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Removal of Cd(II), Cr(VI), Fe(III) and Ni(II) from aqueous solutions by an *E. coli* biofilm supported on kaolin

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ABSTRACT

The pollution caused by heavy metals is one of the major environmental problems that is imperative to be solved. New technologies, easy to implement and to adapt to any system, deserve special attention and are a focus of this work. The ability of a biofilm of *Escherichia coli* supported on kaolin to remove Cr(VI), Cd(II), Fe(III) and Ni(II) from aqueous solutions was investigated in batch assays for the treatment of diluted aqueous solutions. The biosorption performance, in terms of uptake, followed the sequence: Fe(III) > Cd(II) > Ni(II) > Cr(VI). The equilibrium data in batch systems were described by Langmuir, Sips and Redlich–Peterson model isotherms. The best fit for chromium and nickel was obtained with the Redlich–Peterson model and for cadmium the best fit was obtained with the Sips model. The presence of functional groups on the cell wall surface of the biomass that may interact with the metal ion was confirmed by FTIR. Kaolin samples have been fully characterized by chemical analyses. The results obtained are very promising for the removal of metal ions from effluents.

1. Introduction

Heavy metals pollution represents a serious problem for human health and for life in general. The disposal of heavy metals is a consequence of several activities like chemical manufacturing, painting and coating, mining, extractive metallurgy, nuclear and other industries. Those metals exert a deleterious effect on fauna and flora of lakes and streams [1].

The removal of toxic metals is often performed by oxidation–reduction processes, filtration, electrochemical treatment, evaporation, ion exchange or reverse osmosis [2]. But these methods present several disadvantages like high energy and chemical requirements, low efficiency and usually produce large amounts of sludge.

In this context, the search for new technologies is strongly recommended. Biosorption is a process that involves the use of biological materials that form complexes with metal ions using their functional groups [3]. The mechanism of biosorption usually involves the chemical link between functional groups on the biosorbent and the metal ions or an ion-exchange reaction due to the high ion exchange capacity of the biosorbent [4]. One of the major advantages of a biosorption system is its economical nature. Moreover, it presents an ecofriendly behaviour, the regeneration of biosorbents for multiple uses is easy, it shows selectivity towards different metals and it presents high efficiency [5]. Bacteria have a high surface area-to-volume ratio that can provide a large contact interface, and this allows the interaction with metals from the surrounding environment [6]. On the other hand, the use of natural adsorbents like clays and zeolites is also very promising due to their availability, low cost, simplicity of extraction and retention capacity [7]. The large surface area of natural clays, helped by edges and faces of clay particles, accounts for the excellent capacity of the clay minerals to adsorb heavy metals [8]. A system that uses clays covered with a microorganism allows to combine the features of both for an even more efficient heavy metals removal.

Kaolin is a 1:1 alumina silicate structure comprising a tetrahedral silica sheet bonded to an octahedral alumina sheet through the sharing of oxygen atoms between silicon and aluminum in adjacent sheets [9], with the ideal formula of $Al_2Si_2O_5(OH)_4$ [10]. This clay is found as a common constituent of soils and sediments and the exchange sites are located on the surface of kaolin with no interlayer exchange sites. Accordingly with Sari et al. [10], the sorption properties of this clay are determined by the nature of its surface and edges. Kaolin clay possesses a variable charge that can be related to the reactions between ionisable surface groups located at the edges and the ions present in aqueous solution. Miranda-Trevino and Coles [11] affirmed that the use of kaolin reveals great advantages in many processes due to its high chemical stability and low expansion coefficient.

Bacteria are known to produce macromolecules usually called extracellular polymeric substances (EPSs). EPSs are metabolic products of bacteria and may derive from their lysis or hydrolysis. They are also associated with the organic matter present in the effluent to be treated [12]. EPSs are mainly composed by polysaccharides,

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Table 1
Chemical composition of kaolin by XRF.

Constituent	%
SiO_2	48.1
Al_2O_3	35.5
Fe_2O_3	1.70
CaO	0.13
MgO	0.30
Na_2O	0.20
K_2O	2.00
TiO_2	0.20

proteins, humic substances and uronic acid [13,14] which contain several functional groups like carboxyl, phosphoric, amine and hydroxyl groups. EPSs have various functions, the more important is the induction of cell aggregation [15], producing a protective barrier for cells against harmful products and allowing the sorption of inorganic ions from the environment [16].

The use of kaolin for the treatment of heavy metals contaminated aqueous solutions was studied by several authors. Covelo et al. [17] studied the sorption of Cd, Cr, Cu, Ni, Pb and Zn while Asçi et al. [18] studied the sorption of Cd(II) onto kaolin. Ohnuki et al. [19] investigated the interactions between Pu(VI) and *Bacillus subtilis*, kaolin clay and a mixture of the two. The use of biofilms to remove heavy metals was well studied using bacterial biofilms supported on GAC [20–25] and on NaY [26].

This work aims to investigate the biosorption behaviour of a biofilm of *Escherichia coli* supported on kaolin clay for the treatment of cadmium, iron, nickel and chromium aqueous solutions. The effect of initial heavy metal concentrations was studied and the relationship between pH and removal efficiency was analysed. For comparison purposes, the same study was carried out without the bacterial biofilm. Experimental results were analysed using the Langmuir, Redlich–Peterson and Sips adsorption isotherms. The presence of functional groups in the suspended biomass that may have a role in the biosorption process was confirmed by FTIR. The kaolin samples were characterized by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).

2. Materials and methods

2.1. Materials

The bacterium *E. coli* was obtained from the Spanish Type Culture Collection of the University of Valência. Stock heavy metals solutions with the appropriate concentrations (10, 25, 50, 70, 80, and 100 mg/L) were prepared by dissolving $K_2Cr_2O_7$, $FeCl_3{\cdot}(6H_2O)$, $CdSO_4{\cdot}(8/3H_2O)$ and $NiCl_2{\cdot}(6H_2O)$, in distilled water. Atomic absorption spectrophotometric standards were prepared from 1000 mg/L metal solutions.

The kaolin was obtained from Minas de Barqueiros, S.A. (Apúlia, Portugal). The chemical compositions of kaolin as analysed by X-ray fluorescence (XRF) is given in Table 1. The analysis of this material revealed that it contains mainly silica and alumina, which account for 86.6% of the total weight.

Glassware used for experimental purposes was washed in 10% nitric acid and rinsed with deionised water to remove any possible interference by other metals.

2.2. Methods

2.2.1. Preparation of the biofilm supported on kaolin

A medium with 5 g/L of beef extract, 10 g/L of peptone and 5 g/L of NaCl, pH 7.2, was used for the microorganism growth. The medium was sterilized at 121 °C for 20 min, cooled to room temperature, inoculated with bacteria and kept at 37 °C for 24 h with moderate stirring in a incubator.

2.2.2. Biosorption assays

Batch experiments were conducted using 1 g of the kaolin clay with 15 mL of *E. coli* culture media and 150 mL of the different heavy metal solutions (10, 25, 50, 70, 80, and 100 mg/L) in 250-mL Erlenmeyer flasks. Experiments were repeated without the bacterium for comparison purposes. The whole experimental work was conducted in duplicate. The pH of the initial solutions was measured (pH meter ORION 720 A). The Erlenmeyer flasks were kept at 37 °C, with moderate stirring for about 10 days. Samples of 1 mL were taken, centrifuged and analyzed for metals using atomic absorption spectrophotometry. Three isotherm equations have been tested in the present study and are presented in Table 2.

2.2.3. Characterization procedures

Total metal ions concentrations during the experiments were measured using a Varian Spectra AA-400, an Atomic Absorption Spectrophotometer (AAS). Infrared spectra of the unloaded biomass and the metal loaded biomass, both in suspension, were obtained using a Fourier transform infrared spectrometer (FTIR BOMEM MB 104). For the FTIR study, biomass was centrifuged and dried, followed by weighting. Then, 10 mg of finely ground biomass was encapsulated in 100 mg of KBr in order to prepare translucent sample disks. Elemental chemical analyses (Si, Al, Na, Cr, Cd, Fe and Ni) were performed by ICP-AES using a Philips ICP PU 7000 Spectrometer.

2.2.4. Effect of pH on metal ions biosorption

Several factors influence a biosorption process and the solution pH is one of the most important. This is so, partly because hydrogen ions themselves are strongly competing with metal ions and because pH determines the valence state of the ions and their precipitation process. Comte et al. [12] affirm that the deprotonated form of the reactive sites, mainly carboxylic, phosphoric and amino groups, is primarily responsible for the binding of metal ions to EPS. The solution pH affects the ionization state of these functional groups.

It is well known that the hydrolysis of Fe(III) occurs at pH values higher than 3. However, the adsorption of Fe(III) increases

Table 2
Isotherm models used to represent the equilibrium of biosorption.

Isotherm model	Equation	Nomenclature	Reference
Langmuir	$Q_e = (Q_{max}\, b\, C_e)/(1 + b\, C_e)$	Q_e (mg/g) is the amount of metal ion sorbed by the biofilm at the equilibrium, Q_{max} (mg/g) is the maximum metal sorption, C_e (mg/L) is the concentration of metal in solution at the equilibrium, b (l/mg) is the Langmuir adsorption equilibrium constant	Langmuir [27]
Sips	$Q_e = (K_S\, C_e^{1/b_S})/(1 + a_S\, C_e^{1/b_S})$	K_S ($l^{b_S}\ mg^{1-b_S}/g$), a_S $(l/mg)^{b_S}$ and b_S are the Sips isotherm parameters	Sips [28]
Redlich–Peterson	$Q_e = (K_R C_e)/(1 + a_R C_e^{\beta})$	K_R (l/g), a_R (l/mg) and β represents the Redlich–Peterson constants and are empirical parameters without physical meaning. β varies between 0 and 1	Redlich and Peterson [29]

until this value of pH is reached [30]. Usually, an increase in pH results in an increased in Cd(II) adsorption. Hetzer et al. [31] justified this fact with the deprotonation of cell wall functional groups that occurs with increasing pH, progressively resulting in increasing Cd(II) biosorption until saturation of binding sites is reached. However, several authors affirm that the optimal pH for the biosorption of cadmium is situated around 6 [18,32] as the formation of anionic hydroxide complexes and their competition with the active sites affects dramatically the Cd biosorption process at higher pH values [33]. The same authors affirm that at pH lower than 6 the poor biosorption could be due to competition with the H^+ ions for metal binding sites on the microbial cell.

Bhattacharyya and Sen Gupta [8] studied the adsorption of Ni(II) on kaolinite and montmorillonite and concluded that it was not possible to carry out adsorption experiments with pH higher than 8 due to precipitation of that ion as an hydroxide. Like for several other ions, an increase in pH had a positive impact on the adsorption of nickel. Sari et al. [34] reported a maximum biosorption for nickel at pH 5–6 with low Ni(II) adsorption at lower pH values. This was due to the effect of pH on metal binding sites of the biomass surface and on metal speciation in aqueous solutions. The increased number of protons on the sites of biomass surface restricted the approach of metal cations as a result of repulsive forces.

The biosorption of Cr(VI) is strongly affected by the pH of the aqueous solutions. For pH values higher than 6 the biosorption of Cr(VI) on *Sargassum* sp. become negligible [35]. Bhattacharyya and Sen Gupta [36] defend that the formation of hydroxyl complexes of chromium may be responsible for that decrease in adsorption because it hinders the diffusion of Cr(VI) ions into the clay surface. The same authors affirm that at low pH values, the biosorption decreases due to the competition with ions like NO_3^- introduced in the form of nitric acid to maintain the pH low. Contrarily, Murphy et al. [37] revealed that as solution pH decreases the biosorption increases because the amino and carboxyl groups may become protonated, thus making the biomass more positively charged and hence creating an electrostatic attraction with Cr(VI) species.

In this study, pH values between 4.6 and 5.1 (for chromium), 2.7 and 3.5 (for iron), 5.6 and 6.0 (for cadmium) and 5.7 and 6.2 (for nickel) were used for the reasons discussed above.

3. Results and discussion

3.1. Batch biosorption assays

Batch biosorption data showed differences on the biosorption performance between the four metals studied (Table 3). Kaolin surface in water had a net negative charge [9] and the bacteria have also a net negative charge [26]. These negative surfaces obviously favour the biosorption of cations. In addition to the valence state, the electronegativity [38], atomic weight and ionic radius are also characteristics that influence the biosorption efficiency [21].

In accordance to studies performed by Krishnani et al. [3] the carboxyl groups are the main groups responsible for the binding of metal ions. At low pH values these groups retain their protons hence reducing the possibility to bind any positively charged ions. On the other hand, at high pH values, the carboxyl groups are deprotonated and become negatively charged, which attract positively charged metal ions. In this way, the metal binding to the biomass is a mechanism involving electrostatic interaction between metal ions and the biomass. Recently Xu and Liu [39] studied the mechanism of biosorption of Cd(II) and Ni(II) and concluded that the main contribution for the process comes from an ion exchange process, followed by extracellular polymers binding and chemical precipitation. Baral et al. [40] investigated the biosorption of Cr(VI) by the weed *Salvinia cucullata* and found that the mechanism is purely adsorption with three stages: mass transfer (boundary layer/film diffusion), followed by intraparticle diffusion and, finally, sorption of ions.

Table 3
Uptake values (mg/g) and removal percentage values for Cr(VI), Cd(II), Fe(III) and Ni(II), obtained for different initial concentrations of metal (37 °C, 150 rpm), for the biofilm of *Escherichia coli* supported on kaolin.

C_0 (mg/L)	Uptake (mg/g)	R_p (%)
Cr(VI)		
8	1.2	100
30	1.1	23.6
62	2.1	22.9
85	2.6	20.2
97	3.4	23.2
116	4.6	26.2
Cd(II)		
13	1.5	78.9
25	2.7	69.6
50	5.8	77.8
70	7.7	73.0
78	7.8	67.1
97	10.3	71.3
Fe(III)		
7	1.0	100
22	3.4	100
53	7.9	98,7
78	11.7	100
87	13.0	100
110	16.5	100
Ni(II)		
12	1.4	74.2
29	3.1	71.0
56	4.2	49.9
76	4.6	40.6
86	5.1	39.4
101	6.9	45.3

It is observable in Table 3, that as the initial metal ion concentration increases, the metal uptake, defined as the mass of metal ion over the mass of sorbent, increased as well and this is true for all the metal ions tested and for the range of concentrations used. Hanif et al. [41] justified this behaviour with the fact that surface saturation was dependent on the initial metal ion concentration. At low concentrations, the available adsorption sites are more easily occupied and, at higher concentrations, the metal ions have to diffuse into the biomass surface by intraparticle diffusion and this process is slow. The sequence in terms of uptake values by the *E. coli* biofilm supported on kaolin was Fe > Cd > Ni > Cr, confirmed by ICP-AES. The preference of a sorbent for a metal may be explained on the basis of electronegativity of the metal ions (Fe, 1.96 Pauling; Ni, 1.8 Pauling; Cr, 1.6 Pauling [42] and Cd, 1.69 Pauling [43]), molecular weight (Fe, 55.8 u; Cd, 112.4 u; Ni, 58.7 u, and Cr, 51.99 u), ionic radius (Fe, 55 pm; Cd, 95 pm; Ni, 69 pm and Cr, 44 pm [44]) and with the cation/anion state. The lowest biosorption capacity for chromium is justified by its anionic state. Comparing the results obtained for the cationic metals, better retentions were obtained for iron and this can be justified with the higher electronegativity and smaller ionic radius which promote the penetration into the polymeric net. Nickel ion has high electronegativity, low atomic weight and lower ionic radius and these characteristics allowed good qualities for entrapment by a biofilm. However, better results were obtained for cadmium. A possible explanation for these unexpected results is a stronger xenobiotic effect caused by the nickel on the biofilm in comparison to cadmium. In a biofilm–kaolin system both materials contribute to the biosorption performance and the retention by the biofilm includes a metabolic path that is more sensitive to the toxicity of nickel than to the toxicity of cadmium.

Table 4
Chemical analysis of unloaded and metal loaded kaolin samples by ICP-AES.

	% Cr	% Fe	% Cd	% Ni	Si/Al
Kaolin	–	–	–	–	1.50
Kaolin + biofilm + Cr	0.05	–	–	–	1.52
Kaolin + biofilm + Fe	–	2.54	–	–	1.52
Kaolin + biofilm + Cd	–	–	0.72	–	1.58
Kaolin + biofilm + Ni	–	–	–	0.52	1.45

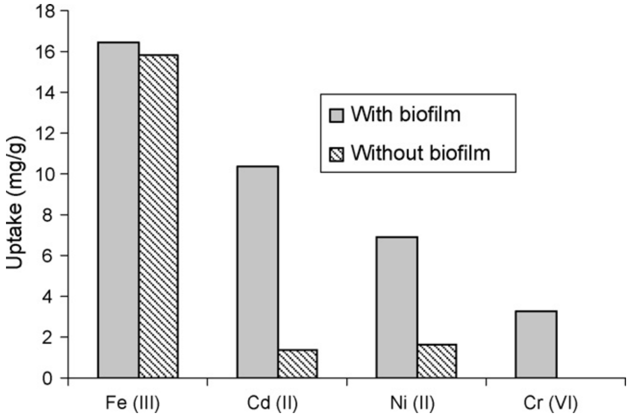

Fig. 1. Uptake values for four different metal ions by kaolin and by kaolin with biofilm for the highest concentration tested (ca. 100 mg/L).

In terms of removal percentage, the results showed around 100% for iron and for the range of concentrations tested. For cadmium the removal percentage remains around 70% for all the initial concentrations tested (between 67.1% and 78.9%) and for nickel the removal percentage is around 70% for the two lower concentrations (12 and 29 mg/L) and around 40% for higher concentrations (56, 76, 86 and 101 mg/L), confirming the xenobiotic effect caused by nickel ion. For chromium, the removal was 100% for the lower concentration tested (8 mg/L) and around 20% for the higher ones. The results were confirmed by chemical analyses (Table 4) where kaolin-supported biofilm showed highest iron concentration compared to cadmium, nickel and chromium.

The accumulation of each of the four metal ions by kaolin with and without the bacterium biofilm was compared and systematically showed better results when the supported biofilm was used, for the whole range of initial concentrations. Fig. 1 shows the metal uptake values with and without biofilm, for the highest concentrations tested. These results confirm the important role of the biofilm on the biosorption process.

3.2. *Modelling batch biosorption data*

Table 5
Adsorption isotherm constants for the best fit models, for Cr(VI), Cd(II) and Ni(II) onto a biofilm supported on kaolin.

Cr(VI)				
Best fit model	K_R	a_R	β	R^2
Redlich–Peterson	0.615	8.629	0.143	0.984
Cd (II)				
Best fit model	K_S	a_S	b_S	R^2
Sips	0.019	0.002	2.750	0.977
Ni (II)				
Best fit model	K_R	a_R	β	R^2
Redlich–Peterson	0.733	0.136	1	0.994

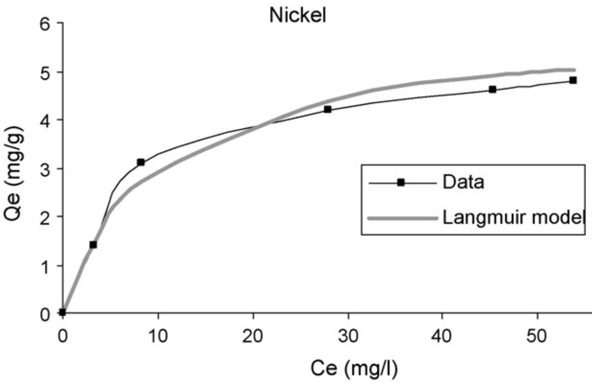

Fig. 3. Nickel sorption isotherm, adjusted by Langmuir model (... experimental data; — model).

Through the modelling of equilibrium data it is possible to characterize biosorbents under various operational conditions and this point is essential for future industrial applications. For the biosorbent used (biofilm + kaolin), sorption isotherms were experimentally determined. Three different models – Langmuir, Sips and Redlich–Peterson – were fitted and constants calculated for the best fit are presented in Table 5. The comparison between the experimental results and those predicted by the best fitted model is shown in Fig. 2. For the range of initial concentrations tested, iron was totally and instantaneously biosorbed so the concentration at equilibrium was always 0 and consequently it was not relevant to represent C_e (mg/L) vs. Q_e (mg/g). The best fit for chromium and nickel was obtained with the Redlich–Peterson model isotherm and for cadmium the best fit was the obtained with the Sips model. The Redlich–Peterson model incorporated the features of both Langmuir and Freundlich isotherms models but in a single equation. At low concentrations, the Redlich–Peterson isotherm approximates to Henry's law and at high concentrations its behaviour approaches that of the Freundlich isotherm. The exponent β lies between 0 and 1. For $\beta = 1$, the isotherm converts

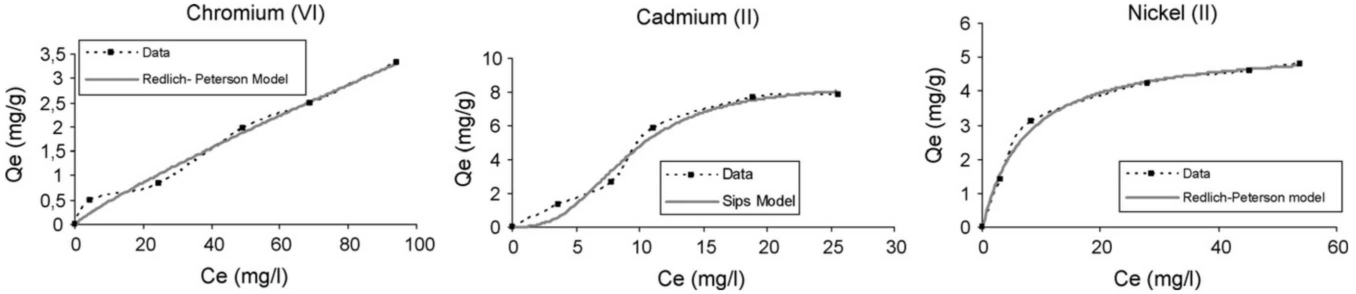

Fig. 2. Best fit sorption isotherms for Cr(VI), Cd(II) and Ni(II) (... experimental data; — model).

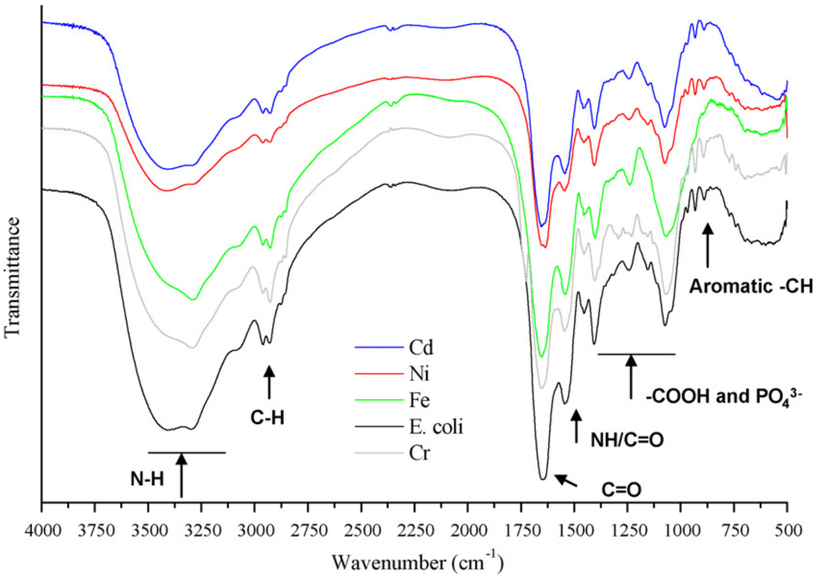

Fig. 4. FTIR spectra of *Escherichia coli* before and after metal loading.

to the Langmuir form. This is confirmed by the satisfactory fit of the Langmuir model to the data, for nickel (Fig. 3).

3.3. FTIR spectral analysis

The FTIR spectra of unloaded and metal-loaded *E. coli* biomass, in the range of 500–4000 cm^{-1}, were taken to confirm the presence of functional groups that are usually responsible for the biosorption process (Fig. 4). The unloaded biomass displays a number of absorption peaks, reflecting the complex nature of the biomass. A peak at 3500–3200 cm^{-1} region is due to the stretching of the N–H bond of amino groups and indicative of bonded hydroxyl group [45]. A change in peak position in the spectrum of the chromium and iron-loaded samples indicates the binding of chromium and iron with amino and hydroxyl groups. The absorption peaks at 2900–3000 cm^{-1} are ascribed to the asymmetric stretching of γC–H bond of the $-\mathrm{CH_2}$ groups combined with that of the $\mathrm{CH_3}$ groups [46]. The nickel and cadmium-loaded samples present slight changes in this region. In the unloaded spectrum, the γC=O of amide I and γNH/γC=O combination of the amide II bond were present at 1650 and 1544 cm^{-1}, respectively, indicating the presence of carboxyl groups. Interestingly, the 1544 cm^{-1} peak increased with the presence of chromium and iron suggesting an interaction of Cr and Fe with carboxyl groups. The peaks in the range 1300–1067 cm^{-1} are attributable to the presence of carboxyl and phosphate groups [47] and at 861 cm^{-1}, to the presence of aromatic –CH stretching peak [48]. Pradhan et al. [42] and Volesky [49] affirm that the main functional groups responsible for a biosorption process are the hydroxyl, carbonyl, carboxyl, sulfonate, amide, imidazole, phosphonate and phosphodiester groups. Some of these groups are present on the *E. coli* biomass and may interact with the metal ions.

4. Conclusions

It was demonstrated that a biofilm of *E. coli* supported on kaolin is able to remove Cr(VI), Cd(II), Ni(II) and Fe(III) from aqueous solutions. The best isotherm fit for chromium and nickel was obtained with the Redlich–Peterson model while the Sips model was the best option for cadmium. Complete removal of iron was achieved followed by cadmium (70%), nickel (between 74 and 40%) and chromium (between 100% and 20%). FTIR analyses showed that functional groups on the biomass, such as hydroxyl, carboxyl and phosphate groups, would be the main binding sites for biosorption of the studied heavy metals by *E. coli*. The presence of the biofilm increased the uptake efficiency of the kaolin. Finally, the metal affinity to the biofilm was found to be in the sequence Fe > Cd > Ni > Cr and the preference of this sorbent for a metal may be explained on the basis of electronegativity of the metal ions and on the basis of their cation/anion state.

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References

[1] A. Sayari, S. Hamoudi, Y. Yang, Applications of pore-expanded mesoporous silica. 1. Removal of heavy metal cations and organic pollutants from wastewater, Chem. Mater. 17 (2005) 212–216.
[2] V. Radhika, S. Subramanian, K.A. Natarajan, Bioremediation of zinc using *Desulfotomaculum nigrificans*: bioprecipitation and characterization studies, Water Res. 40 (2006) 3628–3636.
[3] K.K. Krishnani, X. Mengb, C. Christodoulatos, V.M. Bodduc, Biosorption mechanism of nine different heavy metals onto biomatrix from rice husk, J. Hazard. Mater. 153 (2008) 1222–1234.
[4] E.I. Unuabonah, K.O. Adebowale, B.I. Olu-Owolabi, Kinetic and thermodynamic studies of the adsorption of lead (II) ions onto phosphate-modified kaolinite clay, J. Hazard. Mater. 144 (2007) 386–395.
[5] K. Akhtar, M.W. Akhtar, A.M. Khalid, Removal and recovery of uranium from aqueous solutions by *Trichoderma harzianum*, Water Res. 41 (2007) 1366–1378.
[6] A.I. Zouboulis, M.X. Loukidou, K.A. Matis, Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils, Process Biochem. 39 (2004) 909–916.
[7] W.A. Carvalho, C. Vignado, J. Fontana, Ni(II) removal from aqueous effluents by silylated clays, J. Hazard. Mater. 153 (2008) 1240–1247.
[8] K.G. Bhattacharyya, S. Sen Gupta, Influence of acid activation on adsorption of Ni(II) and Cu(II) on kaolinite and montmorillonite: kinetic and thermodynamic study, Chem. Eng. J. 136 (2008) 1–13.
[9] P. Turan, M. Dogan, M. Alkan, Uptake of trivalent chromium ions from aqueous solutions using kaolinite, J. Hazard. Mater. 148 (2007) 56–63.

[10] A. Sari, M. Tuzen, D. Citak, M. Soylak, Equilibrium, kinetic and thermodynamic studies of adsorption of Pb(II) from aqueous solution onto Turkish kaolinite clay, J. Hazard. Mater. 149 (2007) 283–291.

[11] J.C. Miranda-Trevino, C.A. Coles, Kaolinite properties, structure and influence of metal retention on pH, Appl. Clay Sci. 23 (2003) 133–139.

[12] S. Comte, G. Guibaud, M. Baudu, Biosorption properties of extracellular polymeric substances (EPS) towards Cd, Cu and Pb for different pH values, J. Hazard. Mater. 151 (2008) 185–193.

[13] E.D. Van Hullebusch, M.H. Zandvoort, P.N.L. Lens, Metal immobilization by biofilms: mechanisms and analytical tools, Rev. Environ. Sci. Bio/Technol. 2 (2003) 9–33.

[14] A. Omoike, J. Chorover, Spectroscopic study of extracellular polymeric substances from *Bacillus subtilis*: aqueous chemistry and adsorption effects, Biomacromolecules 5 (2004) 1219–1230.

[15] K.E. Eboigbodin, C.A. Biggs, Characterization of the extracellular polymeric substances produced by *Escherichia coli* using infrared spectroscopic, proteomic, and aggregation studies, Biomacromolecules 9 (2008) 686–695.

[16] S. Comte, G. Guibaud, M. Baudu, Biosorption properties of extracellular polymeric substances (EPS) resulting from activated sludge according to their type: soluble or bound, Process Biochem. 41 (2006) 815–823.

[17] E.F. Covelo, F.A. Vega, M.L. Andrade, Competitive sorption and desorption of heavy metals by individual soil components, J. Hazard. Mater. 140 (2007) 308–315.

[18] Y. Asçi, M. Nurbas, Y. Sag Açikel, Sorption of Cd(II) onto kaolin as a soil component and desorption of Cd(II) from kaolin using rhamnolipid biosurfactant, J. Hazard. Mater. B 139 (2007) 50–56.

[19] T. Ohnuki, T. Yoshida, T. Ozaki, N. Kozai, F. Sakamoto, T. Nankawa, Y. Suzuki, A.J. Francis, Chemical speciation and association of plutonium with bacteria, kaolinite clay, and their mixture, Environ. Sci. Technol. 41 (2007) 3134–3139.

[20] C. Quintelas, T. Tavares, Removal of chromium (VI) and cadmium (II) from aqueous solution by a bacterial biofilm supported on granular activated carbon, Biotechnol. Lett. 23 (2001) 1349–1353.

[21] C. Quintelas, T. Tavares, Lead (II) and iron (II) removal from aqueous solution: biosorption by a bacterial biofilm supported on granular activated carbon, J. Res. Environ. Biotechnol. 3 (2002) 196–202.

[22] C. Quintelas, E. Sousa, F. Silva, S. Neto, T. Tavares, Competitive biosorption of ortho-cresol, phenol, chlorophenol and chromium (VI) from aqueous solution by a bacterial biofilm supported on granular activated carbon, Process Biochem. 41 (2006) 2087–2091.

[23] C. Quintelas, B. Fernandes, J. Castro, H. Figueiredo, T. Tavares, Biosorption of Cr (VI) by a *Bacillus coagulans* biofilm supported on granular activated carbon (GAC), Chem. Eng. J. 136 (2008) 195–203.

[24] C. Quintelas, B. Fernandes, J. Castro, H. Figueiredo, T. Tavares, Biosorption of Cr (VI) by three different bacterial species supported on granular activated carbon—a comparative study, J. Hazard. Mater. 153 (2008) 799–809.

[25] S. Lameiras, C. Quintelas, M.T. Tavares, Development of a biosorption system for chromium (VI) using a *Arthrobacter viscosus* biofilm supported on granular activated carbon and on natural zeolites, Bioresour. Technol. 99 (2008) 206–801.

[26] B. Silva, H. Figueiredo, C. Quintelas, T. Tavares, I.C. Neves, Zeolites as supports for the biorecovery of hexavalent and trivalent chromium, Micropor. Mesopor. Mater. 116 (2008) 555–560.

[27] I. Langmuir, Adsorption of gases on plane surfaces of glass, mica and platinum, J. Am. Chem. Soc. 40 (1918) 1361–1403.

[28] R. Sips, Combined form of Langmuir and Freundlich equations, J. Chem. Phys. 16 (1948) 490–495.

[29] O. Reddlich, D.L. Peterson, A useful adsorption isotherm, J. Phys. Chem. 63 (1959 1024).

[30] K.N. Ghimire, K. Inoue, K. Ohto, T. Hayashida, Adsorption study of metal ions onto crosslinked seaweed *Laminaria japonica*, Bioresour. Technol. 99 (2008) 32–37.

[31] A. Hetzer, C.J. Daughney, H.W. Morgan, Cadmium ion biosorption by the thermophilic bacteria *Geobacillus stearothermophilus* and *G. thermocatenulatus*, Appl. Environ. Microbiol. 72 (2006) 4020–4027.

[32] N. Rangsayatorn, P. Pokethitiyook, E.S. Upatham, G.R. Lanza, Cadmium biosorption by cells of *Spirulina platensis* TISTR 8217 immobilized in alginate and silica gel, Environ. Int. 30 (2004) 57–63.

[33] A. Sari, M. Tuzen, Biosorption of cadmium(II) from aqueous solution by red algae (*Ceramium virgatum*): equilibrium, kinetic and thermodynamic studies, J. Hazard. Mater. 157 (2008) 448–454.

[34] A. Sari, M. Tuzen, O.D. Uluozlu, M. Soylak, Biosorption of Pb(II) and Ni(II) from aqueous solution by lichen (*Cladonia furcata*) biomass, Biochem. Eng. J. 37 (2007) 151–158.

[35] L. Yang, J.P. Chen, Biosorption of hexavalent chromium onto raw and chemically modified *Sargassum* sp, Bioresour. Technol. 99 (2008) 297–307.

[36] K.G. Bhattacharyya, S. Sen Gupta, Adsorption of chromium(VI) from water by clays, Ind. Eng. Chem. Res. 45 (2006) 7232–7240.

[37] V. Murphy, H. Hughes, P. McLoughlin, Comparative study of chromium biosorption by red, green and brown seaweed biomass, Chemosphere 70 (2008) 1128–1134.

[38] J.A. Allen, P.A. Brown, Isotherm analyses for single component and multi-component metal sorption onto lignite, J. Chem. Technol. Biotechnol. 62 (1995) 17–24.

[39] H. Xu, Y. Liu, Mechanisms of Cd^{2+}, Cu^{2+} and Ni^{2+} biosorption by aerobic granules, Sep. Purif. Technol. 58 (2008) 400–411.

[40] S.S. Baral, S.N. Das, G.R. Chaudhury, P. Rath, Adsorption of Cr (VI) by treated weed *Salvinia cucullata*: kinetics and mechanism, Adsorption 14 (2008) 111–121.

[41] M.A. Hanif, R. Nadeem, H.N. Bhatti, N.R. Ahmad, T.M. Ansari, Ni(II) biosorption by *Cassia fistula* (Golden Shower) biomass, J. Hazard. Mater. B 139 (2007) 345–355.

[42] S. Pradhan, S. Singh, L.C. Rai, Characterization of various functional groups present in the capsule of *Microcystis* and study of their role in biosorption of Fe, Ni and Cr, Bioresour. Technol. 98 (2007) 595–601.

[43] R. Shawabkeh, A. Al-Harahsheh, M. Hami, A. Khlaifat, Conversion of oil shale ash into zeolite for cadmium and lead removal from wastewater, Fuel 83 (2004) 981–985.

[44] R.D. Shannon, Revised effective ionic radii and systematic studies of interatomic distances in halides and chalcogenides, Acta Cryst. A 32 (1976) 751–767.

[45] D. Park, Y.-S. Yun, J.M. Park, Studies on hexavalent chromium biosorption by chemically-treated biomass of *Ecklonia* sp, Chemosphere 60 (2005) 1356–1364.

[46] S. Kazy, S.K. Das, P. Sar, Lanthanum biosorption by a *Pseudomonas* sp.: equilibrium studies and chemical characterization, J. Ind. Microbiol. Biotechnol. 33 (2006) 773–783.

[47] A. Pistorius, Biochemical applications of FT-IR spectroscopy, Spectrosc. Europe 7 (1995) 8–15.

[48] S. Tunali, A. Çabuk, T. Akar, Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil, Chem. Eng. J. 115 (2006) 203–211.

[49] B. Volesky, Biosorption and me, Water Res. 41 (2007) 4017–4029.