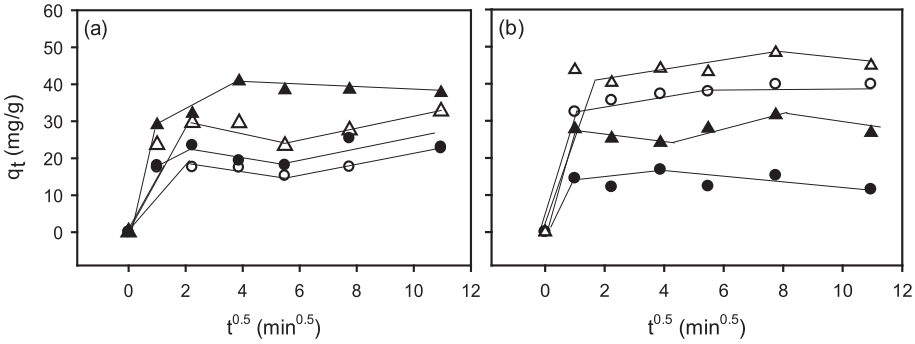

Fig. 2. Experimental data for (a) Cu^{2+} and (b) Ni^{2+} biosorption onto four different types of *A. platensis* biomass. The curves represent the best-fitted intra-particle diffusion model. (Here, ○: TDB, ●: CDB, △: TLB, and ▲: CLB.)

Table 3
Isotherm model parameters for Cu^{2+} and Ni^{2+} sorption onto *A. platensis*.

		Cu^{2+}				Ni^{2+}			
		TLB	CLB	TDB	CDB	TLB	CLB	TDB	CDB
Langmuir	q_{max} (mg/g)	40.65	35.59	17.3	33.44	90.91	63.29	52.63	56.82
	q_{calc} (mg/g)	38.6	32.71	14.09	20.05	40.59	43.88	31.15	34.61
	b (L/mg)	0.225	0.135	0.048	0.0165	0.0097	0.028	0.0169	0.0184
	R_L[a]	0.043	0.069	0.234	0.377	0.508	0.263	0.372	0.352
	R^2	0.709	0.908	0.799	0.976	0.968	0.992	0.985	0.947
	CFEF	20.63	3.07	5.77	1.09	7.42	3.87	1.17	3.76
	χ^2	34.88	3.66	7.33	1.3	8.66	4.64	1.29	2.85
Freundlich	q_{calc} (mg/g)	46.14	34.49	15.68	18.78	39.84	43.67	29.5	31.59
	K_F ((mg/g)(L/g)^n)	11.303	9.206	2.036	1.513	1.526	3.902	2.192	1.639
	n	3.148	3.356	1.71	1.79	1.355	1.818	1.712	1.5
	R^2	0.802	0.987	0,911	0.907	0.962	0.98	0.941	0.859
	CFEF	10.93	0.3	1.68	2.31	3.13	1.24	2.36	6.88
	χ^2	10.65	0.29	1.4	2.9	3.24	1.21	2.91	7.88
Dubinin–Radushkevich	K_{DR} (mol^2/kJ^2)	3.14×10^{-9}	3.09×10^{-9}	5.31×10^{-9}	6.2×10^{-9}	7.99×10^{-9}	5.91×10^{-9}	6.46×10^{-9}	7.49×10^{-9}
	q_s (mol/g)	1.72×10^{-3}	1.28×10^{-3}	1.05×10^{-4}	1.61×10^{-3}	5.97×10^{-3}	3.79×10^{-3}	2.89×10^{-3}	4.16×10^{-3}
	E (kJ/mol)	12.6	12.7	9.7	8.9	7.9	9.2	8.8	8.2
	R^2	0.771	0.981	0.887	0.93	0.955	0.983	0.959	0.892
	CFEF	1.95×10^{-4}	7.00×10^{-6}	3.30×10^{-5}	2.80×10^{-5}	7.90×10^{-5}	2.40×10^{-5}	2.90×10^{-5}	9.20×10^{-5}
	χ^2	2.04×10^{-4}	7.00×10^{-6}	2.70×10^{-5}	3.50×10^{-5}	8.30×10^{-5}	3.40×10^{-5}	3.50×10^{-5}	1.05×10^{-4}

[a] The R_L values correspond to C_o = 100 mg/L.

n describes the adsorption intensity or surface heterogeneity, which increases (becomes more heterogeneous) as the n values increase [25]. The estimated n values for copper biosorption onto living biomass were twofold greater than those obtained for copper biosorption onto dry biomass, suggesting that the surfaces of living biomass are more heterogeneous than those of dry biomass. This surface heterogeneity probably could be attributed to the various cell membrane layers and membrane-bound enzymes that in some extend control the adsorption process [22]. In contrast, the n values for nickel biosorption were no significant different between the four biomass types investigated here. Overall, *A. platensis* is considered to be a good biosorbent for copper and nickel removal from aqueous solutions. This observation is in agreement with numerous earlier studies [11,15,17,21,23,28].

Based on the typical range of E values for chemisorption (8–16 kJ/mol) reported by Ho et al. [29], the calculated E values for all types of biomass used in this study (see Table 2) suggest that chemisorption played a significant role as a biosorption mechanism. As shown in Table 3 the E values for the dried biomass were in general lower than those of the living biomass, suggesting that a weaker chemisorption mechanism is involved with the dried biomass.

3.3. Effect of biomass type on biosorption capacity

Fig. 4a shows that the biosorption of copper was significantly positively affected by the accumulation of carbohydrates in the dry biomass; however, it was significantly negatively affected in the living biomass. Living biomass exhibited a higher biosorption capacity for copper than the dry biomass. Fig. 4b shows that for the case of nickel, biomass alteration contributed to a small enhancement of biosorption onto the dry biomass, and to a substantial reduction of biosorption (about 30% lower biosorption capacity) onto the living biomass. Furthermore, the living biomass, compared to dry biomass, exhibited a higher biosorption capacity for nickel due to bioaccumulation, i.e., the uptake of the metal ions intracellularly. Also, carbohydrate-enriched living biomass exhibited lower biosorption capacity than the TLB due to the relatively low capability of the cells to uptake metals energetically. A possible explanation for this is the apparent decrease of the phosphorus containing, energy transfer molecule, adenosine triphosphate (ATP), which is known to be affected by intracellular phosphorus [14,30].

It should be noted that in relatively similar study conducted by Hernández and Olguín [9] who studied the biosorption of Pb^{2+},

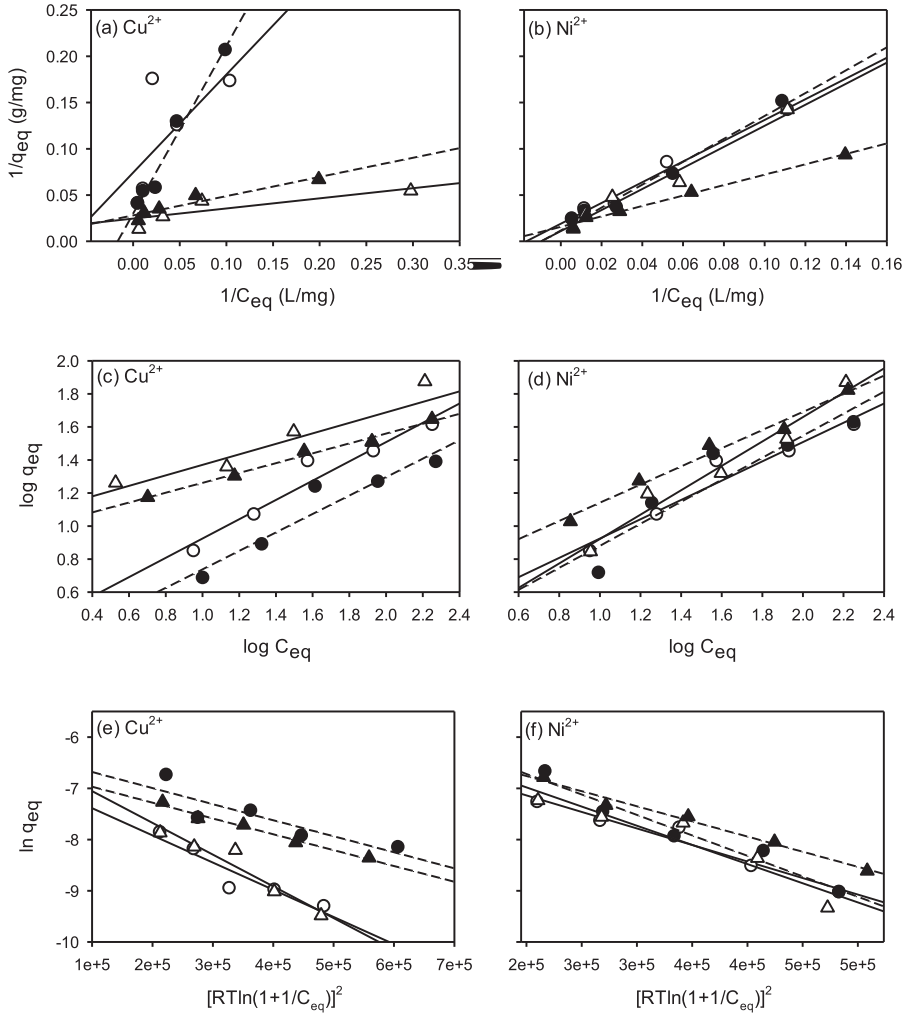

Fig. 3. Linearized experimental data fitted with: (a and b) Langmuir, (c and d) Freundlich, and (e and f) DR isotherms. (Here, ○: TDB, ●: CDB, △: TLB, and ▲: CLB, solid lines: typical biomass, and dashed lines: carbohydrate-enriched biomass.)

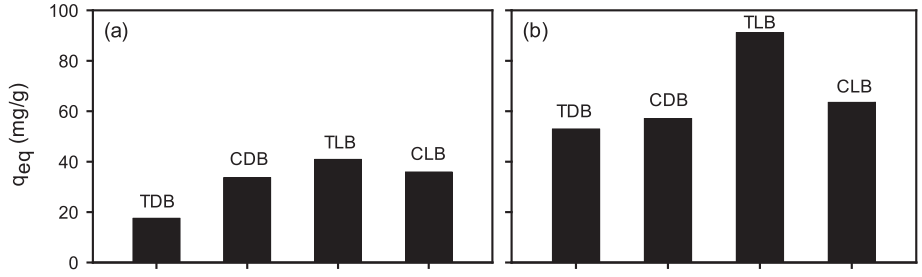

Fig. 4. Maximum biosorption capacity of: (a) copper, and (b) nickel for the four different types of biomass used in this study, as predicted by the corresponding Langmuir isotherms.

Cd^{2+} and Cr^{6+}, onto dry biomass of *A. platensis* with different compositions, it was reported that due to the increase of the carbohydrate content in *A. platensis* dry biomass from 7–9% to 27%, the q_{max} for $Pb^{2+}$and Cd^{2+} increased almost 150% and 40%, respectively, while for Cr^{6+} q_{max} was decreased around 1100%. Consequently, the degree that carbohydrate accumulation can affect the biosorption capacity is dependent on the metal ion. However, it is not clear from the present study, whether the accumulated carbohydrates were located intracellularly or extracellularly, which may affect the various functional groups of the cell walls or even the overall biosorption behavior of the cells.

Although nickel exhibited higher biosorption onto living biomass than copper, chlorosis of *A. platensis* occurred with copper, but not with nickel. This suggests that the bioaccumulation of copper may be more toxic to the cells than the bioaccumulation of nickel. This observation is valid only for the typical biomass, because in the carbohydrate-enriched biomass, chlorosis is expected to occur due to nutrient limitation during cultivation.

The metabolic grow pathway (heterotrophy, mixotrophy, and autotrophy) can significantly affect the morphology of the microalgal and cyanobacterial cells, which in turn can influence the biosorption capacity [20]. Furthermore, it is expected that various

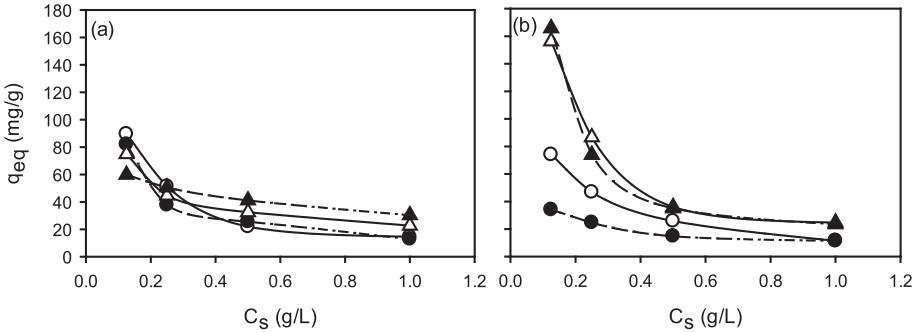

Fig. 5. Effect of biosorbent dosage on biosorption capacity for: (a) copper, and (b) nickel. (Here, ○: TDB, ●: CDB, △: TLB, ▲: CLB, solid curves: typical biomass, and dashed curves: carbohydrate-enriched biomass.)

cultivation conditions (temperature, light intensity, nutrient depletion, salt stress, etc.), which affect the biomass composition, will have a strong impact on the biosorption capacity. Although the sorption of heavy metals on biological materials is widely studied, it has not yet been really used in commercial scale. However, biosorption is a potent alternative not only for treating wastewaters contaminated with metals but also with other contaminants such as organics or pharmaceuticals [31,32]. Given that plenty different methods exist to alter the biomass composition of cyanobacterial or microalgal biomass by applying stress factors, this topic generates new potentials to find selective and eco-friendly biosorbents.

3.4. Effect of biosorbent dosage

Fig. 5 illustrates the effect of biosorbent dosage (C_s) on the biosorption capacity at equilibrium (q_{eq}). The results show that for all cases examined here, q_{eq} decreases with increasing C_s. Clearly, this relation is more pronounced for the case of nickel with living biomass. Decreasing sorption capacity with increasing C_s is frequently reported in the literature, and indicates that metal ion sorption onto biomass is not strictly a surface phenomenon [19] and that some sorption sites may remain unsaturated during the biosorption process due to possible electrostatic interactions between binding sites, ineffective mixing of metal solutions, and high sorbent dosages [33–37]. However, the increased metal sorption capacity as sorbent dosage decreases, could be an attractive procedure for the removal of heavy metals from wastewaters, if used in a sequential instead of a single process [37].

3.5. Biosorption mechanism

The results from the methylene blue method are presented in Table 4, and show that the calculated metal sorption capacity due to physical adsorption was very low. The estimated sorption capacity for copper was estimated to be in the range 2.33–3.08 mg/g, and for nickel in the range 2.14–2.84 mg/g. This indicates that the physical adsorption mechanism represents less than 15% of q_{max}. Biomass alteration did not affect the methylene blue sorption, which indicates that the phosphorus limitation did not change the surface area of the cells. It should be noted that low adsorption values for typical dry biomass of *A. platensis* have also been recorded by Chojnacka et al. [15]. This observation is in agreement with the results obtained by the DR isotherm analysis of this study.

The results from the ion-exchange experiments are listed in Table 5. Clearly, based on the sodium, potassium, and magnesium concentrations measured in the solution, it is evident that one of the major sorption mechanisms involved is the ion exchange. Note that living biomass exhibited higher ion-exchange capacity,

Table 4
Physical biosorption of metal ions based on the methylene blue method.

	TDB	CDB	TLB	CLB
Cu^{2+} (mg/g)	2.44	2.33	3.08	3.08
Ni^{2+} (mg/g)	2.25	2.14	2.84	2.84

Table 5
Exchanged ions from 0.5 g/L biomass solutions.

		Cu^{2+} (meq/g)		Ni^{2+} (meq/g)	
		Living	Dry	Living	Dry
	Biomass composition type				
Na^+ (meq/g)	Typical	0.22	0.153	0.193	0.165
	Carbohydrate-enriched	0.164	0.229	0.071	0.248
K^+ (meq/g)	Typical	0.341	0.263	0.328	0.262
	Carbohydrate-enriched	0.181	0.102	0.14	0.109
Mg^{2+} (meq/g)	Typical	0.196	0.174	0.224	0.216
	Carbohydrate-enriched	0.138	0.122	0.178	0.096
Total (meq/g)	Typical	0.757	0.590	0.745	0.643
	Carbohydrate-enriched	0.483	0.453	0.389	0.453

perhaps due to ion homeostasis. The three ion concentrations were lower in the carbohydrate-enriched biomass, indicating that this biomass type might have fewer ions available for exchange. Also it was observed that the pH of the samples decreased by approximately 0.8 units (data not shown), indicating that an exchange of H^+ with metal cations occurred. For the desorption experiments with 1 M NH_4NO_3, 0.1 M Na_2-EDTA, and 0.1 M $CaCl_2$ (data not shown), the only significant observation was that copper desorption by Na_2-EDTA was significantly higher than that of nickel. This observation suggested that copper biosorption was controlled by a complexation mechanism [11], and that nickel was more strongly attached to the biomass than copper. The desorption of both metals in DI water was observed to be very low (~1.7%), suggesting that the contribution of physical adsorption was minor [11].

Based on the experimental data of this study, it is evident that although the CDB biomass displayed a weak ion-exchange mechanism with both metals, it exhibited higher biosorption capacity than the typical biomass. This observation indicates that more sorption mechanisms (such as complexation, entrapment in inter and intrafibrillar capillaries and spaces of the structural polysaccharides) may be involved with CDB than TDB [2]. Certainly, the biosorption capacity is affected by the quality and not the quantity of carbohydrates [8].

The results of this study that copper and nickel biosorption onto *A. platensis* biomass is governed by ion-exchange, complexation, and to lesser degree by physical adsorption are generally in accord to results presented in the literature for similar but yet quite

different studies employing typical dry biomass [11,15,23]. However, although the same sorption mechanisms may be involved in various biomass types, the type of biomass used controls the degree of metal sorption.

4. Conclusions

This study employed for the first time carbohydrate-enriched dry and living biomass of *A. platensis* as biosorbent for copper or nickel ions. The results show that the various biomass compositions of *A. platensis* obtained from cultivation under different phosphorus concentrations had a diverse effect on Cu^{2+} and Ni^{2+} biosorption capacity. The experiments with dry biomass show that the accumulation of carbohydrates slightly improved the biosorption capacity for nickel, but significantly improved the biosorption capacity for copper. In contrast, the experiments with living biomass show that the accumulation of carbohydrates decreased the biosorption capacity for both metals. *A. platensis* showed greater biosorption capacity for Ni^{2+} than for Cu^{2+}. For all biomass types investigated, the pseudo-second order kinetics model fitted better the experimental data, but underestimated slightly the early time data (first 15 min). It was observed that the intra-particle diffusion was not a limited process, and that intra-particle diffusion model described well the experimental data for the first 15 min. Also, it was observed that the Freundlich model represents better the biosorption onto living biomass due to the presence of possible bioaccumulation, whereas, the Langmuir model fits better the experimental data with dry biomass. The results show that the main sorption mechanisms involved were ion exchange and complexation. In general, the carbohydrate-enriched biomass contributed to a weak ion-exchange sorption.

It was concluded that the carbohydrate-enriched dry biomass has a great potential for removal of copper and nickel from aqueous solutions. Nevertheless, the biosorption capacity is strongly affected by the affinity of the carbohydrates or other cell wall compounds to bind specific metals. Given that plenty different methods exist to alter the biomass composition of cyanobacterial or microalgal biomass by applying stress factors, this topic deserves further research in order to find selective and eco-friendly biosorbents.

Acknowledgement

Dr. D. Gasparatos is kindly acknowledged for his valuable help with the operation of the atomic absorption spectrophotometer.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cej.2014.08.037.

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