Biosorption of copper and nickel on biomass of cyanobacterium *Arthrospira platensis*

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***Abstract* – In this Master work, the effect of *Arthrospira (Spirulina) platensis* biomass composition alteration on biosorption capacity of copper and nickel ions was studied. Two types of dry and living *A. platensis* biomass were used as biosorbents. The carbohydrate-enriched biomass was produced using phosphorus limitation cultivation mode. The batch biosorption kinetics of both heavy metals was very fast and equilibrium was achieved in 30-60 min. Equilibrium data were well described by the Langmuir, Freundlich, Temkin and Dubinin-Radushkevich isotherm models. Biosorption capacity of Cu^{2+} and Ni^{2+} on the dry biomass was positively affected by the alteration of biomass composition, but decreased in case of living biomass. The cell metabolism depended biomaccumulation contributed to biosorption capacity of the living biomass. The main biosorption mechanisms were ion-exchange and complexation, and less physical adsorption.**

Key words: heavy metals, *Arthropsira platensis*, biosorption, isotherms, kinetics

I. INTRODUCTION

Various industrial processes generate wastewater contaminated with heavy metals which may cause environmental problems. Among the most frequently contained heavy metals in industrial wastewater are copper and nickel. These metals are known to cause several health problems. Numerous physical, chemical or biological technologies are currently used for the treatment of wastewaters contaminated by heavy metals. However, each technology has its disadvantages such as high operational costs, low efficiency and production of undesirable secondary sludge. Dry or living biomass is considered to be a promising cost-effective material for the sorption of heavy metals, especially for wastewater with relative low metal concentrations (< 100 mg/L). 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 medium (Markou & Nerantzis, 2013), their high sorption capacities, and their strong metal ion sorption selectivity (Wang & Chen, 2009).

Metal sorption is affected by several parameters such as the solution pH, temperature and ionic strength. In addition, metal sorption capacity is significantly affected by the functional groups of the biomass surface (cell wall), such as carboxyl, amide/amine, hydroxide, phosphate groups, which are related to biomass macro-molecules like carbohydrates, proteins and lipids. However, the relative content of these macro-molecules is significantly affected by the grow conditions of microalgae or cyanobacteria.

Stress grow conditions trigger the accumulation of lipids, carbohydrates, and other secondary metabolites in the biomass (Markou & Nerantzis, 2013). Nevertheless, there is little known about the effect of the biomass composition alteration, due to stress conditions, on metal ions biosorption capacity (Hernández & Olguín, 2002). No previous published work has explored Cu^{2+} or Ni^{2+} 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-enriched biomass of *Arthrospira platensis*, produced by a phosphorus limitation process.

II. METHODOLOGY

A. *Cultivation and preparation of biomass*

The cyanobacterium *A. platensis* was cultivated in Zarrouk medium within 10 L plastic cubical photobioreactors (Figure 1), which were kept at 30 ± 2 °C in semi-continuous cultivation mode with a dilution rate of 0.1 1/d. The carbohydrate-enriched biomass was produced by cultivation in a modified Zarrouk medium which contained less phosphorus (K_2HPO_4) compared to the typical Zarrouk medium. Typical composition biomass contained 45-55% proteins, 10-20% carbohydrates and 5-7% lipids, while the P-limited type (or carbohydrate-enriched) biomass contained 25-28% proteins, 50-60% carbohydrates and 4-6% lipids.

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 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. Then, the dried biomass was milled, sieved through a metal sieve (300 μm pore size), and stored in plastic containers inside an exsiccator containing silica gel to prevent moisture absorption by the biomass.

Figure 1. Cultivation of the cyanobacterium *A. platensis* in photobioreactors of 10 L volume (left: carbohydrate-enriched biomass, right: conventional composition biomass).

B. Biosorption experiments and analytical methods

The present study consisted of three experimental series. Four types of *A. platensis* biomass were employed as biosorbents, namely: (1) typical dry biomass (TDB), (2) carbohydrate-enriched dry biomass (CDB), (3) typical living biomass (TLB), and (4) carbohydrate-enriched living biomass (CLB).

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 not shown). In the second experimental series, five different metal concentrations (12.5- 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 48 h in order to include the possible metal bioaccumulation. In the third experimental series, four different biomass concentrations (0.125, 0.25, 0.5 and 1 g/L) were used to investigate the effect of biosorbent dosage on biosorption capacity.

Biosorption experiments were performed in batch system 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 and Ni 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 was adjusted to 5 and 6 for Cu and Ni, respectively, in order to avoid metal precipitation. These pH values are very low and could have a negatively impact on the living cells of *A. platensis*. However, the macro- but as well the microscopical examination showed 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 HNO_3 and/or 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.

All Cu, Ni, and Mg 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 time intervals, 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.

The adsorption capacity of *A. platensis* biomass for metal at time *t* and equilibrium, q_t and q_e (mg/g), respectively, were calculated with the following equations:

$$q_t = (C_0 - C_t)V/m \tag{1}$$

$$q_e = (C_0 - C_e)V/m \tag{2}$$

where C_o, C_e and C_t are the initial metal concentration, the metal concentration at equilibrium and at time *t* (mg/L), respectively; *V* is the volume of the working solution (L) and *m* is the adsorbent mass (g).

C. Error analyis

The fit goodness of the kinetic and isotherm models applied to the experimental data was evaluated by the coefficient of determination (R^2) through linear regression analysis and by the Composite Fractional Error Function (*CFEF*). The latter function measures the difference between experimental and model predicted data, and can be expressed by the following equation (Foo & Hameed, 2010):

$$CFEF = \sum_{i=1}^{n}\left[\frac{(q_{e,exp} - q_{e,cal})^2}{q_{e,exp}}\right] \tag{3}$$

where $q_{e,exp}$ and $q_{e,cal}$ (mg/g) are the experimental and model calculated values of adsorption capacity, respectively, and *n* is the number of experimental samples. The smaller the values of *CFEF*, the more similar are the calculated data to the experimental one.

III. SELECTED RESULTS

A. Biosorption kinetics

Figure 2 illustrates the kinetics of Cu and Ni on *A. platensis* biomass. For all biomass types studied, the kinetic of Cu and Ni was very fast reaching equilibrium within 30-60 min. Most of the metal biosorption capacity was filled in the first 15-30 min of the process. Cultivation conditions (P repletion or depletion) did not affect the pattern of biosorption kinetics (Figure 1).

Kinetic experimental data were fitted with five different models: first order, second order, pseudo-first order model of Lagergren, pseudo-second order model of Ho and intra-particle diffusion model of Weber-Morris (Ho & McKay, 1999; Kumar & Gaur, 2011). According to the linear regression analysis, the pseudo-second order model fitted best the biosorption time data with $R^2 > 0.976$. This model assumes that two serial or parallel reactions are taking place during the process: a fast first one and a slower second one (Anagnostopoulos, Manariotis, Karapanagioti, & Chrysikopoulos, 2012).

The application of the intra-particle diffusion model showed that in most of the cases studied, three distinct linear time-fractions exist. The presence of multi-linear plot sections in conjunction with $I \neq 0$, suggested that the biosorption process was not limited by intra-particle diffusion, and that additional sorption processes such as external mass transfer could be involved (Kumar & Gaur, 2011).

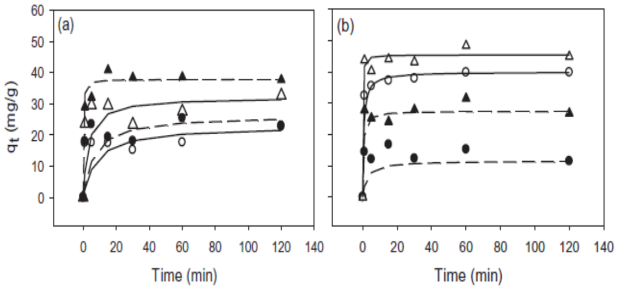


Figure 2. Biosorption kinetics of Cu^{2+} (left) and Ni^{2+} (right) on the four types of *A. platensis* biomass. Points represent the experimental data and curves the best fitted pseudo-second order model. TDB: typical dry biomass, CDB: carbohydrate-enriched dry biomass, TLB: typical living biomass, CLB: carbohydrate-enriched living biomass.

B. *Biosorption isotherms*

Table 1 and 2 present the estimated parameters of Langmuir and Freundlich isotherm, respectively. Based on R^2 values, in most of the cases, Freundlich isotherm fitted best the copper, while the Langmuir isotherm fitted best the nickel biosorption linear data. However, the calculated *CFEF* values suggest that the Langmuir model represented better the dry biomass case, while the Freundlich model represented 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 a heterogeneous biosorbent surface without a uniform distribution of affinities, which may be a better representation of living biomass 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, a description which could be more suitable to dry biomass (Foo & Hameed, 2010).

The values of the Langmuir constant *b* (Table 1) showed 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 values of the Freundlich constant *n* were > 1 (Table 2) for all biomass types studied here, indicating a favorable sorption for both metals (Foo & Hameed, 2010). Note that the constant

The Dubinin-Radushkevich isotherm model was used to calculate the adsorption mean free energy *E* (kJ/mol). For all types of biomass used in this study, *E* values were in the range of 8-16 kJ/mol, suggested that chemisorption played a significant role as a biosorption mechanism. 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.

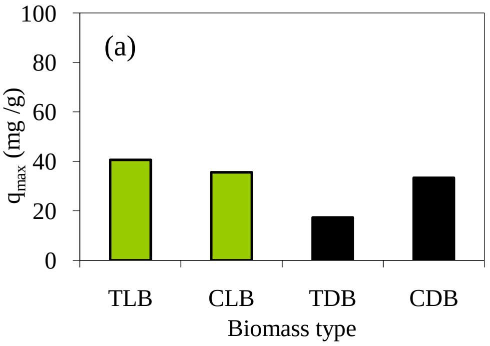
TABLE 1. PARAMETERS OF LANGMUIR ISOTHERM MODEL.

	Biomass type	q_{max} (mg/g)	b (L/mg)	R^2	CFEF
Cu	TLB	40,65	0,225	0,709	20.63
	CLB	35,59	0,135	0,908	3.07
	TDB	17,30	0,048	0,799	5.77
	CDB	33,44	0,017	0,976	1.09
Ni	TLB	90,91	0,010	0,968	7.42
	CLB	63,29	0,028	0,992	3.87
	TDB	52,63	0,017	0,985	1.17
	CDB	56,82	0,018	0,947	3.76

TABLE 2. PARAMETERS OF FREUNDLICH ISOTHERM MODEL.

	Biomass type	K_F (mg/g)	n	R^2	CFEF
Cu	TLB	11,303	3,148	0,802	10,93
	CLB	9,206	3,354	0,987	0,30
	TDB	2,036	1,710	0,911	1,68
	CDB	1,513	1,790	0,907	2,31
Ni	TLB	1,526	1,355	0,962	3,13
	CLB	3,902	1,818	0,980	1,24
	TDB	2,192	1,713	0,941	2,36
	CDB	1,639	1,500	0,859	6,88

Figure 3 shows the values of the theoretical maximum biosorption capacity (q_{max}) estimated by Langmuir model. As can be seen (Figure 3a), q_{max} for copper was positively affected by the accumulation of carbohydrates in the dry biomass, while it was negatively affected in the living biomass. Living biomass exhibited a higher q_{max} for copper than the dry biomass. For the case of Ni (Figure 3b), biomass alteration contributed to a small enhancement of q_{max} onto the dry biomass, and to a substantial reduction of biosorption (about 30% lower q_{max}) onto the living biomass. Furthermore, the living biomass, compared to dry biomass, exhibited a higher biosorption capacity for Ni due to bioaccumulation, i.e., the uptake of the metal ions intracellularly.



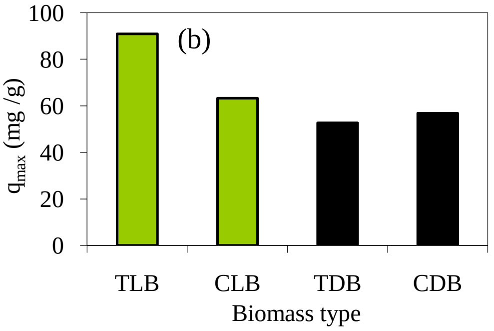


Figure 3. Maximum biosorption capacity of (a) Cu and (b) Ni ions for the four biosorbents used, as predicted by the Langmuir isotherm. TDB: typical dry biomass, CDB: carbohydrate-enriched dry biomass, TLB: typical living biomass, CLB: carbohydrate-enriched living biomass.

C. *Effect of biosorbent dosage*

The biosorption capacity at equilibrium (q_e) decreased with increasing biosorbent dose (C_s, g/L). Decreasing sorption capacity with increasing C_s is frequently reported in the literature, and indicates that adsorption of heavy metals onto biomass is not strictly a surface phenomenon (Anagnostopoulos et al., 2012) 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 (Al-Rub, El-Naas, Ashour, & Al-Marzouqi, 2006).

D. *Biosorption mechanism*

The concentrations of Na, K and Mg ions measured in the solution showed that one of the major sorption mechanisms involved is the ion-exchange. Living biomass exhibited higher ion-exchange capacity, 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. Moreover, it was observed that the equilibrium pH of the samples decreased by approximately 0.8 units compared to the initial pH, indicating that an exchange of H^+ with metal cations occurred.

E. *Desorption tests*

Desorption experiments of the metal loaded biomass with 1 M NH_4NO_3, 0.1 M Na_2-EDTA and 0.1 M $CaCl_2$, showed 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. On the other hand, the contribution of physical adsorption was minor because the desorption of both metals with DI water was observed to be very low (1.7%).

IV. CONCLUSIONS

In this study, carbohydrate-enriched dry and living biomass of the cyanobacterium *A. platensis* were used for first time as biosorbent for copper and nickel removal. The results of the experiments with dry biomass showed that the biosorption capacity of carbohydrate-enriched biomass for copper was significantly improved, while it slightly increased for nickel. In contrast, the experiments with living biomass showed that the biosorption capacity for both metals decreased due to the accumulation of carbohydrates. *A. platensis* showed higher biosorption capacity for Ni^{2+} than for Cu^{2+}. Freundlich model described better the biosorption onto living biomass due to the presence of possible bioaccumulation, whereas, the Langmuir model fitted better the experimental data with dry biomass. The results showed that the main sorption mechanisms involved were ion-exchange and complexation. In general, a weak ion-exchange occurred in case of the carbohydrate-enriched biomass.

It was concluded that the carbohydrate-enriched dry biomass has a great potential for removal of copper and nickel from aqueous solutions. The biosorption capacity was 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.

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