Available online at www.sciencedirect.com
ScienceDirect
Journal of Hazardous Materials 153 (2008) 799–809
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Biosorption of Cr(VI) by three different bacterial species supported on granular activated carbon—A comparative study

C. Quintelas *, B. Fernandes, J. Castro, H. Figueiredo, T. Tavares

IBB-Institute for Biotechnology and Bioengineering, Centre for Biological Engineering, Universidade do Minho, Campus de Gualtar, Braga 4710–057, Portugal

Received 7 February 2007; received in revised form 5 September 2007; accepted 5 September 2007
Available online 12 September 2007

Abstract

The ability of three different bacterial species supported on granular activated carbon (GAC) to remove hexavalent chromium from low concentration liquid solutions was investigated, in batch and column studies. The microorganisms tested were Cr(VI) reducing types: *Streptococcus equisimilis* (CECT 926), *Bacillus coagulans* (CECT 12) and *Escherichia coli* (CECT 515). The results showed metal uptake values of 5.82, 5.35 and 4.12 mg/$g_{bios.}$, respectively, for *S. equisimilis*, *B. coagulans* and *E. coli*, for an initial metal concentration of 100 mg/l. In the same order and for the initial concentration of 50 mg/l, metal uptake values were 2.33, 1.98 and 3.60 mg/$g_{bios.}$. Finally, for the initial metal concentration of 10 mg/l, those values were, respectively, 0.66, 1.51 and 1.12 mg/$g_{bios.}$. Studies made with an industrial effluent, with the aim of testing these biofilms in a real situation, showed values of Cr uptake of 0.083, 0.090 and 0.110 mg/$g_{bios.}$, respectively, for *S. equisimilis*, *B. coagulans* and *E. coli*, for an initial concentration of 4.2 mg/l of total Cr. The quantification of polysaccharides, playing a key role in the whole process, was made and it was concluded that the production of polysaccharides is higher for *B. coagulans* followed by *S. equisimilis* and *E. coli* (9.19, 7.24 and 4.77 mg/$g_{bios.}$).

The batch studies data were described using the Freundlich, Langmuir, Redlich–Peterson, Dubinin–Radushkevich, Sips and Toth model isotherms. The best fit was obtained with Sips and Toth model isotherms, respectively, for the *S. equisimilis* and for the *B. coagulans* biofilms. For the *E. coli* biofilm the Freundlich, Redlich–Peterson, Sips and Toth models fitted very well to the experimental data. The Adams–Bohart, Wolborska and Yoon and Nelson models were applied to column studies data. Those models were found suitable for describing the dynamic behaviour of the columns with respect to the inlet chromium concentration. Obtained results showed that the biofilms tested are very promising for the removal of Cr(VI) in diluted industrial wastewater. Despite differences in the cell wall structure and composition, the three bacteria exhibit comparable sorption affinities towards chromium, in the open systems studies. The Gram-positive bacteria tested (*B. coagulans* and *S. equisimilis*) presented best metal removal percentages in batch studies.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Activated carbon; Bacteria; Biofilm; Biosorption; Chromium(VI)

1. Introduction

Chromium is a heavy metal with large industrial applications, such as in textile dyeing, chemicals and pigments production, wood preservation, tanning activity and electroplating for surface treatment [1]. This metal is the second most common inorganic contaminant of groundwater at hazardous waste sites [2]. The hexavalent form of chromium, usually present in the form of chromate (CrO_4^-) and dichromate ($Cr_2O_7^-$), possesses significant higher levels of toxicity than other valence states [3].

The conventional methods for heavy metal removal from industrial effluents are precipitation, coagulation, ion exchange, cementation, electro-dialysis, electro-winning, electro-coagulation, reverse osmosis [4], evaporation, solvent extraction and membrane separation [1]. These processes are expensive and present some technological problems, mainly when applied to diluted metal solutions and, subsequently, the search for clean and competitive technologies is strongly recomended. Biosorption is a process in which certain types of biomasses, viable or dead, may bind and concentrate heavy metals from aqueous solutions [5]. The mechanisms of biosorption may involve intracellular uptake and storage via active cationic transport systems, surface binding or same unidentified mechanisms. The chemical and biological characteristics of these

* Corresponding author. Tel.: +351 253604400; fax: +351 253678986.
E-mail address: cquintelas@deb.uminho.pt (C. Quintelas).

0304-3894/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2007.09.027

Nomenclature

Q_e	is the amount of metal ion sorbed by the biofilm at the equilibrium (mg/g)
Q_{max}	is the maximum metal sorption (mg/g)
C_e	is the concentration of metal in solution at the equilibrium (mg/l)
b	is the Langmuir adsorption equilibrium constant (l/mg)
K_f	represents the capacity of adsorption
n	represents the intensity of adsorption
K_R (l/g), a_R (l/mg) and β	represents the Redlich–Peterson constants and are empirical parameters without physical meaning [38]. β varies between 0 and 1
K_S (l^{bs} mg^{1-bs}/g), a_S $(l/mg)^{bs}$ and b_S	are the Sips isotherm parameters
K_t (mg/g), a_t and t	represents the Toth isotherm constants
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)
R	is the universal gas constant
k_{AB}	is the kinetic constant (l/(mg min)) for the Adams–Bohart model
N_0	is the saturation concentration (mg/l) for the Adams–Bohart model
C_0	is the inlet chromium concentration (mg/l)
C	are the effluent chromium concentrations (mg/l)
C_s	is the chromium concentration at the solid/liquid interface (mg/l)
D	is the axial diffusion coefficient (cm^2/min)
v	is the migration rate (cm/min)
β_a	is the kinetic coefficient of the external mass transfer (1/min)
β_0	is the external mass transfer coefficient with a negligible axial dispersion coefficient D
k_{YN}	is the rate constant (1/min)
τ	is the time required for 50% adsorbate breakthrough (min)
t	is the breakthrough time (min)

processes of uptake are important for understanding the role of metallic ions in basic cellular functions and also for detoxification of metal-polluted industrial effluents by application of biomass [6]. 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 [7].

In spite of being extremely small, bacteria have the largest surface area to volume ratio of any independent life form. The structural polymers in the bacteria cell wall provide acidic functional groups like carboxyl, phosphoryl and amino groups that are directly responsible for the reactivity of bacterial cells [8]. All the surfaces of bacteria are intrinsically reactive towards dissolved metals, despite the different surface formats between the different types of bacteria. It has been proved that, in some cases, growing cells are able to remove metals continuously through internal detoxification mechanisms [9].

Biofilms can be defined as communities of microorganisms attached to a surface [10,11]. The binding of metal ions with supported biofilms is relayed by several authors: Quintelas and Tavares [12,13] used a biofilm of *Arthrobacter viscosus* to remove Cr(VI), Cd(II), Pb(II) and Fe(II), Kang et al. [14] applied a biofilm of *Pseudomonas aeruginosa* to the removal of Cr(III), Ni(II) and Co(II), Leonhauser et al. [15] studied the behaviour of a biofilm of *A. hydrophila* and *P. putida* in mercury removal. The bacterial extracellular polymeric substances (EPS) synthesis is an aspect of special relevance in the development of biofilms.

The main composition of EPS includes a complex mixture of macromolecular biopolymers such as polysaccharides, proteins [16], lipids or humic substances [17]. On the other hand, these EPS building molecules contain ionisable functional groups such as carboxyl, phosphoric, amine and hydroxyl groups [17]. Several important functions can be attributed to EPS including adhesion to surfaces, aggregation of bacterial cells 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 exogenous organic compounds for the accumulation of nutrients from the environment and accumulation of enzymatic activities, such as digestion of exogenous macromolecules for nutrient acquisition, aiding the cells in uptaking metal nutrients [18]. Some of these functions play a key role in biosorption procedures.

Cr(VI) reduction by different microrganisms has been well documented in different studies [19]. Activated carbon is widely used as an adsorbent in industry due to its high adsorption capacity. This capacity is related to the pore structure and chemical nature of the carbon surface in connection with preparation conditions [20]. The behaviour of a biofilm supported on granular activated carbon (GAC) for the removal of heavy metals and organic compounds was studied by Quintelas el al. [21] and Quintelas and Tavares [12,13].

The investigation presents a comparative study of the behaviour of three different biofilms supported on granular activated carbon (GAC) in terms of the removal of chromium(VI). For that purpose *Bacillus coagulans*, *Streptococcus equisimilis* (both Gram-positive bacteria) and *Escherichia coli* (Gram-negative) were selected. The relationship between the Gram-positivity or negativity and the removal of Cr(VI) was also evaluated. 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 each one of the bacterial biofilms, 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 were quantified and the application of these systems to the treatment of a specific industrial effluent was made. All the equilibrium isotherms for the adsorption of Cr(VI) on the biofilms were described by Freundlich, Langmuir, Redlich–Peterson, Dubinin–Radushkevich, Sips and Toth isotherms. The dynamic behaviour of the columns with respect to the inlet chromium concentration was analysed by the Adams–Bohart, Wolborska and Yoon and Nelson models.

2. Materials and methods

2.1. Materials

The microorganisms used were the Cr(VI) reducing species *S. equisimilis* (CECT 926), *B. coagulans* (CECT 12) and *E. coli* (CECT 515) (Spanish Type Culture Collection, University of Valencia). 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 mgCr l^{-1} 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 completed in triplicate. GAC was placed in Erlenmeyer flasks of 250 ml with 150 ml of distilled water. It was sterilised at 120 °C for 20 min to release the air inside the pores. Then, it was placed in mini-columns (internal diameter = 2 cm, h = 30 cm) for open system studies. The microorganism culture and the nutrient broth were pumped through at a flow rate of 25 ml/min. A media 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) for the *B. coagulans* and with 5 g/l of beef extract (HIMEDIA), 10 g/l of peptone (Riedel), 5 g/l of NaCl (Prolabo) for *E. coli*, both with pH of 7.2, were used to grow the microorganisms for 3 days, aiming the optimisation of the adhesion. The same procedure was used for the *S. equisimilis* with the media Brain Heart Infusion CM0225 (Oxoid, 37 g/l, pH 7.4). All growth media were prepared accordingly to collection instructions. The experimental assays were made, during the biofilm formation and passage of the Cr solutions, at a temperature of 37 °C, once more accordingly to collection instructions. The high flow rate used (25 ml/min) allows the formation of a compact biofilm, resistant to the erosion stress promoted by hydrodynamic forces. The extensive coverage of GAC surface by the biofilm was confirmed (after dehidratation with different concentration of ethanol) by SEM (Leica Cambridge S360) and is shown in Fig. 1(a)–(c). The samples were gold coated prior to SEM observation. These figures intend to show that the biofilm uniformly covered the GAC surface for all the microorganisms and each of them is an example of many pictures taken at various zoomed areas.

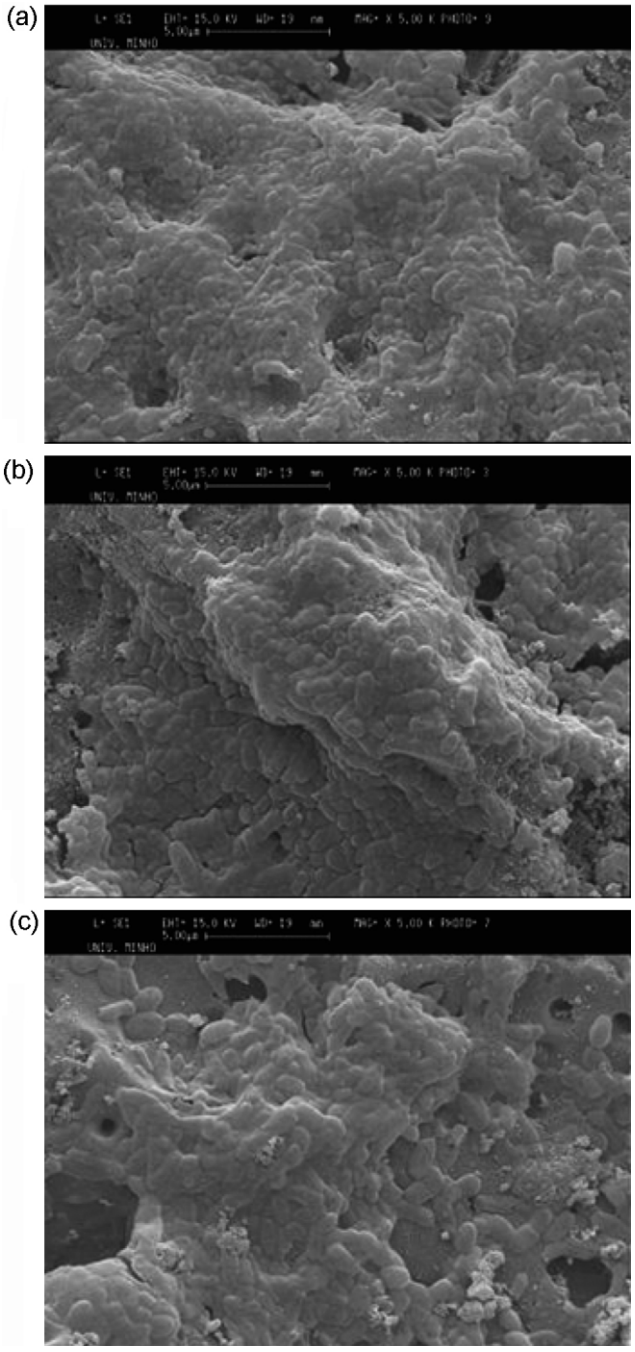

Fig. 1. SEM image of: (a) *Bacillus coagulans* biofilm supported on GAC, (b) *Escherichia coli* biofilm supported on GAC and (c) *Streptococcus equisimilis* biofilm supported on GAC.

After biofilm formation, beds were washed out and the metal solutions with Cr concentrations between 10 and 100 mg/l (prepared on laboratory) and a Cr concentration of 4.2 mg/l (industrial effluent), with pH ranging from 4.5 to 5.5, were passed through the columns with 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. Total Cr 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. The metal ionization state was confirmed by the diphenylcarbazide method.

2.2.2. Batch biosorption studies

The biofilm formation experiment was setup according to the previous section (see Section 2.2.1). The adsorption isotherms for chromium on GAC with biofilm were obtained from batch experiments at 37 °C. The experiments were performed with 250 ml Erlenmeyer flasks containing 150 ml of chromium solution. 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 for determining the time needed for equilibrium to be reached. Five millilitres samples were taken after reaching equilibrium, centrifuged at 4000 rpm during 5 min and the supernatant liquid was analysed for chromium ions.

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 [22]. It consists of three steps: (i) solubilization of the polysaccharide and polymeric net with a solution of glutardialdehyde, (ii) dialysis of the obtained solution and (iii) precipitation of the dialyzed. 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.

3. Modelling

3.1. Modelling column biosorption studies

3.1.1. The Adams–Bohart, Wolborska and Yoon and Nelson models

The prediction of the concentration–time profile or breakthrough curve for the effluent is one of the requirements for a successful design of a column adsorption process. The Adams–Bohart, Wolborska and Yoon and Nelson models can be used to predict the behaviour of breakthrough curves. All parameters are referred to in Nomenclature, in order of appearance.

3.1.1.1. The Adams–Bohart model. In 1920, Adams and Bohart [23] established the fundamental equations that described the relationship between C/C_0 and t in a flowing system for the adsorption of chlorine on charcoal. The model proposed assumes that the adsorption rate is proportional to the residual capacity of the activated carbon and to the concentration of the sorbing species. The mass transfer rates obey the following equations:

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

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

Two assumptions are made for the solution of these differential equation systems: $t \to \infty$ and $q \to N_0$. When the differential equations system is solved, the following equation is obtained with parameters k_{AB} and N_0:

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

From this equation values describing the characteristic operational parameters of the column can be determined from a plot of $\ln C/C_0$ against t at a given bed height and flow rate.

3.1.1.2. The Wolborska model. Wolborska [24] proposed another model for the description of adsorption dynamics using mass transfer equations for diffusion mechanisms in the range of the low-concentration breakthrough curve. The mass transfer in the fixed bed sorption is described by the following equations:

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

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

Three assumptions are made for the solution of this differential equation:

$C_s \ll C_b$, $\nu \ll U_o$ and axial diffusion negligible $D \to 0$ as $t \to 0$. The solution can be approximated to:

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

with

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

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

$$\nu = \frac{U_o C_o}{(N_o + C_o)} \tag{8}$$

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.

3.1.1.3. The Yoon and Nelson model. In 1984, Yoon and Nelson [25] developed a simple model for describing the adsorption and breakthrough of adsorbates with respect to activated carbon. The basis of this model is the assumption that the rate of decrease

Table 1
Isotherm models used to represent the equilibrium of biosorption

Isotherm model	Equation	Theory	Reference
Langmuir	$Q_e = (Q_{max} b C_e)/(1 + bC_e)$	Established a relationship between the amount of gas sorbed on a surface and the pressure of gas. Assumes monolayer coverage of adsorbate over a homogenous adsorbent surface.	Langmuir [27]
Freundlich	$Q_e = K_f C_e^{1/n}$	This exponential equation assumes that as the adsorbate concentration increases so too does the concentration of adsorbate on the adsorbent surface. Can be applied to non-ideal sorption on heterogeneous surfaces as well as multilayer sorption.	Freundlich [28]
Redlich–Peterson	$Q_e = (K_R C_e)/(1 + a_R C_e^{\beta})$	This isotherm model incorporates features of both the Langmuir and Freudlich isotherms and may be used to represent adsorption equilibria over a wide concentration range.	Redlich and Peterson [29]
Sips	$Q_e = (K_S C_e^{1/bs})/(1 + a_S C_e^{1/bs})$	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 a monolayer sorption capacity characteristics of the Langmuir isotherm.	Sips [30]
Toth	$Q_e = (K_t C_e)/[(a_t + C_e)^{1/t}]$	Derived from potencial theory 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.	Toth [31]
Dubinin–Radushkevich	$Q_e = q_D \exp(-B_D[RT \ln(1 + 1/C_e)]^2)$	The characteristic sorption curve is related to the porous structure of the sorbent.	Dubinin and Radushkevich [32]

in the probability of adsorption for each adsorbate molecule is proportional to the probability of adsorbate adsorption and to the probability of adsorbate breakthrough on the adsorbent. The Yoon and Nelson model can be expressed by the following equation:

$$\ln\left(\frac{C}{(C_0 - C)}\right) = k_{YN}t - \tau k_{YN} \quad (9)$$

The values values of k_{YN} and τ can be calculated from the plot of $\ln(C/(C_0 - C))$ versus t.

3.2. Adsorption isotherm models

The distribution of Cr(VI) between the liquid phase and the solid adsorbent phase is a measure of the position of equilibrium in the adsorption process [26] and can be expressed by isotherm models. Six isotherm equations have been tested in the present study and are represented in Table 1. The simplest method to determine isotherms constants for two parameter isotherms (Langmuir, Freundlich and Dubinin–Radushkevich) is to convert the equation to a linear form and then to apply linear regression. For the other equations, the model parameters were estimated by non-linear regression using MATLAB and EXCEL softwares.

4. Results and discussion

It was confirmed that wall characteristics are determinant for good biosorption performances, depending on surface functional groups and mainly on the ability of the bacterium to protect itself from the xenobiotic environment. These preliminary results reveal that further studies are needed to establish the basic characteristics of biomass to define a robust and effective biosorbent, for different ranges of ions concentrations.

Biosorption investigations on bacteria in the acidic pH range have demonstrated a reduction in the available metal-binding sites due to protonation or interaction between cations and negative charges of acidic functional groups of polysaccharides [6]. The studies made by this group were performed at pH of 7.2–7.4 (during the biofilm formation) which proves that all the metal-binding sites are available at the beginning of the biosorption process.

The inclusion of an industrial effluent in this study aims to evaluate the behaviour of these biosorption systems in the presence of an industrial effluent obtained from a tannery facility, known to include several metal ions and organic substances in its composition that may compete with Cr for the same active sites on the biosorbent surface.

4.1. Column studies

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

The results showed metal uptake values of 5.82, 5.35 and 4.12 mg/$g_{bios.}$, respectively, for *S. equisimilis*, *B. coagulans* and *E. coli*, for the initial concentration of 100 mg/l; 2.33, 1.98 and 3.60 mg/$g_{bios.}$ for the initial concentration of 50 mg/l; and 0.66, 1.51 and 1.12 mg/$g_{bios.}$ for the most diluted initial concentration used, 10 mg/l (Fig. 2). The performances of each of the bacteria are comparable, despite the differences between their cell walls structure and composition. Kulczycki et al. [8] explained this with the specific chemical reactivity of functional groups (e.g., carboxyl and phosphoryl groups) that occur within the structural polymers of cell walls of all bacteria. Industrial effluent was also

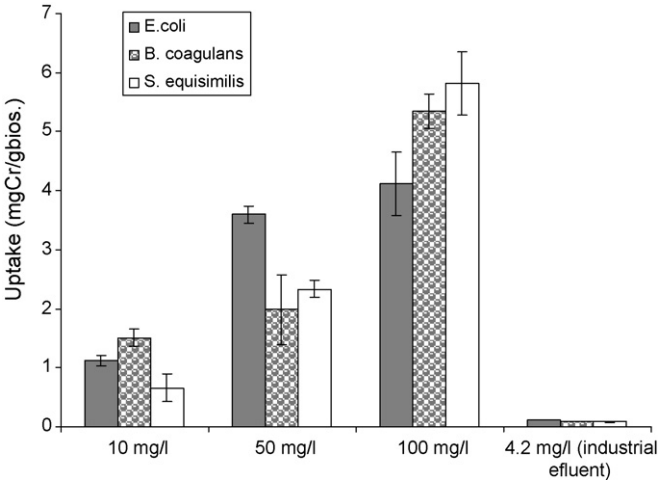

Fig. 2. Uptake values obtained for the column studies as a function of the inlet chromium concentration, for all the different biofilms tested.

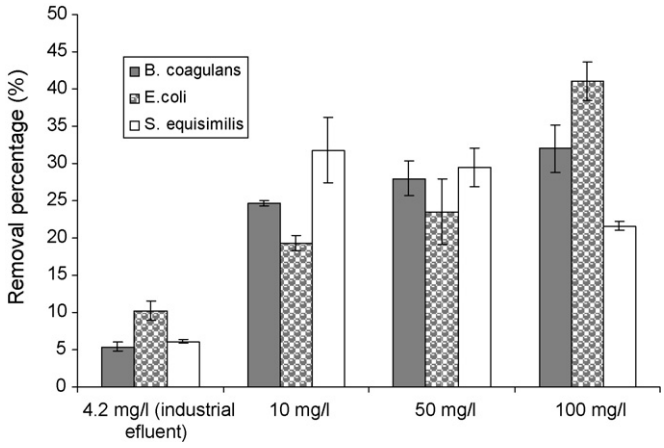

Fig. 4. Removal percentage values after 10 h of experimental assay for all the concentrations tested and for the industrial effluent, for all the biofilms tested.

included in this approach, for comparison purposes, reaching an uptake never higher than 0.12 mg/g_{bios}.

Fig. 3 illustrates the resulting breakthrough curves for Cr at different inlet concentrations and for all the different biofilms tested. From its analysis it can be concluded that the biosorption process occurs in two stages, the first one is very fast and reversible and is mainly based on physical adsorption, ion exchange and chemisorption. The second one is a much slower and irreversible metal binding process that may include covalent bonding, surface precipitation, redox reactions or crystallization on the cell surface [33]. Padmesh et al. [34] affirm that the biosorption capacity of the biofilm increases with increasing initial + metal concentration and suggesting that the driving force for biosorption is the concentration difference between the chromium concentration in the solution and the chromium concentration on the biosorbent. Thus, the high driving force due to the high chromium concentration in the liquid phase results in better column performance. The present study confirms this statement in terms of uptake (Fig. 2), the removal percentage after 10 h of experimental assay shows that the removal capacity increased with the concentration for the *E. coli* and the *B. coagulans* biofilms. For the *S. equisimilis* biofilm, the removal capacity decreases with the increase of concentration, for the solutions of 10, 50 and 100 mg/l, Fig. 4. A possible explanation could be that this bacterium is more sensitive to the xenobiotic effect caused by the increase on the concentration of chromium. Industrial effluent was also included in this approach, with best removal percentage achieved with *E. coli*.

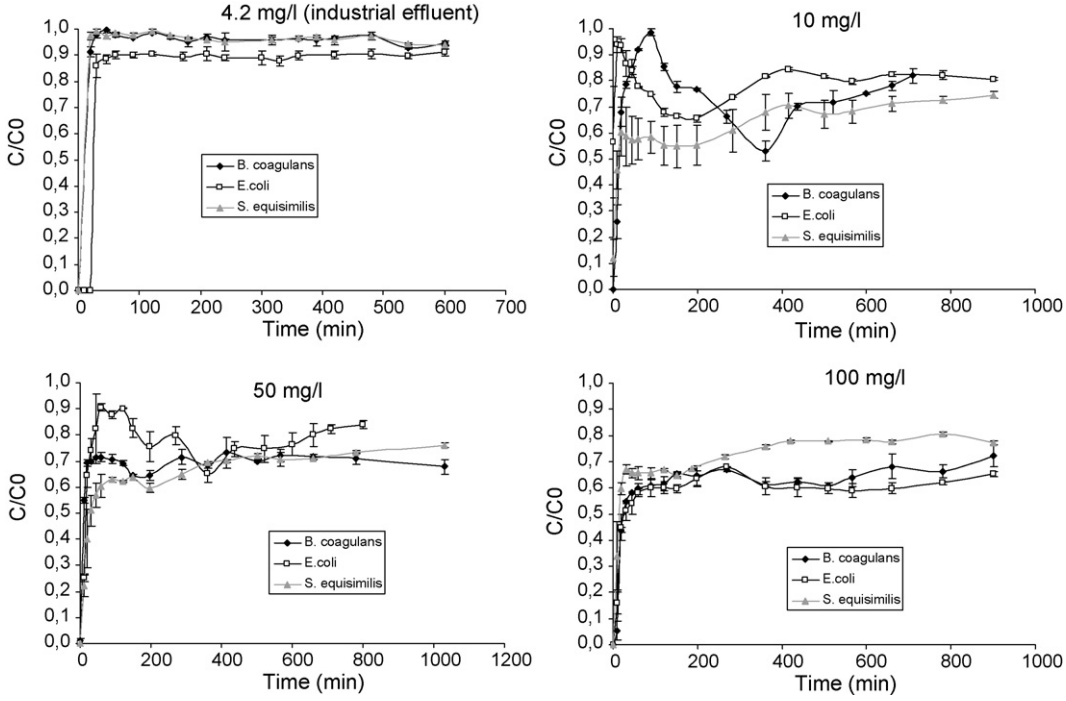

Fig. 3. Breakthrough curves for Cr(VI) biosorption onto *B. coagulans*, *E.coli* and biofilms supported on GAC at different inlet metal concentrations.

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

The Adams–Bohart and the Wolborska sorption models were used to describe the experimental data. This approach was focused on the estimation of characteristic parameters, such as maximum adsorption capacity (N_0) and kinetic constant (k_{AB}) of Adams–Bohart model and kinetic coefficient of the external mass transfer (β_a) of 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 concentration as presented in Table 2 together with the respective correlation coefficients. These models were applied to all the different biofilms. The value of maximum adsorption capacity (N_0) increased with increasing inlet chromium concentration, as expected. The predicted and experimental breakthrough curves, with respect to inlet chromium concentration, are shown in Fig. 5. For the most diluted concentration used, the discrepancies are relatively higher (data not shown, for the concentration of 10 mg/l) for the *B. coagulans* and *E. coli* biofilms. The relatively higher errors obtained for the lower concentrations of metal seem to be related to the metabolic activity which is not quantified and consequently is not introduced in the model. The *S. equisimilis* data were well fitted with this model. A probable reason for this evidence could be that the metabolic activity for this microorganism remains the same for all ranges of concentration used with little influence in the overall process. Although the Adams–Bohart (or Wolbraska) model provides a simple and comprehensive approach to running and evaluating sorption-column tests, its validity is limited to the range of conditions used.

4.1.3. Application of the Yoon and Nelson model

A simple theoretical model developed by Yoon–Nelson was applied to describe the breakthrough behaviour of chromium on three different biofilms supported on GAC. The values of k_{YN} and τ were determined from $\ln [C/(C_0-C)]$ against t plots at different inlet chromium concentrations varied between 10 and 100 mg/L. These values are listed in Table 2. The theoretical curves are compared with the corresponding experimental data in Fig. 6, and it could be concluded that the experimental breakthrough curves were followed very closely by those predicted by the Yoon–Nelson model for all the concentrations tested, for the biofilm of *S. equisimilis* supported on GAC. For the biofilm of *B. coagulans* there is a good agreement between the experimental and predicted values for the higher concentrations used. As with the models of Adams–Bohart and Wolborska, the most diluted concentration showed large discrepancies, most probably for the same reason. For the biofilm of *E. coli*, there is good agreement between the experimental and predicted values for periods of time longer than 300 min at the higher concentrations studied. Despite some discrepancies found between the experimental results and data regression, the model proposed by Yoon–Nelson provided a good correlation of the effects of inlet chromium concentration.

4.1.4. Effects of other ions presents on the solution

Studies made with the industrial effluent, containing several different ions, showed values of Cr uptake of 0.083, 0.110 and 0.090 $mg/g_{bios.}$, respectively, for *S. equisimilis*, *E. coli* and *B. coagulans* for an initial concentration of 4.2 mg/l (Fig. 2). As with the uptake value, after 10 h of experimental assay, the best removal value was obtained with the biofilm of *E. coli* (10.2%, Fig. 4). The value obtained for the removal percentage with the most diluted solution used (10 mg/l) was of 19.3%, 24.7% and 31.8% (after 10 h of experiment), respectively, for the *E. coli*, *B. coagulans* and *S. equisimilis* biofilms. These values were much higher than the values obtained with the industrial effluent (10.2%, 5.4% and 6.1%, respectively, for *E. coli*, *B. coagulans* and *S. equisimilis* biofilms), for the same period of time. As it was shown in Figs. 3 and 4, the process of metal removal is inhibited by the presence of other ions.

4.2. Batch studies

The pH dependence of metal adsorption can largely be related to type and ionic state of the functional group present in the adsorbent and also to the metal chemistry in the solution. High adsorption of Cr(VI) at low pH can be explained by Cr species

Table 2
Parameters predicted from the Adams–Bohart, Wolborska and Yoon and Nelson models at different inlet chromium concentrations and for the three different biofilms

C_0 (mg/l)	N_0 (mg/l)	k_{AB} (l/(mg min))	β_a (1/min)	R^2	τ (min)	k_{YN} (min^{-1})	R^2
Bacillus coagulans							
10	658.5	4.44E−5	0.029	0.94	61.11	0.0018	0.91
50	10436.7	1.76E−6	0.018	0.89	519.25	0.0004	0.83
100	13091.3	2.15E−6	0.028	0.89	554.83	0.0006	0.84
Escherichia coli							
10	8049.8	6.22E−5	0.50	0.84	358.36	0.0014	0.84
50	44801.6	1.26E−5	0.57	0.87	284.21	0.0014	0.85
100	495370.4	1.08E−6	0.54	0.86	186.2	0.0005	0.91
Streptococcus equisimilis							
10	20424.8	2.66E−5	0.54	0.89	10	269.11	0.0009
50	67768.9	7.53E−6	0.51	0.83	50	490.38	0.0008
100	138421.1	3.23E−6	0.48	0.86	100	463.33	0.0012

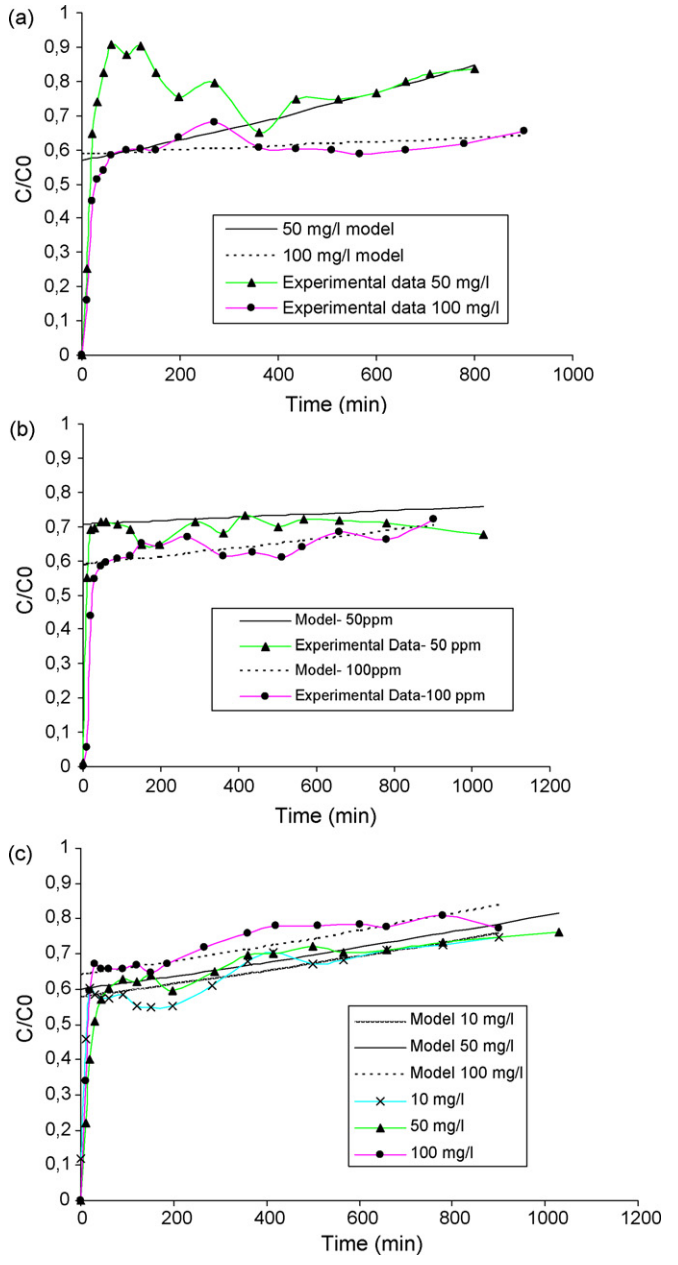

Fig. 5. Comparison between the experimental results and those predicted by the models for the inlet solute concentration of 50 and 100 mg/l, according to the Adams–Bohart (or Wolbraska) model, for all the biofilms. (a) *E. coli* biofilm supported on GAC, (b) *B. coagulans* biofilm supported on GAC, (c) *S. equisimilis* biofilm supported on GAC.

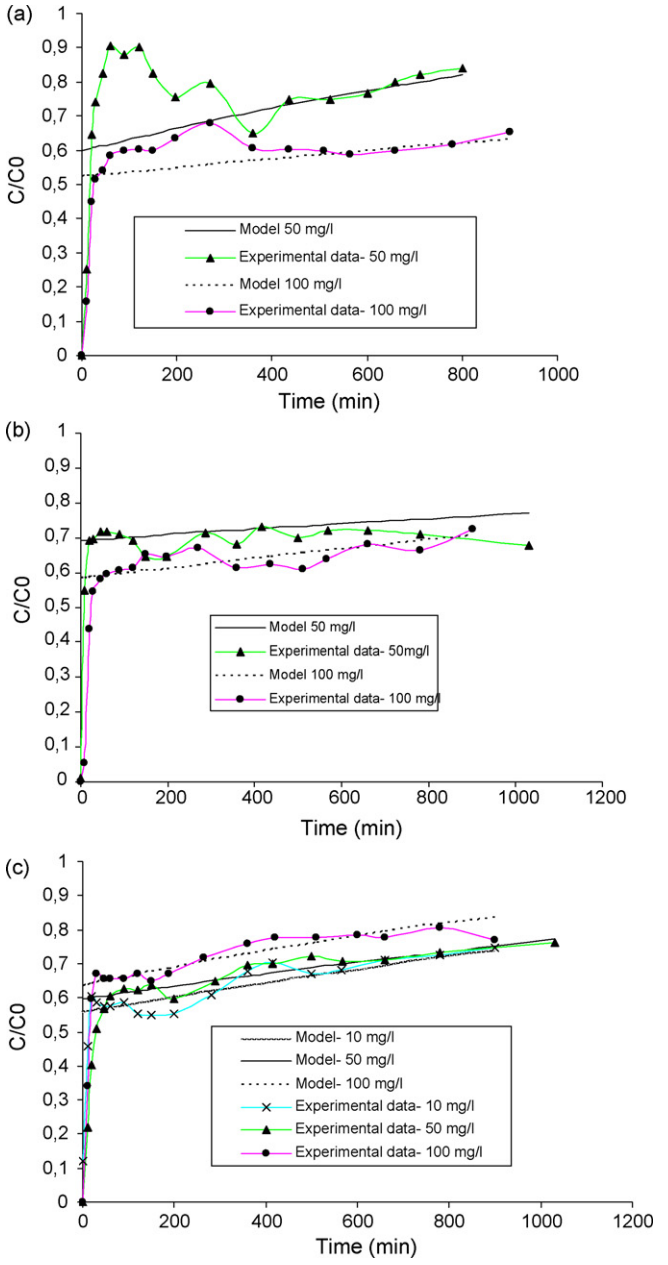

Fig. 6. Comparison between the experimental data and those predicted by the models for the inlet solute concentration of 50 and 100 mg/l, according to the Yoon and Nelson model, for all the biofilms. (a) *E. coli* biofilm supported on GAC, (b) *B. coagulans* biofilm supported on GAC, (c) *S. equisimilis* biofilm supported on GAC.

and adsorbent surface characteristics. Above pH 8, only CrO_4^{2-} is stable and as the pH decreases into the region 3–6, the equilibrium shifts to dichromate according to the overall equilibrium:

$$2CrO_4^{2-} + 2H^+ \rightarrow Cr_2O_7^{2-} + H_2O \quad (10)$$

Decreasing pH results in the formation of more polymerised Cr oxide species. The surface of the adsorbent becomes highly protonated and favours the uptake of Cr(VI) in the anionic form, under acidic conditions. With increase in pH, the degree of protonation of the surface reduces gradually and hence adsorption is decreased. Furthermore, as pH increases there is competition between OH^- and chromate ions (CrO_4^{2-}), the former being the dominant species at higher pH values. The net positive surface potential of sorbent decreases, resulting in the weakening of electrostatic forces between sorbent and sorbate, which ultimately leads to reduced sorption capacity [26]. The present study used solutions with pH ranging from 4.5 to 5.5.

Adsorption experiments at initial Cr(VI) concentrations from 50 to 1000 mg/l were performed with fixed doses (1.5 g/150 ml) of GAC covered with biofilm. The results indicated that the percentage of Cr(VI) removal decreased as the initial concentration

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

	Bacillus coagulans		*Escherichia coli*		*Streptococcus equisimilis*	
C_0 (mg/l)	C_{eq} (mg/l)	Rp (%)	C_{eq} (mg/l)	Rp (%)	C_{eq} (mg/l)	Rp (%)
73.2	38.9	46.9	46.4	36.6	20.5	72.0
105.9	62.6	40.9	74.8	29.4	37.8	64.3
247.0	169.5	31.4	198.8	19.5	138.2	44.1
365.2	251.5	31.1	303.0	17.0	222.4	39.1
546.9	393.0	28.1	462.9	15.4	355.2	35.1
743.6	579.8	22.0	655.1	11.9	498.8	32.9
947.4	784.9	17.2	845.3	10.8	508.5	46.3

of Cr(VI) was increased (Table 3). Cr(VI) removal ranged from 46.9% to 17.2% at initial Cr(VI) concentration of 50–1000 mg/l, for the biofilm of *B. coagulans*, from 36.6% to 10.8%, for the *E. coli* biofilm and from 72% to 46.3%, to the *S. equisimilis* biofilm. Padmesh et al. [34] explain this decrease based on the fact that at lower concentration, the ratio of the initial moles of chromium to the available surface area may be lower and subsequently the fractional sorption independent of the initial concentrations. On the other hand, at higher concentration the available sites become fewer compared to the moles of chromium present and hence the removal percentage of chromium is dependent of the initial percentage. Horsfall Jr. et al. [3] reinforce this idea and affirm that at higher concentrations, the reduced average distance between the adsorbing species affects the charge distribution of its neighbours, thus altering the ability of the species to migrate to the biomass surface, which results in reduced adsorption.

In terms of removal percentage, the Gram-positive bacteria tested (*B. coagulans* and *S. equisimilis*) presented the best results, in the batch studies (Table 3). This seems to be related to the higher contact time of the chromium solution with the biofilm in batch studies compared to the residence time in column studies.

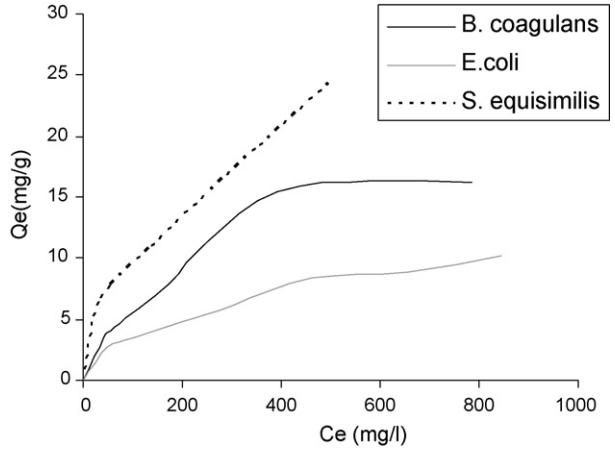

Fig. 7. Chromium adsorption isotherms at 37 °C using the three different biofilms supported on GAC.

4.2.1. Adsorption isotherm studies

The process of adsorption of a substance from one phase to a surface of another in a specific system leads to a thermodynamically defined distribution of that substance between the two 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 solution [35]. For the biosorbent used (biofilm + GAC), adsorption isotherms were experimentally determined and results are shown in Fig. 7. Six different models – Langmuir, Freundlich, Redlich–Peterson, Dubinin–Radushkevich, Sips and Toth – were fitted and constants calculated are presented in Table 4 and the comparison between the experimental results and those predicted by different models are shown in Fig. 8. For the biofilm of *E. coli*, the worst fit was obtained with the Langmuir and Dubinin–Radushkevich models and for the other four models used the fit was very good. For the *B. coagulans* biofilm the best fit was obtained with the

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

	Q_{max}	b	R^2
Langmuir parameters			
E. coli	9.533	0.0077	0.943
Bacillus	19.455	0.0052	0.976
Streptococcus	16.077	0.0220	0.931
	K_f	n	R^2
Freundlich parameters			
E. coli	0.408	2.091	0.991
Bacillus	0.431	1.751	0.970
Streptococcus	1.226	2.147	0.984
	q_D	B_D	R^2
Dubinin–Radushkevich parameters			
E. coli	9.677	45.862	0.928
Bacillus	17.264	36.438	0.989
Streptococcus	14.415	1.951	0.740

	K_R	a_R	β	R^2
Redlich–Peterson parameters				
E. coli	0.693	1.500	0.537	0.992
Bacillus	0.088	0.0038	1.000	0.980
Streptococcus	2.163E + 5	2.415E + 5	0.475	0.989
	K_S	a_S	b_S	R^2
Sips parameters				
E. coli	0.321	0.0057	0.543	0.992
Bacillus	0.051	0.0024	1.120	0.981
Streptococcus	1.744	−0.279	0.169	0.999
	K_t	a_t	t	R^2
Toth parameters				
E. coli	0.402	−0.205	1.928	0.992
Bacillus	2.788E + 7	1381	0.366	0.988
Streptococcus	0.578	−13.880	2.480	0.995

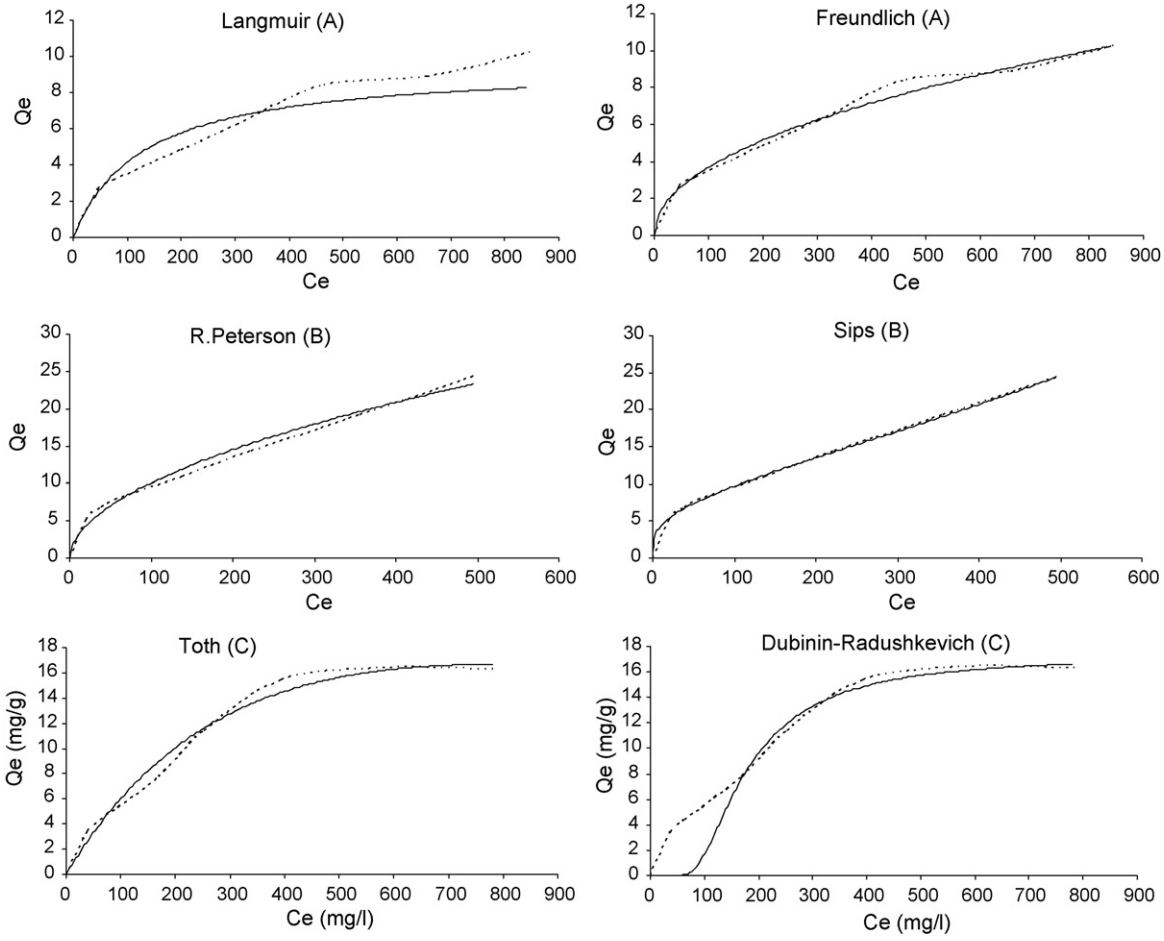

Fig. 8. Comparison between the experimental results and those predicted by different models for the chromium adsorption isotherms, (A) for the biofilm of *E. coli*, (B) for the biofilm of *S. equisimilis*, (C) for the biofilm of *B. coagulans*, all supported on GAC (—, model; - - -, experimental data).

Toth model isotherm and for the *S. equisimilis* biofilm the best fit was the obtained with the Sips model. The fact that the fit obtained, for all the biofilms, with Langmuir model represents the worst results suggests that the binding of chromium do not occur as a monolayer on the surface of the biomass and this surface is far from energetically homogeneous.

4.3. Quantification of polysaccharides and total polymers

The attachment of bacteria to a solid surface forming a biofilm can be described by a process with two steps: first, the microorganisms come close enough to the surface to be weakly held by electrostatic forces (at this point the cells can be easily removed from the surface) and second, the attached microorganisms are harder to remove from the surface, as the bacteria produce exopolysaccharides which eventually form the biofilm matrix which is firmly adherent to the substract [36]. The polyssacharide and polymeric net give important informations about the capacity of biofilm formation by the microrganism. 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 [37].

The production of polysaccharides is higher for *B. coagulans* follow by *S. equisimilis* and *E. coli* (9.19, 7.24 and 4.77 mg/$g_{bios.}$). The results are from the same order of magnitude and it can be concluded that, in all the cases, bacteria have a very good adhesion of to GAC and good qualities for metal ions entrapment.

5. Conclusions

- Three different bacterial species (*S. equisimilis*, *B. coagulans* and *E. coli*) supported on GAC were tested to remove hexavalent chromium from aqueous solutions and the overall results were very promising.
- The quantification of EPS for the three biofilms showed that their production is of the same order of magnitude and it can be concluded that, in all the cases, bacteria have a very good adhesion to GAC and promising qualities for metal ions entrapment.
- These studies were focused in hexavalent chromium fixation but it was concluded that the presence of other ions does affect the uptake and removal efficiency of the biosorbent. Still, the procedure optimization will allow good results in industrial applications.
- The equilibrium data were well described by the Sips and Toth model for the *S. equisimilis* and for *B. coagulans* biofilms. The Freundlich, Redlich–Peterson, Sips and Toth isotherms

fitted very well to the experimental equilibrium data obtained with *E. coli* biofilm. This difference in the sorbent properties may result from the fact that *B. coagulans* and *S. equisimilis* are Gram-positive, while *E. coli* is Gram-negative, with the consequent difference in the sorption properties. The possible reduction of Cr(VI), negatively charged, to Cr(III), a cation, enhances the sorption ability on the Gram-negative *E. coli*.

- The Adams–Bohart, Wolborska and Yoon and Nelson models were applied to column studies data with a good correlation between the predicted and the experimental data.

Acknowledgement

The authors would like to gratefully acknowledge the financial support of this project by the Fundação para a Ciência e Tecnologia, Ministério da Ciência e Tecnologia, Portugal (POCTI/CTA/44449/2002), financed by FEDER.

References

[1] A. Agrawal, V. Kumar, B.D. Pandey, Remediation options for the treatment of electroplating and leather tanning effluent containing chromium—a review, Miner. Process. Extr. Metall. Rev. 27 (2006) 99–130.
[2] R. Horton, W.A. Apel, V.S. Thompson, P.P. Sheridan, Low temperature reduction of hexavalent chromium by a microbial enrichment consortium and a novel strain of Arthrobacter aurescens, BMC Microbiol. 6 (2006) 1–8.
[3] M. Horsfall Jr., F. Ogban, E.E. Akporhonor, Sorption of chromium(VI) from aqueous solution by cassava (*Manihot sculenta* CRANZ) waste biomass, Chem. Biodivers. 3 (2006) 161–173.
[4] S.S. Ahluwalia, D. Goyal, Microbial and plant derived biomass for removal of heavy metals from wastewater, Bioresource Technol. 98 (2007) 2243–2257.
[5] G. Naja, B. Volesky, Behavior of the mass transfer zone in a biosorption column, Environ. Sci. Technol. 40 (2006) 3996–4003.
[6] M. Prado Acosta, E. Valdman, S.G.F. Leite, F. Battaglini, S.M. Ruzal, Biosorption of copper by *Paenibacillus polymyxa* cells and their exopolysaccharide, World J. Microb. Biotechnol. 21 (2005) 1157–1163.
[7] 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.
[8] E. Kulczycki, F.G. Ferris, D. Fortin, Impact of cell wall structure on the behaviour of bacterial cells as sorbents of cadmium and lead, Geomicrobiol. J. 19 (2002) 553–565.
[9] B. Godlewska-Zylkiewicz, Microorganisms in inorganic chemical analysis, Anal. Bioanal. Chem. 384 (2006) 114–123.
[10] G. OïToole, H.B. Kaplan, R. Kolter, Biofilm formation as microbial development, Annu. Rev. Microbiol. 54 (2000) 49–79.
[11] P. Le Cloirec, Y. Andrès, C. Faur-Brasquet, C. Gérente, Engineered biofilms for metal ion removal, Rev. Environ. Sci. Bio/Technol. 2 (2003) 177–192.
[12] C. Quintelas, T. Tavares, Lead(II) and iron(II) removal from aqueous solution: biosorption by a bacterial biofilm supported on granular activated carbon, J. Resource Environ. Biotechnol. 3 (2002) 196–202.
[13] C. Quintelas, T. Tavares, Removal of chromium(VI) and cadmium(II) from aqueous solution by a bacterial biofilm supported on granular activated carbon, Biotech. Lett. 23 (2001) 1349–1353.
[14] S.Y. Kang, P.J. Bremer, K.W. Kim, A.J. McQuillan, Monitoring metal ion binding in single-layer *Pseudomonas aeruginosa* biofilms using ATR-IR spectroscopy, Langmuir 22 (2006) 286– 291.
[15] J. Leonhauser, M. Rohricht, I. Wagner-Dobler, W.D. Deckwer, Reaction engineering aspects of microbial mercury removal, Eng. Life Sci. 6 (2006) 139–148.
[16] A. Omoike, J. Chorover, Spectroscopic study of extracellular polymeric substances from *Bacillus subtilis*: aqueous chemistry and adsorption effects, Biomacromolecules 5 (2004) 1219–1230.
[17] 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.
[18] 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.
[19] D.R. Lovley, E.J.P. Phillips, Reduction of chromate by *Desulfovibrio vulgaris* and its c_3 cytochrome, Appl. Environ. Microbiol. 60 (1994) 726–728.
[20] I. Uzun, F. Guzel, Adsorption of some heavy metal ions from aqueous solution by activated carbon and comparison of percent adsorption results of activated carbon with those of some other adsorbents, Turk. J. Chem. 24 (2000) 291–297.
[21] 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.
[22] R. Oliveira, J. Azeredo, A new method for precipitating bacterial exopolysaccharides, Biotechnol. Tech. 5 (1996) 341–344.
[23] G. Bohart, E.Q. Adams, Some aspects of the behaviour of charcoal with respect to chlorine, J. Am. Chem. Soc. 42 (1920) 523–544.
[24] A. Wolborska, Adsorption on activated carbon of *p*-nitrophenol from aqueous solution, Water Res. 23 (1989) 85–91.
[25] Y.H. Yoon, J.H. Nelson, Application of gas adsorption kinetics—II. A theoretical model for respirator cartridge service life and its practical applications, Am. Ind. Hyg. Assoc. J. 45 (1984) 509–516.
[26] S. Mor, K. Ravindra, N.R. Bishnoi, Adsorption of chromium from aqueous solution by activated alumina and activated charcoal, Bioresource Technol. 98 (2007) 954–957.
[27] I. Langmuir, Adsorption of gases on plane surfaces of glass, mica and platinum, J. Am. Chem. Soc. 40 (1918) 1361–1403.
[28] H. Freundlich, Adsorption in solutions, Phys. Chem. 57 (1906) 384–410.
[29] O. Reddlich, D.L. Peterson, A useful adsorption isotherm, J. Phys Chem. 63 (1959) 1024.
[30] R. Sips, Combined form of Langmuir and Freundlich equations, J. Chem. Phys. 16 (1948) 490–495.
[31] J. Toth, State equations of the solid gas interface layer, Acta Chim. Acad. Sci. Hung. 69 (1971) 311–317.
[32] M.M. Dubinin, L.V. Radushkevich, Equation of the characteristic curve of activated charcoal, Chem. Zentr. 1 (1947) 875.
[33] R. Munoz, M.T. Alvarez, A. Munoz, E. Terrazas, B. Guieysse, B. Mattiasson, Sequencial removal of heavy metals ions and organic pollutants using an algal-bacterial consortium, Chemosphere 63 (2006) 903–911.
[34] T.V.N. Padmesh, K. Vijayaraghavan, G. Sekaran, M. Velan, Batch and column studies on biosorption of acid dyes on fresh water macro alga *Azolla filiculoides*, J. Hazard. Mater. B 125 (2005) 121–129.
[35] P. Lodeiro, B. Cordero, Z. Grille, R. Herrero, M.E. Sastre de Vicente, Physicochemical studies of cadmium (II) biosorption by the invasive alga in europe *Sargassum muticum*, Biotechnol. Bioeng. 88 (2004) 237–247.
[36] S.G. Parkar, S.H. Flint, J.S. Palmer, J.D. Brooks, Factors influencing attachment of thermophilic bacilli to stainless steel, J. Appl. Microbiol. 90 (2001) 901–908.
[37] H. Liu, H.P. Fang, Characterization of electrostatic binding sites of extracellular polymers by linear programming analysis of titration data, Biotechnol. Bioeng. 80 (2002) 806–811.
[38] V.J.P. Vilar, C.M.S. Botelho, R.A.R. Boaventura, Equilibrium and kinetic modelling of Cd (II) biosorption by algae *Gelidium* and agar extraction algal waste, Water Res. 40 (2006) 291–302.