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Biosorption of Cr(VI) by a *Bacillus coagulans* biofilm supported on granular activated carbon (GAC)

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Abstract

The ability of a biofilm of *Bacillus coagulans* supported on granular activated carbon (GAC) to biosorb Cr(VI) was investigated in batch and column studies so it may be applied to low metal concentration wastewater treatment. The quantification of polysaccharides and polymeric net revealed a value of 9.19 mg/$g_{biosorbent}$ for the polysaccharides and 75 mg/$g_{biosorbent}$, for the polymeric net. The results obtained with open systems showed uptake values of 1.50, 1.98 and 5.34 mg/$g_{biosorbent}$, respectively, for initial concentrations of 10, 50 and 100 mg/L of Cr(VI). Column studies performed with an industrial effluent showed values of Cr uptake of 0.090 mg/$g_{biosorbent}$, for an initial concentration of 4.2 mg/L. The presence of functional groups on the cell wall surface of the biomass that may interact with the metal ion, was confirmed by FTIR. The equilibrium studies in batch systems were described by Freundlich, Langmuir, Reddlich–Peterson, Dubinin–Radushkevich, Sips and Toth model isotherms. Best fit was obtained with Toth model isotherm. Data from column studies were described by Adams–Bohart and Wolborska models. These models were found suitable for describing the dynamic behaviour of the columns with respect to the inlet chromium concentration. The whole study showed that the biofilm tested is very promising for the removal of Cr(VI) in industrial wastewater.

Keywords: Activated carbon; *Bacillus coagulans*; Biofilm; Biosorption; Chromium(VI)

1. Introduction

The accumulation of heavy metals on the environment is a serious problem that needs to be solved. The conventional methods for heavy metal removal from industrial effluents are precipitation, coagulation, ion exchange, cementation, electro-dialysis, electro-winning, electro-coagulation and reverse osmosis [1]. However, the application of these treatment processes to low metal concentration wastewater is sometimes restricted, due to technological or economical reasons. The search for novel technologies has been directed to the application of biosorption.

Biosorption consists of several mechanisms, mainly ion exchange, chelation, adsorption and diffusion through cell walls and membranes, which differ from each other depending on the species used, the origin and the processing of the biomass and on the solution chemistry [2]. Microorganisms have a high surface area-to-volume ratio because of their small size and therefore, they can provide a large contact interface, which would interact with metals from the surrounding environment [3]. Bacteria are classified as Gram-positive or Gram-negative and this classification divides bacteria into two main groups that differ in their cell wall characteristics. In general, Gram-positive bacteria have a greater sorptive capacity due to their thicker layer of peptidoglycan which contains numerous sorptive sites [4]. The bacterial ability for Cr(VI) reduction does not require high-energy input nor toxic chemical reagents and it allows the use of native, non-hazardous strains [5]. These factors constitute a major advantage over classical processes for treatment of Cr(VI) wastewater.

Biofilms can be defined as communities of microorganisms attached to a surface [6,7]. There are four potential incentives for the biofilm formation: defense (protection from harmful conditions), colonization (biofilm formation as a mechanism to remain in a favorable niche), community (utilization of cooperative benefits) and default mode of growth. Bacteria spend the majority of their natural existence growing as a biofilm. It is possible that the presence of a suitable substrate for attachment is all that is required to trigger biofilm formation [8]. The synthesis of EPS,

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bacterial extracellular polymeric substances, is important in the development of biofilms in general.

The EPS are a complex mixture of macromolecular polyelectrolytes including polysaccharides, proteins, nucleic acids [9], lipids or humic substances [4]. These EPS building molecules contain ionisable functional groups such as carboxyl, phosphoric, amine and hydroxyl groups [4]. The most important functions of EPS are adhesion to surfaces, aggregation of bacterial cell in flocs, stabilization of the floc structure, formation of a protective barrier that provides resistance to biocides or other harmful effects, retention of water, sorption of exogeneous organic compounds for the acumulation of nutrients from environment and accumulation of enzymatic activities, such as digestion of exogeneous macromolecules for nutrient acquisition, aiding the cells in uptaking metal nutrients [10]. The adhesion to surfaces and the accumulation of elements from environment are two key functions of EPS on supported biosorption processes.

The mechanisms of interactions between metal ions and biofilms are well described by Le Cloirec et al. [7] and can be resumed as follows: bulk diffusion (the metal ions present in solution diffuse to the external surface of the biofilm), external mass transfer (the mass transfer occurs through the high concentration layer around the biofilm), fast interactions of the metal ion with solid surface and especially with the bacteria wall (these interactions can be bioaccumulation, oxidation and/or reduction, enzyme production, extracellular precipitation by metabolites produced by bacteria, extracellular complexation and biosorption on the surface of the bacteria), slow surface diffusion, diffusion into the biofilm before the interaction reaction with bacteria and finally, interactions with bacteria present inside the biofilm.

Activated carbons, with their high surface area, microporous character and chemical nature of their surface, their high adsorption capacity and fast adsorption kinetics are potential adsorbents for the removal of heavy metals from industrial wastewater [11,12].

The aim of this work is the investigation of the biosorption properties of a *Bacillus coagulans* (CECT 12) biofilm supported on granular activated carbon (GAC) for the removal of chromium(VI) from wastewater. Activated carbon might be able to retain chromium from liquid solutions in certain conditions but the fact that the hexavalent ion is negatively charged as chromate (CrO_4^-) or dichromate ($Cr_2O_7^{2-}$) and strongly hydrated, drastically reduces the uptake of this metal by GAC. On the other hand, the cationic form Cr^{3+} is easily retained by the adsorbent. The novelty of this study is the synergetic effect of the combination between the *B. coagulans* biofilm, able to reduce Cr^{6+} to Cr^{3+}, and GAC, able to retain this last ion on its surface. The biofilm by itself would not be able to retain appreciable amounts of Cr, but the carbon matrix will allow Cr accumulation for downstream processing. Without the biofilm, GAC would not adsorb the chromate or dichromate ions due to ionic repulsions and sterical limitations. The effect of the initial concentration of metal was tested, the polysaccharide and polymeric net of the *Bacillus* were quantified and the presence of functional groups in the suspended biomass that may have a role in biosorption process was confirmed by FTIR. It was studied the application of this system to the treatment of a real effluent provided by a tannery. Equilibrium isotherms for the adsorption of Cr(VI) on the biofilm were described by Freundlich, Langmuir, Reddlich–Peterson, Dubinin–Radushkevich, Sips and Toth models. The dynamic behaviour of the columns with respect to the inlet chromium concentration were analysed by the Adams–Bohart and Wolborska models.

2. Materials and methods

2.1. Materials

The bacterium *Bacillus coagulans* (CECT 12) was obtained from the Spanish Type Culture Collection of the University of Valência. Aqueous chromium solutions were prepared by diluting $K_2Cr_2O_7$ (Riedel) in distillated water. All glassware used for experimental purposes was washed in 60% nitric acid and subsequently rinsed with deionised water to remove any possible interference by other metals. Atomic absorption spectrometric standards were prepared from 1000 mg_{Cr}/L solution.

The support was granular activated carbon (GAC) from MERCK with an average particle size of 2.5 mm, characterised by N_2 adsorption (77 K) with an ASAP Micromeritics 2001, which indicated a Langmuir area of 1270 $m^2\ g^{-1}$ and an average pore diameter of 2 nm.

2.2. Methods

2.2.1. Column biosorption

The whole experimental work was conducted in duplicate. GAC (15 g) was placed in Erlenmeyer flasks of 250 mL with 150 mL of distilled water and these were sterilised at 120 °C for 20 min to release the air inside the support pores. Then, those materials were placed in mini-columns (internal diameter = 2 cm, ht = 30 cm) for open system studies. The microorganism culture and the nutrient broth were pumped through (upflow) at a flow rate of 25 mL/min. Afterwards, medium with 5 g/L of beef extract (HIMEDIA), 10 g/L of peptone (Riedel), 5 g/L of NaCl (Prolabo) and 10 mg/L of $MnSO_4{\cdot}H_2O$ (Panreac), as suggested by the original collection, was used to grow the microorganism on the support for 3 days, aiming the optimisation of the adhesion. The high flow rate used allows the formation of a compact biofilm and consequently a resistant one to the erosion stress resultant from the hydrodynamic forces [13]. The biofilm supported on GAC was observed (after dehydratation with different concentrations of ethanol) by SEM (Leica Cambridge S360) and is shown in Fig. 1. The sample was gold coated prior to SEM observation. This picture is an example of many similar ones taken at various zoomed areas and they all show that the biofilm covered uniformly the GAC.

After the biofilm formation, the beds were washed out and the metal solutions with concentrations between 10 and 100 mg/L (prepared on laboratory) and a concentration of 4.2 mg/L (industrial effluent), with pH naturally ranging from 4.5 to 5.5 and a temperature of 37 °C, were passed through the columns with

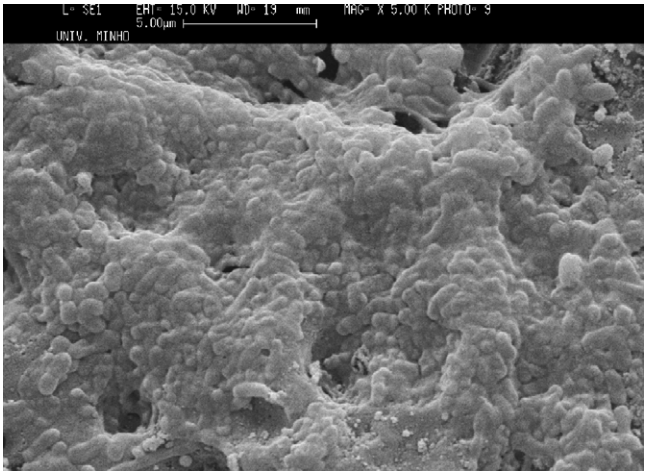

Fig. 1. SEM image of *Bacillus coagulans* biofilm supported on GAC.

a flow rate of 5 mL/min. At the end of each run, columns were washed out and samples of the effluent were seeded in Petri plates with nutrient agar to assess the metabolic activity of the microorganism. Cr(VI) concentration at the inlet and at the outlet of the columns was measured by Atomic Absorption Spectroscopy, Varian Spectra AA-250 Plus, by acetylene flame emission and wavelengths of 357.9, 425.4 and 520.8 nm. Carbon samples were taken and prepared for SEM analysis.

2.2.1.1. The Adams–Bohart and the Wolborska models. Successful design of a column adsorption process requires prediction of the concentration–time profile or breakthrough curve for the outlet. Traditionally, the Adams–Bohart and the Wolborska models are used to fulfil the purpose.

2.2.1.1.1. The Adams–Bohart model. Bohart and Adams [14] established the fundamental equations that describe the relationship between C/C_0 and time in an open system for the adsorption of chlorine on charcoal. In spite of the fact that the original studies of Adams–Bohart were performed with the gas–charcoal adsorption system, its overall approach can be applied successfully in quantitative description of other systems. The model proposed assumes that the adsorption rate is proportional to both the residual capacity of the activated carbon and the concentration of the sorbing species. The mass transfer rates obey the following equations:

$$\frac{\partial q}{\partial t} = -k_{AB} q C_b \tag{1}$$

$$\frac{\partial C_b}{\partial Z} = -\left(\frac{k_{AB}}{U_0}\right) q C_b \tag{2}$$

k_{AB} is the kinetic constant ($L\,mg^{-1}\,min^{-1}$), C_b the bulk chromium concentration in the solution in the column ($mg\,L^{-1}$), q represents the chromium concentration in the solid phase in the column at any time ($mg\,L^{-1}$), U_0 the superficial velocity ($cm\,min^{-1}$) and Z is the height of the column (cm). For the solution of these differential equations system two assumptions are made: $t \to \infty$ and $q \to N_0$, (where N_0 is the saturation concentration ($mg\,L^{-1}$)). When the differential equations system is solved, the following equation is obtained with parameters k_{AB} and N_0:

$$\ln\left(\frac{C}{C_0}\right) = k_{AB} C_0 t - k_{AB} N_0 \left(\frac{Z}{U_0}\right) \tag{3}$$

C_0 and C are the inlet and outlet chromium concentrations ($mg\,L^{-1}$), respectively. From this equation, values describing the characteristic operational parameters of the column can be determined from a plot of $\ln C/C_0$ versus t at a given bed height and flow rate.

2.2.1.1.2. The Wolborska model. Another model used for the description of adsorption dynamics using mass transfer equations for diffusion mechanisms in the range of the low-concentration breakthrough curve is the Wolborska model [15]. The mass transfer in the fixed bed sorption is described by the following equations:

$$\frac{\partial C_b}{\partial t} + U_0\left(\frac{\partial C_b}{\partial Z}\right) + \left(\frac{\partial q}{\partial t}\right) = D\left(\frac{\partial^2 C_b}{\partial^2 Z}\right) \tag{4}$$

$$\frac{\partial q}{\partial t} = -v\left(\frac{\partial q}{\partial Z}\right) = \beta_a (C_b - C_s) \tag{5}$$

C_s is the chromium concentration at the solid/liquid interface ($mg\,L^{-1}$), D the axial dispersion coefficient ($cm^2\,min^{-1}$), v the migration rate ($cm\,min^{-1}$) and β_a is the kinetic coefficient of the external mass transfer (min^{-1}). For the solution of these differential equations system some assumptions are made: $C_s \ll C_b$, $v \ll U_0$ and axial dispersion negligible, $D \to 0$ as $t \to 0$. The solution can be approximated to:

$$\ln\left(\frac{C}{C_0}\right) = \left(\frac{\beta_a C_0}{N_0}\right) t - \beta_a (C_b - C_s) \tag{6}$$

with

$$\beta_a = \frac{U_0^2}{2D}\sqrt{\left(\left(\frac{(1 + 4\beta_0 D)}{U_0^2}\right) - 1\right)} \tag{7}$$

β_0 is the external mass transfer coefficient with a negligible axial dispersion coefficient D. The author observed that in short beds or at high flow rates of solution through the bed, the axial dispersion is negligible and $\beta_a = \beta_0$. The migration rate of the steady-state front satisfies the Wicke's law:

$$v = \frac{U_0 C_0}{(N_0 + C_0)} \tag{8}$$

The expression of the Wolborska model is equivalent to the Adams–Bohart relation if the coefficient k_{AB} is equal to β_a/N_0. So, the drawing of $\ln C/C_0$ versus t would also give information on this model.

2.2.2. Batch biosorption studies

The biofilm formation was prepared accordingly to the previous section (see Section 2.2.1). The adsorption isotherm for chromium solution on GAC with biofilm was obtained from batch experiments at 37 °C. The experiments were performed with 250 mL Erlenmeyer flasks containing 150 mL of chromium solution and 1.5 g of GAC covered with biofilm. The initial

chromium solutions varied between 50 and 1000 mg/L. The flasks were rotated at a constant shaking rate of 150 rpm until equilibrium was reached. Previous studies were made to determine the time needed for equilibrium to be reached. Samples of 5 mL were taken after reaching equilibrium, centrifuged at 4000 rpm during 5 min and the supernatant liquid was analysed for chromium ion.

2.2.2.1. Adsorption isotherm models. Adsorption isotherm equations are used to describe experimental sorption data and, on the other hand, to describe how adsorbates interact with the adsorbents. Six isotherm equations have been tested in the present study.

2.2.2.1.1. Langmuir isotherm. Langmuir [16] developed a theoretical equilibrium isotherm that established a relationship between the amount of gas sorbed on a surface and the pressure of gas. This isotherm assumes monolayer coverage of adsorbate over a homogeneous adsorbent surface. The general Langmuir sorption model is expressed by:

$$Q_e = \frac{(Q_{max} b C_e)}{(1 + bC_e)} \quad (9)$$

Q_e (mg/g) is the amount of metal ion sorbed by the biofilm at the equilibrium, Q_{max} (mg/g) the maximum metal sorption, C_e (mg/L) the concentration of metal in solution at the equilibrium and b (L/mg) is the Langmuir adsorption equilibrium constant.

2.2.2.1.2. Freundlich isotherm. Freundlich [17] presented the earliest isotherm equation, an exponential equation, and assumes that as the adsorbate concentration in solution increases so, does the concentration of adsorbate on the adsorbent surface. This empirical model can be applied to non-ideal sorption on heterogeneous surfaces as well as to multilayer sorption and is expressed by:

$$Q_e = K_f C_e^{1/n} \quad (10)$$

Q_e and C_e are the same as in the Langmuir equation, and K_f and n relate to the capacity and intensity of adsorption, respectively.

The Freundlich equation agrees well with the Langmuir over moderate concentration ranges but, unlike the Langmuir expression, it does not reduce to the linear isotherm (Henry's Law) at low surface coverage. Both these theories suffer from the disadvantage that the equilibrium data over a wide concentration range cannot be fitted with a single set of constants [18,19].

2.2.2.1.3. Reddlich–Peterson isotherm. Reddlich and Peterson [20] proposed the first three-parameter isotherm model that incorporates features of both the Langmuir and Freudlich isotherms. This equation may be used to represent adsorption equilibria over a wide concentration range and it can be described as follows:

$$Q_e = \frac{(K_R C_e)}{(1 + a_R C_e^{\beta})} \quad (11)$$

K_R (L/g), a_R (L/mg) and β (varied between 0 and 1) are empirical parameters without physical meaning [21]. At low concentrations, the Reddlich–Peterson isotherm approximates to Henry's law and at high concentrations its behaviour approaches that of the Freundlich isotherm.

2.2.2.1.4. Sips isotherm (or Langmuir–Freundlich isotherm). Sips [22] proposed a new equation that can be expressed by:

$$Q_e = \frac{(K_S C_e^{1/b_S})}{(1 + a_S C_e^{1/b_S})} \quad (12)$$

K_S (L^{b_S} mg^{1-b_S}/g), a_S $(L/mg)^{b_S}$ and b_S are the Sips isotherm parameters. This equation is also called Langmuir–Freundlich isotherm and the name derives from the limiting behaviour of the equation. At low sorbate concentrations it effectively reduces to a Freundlich isotherm and thus does not obey Henry's law. At high sorbate concentrations, it predicts the monolayer sorption capacity characteristics of the Langmuir isotherm.

2.2.2.1.5. Toth isotherm. Derived from potential theory, the Toth equation [23] is used in heterogeneous systems. It assumes a quasi-Gaussian energy distribution, i.e. most sites have an adsorption energy lower than the peak of maximum adsorption energy. The model can be represented by the following equation:

$$Q_e = \frac{(K_t C_e)}{[(a_t + C_e)^{1/t}]} \quad (13)$$

K_t (mg/g), a_t and t represents the Toth isotherm constants.

2.2.2.1.6. Dubinin–Radushkevich isotherm. Dubinin and Radushkevich [24] have reported that the characteristic sorption curve is related to the porous structure of the sorbent. The Dubinin–Radushkevich equation is generally expressed as follows:

$$Q_e = q_D \exp\left(-B_D\left[RT \ln\left(1 + \frac{1}{C_e}\right)\right]^2\right) \quad (14)$$

The constant, B_D, is related to the mean free energy of sorption per gram of the sorbate as it is transferred to the surface of the solid from infinite distance in the solution. T is the temperature (K) and R is the universal gas constant.

The simplest method to determine isotherms constants for two parameter isotherms (Langmuir, Freundlich and Dubinin–Radushkevich) is to transform the isotherms parameters so that the equation presents linear form and then linear regression is applied. For the other equations, the model parameters were estimated by non-linear regression using MATLAB and EXCEL softwares.

2.2.3. Quantification of polysaccharides and total polymers

The method used for the quantification of polysaccharides and total polymers was first described by Oliveira and Azeredo [25]. It consists of three steps: (i) solubilization of the polysaccharide and polymeric net with glutardialdehyde for 2 days in smooth rotating speed, (ii) dialysis of the obtained solution and (iii) precipitation of the dialysed. This precipitation step is achieved with phenol and sulphuric acid for quantification of polysaccharides, which is performed by spectrometry at 440 nm. The quantification of total polymers is achieved by precipitation of the dialysed with nitron solution, followed by centrifugation and drying. The residual material is finally weighted.

2.2.4. Fourier transform infrared spectroscopy (FTIR)

Infrared spectra of the unloaded biomass and chromium-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 20 mg of finely ground biomass was encapsulated in 200 mg of KBr (Riedel) in order to prepare translucent sample disks. Background correction for atmospheric air was used for each spectrum. The resolution was 4 cm^{-1} and the number of scans were a minimum of 5 scans for each spectrum and the range was 500–4000 wavenumbers.

3. Results and discussion

The uptake of metal ions by *Bacillus coagulans* biofilm, applying batch systems or open studies (column), occurs in two consecutive stages: an initial stage (mainly due to passive uptake), followed by a slower stage (due to active uptake) [3]. Other authors suggested [26] that Cr(VI) can be reduced to Cr(III) by the biomass through two different mechanisms. In the first mechanism, Cr(VI) is directly reduced to Cr(III) in the aqueous phase by contact with the electron-donor groups of the biomass, i.e. groups having lower reduction potential values than that of Cr(VI) (+1.3 V). The second mechanism consists of three steps: the binding of anionic Cr(VI) ion species to the positively charged groups present on the biomass surface, the reduction of Cr(VI) to Cr(III) by adjacent electron-donor groups and the release of the Cr(III) ions into the aqueous phase due to electronic repulsion between the positively charged groups and the Cr(III) ions, or the complexation of the Cr(III) with adjacent groups capable of Cr-binding.

This bacterium was chosen because several authors used *Bacillus sp*. in heavy metals removal processes with very good results. It is highlighted the work of Zouboulis et al. [3], with *B. lichenformis* and *B. laterosporus* for the removal of Cd(II), the work of Salehizadeh and Shojaosadati [27], with *B. firmus* for the removal of Pb(II), Cu(II) and Zn(II) and the work of Green-Ruiz [28] with *Bacillus sp*. for the removal of Hg(II).

3.1. Quantification of polysaccharides and total polymers

The attachment of bacteria to a solid surface is the first and more important stage in the formation of a biofilm. This attachment stage is generally described as a two-step process. In the first step, the microrganisms come close enough to the surface to be weakly held by electrostatic forces. This step can be named "reversible attachment" because the cells can be easily removed from the surface. In the second step, called "irreversible step", the attached microorganisms are more difficult to remove from the surface, as the bacteria produce exopolysaccharides that eventually form the biofilm matrix, which is firmly adherent to the substrate [29]. The quantification of polysaccharides and polymeric net revealed a value of 9.19 $mg/g_{biosorbent}$ for the polysaccharides and 75 $mg/g_{biosorbent}$, for the polymeric net and these are quite relevant values. The polyssacharide and polymeric net give important informations about the capacity of biofilm formation by the microorganism which was confirmed in this case. These results revealed a very good adhesion of the bacteria to the GAC. The presence of binding sites enables EPS not only to sequester minerals and nutrients for microbial growth, but also to remove toxic metals in biological treatment of wastewater [30].

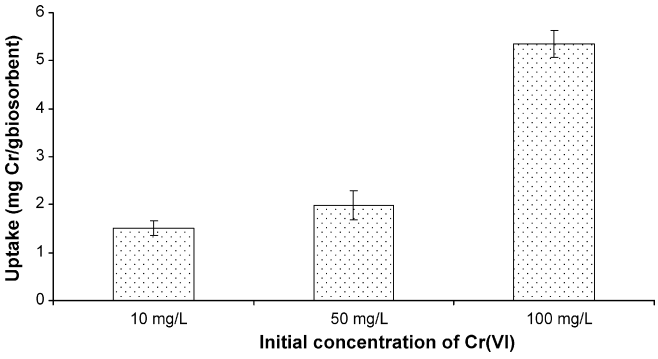

Fig. 2. Uptake values obtained for column studies as a function of the inlet chromium concentration.

3.2. Column studies

3.2.1. Effects of initial concentration of metals ions on the biosorption capacity

The results showed uptake values of 1.50, 1.98 and 5.34 $mg/g_{biosorbent}$, respectively, for the initial concentration of 10, 50 and 100 mg/L (Fig. 2). Fig. 3 illustrates the resulting breakthrough curves for Cr at different inlet concentrations. It

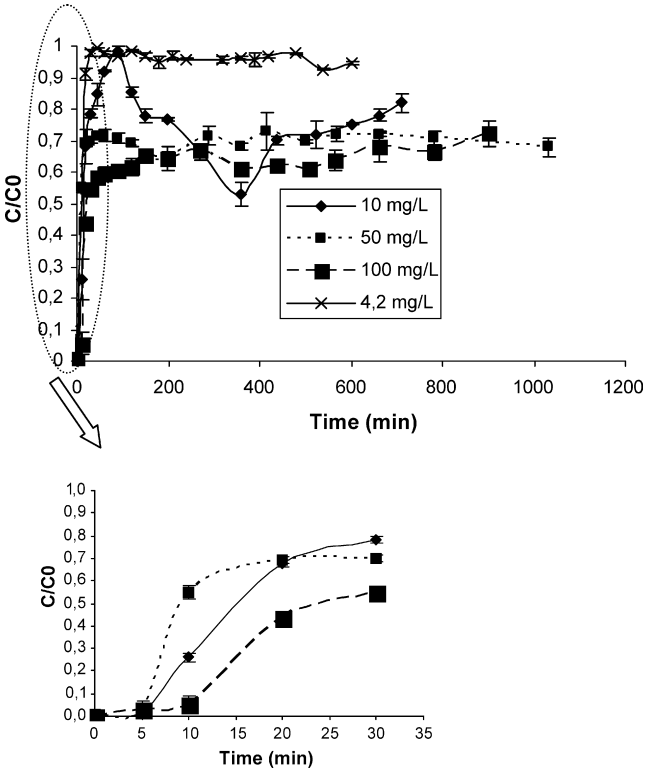

Fig. 3. Breakthrough curves for Cr(VI) biosorption onto *Bacillus* biofilm supported on GAC at different inlet metal concentrations; zooming for initial concentrations of 10, 50 and 100 mg/L.

Table 1
Removal percentage values, for the Cr(VI), after 10 h of experimental assay (open systems) for all the concentrations tested and for the industrial effluent

C_0 (mg/L)	Removal percentage (%)
4.2 (industrial effluent)	5.4
10	24.7
50	28.0
100	32.0

Table 2
Parameters predicted from the Adams–Bohart and Wolborska models at different inlet chromium concentrations

C_0 (mg/L)	N_0 (mg/L)	k_{AB} (L/(mg.min))	β_a (L/min)	R^2
10	658.5	4.44E−5	0.029	0.94
50	10436.7	1.76E−6	0.018	0.89
100	13091.3	2.15E−6	0.028	0.89

can be seen from this figure that there was a period of time (very short) where the heavy metal concentration in the column effluent remained zero and then the concentration of the metal started to increase. This is due to the formation of the mass transfer zone in the column. Once the solution containing the heavy metal becomes exposed to the fresh layer of the biomass, the metal ions are sequestred by the biomass until the retained amount is in equilibrium with the influent concentration. At this time, the biomass is loaded to its full capacity and that portion of the biomass becomes exhausted. Above this line which is progressing in the direction of the flow, adsorption is occurring and the metal ion is being actively transferred from the liquid onto the biomass. The mass transfer zone will move up through the column until it reaches the effluent port, whereupon the heavy metal concentration in the effluent begins to rise. In this process arrangement, the metal-bearing solution permeates through the bed of active biomass, which would act like a series of batch contactors. Consequently, the biomass would be loaded up to its maximum capacity [31]. The biosorption capacity of the biofilm increased with increasing initial concentrations (Fig. 2 and Table 1). This could be explained by the fact that the driving force for biosorption is the Cr concentration difference between the solution and the biosorbent. Thus, the high driving force due to the high chromium concentration resulted in better column performance [32].

3.2.2. Application of the Adams–Bohart and the Wolborska models

The Adams–Bohart and Wolborska sorption models were applied to experimental data for the description of the breakthrough curve. This approach was focused on the estimation of characteristic parameters, such as maximum adsorption capacity (N_0), kinetic constant (k_{AB}) from Adams–Bohart model and kinetic coefficient of the external mass transfer (β_a) from Wolbraska model. After applying Eq. (3) (or Eq. (6)) to the experimental data for different inlet chromium concentrations, a linear relationship between $\ln C/C_0$ and t was obtained. Respective values of N_0, k_{AB} and β_a were calculated from the $\ln C/C_0$ versus t plots at all inlet chromium concentrations studied and are presented in Table 2 together with the correlation coefficients. As expected, maximum adsorption capacity (N_0) increased with increasing inlet chromium concentration. Predicted and experimental breakthrough curves with respect to inlet chromium concentration are shown in Fig. 4. It is clear from this graph that there is a good agreement between the experimental and predicted values for times higher than 20–30 min, for the higher concentrations used. Discrepancies where found between the experimental and the predicted curves for the first minutes of operation. Although the Adams–Bohart (or Wolbraska) model provides a simple and comprehensive approach to evaluating sorption-column tests, its validity is limited to the range of conditions used. For the most diluted concentration used the discrepancies are higher (data not showed). This can be explained by the fact that the model does not take into account the metabolic activity of *Bacillus coagulans* and the retention of Cr(VI) at high concentrations occurs mainly in GAC as a consequence of a xenobiotic effect for the bacteria. The relatively higher errors obtained for the lower concentrations of metal seem to be related with the metabolic activity which is not quantified and consequently is not introduced in the model.

3.2.3. Effects of other ions presents on the solution

The studies made with the industrial effluent showed values of Cr uptake of 0.090 $mg/g_{biosorbent}$, for an initial concentration of 4.2 mg/L. The value obtained for the removal percentage with the most diluted solution used (10 mg/L) was of 24.7% (after 10 h of experiment) and the value of removal percentage obtained with the industrial effluent was of 5.4%, for the same period of time. As it was showed in Fig. 3, the process of metal removal is inhibited in the presence of other ions. The presence of a multiplicity of metals leads to interactive effect. Salehizadeh and Shojaosadati [27] affirm that these effects can be extremely complex and three types of responses may be expected: (1) the effects of mixture is greater than that of the individual effects of ions in the mixture (synergism); (2) the effects of mixture is less than that of the individual effects of ions in the mixture (antagonism); and (3) no effect of mixture (no interaction) is

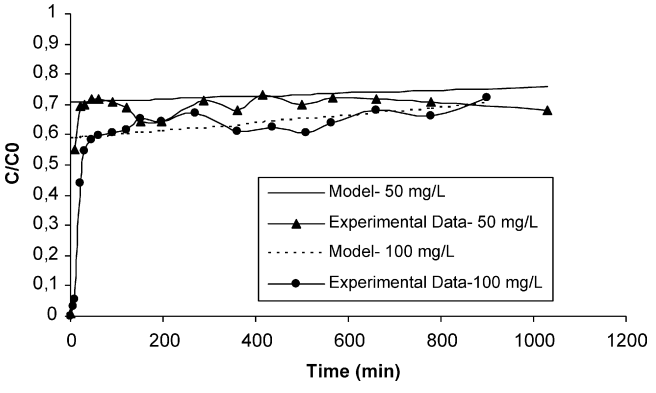

Fig. 4. Comparison between the experimental results and those predicted by the models for the inlet solute concentration of 50 and 100 mg/L, according to the Adams–Bohart (or Wolbraska) model.

observed. In the present case the worst results obtained with the industrial effluent can be explained by the fact that the other metal ions and compounds present in the industrial effluent can compete for the same active sites.

3.3. Batch studies

It was observed that as initial chromium concentration increases, the uptake increases too, but the removal percentage decreases. For instance, on changing the initial chromium concentration from 50 to 1000 mg/L, the amount of chromium biosorbed increased from 38.87 to 784.90 mg/g, but the removal percentage decreased from 46.86 to 17.15 % (Table 3). This could be explained as at lower concentrations, the ratio of the initial moles of chromium to the available surface area is low and subsequently the fractional sorption is independent of the initial concentrations. On the other hand, at higher concentrations the available sites become fewer compared to the number of moles of chromium present and hence the removal percentage of chromium is dependent on the initial percentage [32].

Table 3
Equilibrium adsorbed quantities and removal percentages of Cr(VI) ion obtained at different initial metal ion concentration (37 °C, 150 rpm)

C_0 (mg/L)	q_{eq} (mg/g)	R_p (%)
73.21	38.87	46.86
105.85	62.62	40.89
246.99	169.50	31.38
365.19	251.50	31.13
546.90	393.00	28.14
743.60	579.75	22.04
947.36	784.90	17.15

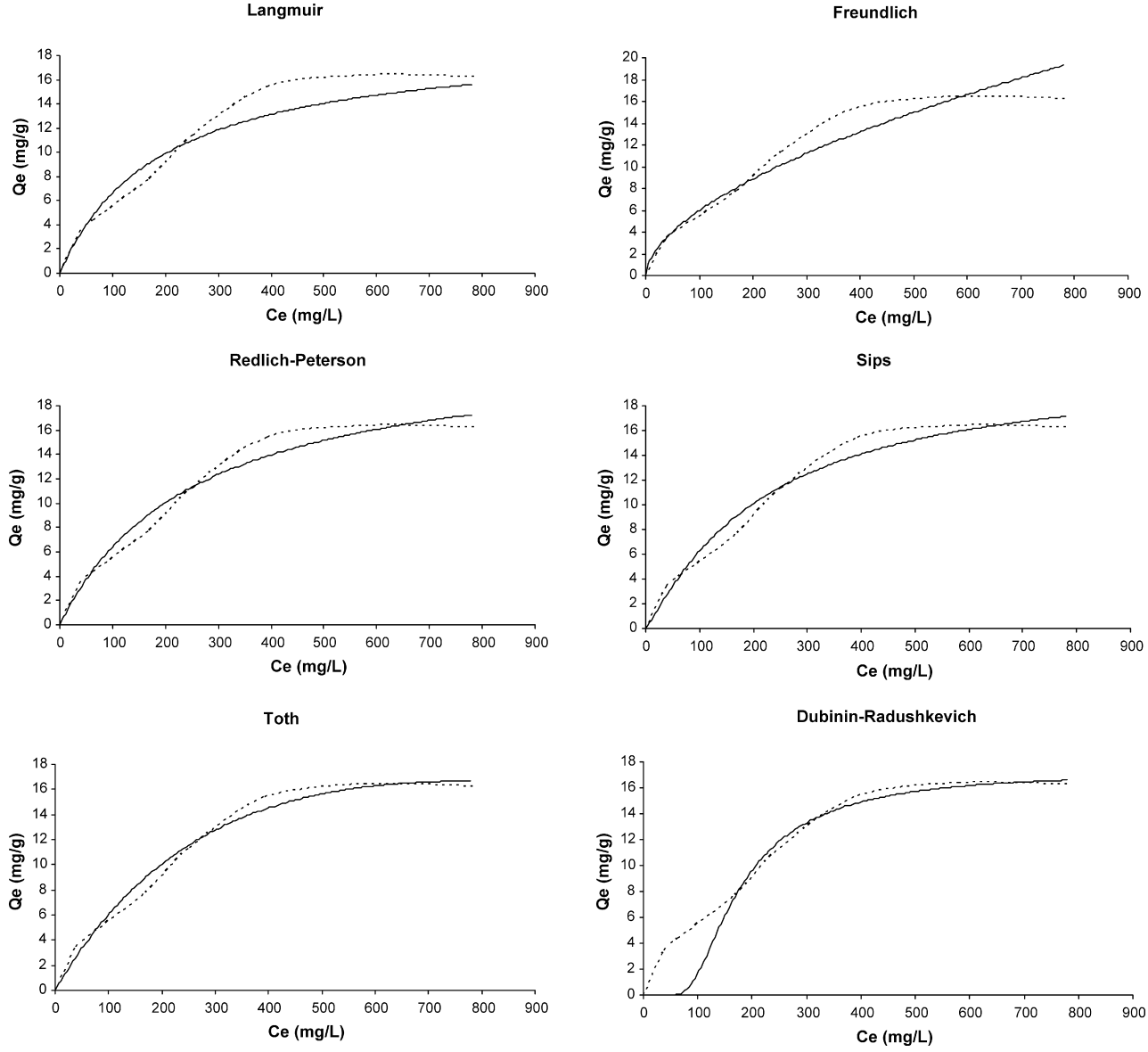

Fig. 5. Comparison between the experimental results and those predicted by the models for the chromium adsorption isotherms for all the six models tested (— model, - - - experimental data).

3.3.1. Adsorption isotherm studies

The adsorption of a substance from one phase to the surface of another in a specific system leads to a thermodynamically defined distribution of that substance between the phases as the system reaches equilibrium. This distribution can be expressed in terms of adsorption isotherms, whereby the metal species, sequestered by the sorbent (biofilm) through a number of several mechanisms, is in equilibrium with its residue left free in the solution [33]. For the biosorbent used (Biofilm + GAC), equilibrium data were experimentally determined. Six different models – Langmuir, Freundlich, Reddlich–Peterson, Dubinin–Radushkevich, Sips and Toth – were fitted and constants calculated are presented in Table 4. All equations fit the data reasonably well (Fig. 5) but the best fit was obtained with the Toth model isotherm. The fact that the fit obtained with Langmuir and Freundlich models showed the worst results suggests that the binding of chromium does not occur as a monolayer on the surface of the biomass. Gerente et al. [34] stressed that equilibrium isotherm equations are used to describe experimental sorption data and, therefore parameters and thermodynamic assumptions of these equilibrium models usually provide some insight into the sorption mechanism, the surface properties and the affinity between sorbent and sorbate. Those authors also stated that the importance of obtaining the best-fit isotherm becomes more and more significant as more applications are developed. As a consequence more accurate and detailed isotherm descriptions are required for the design of wastewater treatment systems.

Table 4
Adsorption isotherm constants for all the isotherm models studied for chromium(VI) onto a biofilm supported on GAC

Parameters	
Langmuir parameters	
$Q_{máx}$	19.455
b	0.0052
R^2	0.976
Freundlich parameters	
K_f	0.431
n	1.751
R^2	0.970
Dubinin–Radushkevich parameters	
q_D	17.264
B_D	36.438
R^2	0.989
Reddlich–Peterson parameters	
K_R	0.088
a_R	0.0038
β	1.000
R^2	0.980
Sips parameters	
K_S	0.051
a_S	0.0024
b_S	1.120
R^2	0.981
Toth parameters	
K_t	2.788E+7
a_t	1381
t	0.366
R^2	0.988

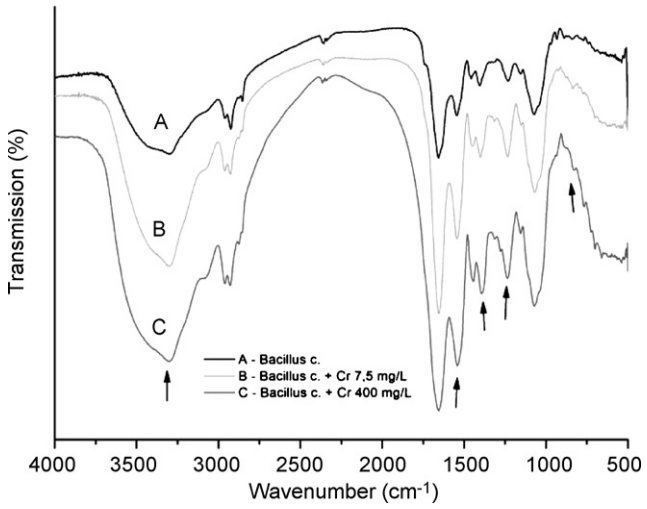

Fig. 6. FTIR spectra of *Bacillus* before and after metal loaded.

The better results obtained with the biofilm used in batch studies compared to open systems seem to be related with the longer contact time of the chromium solution with the biofilm than in the continuous assays.

3.4. FTIR spectral analysis

The FTIR spectra of unloaded and metal loaded *Bacillus coagulans* biomass in the range of 500–4000 cm^{-1} were taken just to confirm the presence of functional groups that might be responsible for the biosorption process and presented in Fig. 6. As seen in this figure unloaded biomass displays a number of absorption peaks, reflecting the complex nature of the biomass. The spectrum pattern of unloaded biomass showed changes of certain bands in the region of 1600–750 and 3000–2800 cm^{-1} as compared to Cr(VI) loaded biomass. Band shifts were observed for the signals at 3350 cm^{-1} (indicative of bonded hydroxyl group and –NH stretching peak) [26], 1546 cm^{-1} (indicative of C–N stretching and N–H deformation), 1398 cm^{-1} (indicative of COO– anions), 1238 cm^{-1} (indicative of $–SO_3$ groups) and at 861 cm^{-1} (aromatic –CH stretching peak) [35]. These changes observed in the spectrum indicated the possible involvement in biosorption process of those functional groups on the surface of the biomass. These band shifts were stronger as the chromium concentration was higher.

4. Conclusions

Batch equilibrium experiments and column studies were conducted to determine the hexavalent chromium adsorption ability of a biofilm of *Bacillus coagulans* supported on granular activated carbon (GAC). The biofilm was studied through the quantification of the polysaccharides, 9.19 $mg/g_{biosorbent}$, and the quantification of the polymeric net, 75 $mg/g_{biosorbent}$. These results are indicative of a good adhesion of the bacteria to the GAC surface. The presence of binding sites enables EPS not only

to sequester minerals and nutrients for microbial growth, but also to remove toxic metals in biological treatments of wastewater. The results obtained with open systems showed uptake values of 1.50, 1.98 and 5.34 $mg/g_{biosorbent}$, respectively, for initial concentrations of 10, 50 and 100 mg/L of Cr(VI). These results allow to conclude that the biosorption capacity of the biofilm increased with increasing initial concentrations and a possible explanation could be the fact that the driving force for biosorption is the Cr concentration difference between the solution and the biosorbent. Thus, the high driving force due to the high chromium concentration resulted in better column performance. Studies made with multiple ions shown worse results than those obtained for the chromium solution. These results can be explained by the fact that the other metal ions and compounds can compete for the same active sites.

Data from column studies were described by Adams–Bohart and Wolborska models. These models were found suitable for describing the dynamic behaviour of the columns with respect to the inlet chromium concentration. The batch equilibrium data were reasonably well fitted by all the equations tested but the best fit was obtained with the Toth model isotherm. The fact that the fits obtained with Langmuir and Freundlich models showed worse results suggests that the binding of chromium does not occur as a monolayer on the surface of the biomass. The presence of functional groups on the cell wall surface of the biomass that may interact with the metal ion, was confirmed by FTIR.

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