Continuous biosorption of Cu and Zn by immobilized waste biomass *Sargassum* sp.

E. Valdman [a], L. Erijman [b], F.L.P. Pessoa [a], S.G.F. Leite [a],*

[a] *Escola de Química, Centro de Tecnologia, Cidade Universitária, Universidade Federal do Rio de Janeiro, Bloco E, Rio de Janeiro, 21941-900, Brazil*
[b] *Instituto de Investigaciones en Ingeniería Genética y Biologia Molecular (INGEBI-CONICET), Vuelta de Obligado 2490, (1428) Buenos Aires, Argentina*

Abstract

Biosorption of copper and zinc by the residual biomass of the alga *Sargassum* sp. was investigated in a mini packed-bed column. The biomass was immobilized in a polymeric matrix to improve its physicochemical properties for continuous biosorption of heavy metals from aqueous solutions. Adsorption breakthrough curves obtained at different flow rates indicated that an increase in flow rate decreased the volume treated until the breakthrough point as well as the service time of the bed and the dynamic capacity of the column. At the lowest flow rate of 1.0 ml/min per cm^2 it was possible to treat 446 and 414 bed volumes of copper and zinc up to their respective breakthrough points. At this flow rate, the dynamic capacity of the column was 11.9 mg Cu/g and 21.0 mg Zn/g. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Copper; Immobilization; Packed-bed column; *Sargassum* sp.; Zinc

1. Introduction

Heavy metals are major pollutants in marine, ground, industrial and even treated wastewaters. Stringent regulations are increasing the demand for new technologies for metal removal from wastewater to attain today's toxicity-driven limits [1].

The use of biological materials for removing and recovering heavy metals from contaminated industrial effluents has emerged as a potential alternative method to conventional techniques, which may be expensive and ineffective [2]. The passive uptake of metals by microbial cells, known as biosorption, is passive in that no energy is required. Indeed, the biomass in this process is often nonviable, some processes relying on nonviable or deliberately killed cells [3]. For industrial-scale processes, the cost of producing biomass may be a serious economic disadvantage. Consequently, it would be desirable to use residual biomass that can be ob-

tained in large quantities from several industrial activities [4–7]. Biosorptive processes are generally rapid and are in theory suitable for the extraction of metal ions from large volumes of water [7].

Although other types of reactors, e.g. batch or continuous stirred tank reactors and fluidized bed columns can be used, packed bed adsorption has a number of advantages. It is simple to operate, attains a high yield and it can be easily scaled up from a laboratory-scale procedure. The stages in the separation protocol can also be automated and high degrees of purification can often be achieved in a single step process [8].

For heavy metal biosorption in packed bed columns, cells are generally immobilized to improve their structural strength, rigidity and porosity [5]. Many biopolymers such as calcium alginate, glutaraldehyde and agarose can be used for biomass immobilization. Biopolymers are non-toxic, selective, efficient and inexpensive, and thus highly competitive with ion exchange resins and activated carbons [9]. The use of live immobilized biomass may present some specific problems: the cell viability, as determined by photosynthetic and res-

---

* Corresponding author. Tel.: + 55-21-5627613; fax: + 55-21-5627567.

E-mail address: selma@eq.ufrj.br (S.G.F. Leite).

piratory activities, should be kept as long as possible. Therefore, immobilization is even more indicated when non-living cells are used, e.g. waste biomass [4].

The aim of this study was to investigate the performance of a packed bed biosorption column for copper(II) and zinc(II) by immobilized *Sargassum* sp. as a function of flow rate. The results obtained for bed volumes treated, breakpoint time and column dynamic capacities were also compared to previous reports.

2. Materials and methods

2.1. Biosorbent

A brown macroalga, *Sargassum* sp., was used in this study; it was obtained as a residual biomass of a polymer extraction industry from Rio de Janeiro, Brazil. It was washed with distilled water, dried at 45°C for 12 h then ground and sieved into different fractions. The 0.56- to 0.85-mm particle size fraction was used in the experiments.

2.2. Biomass immobilization

Crosslinking was the method employed for biomass immobilization. To obtain the immobilized alga, 1.8 g of sieved biomass were embedded in 15% polyethyleneimine (PEI) resin (2 ml) and 15% glutaraldehyde (1 ml) as described previously [4]. The mixture was left overnight at 45°C to form the polymeric matrix followed by distilled water washing for the removal of chemicals. The modified biomass was oven-dried at 45°C.

2.3. Biosorption studies

Biosorption studies were carried out in a fixed-bed mini-column reactor, made of the inert material Kel-F, with an internal diameter of 0.5 cm filled with 0.1 g of immobilized dried cells (packing density, 0.318 g/cm^3; bed volume, 0.314 cm^3). The bed depth was kept at 1.6 cm and the temperature of the column during the experiments was 25°C. Copper and zinc synthetic aqueous solutions (initial concentration, $C_o = 10$ mg/l, pH 4.5) were percolated alternatively through the top of the stationary bed of adsorbent (algae). The flow rate was regulated with a variable peristaltic pump. Samples were taken from the effluent at timed intervals by a fraction collector and analyzed for Cu(II) or Zn(II) ions as described below. The experiments were continued until saturation of the column was observed. Data presented constitute average values from two replicates.

2.4. Analysis of metal ions

The concentration of free Cu(II) or Zn(II) ions in the effluent was determined spectrophotometrically following modified protocols of Smith et al. [10] and Sandell [11], respectively. Bicinchoninic acid (BCA) and dimethylformamide (DMF) were added to samples containing less than 10 mg Cu/l, while ditizone was dissolved in methylcellulose and this resulting solution was added to samples containing less than 1 mg Zn/l. The absorbances of copper- and zinc-colored complex solutions were read at 550 and 520 nm, respectively.

2.5. Column performance evaluation

The evaluation of the column performance was carried out considering the plots of metal ion concentration in the effluent (C_{eff}) as a function of process time (t) and treated (or effluent) volume (V_{eff}). From these plots, it was possible to calculate the capacity of the column for removing heavy metals from aqueous solutions. Another parameter used to compare biosorption column capacity was the bed volume that the column was capable to treat until its breakthrough point.

3. Results and discussion

The focus of this study was the breakthrough point defined as the maximum allowable discharge level for each heavy metal ion. According to the Brazilian standard [12], the environmentally acceptable levels for copper and zinc are 1.0 and 5.0 mg/l, respectively.

3.1. Biosorption of copper and zinc ions as a function of volume treated

The effect of flow rate on the biosorption of copper and zinc ions was investigated in the continuous packed bed mentioned before. For this purpose, the adsorption breakthrough curves were obtained at flow rates of 1.0 and 3.6 ml/min per cm^2 for inlet concentrations of 10 mg/l of Cu(II) or Zn(II) (Fig. 1).

The breakthrough curves drawn in Fig. 1 as a function of treated volume have a gradual shape. These results show that the biosorption of copper and zinc ions is dependent on the flow rate and that this parameter directly affects the volume of treated effluent. It can also be seen from Fig. 1 that the efficiency of the treatment decreases with increasing flow rates.

The bed volume was used as an adimensional parameter to compare the results obtained in this study with others described in the literature. It was defined as the ratio between the treated volume and the volume of the column bed. The results obtained for Zn ion showed that a breakthrough occurred at 414 bed vol-

umes (170 ml) when the flow rate was 1.0 ml/min per cm^2, while attaining 541 bed volumes (130 ml) when the flow rate was increased to 3.6 ml/min per cm^2 (Fig. 1). Taking into account the lowest flow rate, 414 bed volumes treated up to the breakthrough were much higher than other results reported in the literature of 166.7 bed volumes for *Sargassum* sp. [13] and 29 bed volumes for slag — a blast furnace waste material [14].

Considering the Cu ion, breakthrough by immobilized *Sargassum* sp. occurred at 446 bed volumes (140 ml) at a flow rate of 1.0 ml/min per cm^2. However, it was not detected at the higher flow rate of 3.6 ml/min per cm^2, as the concentration of the solution was higher than the copper breakthrough point of 1.0 mg/l at the beginning of the process. Compared to other results cited in literature for the same inlet metal ion concentration (10 mg/l), 446 bed volumes obtained at the lowest flow rate in this study were similar to 400 bed volumes for *Sargassum* sp. [13]. Moreover, it was about two times higher than 231 bed volumes for *Azolla filiculoides* [15] and five times higher than 86 bed volumes for apple residues [16].

3.2. Biosorption of copper and zinc ions as a function of process time

Effluent metal ion levels were also plotted as a function of time in the form of breakthrough curves as illustrated by representative data in Fig. 2. This form of representation clearly highlights the influence of flow rate on metal ion biosorption.

As indicated in Fig. 2, as the flow rate increases the breakthrough curve becomes steeper and the breakpoint time decreases. It can be deduced that the flow rate affects the slope of the linear part of the breakthrough curve. Saturation time is also greatly affected

Fig. 2. Breakthrough curves at different flow rates for Cu(II) or Zn(II) biosorption by immobilized alga *Sargassum* sp. as a function of process time (T, 25°C; C_o, 10 mg/l, pH of inlet metal ion solution: 4.5, C_{eff}, effluent concentration; ■, Cu 1.0 ml/min per cm^2; □, Cu 3.6 ml/min per cm^2; ●, Zn 1.0 ml/min per cm^2; ○, Zn 3.6 ml/min per cm^2).

by the flow rate (not shown). Similar results were obtained to copper sorption by PVF-immobilized *R. arrhizus*, where increasing flow rate decreases the time required for the beginning of column breakthrough [7]. Aksu and Kutsal [8] explained that if the residence time of the solute in the column is not long enough for adsorption equilibrium to be reached at that flow rate, the metal ion solution leaves the column before equilibrium occurs. The highest flow rate used in this study sets an upper limit for sufficient contact time of metal ions with immobilized algae.

As flow rate increased, the time required for detection of the zinc breakthrough point decreased from 23 h at a flow rate of 1.0 ml/min per cm^2 to 5 h for a flow rate of 3.6 ml/min per cm^2. The result obtained at the lowest flow rate (23 h) was almost 42 times higher than that found for slag (0.55 h) [14].

As opposed to zinc solutions, the highest flow rate was not efficient in the treatment of diluted copper solutions. However, the breakpoint time for Cu at 1.0 ml/min per cm^2 was 10.8 h (Fig. 2). This value was higher than those obtained for apple residues [16], protonated *S. fluitans* [2] and *S. kjellmanianum* [17], with values corresponding to 6.6, 2.83 and 0.41 h, respectively.

3.3. Column dynamic capacity

The dynamic capacity of the column is defined as the ratio of the amount of metal ion adsorbed prior to the breakthrough point and the quantity of biomass used in the packed bed. The integration of areas under the curves obtained in Fig. 1 gives the amount of metal not recovered by *Sargassum* sp.; based upon the difference of the quantity of metal fed to the column, this value permits the determination of the amount of metal ad-
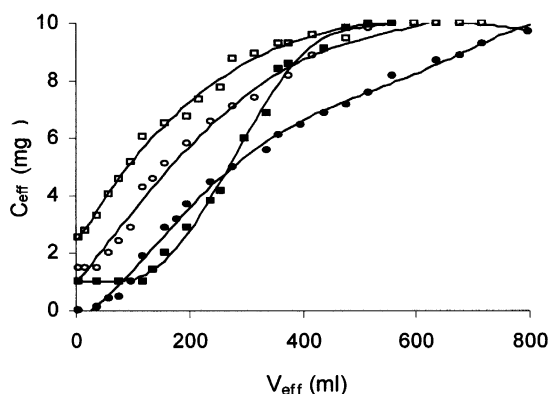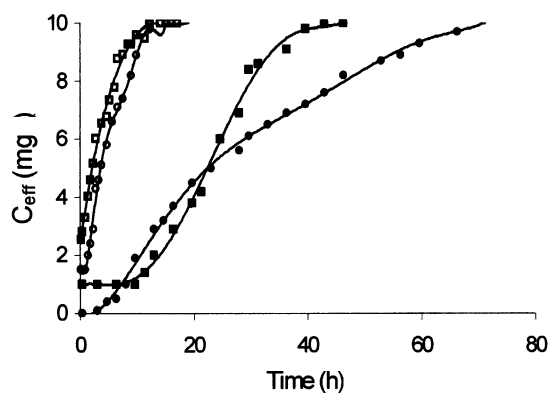

Fig. 1. Breakthrough curves at different flow rates for Cu(II) or Zn(II) biosorption by immobilized alga *Sargassum* sp. as a function of treated volume (T, 25°C; C_o, 10 mg/l, pH of inlet metal ion solution: 4.5; C_{eff}, effluent concentration; V_{eff}, effluent volume; ■, Cu 1.0 ml/min per cm^2; □, Cu 3.6 ml/min per cm^2; ●, Zn 1.0 ml/min per cm^2; ○, Zn 3.6 ml/min per cm^2).

Table 1
Continuous metals biosorption by *Sargassum* sp. up to breakthrough point at different flow rates[a]

Metal	Flow rate (ml/min per cm^2)	Mass metal (mg)			Dynamic capacity (mg/g)
		Inlet	Outlet	Biosorbed	
Cu	1.0	1.28	0.09	1.19	11.9
	3.6	nd	nd	nd	nd
Zn	1.0	2.60	0.50	2.10	21.0
	3.6	1.67	0.45	1.22	12.2

[a] T, 25°C; C_o, 10 mg/l; pH of inlet metal ion solution, 4.5; nd, not determined.

sorbed by the biomass [13]. Bearing in mind the amount of biomass used in the packed bed (0.1 g), it was possible to calculate the dynamic capacity of the column (Table 1).

As shown in Table 1, the dynamic capacity of the column was 21.0 mg Zn/g when the flow rate was 1.0 ml/min per cm^2. This value drops to 12.2 mg Zn/g when the flow rate was increased to 3.6 ml/min per cm^2, that also compares favorably with the dynamic capacity of 2.3 mg Zn/g reported for slag [14].

For copper biosorption, the dynamic capacity was 11.9 mg Cu/g when the flow rate was 1.0 ml/min per cm^2 (Table 1). However, it was not possible to calculate the capacity for copper at a higher flow rate than 3.6 ml/min per cm^2 as the concentration of the outlet solution was higher than the permitted value of 1 mg Cu/l in the first periods of contact (Fig. 2). For the lowest flow rate, the result obtained was similar to 10 mg Cu/g for the alga *S. kjellmanianum* [17] and almost twofold higher (6.6 mg Cu/g) than that of apple residues [16].

The dynamic capacities obtained at 1.0 ml/min per cm^2 were higher for zinc (21.0 mg/g) than for copper (11.9 mg/g). The same order of preference (Zn > Cu) was observed in batch studies using the Langmuir model as described previously by Valdman and Leite [4]. One reason for different metal sorption capacities might be the electrochemical properties of the metal ions [4].

4. Conclusion

Biosorption of heavy metals ions by immobilized alga in a packed bed column is an economically feasible technology for removing metal ions from solution. The successful biomass behaves as an ion exchanger. Adsorption breakthrough curves obtained at different flow rates indicate that an increase in flow rate decreases the volume treated until the breakthrough point and therefore decreases the service time of the bed. At a flow rate of 1.0 ml/min per cm^2, the column was able to treat 446 and 414 bed volumes of copper and zinc, respectively.

At this flow rate, the dynamic capacities of the column were 11.9 mg Cu/g and 21.0 mg Zn/g. At a higher flow rate (3.6 ml/min per cm^2), lower removal capacities were observed, probably due to insufficient contact times for the establishment of adsorption equilibrium between algal cells and metal ions. The application of biosorption by immobilized waste biomass *Sargassum* sp. is potentially well suited for the detoxification of metal-bearing industrial effluents in large-scale columns.

References

[1] Esteves AJP, Valdman E, Leite SGF. Repeated removal of cadmium and zinc from an industrial effluent by waste biomass *Sargassum* sp. Biotechnol Lett 2000;22(6):499–502.

[2] Kratochvil D, Volesky B, Demopoulos G. Optimizing Cu removal/recovery in a biosorption column. Water Res 1997;31(9):2327–39.

[3] Alexander M. Biodegradation and Bioremediation. San Diego, CA: Academic Press, 1999.

[4] Valdman E, Leite SGF. Biosorption of Cd, Zn and Cu by *Sargassum* sp. waste biomass. Bioproc Eng 2000;22(2):171–3.

[5] Lee SH, Jung CH, Chung H, Lee MY, Yang J. Removal of heavy metals from aqueous solutions by apple residues. Proc Biochem 1998;33(2):205–11.

[6] Bustard M, McHale AP. Biosorption of heavy metals by distillery-derived biomass. Bioproc Eng 1998;19:351–3.

[7] Brady JM, Tobin JM, Roux J-C. Continuous fixed bed biosorption of Cu^{2+} ions: application of a simple two parameter mathematical model. J Chem Technol Biotechnol 1999;74:71–7.

[8] Aksu Z, Kutsal T. Determination of kinetic parameters in the biosorption of copper(II) on *Cladophora* sp., in a packed bed column reactor. Proc Biochem 1998;33(1):7–13.

[9] Aksu Z, Egretli G, Kutsal T. A comparative study of copper(II) biosorption on Ca-alginate, agarose and immobilized *C. vulgaris* in a packed-bed column. Proc Biochem 1998;33(4):393–400.

[10] Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. Measurement of protein using bicinchoninic acid. Anal

Biochem 1985;150(1):76–85.

[11] Sandell EB. Colorimetric Determination of Trace Metals. New York: Interscience, 1959.

[12] Ministério de desenvolvimento urbano e meio ambiente. Classificação das águas doces, salobras e salinas do território nacional — Resolução no.20 do Conselho Nacional do Meio Ambiente (CONAMA), 1986, Brasília, Brasil.

[13] da Costa ACA, de França FP. Biosorption of zinc, cadmium, and copper by a brown seaweed (*Sargassum* sp.) in a continuous fixed-bed laboratory reactor. Bioseparation 1997;6:335–41.

[14] Gupta VK, Rastogi A, Dwivedi MK, Mohan D. Process development for the removal of zinc and cadmium form wastewater using slag — a blast furnace waste material. Sep Sci Technol 1997;32(17):2883–912.

[15] Fogarty RV, Dostalek P, Patzak M, Votruba J, Tel-Or E, Tobin JM. Metal removal by immobilised *Azolla filiculoides*. Biotechnol Lett 1999;13:533–8.

[16] Lee S-H, Yang J-W. Removal of copper in aqueous solution by apple wastes. Sep Sci Technol 1997;32(8):1371–87.

[17] Zhou JL, Huang PL, Lin RG. Sorption and desorption of Cu and Cd by macroalgae and microalgae. Environ Pollut 1998;101:67–75.