

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

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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 the focus of this presentation. This paper aims to investigate the biosorption behaviour of a biofilm of *E. coli* supported on kaolin clay for the treatment of cadmium, iron, nickel and chromium aqueous solutions. The effect of metals initial concentrations were studied and the relationship between pH and removal efficiency were analysed. Adsorption characteristics of a biosorbent can be depicted by an adsorption isotherm. Langmuir, Redlich-Peterson and Sips are commonly used for the description of adsorption data and were applied to the experimental results described by this report. The presence of functional groups in the suspended biomass that may have a role in biosorption process was confirmed by FTIR. 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 isotherms were fitted and 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. In terms of removal percentage, the results showed 100% of removal for iron for the whole 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 is higher than the obtained for nickel and chromium. The analyses by FTIR showed that functional groups on the biomass, such as hydroxyl, carboxyl and phosphate groups, may be the main binding sites for biosorption of the studied heavy metals by *E. coli*. Finally, the metal affinity to the biofilm was found to follow the sequence Fe > Cd > Ni > Cr and the preference of a sorbent for a metal may be explained on the basis of electronegativity of the metal ions and on the basis of the cation/anion state.

Materials and Methods

The bacterium *Escherichia coli* was obtained from the Spanish Type Culture Collection of the University of Valência. Heavy metals solutions were prepared by diluting K₂Cr₂O₇, FeCl₃·(6H₂O), CdSO₄·(8/3H₂O) and NiCl₂(6H₂O), in distilled water. Atomic absorption spectrometric standards were prepared from 1000 mg/l metal solutions. The kaolin was obtained from Minas de Barqueiros, S.A. (Apúlia, Portugal).

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

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moderate stirring in a incubator. Then, batch experiments were conducted using 1 g of kaolin clay with 15 ml of *E. coli* culture media and 150 mL of the different metal solutions (10, 25, 50, 70, 80, 100 mg/l) in 250 ml Erlenmeyer flasks. All experimental work was conducted in duplicate. 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, Varian Spectra AA-400.

Infrared spectra of the unloaded biomass and heavy metal loaded biomass, both in suspension, were obtained using a Fourier transform infrared spectrometer (FTIR BOMEM MB 104). For the FTIR study, biomass is 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 Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) using a Philips ICP PU 7000 Spectrometer on samples.

Results and Discussion

The results obtained for the batch biosorption studies showed differences on the biosorption performance between the four metals studied (Table 1). Kaolin surface in water has a net negative surface charge (Turan *et al.*, 2007) and the bacteria have also a net negative charge (Tavares *et al.*, 2006). These negative surfaces obviously favour the biosorption of cations. In addition to the valence state, the electronegativity, atomic weight and ionic radius are also characteristics that influence the biosorption capacity.

Table 1- Uptake values (mg/g) and removal percentage values for Cr (VI), Cd (II), Fe (III) and Ni (II), obtained for the highest initial concentration of metal (37 °C, 150 rpm), for the biofilm of *Escherichia coli* supported on kaolin

Cr (VI)			Cd (II)		
C ₀ (mg/l)	Uptake (mg/g)	Rp (%)	C ₀ (mg/l)	Uptake (mg/g)	Rp (%)
116	4,6	26,2	97	10,3	71,3
Fe (III)			Ni (II)		
C ₀ (mg/l)	Uptake (mg/g)	Rp (%)	C ₀ (mg/l)	Uptake (mg/g)	Rp (%)
110	16,5	100	101	6,9	45,3

The sequence in terms of uptake values by the *E. coli* biofilm supported on kaolin was Fe > Cd > Ni > Cr. The worst biosorption behaviour obtained for chromium is justified by the anionic state. Comparing the results obtained for the cationic metals, better results were obtained for iron and can be justified with the higher electronegativity and reduced ionic radius which promote the penetration into the polymeric net of the biofilm. Considering the ions Cd and Ni, this last one has higher electronegativity, lower atomic weight and ionic radius and these characteristics allows good qualities for metal entrapment. However, better results were obtained for cadmium. A possible explanation for these unexpected results might be the xenobiotic effect of the metal, more accentuated in the nickel case, on the biofilm. In a system biofilm-kaolin both materials contribute to the biosorption performance and if the biofilm is affect by the toxic nickel ion, the biosorption performance is affect too.

It is possible to characterize biosorbents under various operational conditions through the modelling of equilibrium data and this point is essential for future industrial applications. Adsorption isotherms were experimentally determined for the biosorbent used (biofilm + kaolin), and results are shown in Figure 1. Three different models - Langmuir, Sips and Redlich-Peterson- were fitted to the experimental data and constants calculated for the best fit are presented in Table 2. For iron and for the range of initial concentrations tested, the whole metal was immediately and totally biosorbed so the concentration at equilibrium was always zero and consequently it is not possible to represent C_e (mg/l) vs Q_e (mg/g). The best fit for chromium and for nickel was obtained with the Redlich-Peterson model isotherm and for cadmium the best fit was reached with the Sips model.

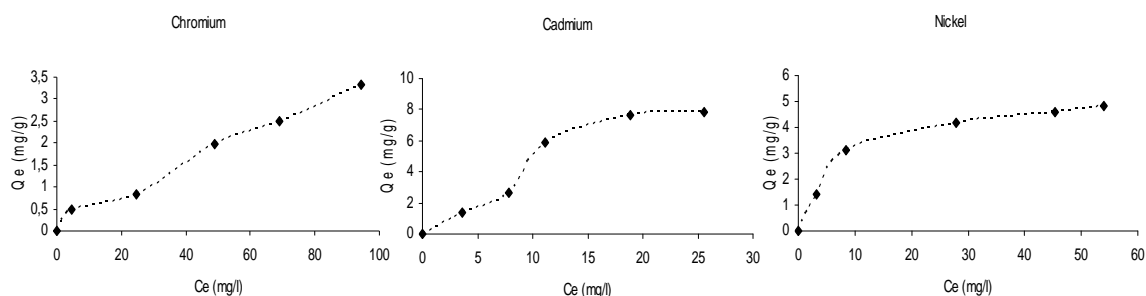


Figure 1- Adsorption isotherms for chromium, cadmium and nickel, at 37 °C, using a biofilm of *Escherichia coli* supported on Kaolin.

Table 2- Adsorption isotherm constants for the isotherm best fit models, for Cr(VI), Cd(II) and Ni(II) onto a biofilm supported on Kaolin

Chromium				
Best fit model	K_R	a_R	β	R^2
Redlich-Peterson	0,615	8,629	0,143	0,984
Cadmium				
Best fit model	K_s	a_s	b_s	R^2
Sips	0,019	0,002	2,750	0,977
Nickel				
Best fit model	K_R	a_R	β	R^2
Redlich-Peterson	0,733	0,136	1	0,994

The FTIR spectra of unloaded and metal loaded *Escherichia coli* biomass in the range of 500–4000 cm^{-1} were taken to confirm the presence of functional groups that may be responsible for the biosorption process and are presented in Figure 2. As it may be seen, unloaded biomass displays a number of absorption peaks, reflecting the complex nature of the biomass. In the unloaded biomass spectrum, 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 [40]. 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 $\gamma\text{C-H}$ bond of the $-\text{CH}_2$ groups combined with that of the CH_3 groups [41]. The nickel and cadmium-loaded samples present slight changes in this region. In the unloaded spectrum, the $\gamma\text{C=O}$ of amide I and $\gamma\text{NH}/\gamma\text{C=O}$ 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 [42] and at 861 cm^{-1} , to the presence of aromatic $-\text{CH}$ stretching peak [43]. Studies developed by Pradhan *et al.* [37] and Volesky (2007) [44] conclude that the main functional groups responsible for a biosorption process are the hydroxyl, carbonyl, carboxyl, sulfonate, amide, imidazole, phosphonate and phosphodiester groups, some of them present on the *Escherichia coli* biomass.

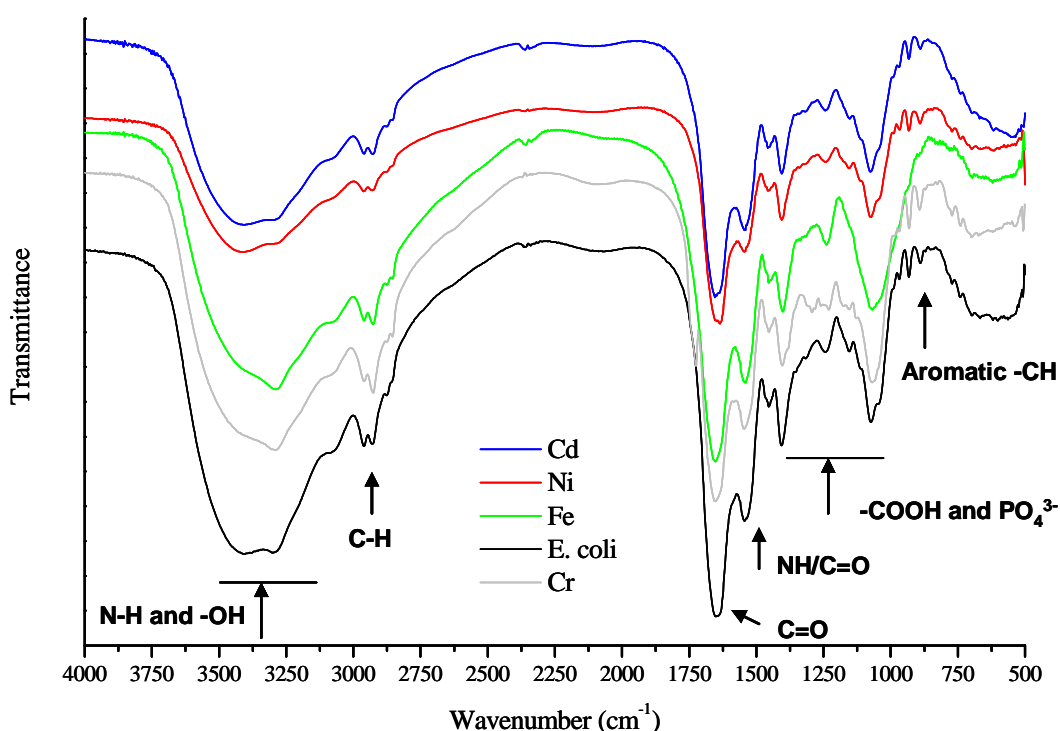


Figure 2- FTIR spectra of *Escherichia coli* before and after metal loading.

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