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Equilibrium and kinetic studies of Cu(II) and Ni(II) sorption on living *Euglena gracilis*

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Abstract Biosorption and bioaccumulation involve the decrease in concentration or removal of metals from aqueous solution through the sequestering of ions by active or metabolically inert biomass. In this study, the potential use of *Euglena gracilis*, a free-floating, flagellated unicellular species of protist, to remove Cu and Ni ions at environmentally relevant levels from aqueous solutions was investigated. Adsorption isotherms were used in a batch system to describe the kinetic and equilibrium characteristics of metal removal. The effects of pH and initial concentration of metal ions on the adsorption of Cu and Ni ions were examined. Results indicate that the sorption reaction occurred quickly (<30 min) in both Cu and Ni monometallic systems, and adsorption followed a pseudo-second-order kinetics model for both metals. Comparable metal sorption was found at pH 5 and 7.5, suggesting that metal sorption onto live *Euglena* cells is not pH dependent. No significant differences in metal sorption were found at pH 5 and 7.5. Removal efficiencies for Cu decreased with higher initial concentrations (3–30 μg L^{-1}) and conformed to the Freundlich sorption model. Ni removal was found to increase with greater initial concentration values (5–110 mg L^{-1}) and conformed to the Freundlich isotherm. The removal efficiency for Cu and Ni was 58 ± 3 and 44 ± 6%, respectively. In bimetallic systems, sorption of both metals were equivalent, suggesting that *E. gracilis* may be appropriate for mining effluent metal removal due to their ability to simultaneously tolerate, sorb, and accumulate multiple metals in solution in a range of environmental conditions.

Keywords Sorption · Metal · Algae · Copper · Nickel · Kinetic · Living algal cells · Euglenophyceae

Introduction

Industrial wastewaters, generated from metal plating, mining, fertilizer production, tannery operations, battery production, pulp and paper, and pesticide production and application, are sources of heavy metal release into the environment which not only pose an ecological threat but also may present serious consequences for human health (Borba et al. 2006; Paulino et al. 2006; Fu and Wang 2011; Kreutzweiser et al. 2013). Consequently, there exists a need for the efficient removal of toxic metals from industrial effluent before any potential exposure to surface and ground waters.

Mining operations have historically contributed to elevated levels of metals in the surrounding environment (Adamo et al. 2002; Keller et al. 2007). Conventional methods of metal removal can include chemical precipitation, resin-based ion exchange, and activated carbons as well as physical methods which utilize filtration, floatation, and coagulation (Fu and Wang 2011). These methods, however, can tend to generate large capital and operational costs, substantial energy requirements, and large volumes of toxic waste materials (Wang and Chen 2009). In addition, these procedures also tend to lack effectiveness at lower (< 100 mg L^{-1}) concentrations and can

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become prohibitively expensive in terms of the volume of wastewater to be treated (Volesky 2001).

The need for an alternative process for the removal of heavy metal ions from aqueous solutions has spawned a substantial amount of research regarding the potential and effectiveness of utilizing biological material to bind (and subsequently remove) contaminants from wastewater (Kratochvil and Volesky 1998; Gadd 2009; Anastopoulos and Kyzas 2015; Gupta et al. 2015). Living microalgae and other eukaryotic organisms have been identified as particularly effective potential biosorbents due to their ability to tolerate high concentrations of metals while growing, low production costs, high surface to area ratios, ubiquity, and ability to remove metals of relatively dilute concentrations (Malik 2004; Perales-Vela et al. 2006; Monteiro et al. 2012). *Euglena gracilis* is a free-floating, flagellated unicellular species of protist which has been found to tolerate and accumulate heavy metals (Rodriguez-Zavala et al. 2007). For example, heterotrophic *E. gracilis* has been shown to efficiently remove toxic metals such as Hg, Cd, Cr(VI), Cr(III), and U (Olaveson and Nalewajko 2000; Avilés et al. 2003; Mendoza-Cozatl et al. 2006; Jasso-Chávez et al. 2010; Lira-Silva et al. 2011; Trendield et al. 2012; Garcia-Garcia et al. 2016 and references therein). Recent work showed that *E. gracilis* grown under anoxic and acidic conditions have the ability to remove Cd (Santiago-Martinez et al. 2015). *Euglena* also have the capacity to tolerate a broad range of environmental circumstances including the extreme conditions (e.g., low pH, high metal concentrations) typically found in acid mine drainage systems (Nakatsu and Hutchinson 1988) and have been found to tolerate pH levels 2.5–7 with no significant impediments to growth (Olaveson and Nalewajko 2000). *Euglena* grown in wastewater have shown faster growth rates as compared to other algal species while concurrently removing substantial amounts of C, N, and P (Mahapatra et al. 2013). Additionally, *E. gracilis* is able to grow under photosynthetic, heterotrophic, and photo-heterotrophic conditions, utilizing carbon from several different sources including glucose, ethanol, and organic acids such as lactate, acetate, and malate (Rodriguez-Zavala et al. 2007; Santiago-Martinez et al. 2015). Together, this supports the proposal of *E. gracilis* as suitable sorbent of metals in metal-rich and acidic environments such as mining effluents.

In the present work, the performance of live *E. gracilis* in sequestering Cu and Ni was tested at acidic and neutral pH to determine adsorption isotherms and the amounts of Cu and Ni taken up by algal cells. The results were compared with a binary-metal system (Cu + Ni) which more realistically reflects actual mining effluent composition. This study was undertaken with the notion of an application to the nickel mining industry where Cu is a typical byproduct and the mitigation of environmental impacts to water quality to which mining has historically contributed (Adamo et al. 2002; Keller et al. 2007; Mudd 2010). Due to the relative inefficiency of conventional treatments of mining waste at concentrations <100 mg L^{-1} (Wang and Chen 2009), sorption kinetics were evaluated at two different initial concentrations for each metal which reflect environmentally relevant conditions.

Materials and methods

Test organism, medium and culture conditions

Euglena gracilis Klebs were obtained from Boreal Laboratory Supplies Ltd. (St. Catharines, ON, Canada). Non-axenic cultures were grown in medium consisting of 0.01 g L^{-1} $CaCl_2$, 1.0 g L^{-1} $CH_3COONa \cdot 3H_2O$, 1.0 g L^{-1} 'Lab-Lemco' powder, 2.0 g L^{-1} tryptone, and 2.0 g L^{-1} yeast extract (Oxoid LDD England). Enumeration of *Euglena* cells was performed with a 0.1 mm Neubauer hemacytometer (Hausser Scientific, USA). All media was prepared using Milli-Q water. The pH of the medium was adjusted using 1 M HCl or NaOH after autoclaving and maintained between pH 3–5 at 20 °C. *Euglena gracilis* were grown under a photoperiod of 18:6 (light/dark) at an intensity of 210 μmol photons m^{-2} s^{-1} in a Conviron (CMP5090) environmental chamber (Controlled Environments Ltd., Winnipeg, MB, Canada). Glassware was immersed in 20% HNO_3 prior to use for at least 24 h and triple-rinsed with Milli-Q water to avoid metal contamination. In addition, any glassware used for culture growth was autoclaved to mitigate bacterial contamination.

Toxicity assays (EC_{50}) of metal toxicity to *Euglena* were performed to establish a 50% response to increasing amounts of both Cu and Ni (Bruce and Versteeg 1992). A four-parameter logistic model was utilized to describe the concentration value at which a 50% mortality response occurs (SigmaPlot Version 12.0). Replicates ($n = 3$) of *Euglena* inocula were exposed to Cu^{2+} (0.064–500 mg L^{-1}) and Ni^{2+} (0.6–140 mg L^{-1}) over a period of 72 h at pH 5. Mortality was quantified via cell count at initial and final time increments from a common inoculum of 1.0×10^6 cells mL^{-1}.

Experimental procedure

Metal solutions

Copper and nickel stock solutions (0.01 mol L^{-1}) were prepared with $CuSO_4 \cdot 5H_2O$ and $NiSO_4 \cdot 6H_2O$, respectively. The pH of working metal solutions was adjusted with 0.1 mol L^{-1} HCl and 0.1 mol L^{-1} NaOH. Actual metal concentrations were determined utilizing inductively coupled plasma mass spectrometry (ICP-MS) (X Series II, ThermoScientific, USA). The accuracy of the ICP-MS measurements was assessed using SLRS-5 reference water (National Research

Council, Canada). The measured Cu and Ni concentrations were within 5% of the certified values.

Sorption kinetics

The biosorption kinetics tests were performed at a constant temperature (20 °C) in 125-mL Erlenmeyer flasks containing *E. gracilis* (biomass concentration 1 g L^{-1}) suspended in growth media spiked with metal solutions of either Cu(II) and/or Ni(II). Actual concentration was normalized to cell density (μg g^{-1} or mg g^{-1}). Kinetic studies were conducted at pH 5.0 and 7.5 and magnetically stirred at 70 rpm for 240 min. Ni and Cu are basic constituents in mine industry pollution (Iakovleva and Sillanpää 2013). Metal concentrations were chosen to correspond with levels (i.e., μg L^{-1} to mg L^{-1}) which have been reported in mining effluent after treatment in a wastewater facility (Nakatsu and Hutchinson 1988; Hruska and Dubé 2004; Mahdavi et al. 2012): 20 and 50 μg L^{-1} for Cu and 3 and 100 mg L^{-1} for Ni in monometallic systems only. Aliquots (7 mL) were immediately filtered using a 0.7 μm glass fiber filter (Merck Millipore, Ireland), acidified with ultrapure HNO_3 to pH 2.0, and stored at 4 °C until ICP-MS analysis. The filters were dried for >4 days in a desiccator and weighted on an analytical balance.

Sorption equilibria

Sorption of Cu and Ni on living *E. gracilis* was examined in batch adsorption-equilibrium experiments (120 min) at a constant temperature (20 °C) in 125-mL Erlenmeyer flasks. Blank trials without algal cells and trials without added metal solution were performed for each tested metal concentration. The effect of target metal concentration was studied at pH 5.0 in monometallic solutions with concentrations ranging from 3 to 40 μg L^{-1} for Cu and from 5 to 110 μg L^{-1} for Ni. Binary metal solutions were prepared with a range of Cu and Ni concentrations (15–40 μg L^{-1} Cu; 7–115 μg L^{-1} Ni) in 125-mL Erlenmeyer flasks. The pH levels of both monometallic and bimetallic solutions were maintained at 5.0 over the duration of the experiments with additions of 0.1 M HCl and/or 0.1 M NaOH. Flasks were magnetically agitated at 70 rpm, and temperature was held constant at 20 °C. Aliquots (7 mL) were immediately filtered using a 0.7 μm glass fiber filter (Merck Millipore, Ireland), acidified with ultrapure HNO_3 to pH 2.0, and stored at 4 °C until ICP-MS analysis. The filters were dried for >4 days in a desiccator and weighted on an analytical balance.

The amount of Cu and Ni adsorbed at equilibrium, q (μg g^{-1}), was calculated with the following equation:

$$q = \frac{\left(C_i - C_{eq}\right)V}{m} \tag{1}$$

where C_i is the initial concentration of the metal ion prior to adsorption (μg L^{-1}) and C_{eq} is the equilibrium concentration of metal ions in the aqueous phase. V is the volume (L) of the aqueous phase and m is the dry weight mass of the adsorbent (g). Each experiment was performed in duplicate.

Data analyses

Adsorption kinetics models were used to evaluate the overall rate of Cu or Ni removal from *Euglena*–single metal solutions. Kinetic studies were carried out for sorption of Cu and Ni as a function of contact time at two initial concentrations for each metal (Cu = 20 and 50 μg L^{-1}; Ni = 3 and 100 mg L^{-1}) at both pH 5 and 7.5 on live *E. gracilis*. Samples were taken at time intervals of 10, 20, 30, 60, 90, 120, and 240 min. Two different, non-linear models were employed: the pseudo-first-order (PFO) and pseudo-second-order (PSO) kinetic equations (Lagergren 1898; Ho et al. 1996).

The Langmuir and Freundlich isotherm models were used to analyze biosorption data. The Langmuir model, which operates on the assumptions that adsorption occurs in a monolayer on the solid, all sites are identical and may sorb only a single molecule, and are independent of adjacent site sorption. The Freundlich isotherm model is an empirical formulation which assumes heterogeneous surface adsorption.

The differences in metal sorption results between different pH levels and different initial metal concentrations were analyzed using paired *t* tests.

Results

Toxicity assays

The EC_{50} assay values in single-metal systems (Fig. 1) were 39.2 and 27.4 mg L^{-1} for Cu and Ni, respectively. These values were similar to previous *E. gracilis* studies (Olaveson and Nalewajko 2000; Willemann 2002; Ahmed and Häder 2010). Lower EC_{50} values were reported for the freshwater algae *Pseudokirchneriella subcapitata* in EDTA-free growth medium (Guéguen et al. 2004; Magdaleno et al. 2014) and the freshwater diatom *Navicula pelliculosa* (Nagai and de Schamphelaere 2016). Together, these results suggest that *E. gracilis* has the capability to effectively cope with high concentrations of Cu and Ni (mg L^{-1} range) not suitable for aquatic life (CCME 2007).

Sorption experiments

The sorption of Cu and Ni to the protist *E. gracilis* in single and binary metal systems was investigated. In single metal systems, the bioaccumulation of both metals

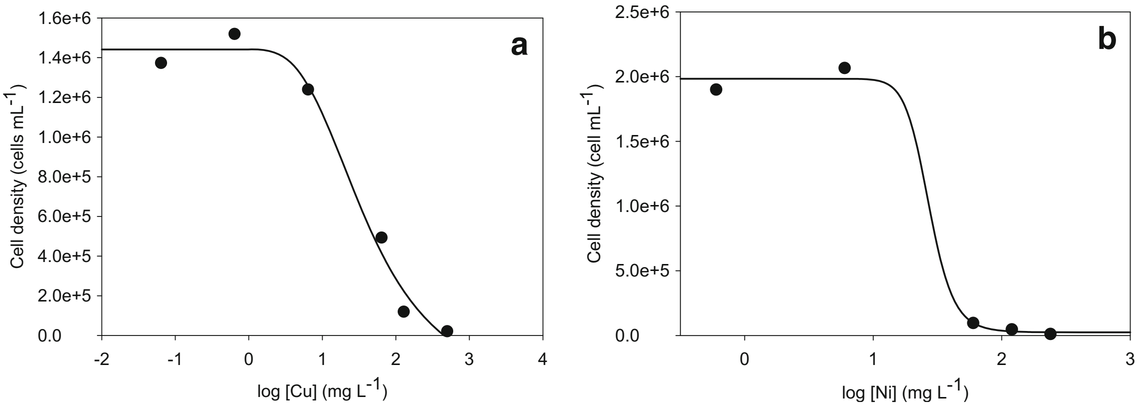

Fig. 1 EC50 toxicity assay on living *Euglena gracilis* for **a** Cu and **b** Ni. The *dotted lines* represent the concentration at EC_{50}

occurred rapidly within 10–30 min and plateaued within 60–90 min regardless of pH (Fig. 2). This fast uptake was congruent with previous biosorption studies (He and Chen 2014; and references therein). The batch sorption experiments were therefore conducted at 2 h to ensure that equilibrium would be obtained.

The sorption uptake increased with increasing equilibrium concentration in single-metal (Fig. 3) and binary solutions (Fig. 4). Compared to single-metal solutions (Fig. 2), Cu and Ni sorption in binary solutions was not significantly different ($p > 0.05$). Total metal uptake of both Cu and Ni in binary solutions increased when compared to single-metal solutions; however, only Cu uptake was significantly higher ($p < 0.05$).

Discussion

Metal sorption and pH effect. Cu kinetics followed the PSO model ($r^2 > 0.98$) whereas Ni exhibited much less consistency

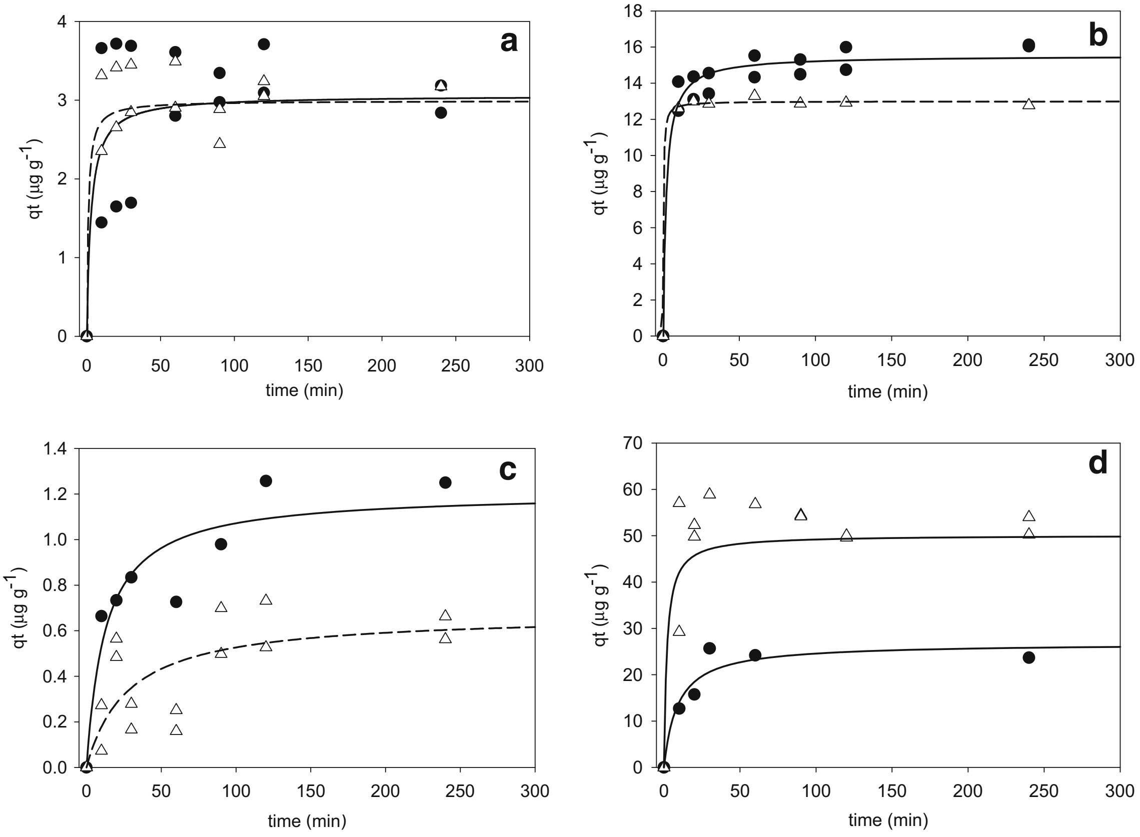

Fig. 2 Cu (**a** 20 μg L^{-1}; **b** 50 μg L^{-1}) and Ni (**c** 3 mg L^{-1} ; **d** 100 mg L^{-1}) sorption kinetics on living *E. gracilis* at pH 5.0 (*black circle*) and 7.5 (*white triangle*). *Curves* represent pseudo-second-order kinetic model (plain pH 5.0; *dotted* pH 7.5)

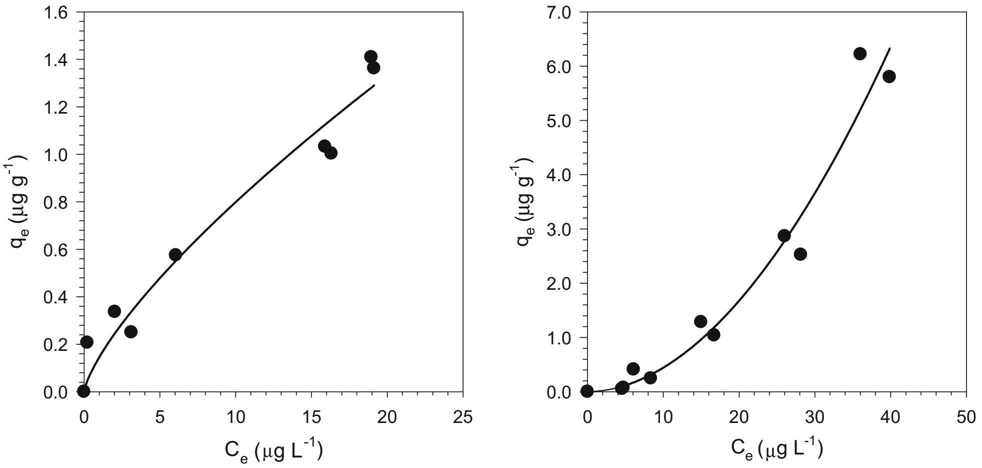

Fig. 3 (left panel) Cu and (right panel) Ni sorption equilibrium on living *E. gracilis. Curves* represent the Freundlich model fit

(r^2 = 0.48–0.92) which could be attributed to metabolically driven cell processes such as active efflux of metal ions or production of metal-binding proteins such as phytochelatins and/or metallothioneins (Rodriguez-Zavala et al. 2007). PFO model fitness (r^2) was weaker than that of the PSO model and thus was not discussed here.

The amount of metal sorbed (q_e) ranged from 3.1 to 15.5 µg g^{-1}and from 1.2 to 50.1 mg g^{-1} for Cu and Ni, respectively (Table 1). The rate constant k_2 varied from 0.03 to 0.33 µg (g min)$^{-1}$ and from 0.004 to 0.06 mg (g min)$^{-1}$ for Cu and Ni, respectively (Table 1). The value of q_e and k were generally found to be in a similar order of magnitude to those reported in the literature based on living biomass (Kizilkaya et al. 2012; Markou et al. 2015). Comparable kinetic parameters were found using other eukaryotes, green algae, and cyanobacteria (Mehta and Gaur 2001; Rajfur et al. 2010; Markou et al. 2015). These parameters are an integral component of designing biologically based water treatment systems in terms of identifying the rate controlling steps (e.g., mass transport processes and/or chemical reactions) which not only determine retention times but also contribute to removal efficacy (Febrianto et al. 2009). These observations (Table 1) conform to published findings regarding the effect of initial metal concentration on algal sorption of Cu and Ni (Mehta and Gaur 2001; Doshi et al. 2008; Kizilkaya et al. 2012; Li et al. 2012).

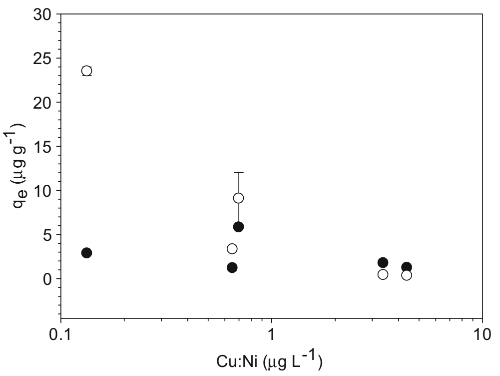

Fig. 4 Cu (left) and Ni (Right) sorption equilibrium from binary-metal solution on living *E. gracilis*. *Error bars* represent standard deviation

Although no difference in sorption rate k_2 was discerned ($p > 0.05$), the amount of Cu and Ni sorbed was found to significantly increase with higher initial concentrations at both pH levels tested ($p < 0.05$). Nickel at higher concentrations however (100 mg L^{-1}) showed a significant increase in the amount of metal sorbed at a higher pH ($p < 0.05$). The initial Ni^{2+} concentration (100 mg L^{-1}) exceeds the EC_{50} obtained in this study (27 mg L^{-1}; Fig. 1) thus it may be assumed that the *Euglena* cells were already under severe stress, modifying metal sorption.

pH is an important environmental parameter affecting the process of biosorption and bioaccumulation in both living and immobilized algal biosorbents (Rao et al. 2005; Al-Rub et al. 2006; He and Chen 2014). Decrease in metal sorption was generally associated with acidic pH (<2–3; He and Chen 2014 and references therein; Mehta and Gaur 2001) and the protonation of functional groups found on cell surfaces. In this study, no significant difference in metal sorption was found between pH 5 and 7.5 ($p > 0.05$; Table 1), suggesting that sorption on live *Euglena* cells was comparable in acid to circumneutral waters (5 < pH < 7.5).

Biosorption equilibrium isotherms. The Freundlich r^2 values (0.96 and 0.88 for Cu and Ni, respectively; $p < 0.001$; Table 2) were significantly higher than those obtained with Langmuir model (0.88 and 0.84, respectively). Freundlich K_f values (0.005 and 0.071 for Ni and Cu, respectively) were lower than those reported for other species of algae at various, and generally much higher, concentrations (Wong et al. 2000; Mehta and Gaur 2001; Tien 2002; Doshi et al. 2008; Markou et al. 2015). For example, *Chlorella*

Table 1 Pseudo-second-order kinetics parameters for the sorption of Cu and Ni on living *Euglena gracilis* cells

Initial metal concentration	pH	q_e	k_2	r^2
Cu 20 μg L^{-1}	5	3.1 ± 1.1 μg g^{-1}	0.12 ± 1.1 μg $(g\ min)^{-1}$	0.98
	7.5	3.0 ± 2.6 μg g^{-1}	0.33 ± 2.6 μg $(g\ min)^{-1}$	0.98
Cu 50 μg L^{-1}	5	15.5 ± 0.03 μg g^{-1}	0.031 ± 0.004 μg $(g\ min)^{-1}$	0.99
	7.5	13.0 ± 10.5 μg g^{-1}	0.33 ± 0.58 μg $(g\ min)^{-1}$	0.99
Ni 3 mg L^{-1}	5	1.2 ± 0.7 mg g^{-1}	0.06 ± 0.05 mg $(g\ min)^{-1}$	0.88
	7.5	0.7 ± 1.2 mg g^{-1}	0.05 ± 0.07 mg $(g\ min)^{-1}$	0.64
Ni 100 mg L^{-1}	5	26.8 ± 11.6 mg g^{-1}	0.004 ± 1.25 mg $(g\ min)^{-1}$	0.92
	7.5	50.1 ± 45.6 mg g^{-1}	0.01 ± 23.1 mg $(g\ min)^{-1}$	0.92

vulgaris K_f values were 1.22 and 1.19 for Cu and Ni (Mehta and Gaur 2001).

The Freundlich 1/*n* value was 0.98 ± 0.24 for Cu sorption on *E. gracilis*, which is comparable to freshwater algae sorption (0.56–1.17; Tien 2002). A less favorable sorption reaction was found for Ni (1.92 ± 0.26). This contrasts with previous studies where more favorable Ni sorption was found on *C. vulgaris* (0.66–0.75; Wong et al. 2000).

The percent removal on living *E. gracilis* increased from 44 ± 6% for Ni to 58 ± 3% for Cu (Table 2). Higher removal of Cu was also reported using live *C. vulgaris* (42.5 vs 37.5%, Mehta and Gaur 2001). The removal efficiency of *E. gracilis* was comparable to living *C. vulgaris* (28 and 37% for Cu and Ni, respectively; Mehta and Gaur 2001) but higher than for living *Chlamydomononas reinhardtii* (28% for Cu; Flouty and Estephane 2012). Our study clearly shows significant removal ability of metal using living *E. gracilis*.

Sorption in binary metal solution. Compared to single-metal solutions (Fig. 2), Cu and Ni sorption in binary solutions was not significantly different ($p > 0.05$). Total metal uptake of both Cu and Ni in binary solutions increased when compared to single-metal solutions; however, only Cu uptake was significantly higher ($p < 0.05$). This suggests that different sorption mechanisms could be involved or that specific binding site exist for each metal (Chong et al. 2000; Mallick 2003). Additionally, transition metals have been reported to share common transporters localized both intracellularly (e.g. P-type ATPase) and at the cell membrane (e.g., NRAMP, CTR) which could account for higher total metal sorption in binary solutions (Blaby-Haas and Merchant 2012). Metal sorption by algal cells has been characterized as an extremely dynamic process in which a number of different mechanisms may operate and thus may prompt both synergistic and antagonistic interactions between metals and binding sites (Flouty and Estephane 2012). The presence of concomitant metals did not significantly influence metal sorption ($p < 0.05$) suggesting that Cu and Ni did not produce inhibitory effects on the sorption of the other metal. This result is contrary to some Cu/Ni sorption studies of other eukaryotes (Mehta et al. 2002; Keshtkar et al. 2015). Sorption of metal ions has been linked with ionic radii and electronegativity which could account for the similar response between Cu and Ni uptake both of which share comparable physiochemical characteristics (Flouty and Estephane 2012). Sorption of both metals appears to be equivalent in binary solutions. This is an important characteristic because the interactive effects of metals can reduce overall metal removal, and the effluent rarely contains only one metal contaminant (Mehta and Gaur 2001; Hruska and Dubé 2004). This suggests that *Euglena* may be more appropriate for mining effluent metal removal than comparable organisms due to their ability to simultaneously tolerate, sorb, and accumulate multiple metals in solution (Devars et al. 1998; Mendoza-Cozatl et al. 2006) in addition to efficiently reducing nutrient levels and organic contaminants (Kobayashi and Rittmann 1982; Mahapatra et al. 2013).

Table 2 Freundlich adsorption isotherm parameters for the biosorption of Cu and Ni on *Euglena gracilis* cells at pH 5

Metal	K_f	1/n	r^2	%removal
Cu	5	0.98 ± 0.24	0.88	58 ± 3
Ni	7.5	1.92 ± 0.26	0.96	44 ± 6

In conclusion, in this study, live *E. gracilis* have been shown to exhibit the capacity to remove Cu and Ni from single and binary solutions in the <100 mg L^{-1} range which, to our knowledge, has not been studied to date. Sorption kinetics followed the PSO model and the single-metal sorption equilibria followed the Freundlich isotherm model which suggests binding sites of living *Euglena* are heterogeneous. Removal of Cu and Ni occurred relatively quickly and increased with reduced pH and increasing initial concentrations of metal; optimizing these conditions could increase *Euglena* affinity for these metals. *Euglena gracilis* showed a greater sorption capacity for Cu as compared to Ni in both single-metal and binary solutions and could be a potentially economic and effective biosorbent for Cu removal. The removal ability of metals using living *E. gracilis* was comparable to higher than previously tested living algae, suggesting that *E. gracilis* constitutes a good biosorbent for Cu and Ni removal from aqueous solutions. Further work should include an assessment of the capacity for non-living *Euglena* biomass to remove Cu and Ni from solution and optimization of operational parameters to maximize removal as well as the evaluation of *E. gracilis* removal performance in actual industrial effluent mixtures of metals, inorganic and organic ligands.

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