



Biosorption of cadmium (II) and lead (II) from aqueous solution using exopolysaccharide and biomass produced by *Colletotrichum* sp.

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ABSTRACT

The biosorption of Cd(II) and Pb(II) ions on biomass and exopolysaccharide (EPS) produced by *Colletotrichum* sp. fungus has been investigated as a function of contact time, initial pH, initial metal ion concentration, and initial adsorbent concentration in a batch system. Adsorption equilibrium was described by Freundlich and Langmuir isotherms. Adsorption was characterized through granulometry, SEM and EDX analysis. Then, studies were performed to regenerate the adsorbent. Biosorption of metals by biomass and EPS were best described by the Langmuir and Freundlich isotherm, respectively. Results of thermodynamic investigations showed that adsorption reactions were spontaneous ($\Delta G^\circ < 0$), exothermic, and mainly physical. The EPS was able to remove 79 and 98% of cadmium and lead, respectively, and the biomass removed 85 and 84% of cadmium and lead, respectively, in a solution with initial concentration 100 mg L^{-1} , and the four adsorption–desorption cycles of all adsorbents showed up with great regenerative capacity and relative stability after these four cycles, the high potential of these biological materials in sorption has been shown.

Keywords: EPS; Biomass; Biosorption; Adsorption isotherms; Lead; Cadmium

1. Introduction

Dumping of contaminated effluents of toxic metals such as cadmium, lead, copper, chromium, mercury and zinc originated from industrial activities has increased metal compound rates in water bodies [1]. Their presence in water sources represents an important ecological issue owing to their high pollutant and contaminant potential, with several toxicological liabilities in a great variety of living beings. Cadmium may

cause in humans kidney dysfunctions, bones damage, cardiovascular diseases, systemic effects, and possibly cancer [2]. As a rule, lead may damage the nervous, digestion and blood systems, with symptoms featuring lethargy, anemia, headaches and bellyaches [3].

The retrieval of heavy metals from industrial effluents may be performed by several conventional and advanced processes such as chemical precipitation, evaporation, reverse osmosis, electrodialysis, adsorption in activated charcoal or alumina, oxy-reduction or ionic exchange, crystallization, coagulation,

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flocculation, ultrafiltration, and others. However, the above processes are generally limited by technical problems, operational costs and materials. Further, the above methods are not efficient in the removal of metals in low concentrations [4].

Sorption is an alternative method to conventional and advanced processes in the removal of heavy metals from liquid effluents. Several advantages may be listed, such as the nonproduction of chemical residues, easy handling, low costs, and efficient removal of pollutants in highly diluted solutions [5,6].

Biological sorption or biosorption is based on the ability of biological materials (dead or alive) to accumulate heavy metals from water through physical and chemical collecting methods. This innovatory depurative process employs biological material such as biomass of bacteria, filamentous fungi, yeasts, algae, microbial metabolism products, such as biogas, biopolymers, such as exopolysaccharides, and residues from industrial biological processes, which are low-cost and naturally abundant [1,7–10].

Several studies focus on the development of new technologies for the treatment of metallic ions-contaminated water by biosorption. Keeping in mind possible damages to biological systems, these studies will broaden perspectives on the solution of the important issue concerning heavy metals in wastewaters. Current research evaluates use of exopolysaccharide (EPS) and biomass produced by the endophytic fungus *Colletotrichum* sp. in the sorption of cadmium and lead.

2. Materials and methods

2.1. Microorganism, submerged fermentation and obtaining adsorbents

A strain of the fungus *Colletotrichum* sp. was donated by the Bioassay and Genetics Laboratory of the Amazonas State University, Brazil. Cultures were performed in 250 mL flasks with 47 mL of basal medium and 3 mL cell solution. Basal medium was composed by minimum medium of Vogel salts [11] 2% (v/v) e glucose 6% (w/v). Cultures were maintained in a rotation shaker at 150 rpm, $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ during 5–6 days, and interrupted by centrifugation (4°C) at 7077 g during 15 min. Supernatants were filtered with gauze (cotton tissue) to separate them from biomass and received previously cooled ethanol 95% (v/v) in the proportion 3:1. The mixture was vigorously stirred and left overnight to precipitate at 4°C . Resulting mixture was centrifuged at 7077 g during 15 min and the supernatant was then dispensed with so that EPS could be obtained. Biomass and EPS produced by the

fungus *Colletotrichum* sp. were dried in a buffer at 60°C , ground and sieved. Adsorbent particles showed a granulometry equal to or less than 500 μm .

2.2. Reagents and apparatuses

Solutions of metallic ions, lead and cadmium, obtained by the dissolution of lead nitrate [$\text{Pb}(\text{NO}_3)_2$] and cadmium chlorate ($\text{CdCl}_2 \cdot 2.5 \text{H}_2\text{O}$) in distilled water were evaluated. Moreover, pH was calculated by a Digimed DM 20 pH meter duly calibrated and adjusted with NaOH and HCl solutions at 1M. Quantification of adsorbed metal in the solution was performed by Flame Atomic Absorption Spectrometry with acetylene gas, GBC spectrophotometer, and model 932 and executed at the Biomass Laboratory, FCAV, UNESP, Brazil [11].

2.3. Procedures for sorption assays

Sorption tests were performed in 250 mL flasks with 50 mL of lead (II) or cadmium (II) solution where the effect of pH (varying between 3 and 12), temperature (ranging between 30 and 60°C), and initial concentrations of metals (between 25 and 150mg L^{-1}) and of adsorbents (between 0.5 and 2.5g L^{-1} in medium with initial concentration 100mg L^{-1}) in sorption was evaluated. Percentage of adsorbed metallic ions was calculated according to formula below:

$$\text{Sorption \%} = (C_i - C_f)/C_i \quad (1)$$

where C_i and C_f correspond to the initial and final concentrations of metals in the solutions, respectively. One gram of EPS or biomass in 50 mL of cadmium or lead solution (100mg L^{-1}) in a time interval of sorption of 48 h was employed in the kinetic and equilibrium studies. All sorption assays were performed in a rotation shaker at 200 rpm, in replicate [9,12–17]. In all experiments (except in the study of the effect of initial concentration of adsorbents in the adsorption), we used concentrations 1g L^{-1} adsorbent.

2.4. Regeneration studies

Regeneration studies were done with three different concentrations of NaOH or H_2SO_4 (0.1, 0.01, 0.001mol L^{-1}).

2.5. SEM and EDX studies

The surface structure of adsorbent was analyzed by scanning electron microscopy (SEM) coupled with

energy dispersive X-ray analysis (EDX) using Quanta 600FEG/FEI. Unloaded and metal-laden adsorbents were mounted on a platinum stub coated with a thin layer of gold under vacuum to increase the electron conduction and to improve the quality of the micrographs [16,17]. The particle size of the samples was determined with SOLOTEST Electromechanical Sieve Shaker and was used sieves with mesh aperture 300, 150, 75, and 20 μm .

2.6. Data analysis

The results of sorption studies were compared by one-way variance analysis (ANOVA) and the analysis was done using the program Excel for Windows 2003, with level of significance maintained at 95% for all tests.

2.7. Equilibrium adsorption isotherms

Adsorption equilibrium data were calculated by Langmuir and Freundlich isotherms. Adsorption in Langmuir's model occurs on a homogeneous surface and not beyond a monolayer. Besides, all adsorption sites are equivalent and the adsorption capacity of a molecule is independent of the neighboring sites. In other words, it is supposed that no interaction occurs between adsorbed molecules [4,10–12]. Langmuir's isotherm is expressed linearly, according to Eq. (2) [12]:

$$Q_e/C_e = 1/(k_1 b) + C_e/k_1 \quad (2)$$

where C_e is the concentration of metal in equilibrium in the solution (mg L^{-1}); Q_e is the quantity of metal adsorbed in the equilibrium (mg g^{-1}); k_1 is Langmuir's constant (mg g^{-1}) (it represents adsorption capacity in the monolayer); and b is a constant which refers to the adsorption energy (L mg^{-1}). Freundlich's model supposes an almost infinite number of adsorption sites with regard to the number of molecules in the solute and is based on the concept that ions are infinitely accumulated on the adsorbent's surface [12]. The widely used Freundlich's equation has been applied for isothermal adsorption, describes energies of heterogeneous surfaces in adsorption multilayers and is expressed linearly according to equation below:

$$\log Q_e = \log k_f + 1/n \log C_e \quad (3)$$

where Q_e is the amount of metal adsorbed in the equilibrium (mg g^{-1}); C_e is the concentration of metal in equilibrium in the solution (mg L^{-1}); k_f ($\text{mg g}^{-1 (1/n)}$)

$1^{1/n} \text{g}^{-1}$) is the adsorbent's adsorption capacity; and $1/n$ represents surface heterogeneity [13].

2.8. Thermodynamics of sorption

The thermodynamics parameters, namely, variation in Gibbs's free energy (ΔG°), enthalpy (ΔH°) and entropy (ΔS°), were determined by the following equations:

$$\Delta G^\circ = -RT \ln K_c \quad (4)$$

$$K_c = Q_e/C_e \quad (5)$$

$$\ln K_c = (\Delta^\circ)/R - (\Delta H^\circ)/RT \quad (6)$$

where R is the universal constant for gases ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$); K_c is the equilibrium constant; T is the temperature (Kelvin); Q_e is the metallic ion concentration on the adsorbent (mg g^{-1}); and C_e is the concentration of metallic ion in the solution in equilibrium (mg L^{-1}). According to Eq. (8), ΔH° and ΔS° rates may be calculated as from the declivity and the interception of the straight line produced in graph K_c as a function of $1/T$ (Kelvin), respectively [14–17].

3. Results and discussion

3.1. Kinetic analysis and adsorption equilibrium

Fig. 1(A) shows percentages of metals adsorbed by EPS and biomass of the fungus *Colletotrichum* sp. during 48 h. Cadmium adsorption equilibrium by the fungus's biomass occurred after 2 h, with the removal of 85% of the cadmium. Initial and final pH varied between 6.8 ± 0.2 (0 h) and 6.7 ± 0.1 (48 h) (data not shown). Lead adsorption equilibrium by the biomass of the fungus *Colletotrichum* sp. occurred after 16 h where 84% of initial lead amount were removed. Initial and final pH did not vary and remained at 6.8 for the 0–48 h-period. Lead adsorption equilibrium by EPS of the fungus occurred after 20 h and 98% of initial concentration was removed. No variation occurred between initial and final pH, which remained at 6.7 for the 0–48 h-period (data not shown). Cadmium adsorption equilibrium by EPS of fungus occurred after 24 h with the removal of 79% of the metal's initial amount. No variation occurred between initial and final pH, which remained at 6.8 for the 0–48 h-period (data not shown). A 48-h period was employed for subsequent assays.

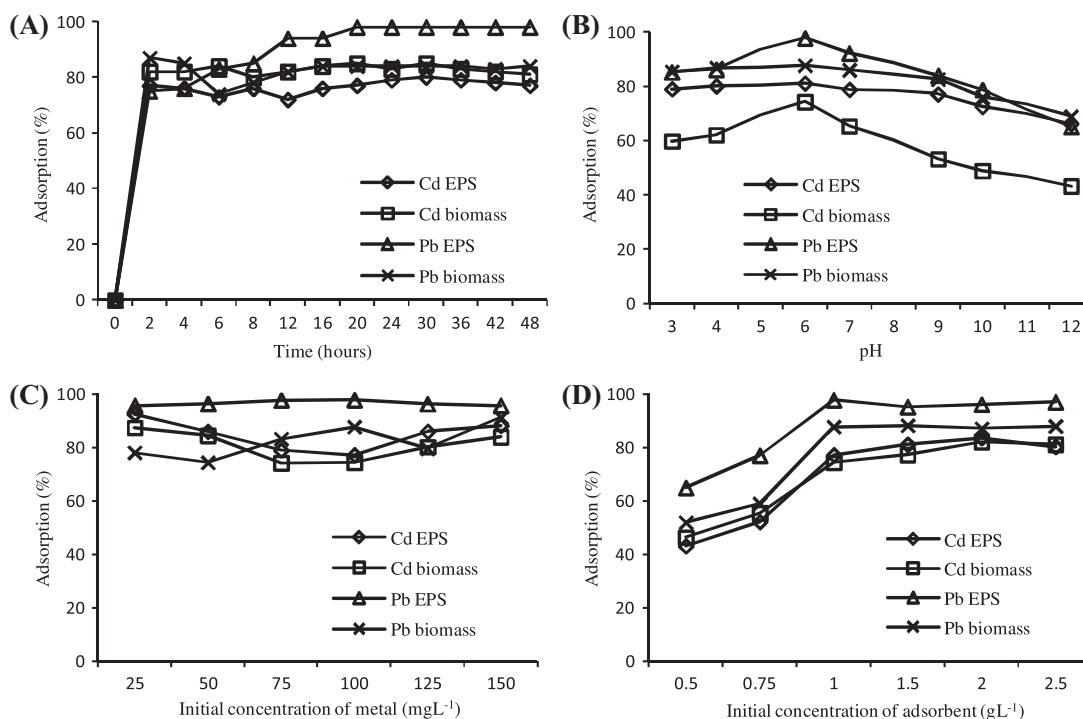


Fig. 1. Percentages of adsorbed metals by EPS and biomass of the fungus *Colletotrichum* sp. during 48 h stirred at 200 rpm, and incubated at 30°C (A), at different pH rates during (B), with different initial concentrations of metals and pH 6 (C), and at different initial concentrations of adsorbents during and pH 6 (D).

3.2. pH effect

Fig. 1(B) shows percentages of adsorbed metals at different pH rates in four adsorption assays (Cd—EPS; Cd—biomass; Pb—EPS; Pb—biomass). There were no statistically significant differences in adsorption Cd—EPS and Pb—Biomass ($p < 0.01$ and 95% confidence interval). However, we observed a behavior similar in the adsorption of Cd—biomass and Pb—EPS, with highest efficiency rates in metal removal occurring in pH 6 medium. In these cases, there was a difference in amount of metal ion adsorbed in the pH 6 and 12 ($p < 0.01$ and 95% confidence interval). Least removal rates occurred in pH 3 and 12 media. Xiao and cols. [18,19] obtained similar results when they analyzed cadmium adsorption by biomass of the endophytic fungus *Microsphaeropsis* sp. LSE10. When initial pH rate increased from 3.5 to 5.5, the above-mentioned authors verified an adsorption increase from 8.6 to 53.3 mg g⁻¹. Increase in adsorption capacity within the above pH range has also been reported with the biomass of the fungi *Pleurotus platypus*, *Agaricus bisporus*, and *Calocybe indica* [20]. Sorption decrease in low pH media is related to competition of hydrogen ions (H⁺) by linking sites in adsorbents. Adsorbents' adsorption capacity depends on available linking sites with a negative charge which are provided by functional groups at the

surface of the adsorbents [21]. High rates of adsorbed metals in pH 6 may be consequently related to a greater availability of linking sites which may not be available in acid pH owing to the concurrence between Cd (II) and Pb²⁺ ions and H⁺. Decrease of the solution's acidity produces the deprotonation of functional acid groups, such as carboxyl, phosphate, and phosphodiester. Deprotonation provides an increase in the attraction between negative charges in the adsorbent and the positively charged metallic ions [18]. On the other hand, pH increase toward base rates may cause the formation of soluble hydroxyl complexes of metallic ions with a decrease of concurrence in active sites of the adsorbent's functional groups and a reduction in sorption [20]. In the case of lead and cadmium ion solutions, EPS was the most efficacious adsorbent in the removal of metals with initial pH 6 medium. The highest efficiency, or rather, 97.81% (lead removal by EPS—data not shown), was reached at the above pH rate. Removal of higher than 80% cadmium and lead, respectively, by EPS and by biomass also occurred in pH 6 medium.

3.3. Effect of variation in initial lead and cadmium concentration in adsorption

Fig. 1(C) shows percentages of metals adsorbed by EPS and biomass of the fungus *Colletotrichum* sp. with

different initial concentrations of metals. Amounts of metal adsorbed in the assay, comprising lead adsorption by EPS, were similar to the initial concentrations of the metal under analysis. Similar behavior was observed in the adsorption of cadmium by biomass and EPS, with higher adsorption of metal ions in the initial concentrations of 25 and 150 mg L⁻¹. However, there was no statistically significant difference in adsorption by biomass or EPS in the initial concentrations of lead or cadmium ($p < 0.01$ and 95% confidence interval). Most metal amounts were adsorbed in medium with initial concentration 100 mg L⁻¹ in lead adsorption by EPS (97.81 mg L⁻¹—data not shown), although not statistically differ from other results, the initial concentration of metal 100 mg L⁻¹ was the reference value chosen in the following step (effect of biomass and EPS's initial concentration).

3.4. Effect of biomass and EPS's initial concentration

Fig. 1(D) shows percentages of adsorbed metals at different initial adsorbent concentrations and at a 100 mg L⁻¹ initial concentration of metal ion. Lead percentages adsorbed by EPS and by biomass remained constant in adsorbent concentrations higher than 1 g L⁻¹. The above proved to be the lowest concentration for the removal of 100 mg L⁻¹ lead initial concentration. Such adsorbent amount provided functional groups with enough active sites for the removal of lead ions [21]. An increase in the initial adsorbent concentration at rates higher than 1 g L⁻¹ did not cause any alterations in the concentrations of removed lead. Further, 2 g L⁻¹ was the minimum concentration of adsorbents EPS and biomass so that the highest amount of cadmium could be removed. However, a high amount of cadmium was removed at 1 g L⁻¹ concentration.

3.5. Isotherms of adsorption equilibrium

Adsorption isotherm was analyzed by placing Langmuir and Freundlich's equations in a linear form. The equation that best adjusted adsorption data was chosen to determine the adsorption parameters. Table 1 shows co-relation coefficients of isotherm models and adsorption constants.

As from the coefficient of linear co-relationship (R^2), the best adjustment of experimental data was obtained by Freundlich's isotherm in the adsorption assays by EPS. Freundlich's adsorption isotherm indicates heterogeneity of the adsorbent's surface and active sites with different energies [12]. Biomass's adsorption capacity (K_f) in adsorption multilayer reached 4.44 and the adsorption constant rate ($1/n$) was 0.10. Further, $1/n$ is an empirical parameter

related to adsorption intensity which varies according to heterogeneity of materials. Rates $0.1 < 1/n < 1.0$ show that cadmium adsorption by the biomass of the fungus *Colletotrichum* sp. is adequate within the assay conditions [22]. The magnitudes of K_f and n (Freundlich constants) show easy adsorption of metal ions from aqueous medium and indicate favorable adsorption [17]. In spite of low rates in R^2 , Freundlich's adsorption isotherm had the best adjustment for lead and cadmium sorption by EPS, which were 0.996 and 0.990, respectively. Adsorption capacity and adsorption constant in lead adsorption assay by EPS were 1.05 and 0.11, respectively. Adsorption capacity and adsorption constant in the cadmium adsorption assay by EPS were, respectively, 2.93 and 0.21. K_f values obtained in this study were relatively high. Akar and Tunali K_f values were 0.81 and 2.59 in the biosorption of Cu(II) and Cd(II), respectively, the biomass of the fungus *Botrytis cinerea* [17]. Langmuir's isotherm had the highest co-relation coefficient for cadmium (0.994) and lead (0.992) sorption by biomass. Langmuir's isotherm shows the homogeneity on the adsorbent's surface [12]. Adsorption capacity (k_1) of set Cd-Biomass reached 35.08 mg g⁻¹ and the affinity capacity was 0.19 L mg⁻¹. Adsorption capacity (k_1) of set Pb-Biomass reached 22.72 mg g⁻¹ and the affinity capacity was 1.76 L mg⁻¹. K_f and b the Langmuir constants related to adsorption capacity and affinity, respectively [17]. Chen et al. obtained k_1 and b values 5.58 mg g⁻¹ and 0.206 L mg⁻¹, respectively, in the biosorption of Cu(II) by byproduct of *Lentinus edodes* [18]. Akar and Tunali obtained k_1 and b values 10.64 mg g⁻¹ and 0.0278 L mg⁻¹, respectively, in the biosorption of Cu(II) by biomass of the fungus *Botrytis cinerea* [17]. Current analysis reported differences in metal links in each adsorbent which may be due to the individual properties of each adsorbent such as structure, functional groups, and surface area [23,12]. Table 2 lists thermodynamic parameters calculated for temperature. Negative rates of ΔH° confirm that sorption processes were exothermal. Further, the processes' free energy decreased in proportion to temperature increase. This fact shows the processes' spontaneity and agrees with results obtained by other authors [12,15]. ΔG° rates indicate that processes under analysis consists of physical adsorption, since chemical adsorption is generally characterized by a variation of ΔG° between 80 and 400 kJ mol⁻¹ in negative (exothermal) rates [12,24].

3.6. Regeneration studies

In order to make the sorption process more economical through repeated use of the adsorbent,

Table 1

Comparison of Langmuir and Freundlich's adsorption constants and co-relation coefficients obtained from adsorption isotherms of cadmium (II) and lead (II) by EPS and biomass of the fungus *Colletotrichum* sp. at 30°C

	Langmuir		Freundlich			
	k_1 (mg g ⁻¹)	b (L mg ⁻¹)	R^2	K_f	$1/n$	R^2
Cd-EPS	14.28	0.92	0.976	2.93	0.21	0.990
Cd-B	35.08	0.19	0.994	1.45	0.29	0.900
Pb-EPS	30.30	2.75	0.926	1.05	0.11	0.996
Pb-B	22.72	1.76	0.992	4.44	0.10	0.938

Note: B = biomass; R^2 = correlation coefficient; k_1 = Langmuir adsorption constant (adsorption capacity of the material); b = Langmuir adsorption constant (adsorption energy); K_f = Freundlich adsorption constant (adsorption capacity of the material); n = Freundlich adsorption constant (efficiency of adsorption).

Table 2

Thermodynamic parameters of the adsorbent's initial concentration at 100 mg L⁻¹

Initial metal concentration (mg L ⁻¹)	ΔG° (kJ mol ⁻¹)			ΔH° (J mol ⁻¹)	ΔS° (J mol ⁻¹ K ⁻¹)
	298.15*	313.15	328.15		
EPS-Cd	-3.72	-3.20	-2.95	-12.22	-45.64
Biomass-Cd	-4.89	-4.05	-3.22	-6.82	-20.12
EPS-Pb	-9.93	-8.38	-6.99	-5.28	-21.20
Biomass-Pb	-4.44	-3.85	-3.17	-3.20	-20.45

Note: ΔG° = change in free energy; ΔH° = change in enthalpy; ΔS° = change in entropy; *temperature in degrees Kelvin.

Table 3

Cycles of cadmium and lead adsorption-desorption in biomass and EPS produced by *Colletotrichum* sp.

Cycles	Adsorption (%)				Desorption (%)			
	Cd-EPS	Cd-B	Pb-EPS	Pb-B	Cd-EPS	Cd-B	Pb-EPS	Pb-B
1	79.0	85.0	98.0	84.0	98.1	98.3	97.1	97.8
2	75.9	83.2	96.1	80.3	94.0	95.0	94.1	94.1
3	72.5	79.0	91.6	75.7	90.2	90.8	90.5	89.5
4	66.8	75.4	87.0	71.6	86.4	86.2	84.5	85.3

Note: B = biomass.

desorption and regeneration potential of EPS and biomass of the fungus *Colletotrichum* sp. were investigated. The regeneration of the biosorbent is one of the key factors for the assessment of its potential for commercial applications [15]. We used the sodium hydroxide and sulfuric acid in the regeneration studies. The sulfuric acid 0.1 M was most efficient effluent used in regeneration studies. The major problem of regeneration process is the disposal of the acid and base solution obtained in large-scale processes [25]. The four adsorption-desorption cycles are shown in Table 3. The set Pb-EPS showed higher adsorption capacity from among the sets evaluated (98%). After four cycles, the set still retained the adsorption

capacity of 87%, higher than the sorption capacity of the first adsorption cycle of the other sets evaluated. All of the adsorbents showed desorption capacity of greater than 97% in the first cycle and desorption retained a capacity of more than 84% after the fourth cycle of desorption. All adsorbents showed up with great regenerative capacity and relative stability after these four cycles.

3.7. Granulometry, SEM, and EDX analysis

Confirmation of adsorption of the metal was done by energy dispersive X-ray analysis (EDX). Fig. 2 shows the EDX pattern for the EPS *Colletotrichum* sp.

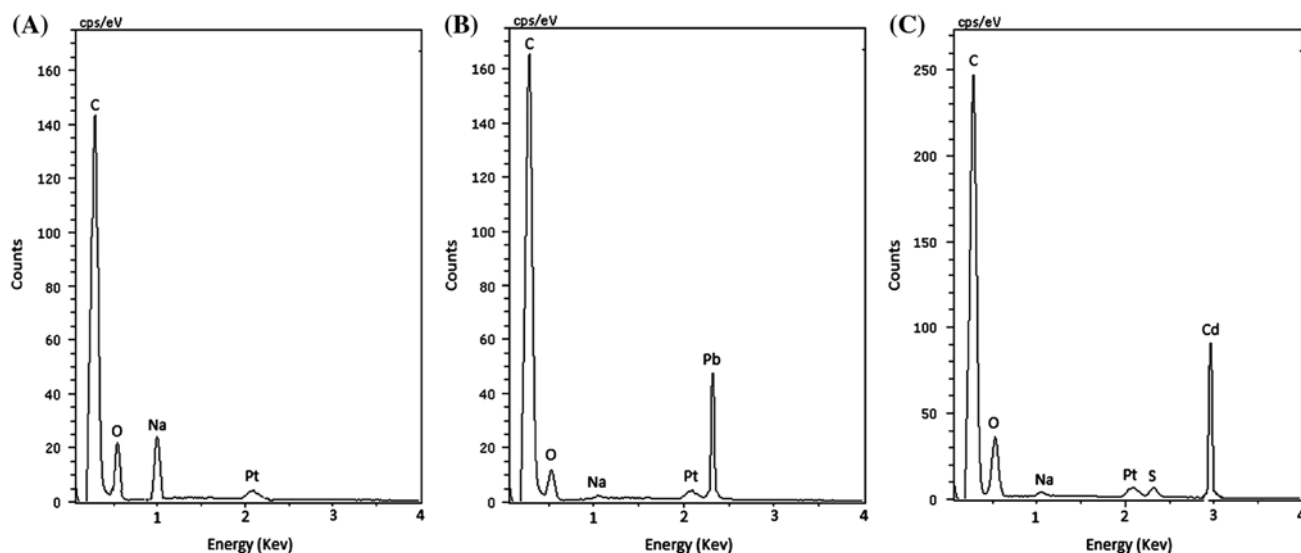


Fig. 2. X-ray energy dispersion analysis spectra of fungus's EPS *Colletotrichum* sp. (a) before metal loaded, (b) Pb(II) loaded, and (c) Cd(II) loaded.

Table 4
Granulometric fraction of adsorbent materials

Mesh opening sizes (μm)	Percentage (%)	
	EPS	Biomass
$S < 20$	22.04	6.35
$20 < S < 75$	17.13	12.88
$75 < S < 150$	15.19	11.22
$150 < S < 300$	11.21	28.34
$300 < S < 500$	34.43	41.23

Note: S = particle size.

before and after the adsorption of cadmium and lead. The EDX pattern (Fig. 2(A)) for the unloaded EPS did not show the characteristic signal of Pb (II) and Cd (II), whereas a clean signal of the presence of lead and cadmium was observed in Pb (II)-loaded and Cd (II)-loaded EPS (Fig. 2(B) and (C)), respectively. Furthermore, the presence of Na^+ has been shown to be

involved in the ion exchange with Pb (II) and Cd (II). The adsorption of lead by the EPS was apparently also favored by the presence of large smaller fragments (Table 4). These fragments may have provided greater contact surface area [26] and also more adsorption sites and favored metals adsorption. These higher adsorption regions observed in EPS present globular format (Fig. 3(B)) and present composition, basically carbon, oxygen, and lead (II) (Fig. 2(B)). These globular particles were also observed on biomass of *Botrytis cinerea* and were called "billiard balls" [16]. Approximately, 20% of the EPS-adsorbent presents particle size less than $20\ \mu\text{m}$, which favored the formation of these globular regions. These findings on EDX analysis indicate the involvement of ion exchange mechanism for the removal of metal ions by EPS produced by *Colletotrichum* sp, similar to that reported in other studies [15,27] (Fig. 4).

Fig. 5 shows the EDX pattern for the EPS *Colletotrichum* sp. before and after the adsorption of

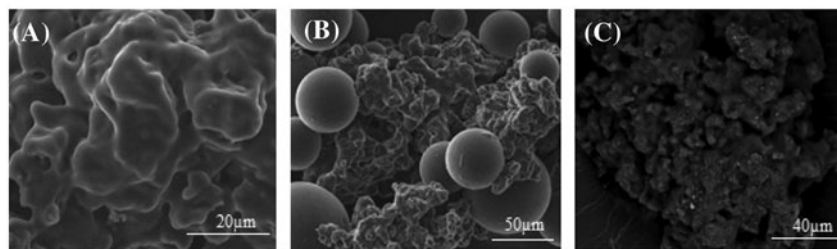


Fig. 3. SEM micrograph of fungus's EPS *Colletotrichum* sp. (a) before metal loaded, (b) Pb(II) loaded, and (c) Cd(II) loaded.

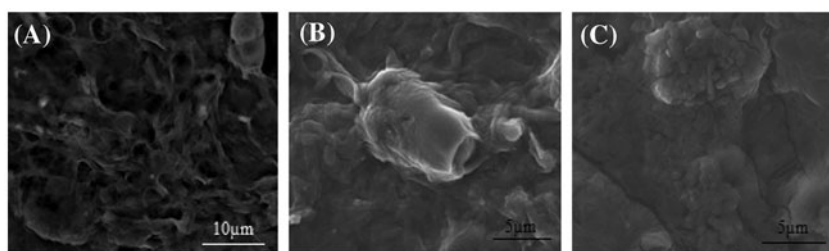


Fig. 4. SEM micrograph of fungus's biomass *Colletotrichum* sp. (A) before metal loaded, (B) Pb(II) loaded, and (C) Cd(II) loaded.

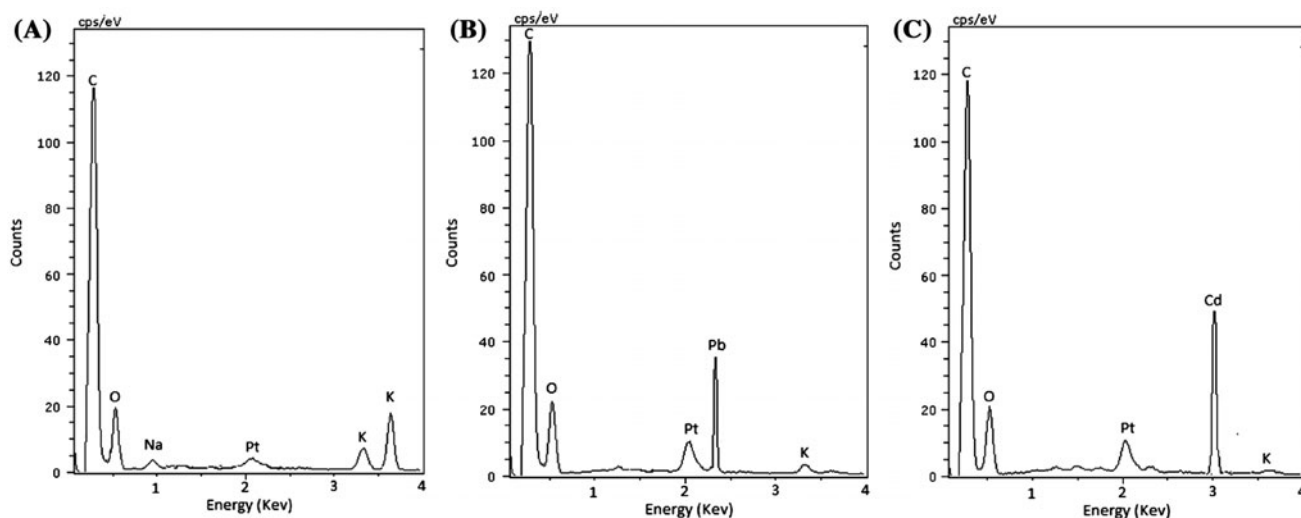


Fig. 5. X-ray energy dispersion analysis spectra of fungus's biomass *Colletotrichum* sp. (A) before metal loaded, (B) Pb(II) loaded, and (C) Cd(II) loaded.

cadmium and lead. Similarly to the EPS, the EDX pattern (Fig. 5(A)) for the unloaded biomass did not show the characteristic signal of Pb (II) and Cd (II), whereas a clean signal of the presence of lead and cadmium was observed in Pb (II)-loaded and Cd (II)-loaded biomass (Fig. 5(B) and (C)), respectively. In EDX analysis, it was observed that the peaks at 1 keV and between 3 and 4 keV corresponding to Na^+ and K^+ (Fig. 5(A)), respectively, disappeared/reduced after Cd(II) and Pb(II) sorption (Fig. 5(B) and (C)). Concomitant with metal sorption,

K^+ , Ca^{2+} , and Mg^{2+} release from biomass is frequently observed [27]. These findings also indicated that sorption process also included ion-exchange mechanism for the removal of both metal ions by these biological materials.

4. Conclusions

Significant removal of initial concentration of metals in solution has been obtained in kinetic studies.

This fact shows that biomass and EPS obtained from fungus *Colletotrichum* sp. are promising biological materials in Pb(II) and Cd(II) sorption. Langmuir and Freundlich models explained cadmium and lead sorption by biomass and EPS, respectively. In this study was observed forming of regions granular with high lead adsorption capacity that may have been motivated by the fragmentation of the EPS. This fragmentation may have provided greater contact surface and also more adsorption sites and favored metals adsorption. In addition, ion exchange mechanism may have acted on the metals adsorption by both biological materials where sodium may have been exchanged from EPS and sodium and potassium from biomass. All adsorbents showed up with great regenerative capacity and relative stability after these four cycles. Lead and cadmium sorption by EPS of the fungus *Colletotrichum* sp. should be evaluated in medium containing other substances (among which metals must be included) to verify its selective capacity. Reactors may optimize EPS and biomass production

to meet large-scale production demands and thus the commercial production of this biological material and its employment as adsorbent will be feasible.

References

- [1] A.S. Luna, A.L.H. Costa, A.C.A. Costa, C.A. Henriques, Competitive biosorption of cadmium(II) and zinc(II) ions from binary systems by *Sargassum filipendula*, *Bioresour. Technol.* 101 (2010) 5104–5111.
- [2] ATSDR, (Agency for Toxic Substances and Disease Registry). Draft Toxicological Profile for Cadmium. US Department of Health and Human Services, Public Health, Atlanta, GA, 2008.
- [3] ATSDR, AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY. Toxicological Profile for Lead. US Department of Health and Human Services, Public Health, Atlanta, GA, 2007.
- [4] B. Volesky, Sorption and Biosorption, BV Sorbex Inc., Montreal-St. Lambert, 2003.
- [5] N. Tewari, P. Vasudevan, B.K. Guha, Study on biosorption of Cr (VI) by *Mucor hiemalis*, *Biochem. Eng. J.* 23 (2005) 185–192.
- [6] G.M. Gadd, Interactions of fungi with toxic metals, *New Phytol.* 124 (1993) 25–60.
- [7] K. Vijayaraghavan, Y.S. Yun, Bacterial adsorbents and biosorption, *Biotechnol. Adv.* 26 (2008) 266–291.
- [8] M.P. Acosta, E. Valdman, S.G.F. Leite, F. Battaglini, S.M. Ruzal, Biosorption of copper by *Paenibacillus polymyxa* cells and their exopolysaccharide, *World J. Microbiol. Biotechnol.* 21 (2005) 1157–1163.
- [9] J. Ferreira, F.L.H. Silva, O.L.S. Alsina, L.S.C. Oliveira, E.B. Cavalcanti, W.C. Gomes, Equilibrium and kinetic study of Pb²⁺ biosorption by *Saccharomyces cerevisiae*, *Quim. Nova* 30 (2007) 1188–1193.
- [10] H.J. Vogel, A convenient growth medium for *Neurospora crassa*, *Genet. Bull.* 13 (1956) 42–46.
- [11] APHA (American Public Health Association), *Methods for Examination of Water and Wasterwater*, 21st ed., Washington, DC, 2005.
- [12] N. Ertugay, Y.K. Bayhan, The removal of copper (II) ion by using mushroom biomass (*Agaricus bisporus*) and kinetic modeling, *Desalination* 255 (2010) 137–142.
- [13] S. Erentürk, E. Malkoc, Removal of lead (II) by adsorption onto *Viscum album* L.: Effect of temperature and equilibrium isotherm analyses, *Appl. Surf. Sci.* 253 (2007) 4727–4733.
- [14] A. Sari, M. Tuzen, Kinetic and equilibrium studies of biosorption of Pb(II) and Cd(II) from aqueous solution by macrofungus (*Amanita rubescens*) biomass, *J. Hazard. Mater.* 164 (2009) 1004–1011.
- [15] R.A. Anayurt, A. Sari, M. Tuzen, Equilibrium, thermodynamic and kinetic studies on biosorption of Pb (II) and Cd (II) from aqueous solution by macrofungus (*Lactarius scrobiculatus*) biomass, *Chem. Eng. J.* 151 (2009) 255–261.
- [16] T. Akar, S. Tunali, I. Kiran, *Botrytis cinerea* as a new fungal adsorbent for removal of Pb (II) from aqueous solutions, *Biochem. Eng. J.* 25 (2005) 227–235.
- [17] T. Akar, S. Tunali, Biosorption performance of *Botrytis cinerea* fungal by products for removal of Cd (II) and Cu (II) ions from aqueous solutions, *Min. Eng.* 18 (2005) 1099–1109.
- [18] G. Chen, G. Zeng, L. Tang, C. Du, X. Jiang, G. Huang, H. Liu, G. Shen, Cadmium removal from simulated wastewater to biomass byproduct of *Lentinus edodes*, *Bioresour. Technol.* 99 (2008) 7034–7040.
- [19] X. Xiao, S.G. Luo, G. Zeng, W. Wei, Y. Wan, L. Chen, H. Guo, Z. Cao, L. Yang, J. Chen, Q. XI, Biosorption of cadmium by endophytic fungus (EF) microsphaeropsis sp. LSE10 isolated from cadmium hyperaccumulator *Solanum nigrum* L., *Bioresour. Technol.* 101 (2010) 1668–1674.
- [20] R. Vimala, N. Nilanjana Das, Biosorption of cadmium (II) and lead (II) from aqueous solutions using mushrooms: A comparative study, *J. Hazard. Mater.* 168 (2009) 376–382.
- [21] A. Saeed, M. Iqbal, Bioremoval of cadmium from aqueous solution by black gram husk (*Cicer arietinum*), *Water Res.* 37 (2003) 3472–3480.
- [22] F. Zha, S. Li, Y. Chang, Preparation and adsorption property of chitosan beads bearing b-cyclodextrin cross-linked by 1, 6-hexamethylene diisocyanate, *Carbohydr. Polym.* 72 (2008) 456–461.
- [23] A. Özer, D. Özer, A. Özer, The adsorption of copper (II) ions on to dehydrated wheat bran (DWB): determination of the equilibrium and thermodynamic parameters, *Process Biochem.* 39 (2004) 2183–2191.
- [24] Z. Aksu, F. Gonen, Z. Demircan, Biosorption of chromium (VI) ions by Mowital®B30H resin immobilized activated sludge in a packed bed: Comparison with granular activated carbon, *Process Biochem.* 38 (2002) 175–186.
- [25] S. Gupta, B.V. Babu, Experimental, kinetic, equilibrium and regeneration studies for adsorption of Cr(VI) from aqueous solutions using low cost adsorbent (activated flyash) *Desalin. Water Treat.* 20 (2010) 168–178.
- [26] K. Kelly-Vargas, M. Cerro-Lopez, S. Reyna-Tellez, E.R. Bandala, J.L. Sanchez-Salas, Biosorption of heavy metals in polluted water, using different waste fruit cortex, *Phys. Chem. Earth, Parts A/B/C* 37 (2012) 26–29.
- [27] D.H. Cho, E.Y. Kim, Characterization of Pb²⁺ biosorption from aqueous solution by *Rhodotorula glutinis*, *Bioprocess. Biosyst. Eng.* 25 (2003) 271–277.