Bioresource Technology 142 (2013) 368–374

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Removal of Ni(II) from aqueous solutions by an *Arthrobacter viscosus* biofilm supported on zeolite: From laboratory to pilot scale

Cristina Quintelas [a,*], Ricardo Pereira [a], Ecem Kaplan [b], Teresa Tavares [a]

[a] IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[b] Department of Bioengineering, Yildiz Technical University, Istanbul, Turkey

HIGHLIGHTS

- In this study we examine the treatment of nickel contaminated solutions.
- For the treatment we used *A. viscosus* supported on zeolite materials.
- The higher removal percentages obtained were 98%, for batch and minicolumns assays.
- For pilot-scale assay, the removal percentage obtained was 89%.

ARTICLE INFO

Article history:
Received 27 March 2013
Received in revised form 14 May 2013
Accepted 16 May 2013
Available online 23 May 2013

Keywords:
Bacteria
Bioreactor
Isotherms
Kinetics
Nickel

ABSTRACT

This study discusses the retention of Ni(II) by *Arthrobacter viscosus* supported on zeolite 13 X in batch mode and in continuous mode, at laboratory scale and at pilot scale. The maximum adsorption capacities of 28.37, 20.21 and 11.13 mg/g were recorded for lab scale batch, for continuous lab scale minicolumns and for pilot scale bioreactors, respectively. The Sips isotherm and pseudo second order kinetics described well the observations registered in batch assays. The Adams–Bohart, Thomas and Yoon–Nelson models were applied to data obtained with the pilot scale bioreactor and a good fit was reached for Adams–Bohart and for Yoon–Nelson models. A fed-batch was performed at lab scale and the applicability of the biofilm in continuous mode for the described purpose was confirmed. The sorption mechanism was investigated in detail through FTIR, SEM and EDX analyses.

1. Introduction

The disposal of heavy metals on water streams is a problem that needs to be solved. Several industrial activities are responsible for this disposal as, for example, metal-plating and electroplating, dyeing operations, mining and metallurgical activities, nuclear power plants operation, aerospace industries, battery manufacturing, paints and pigments production, glass processing and municipal and storm water runoff (Turp et al., 2011). Nickel is one of the heavy metals of environmental concern. Nickel toxicity to humans has received intensive attention due to its carcinogenic behavior (Macomber and Hausinger, 2011). Conventional methods for removing heavy metals from aqueous solutions in industrial wastewaters include chemical precipitation, solvent extraction, coagulation, electrolysis, ion exchange, membrane separation and adsorption (Ramana et al., 2012; Shinde et al., 2012) but most of these methods are expensive or generate harmful wastes.

The use of microorganisms for the removal of nickel had an increment in recent years as a result of the need to find more eco-friendly technologies. Santos Rodrigues et al. (2012) studied the nickel biosorption onto dry biomass of *Arthrospira* (*Spirulina*) *platensis* and *Chlorella vulgaris* and Shinde et al. (2012) investigated the removal of Ni(II) ions from aqueous solutions by biosorption onto two strains of *Yarrowia lipolytica*. The first authors reported that both bacteria are able to remove Ni but have more affinity to lead and zinc. The second authors found that both strains of bacteria displayed an effective performance in removing Ni(II) ions at low as well as at high concentrations. Qian et al. (2012) made a very interesting report about the simultaneous biodegradation of Ni–citrate complexes and removal of nickel from solutions by *Pseudomonas alcaliphila*.

The use of biomaterials as agricultural/forest wastes were also reported. Reddy et al. (2012) optimized the biosorption of Cd(II), Cu(II) and Ni(II) by chemically modified *Moringa oleifera* leaves powder and Ramana et al. (2012) studied the removal of nickel from aqueous solutions by citric acid modified *Ceiba Pentandra* hulls. Both authors found very promising results.

* Corresponding author. Tel.: +351 253604400; fax: +351 253604429.
E-mail address: cquintelas@deb.uminho.pt (C. Quintelas).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.05.059

The adsorption by zeolites is often an ion exchange process. Accordingly to Can et al. (2010), ion exchange is considered to be one of the most cost effective methods. The typical applications for the zeolite types A and X include drying, purification and separation of gases and liquids (Schumann et al., 2012). The use of zeolites for the removal of heavy metals from aqueous media is well known. Turp et al. (2011) analyzed the adsorption of Ni by zeolites and predicted the adsorption efficiency using an artificial neural network (ANN) approach and Can et al. (2010) studied the removal of copper, nickel and cobalt, by batch and column set ups, using a local zeolitic tuff.

The simultaneous use of zeolites and bacteria is a recent topic of research and only a few works are reported. Quintelas et al. (2009) reported the biosorptive performance of an *Escherichia coli* biofilm supported on zeolite NaY for the removal of Cr(VI), Cd(II), Fe(III) and Ni(II) and more recently, Pazos et al. (2010) refer the removal of Cr(VI) from aqueous solutions by a bacterial biofilm supported on zeolite. Quintelas et al. (2011) optimized the production of extracellular polymeric substances by *Arthrobacter viscosus* and studied their interaction with a zeolite for the biosorption of Cr(VI). However, there are no reports on nickel entrapment by an *A. viscosus* biofilm supported on 13 X zeolites and, therefore, this research may implement the competitiveness of the process, as well as it may add some input to the overall synergism between bacteria, zeolite and metal.

Adsorption isotherms illustrate the adsorbate distribution between the solid and liquid phase and are obtained by changing experimental parameters such as the amount of adsorbent used. Adsorption isotherms are considered as a critical piece of information for designing sorption systems and represent a first step for their industrial application.

This research work aims to investigate the biosorption behaviour of a biofilm of *A. viscosus* supported on 13 X zeolite towards nickel. The adsorption characteristics of the biosorbent were assessed by an adsorption isotherm. For performance comparison, the same protocol was carried out for the zeolite and for the bacterium biofilm, separately. Different models as Langmuir, Freundlich, Sips and Dubinin–Raduskevich are commonly used for the description of adsorption equilibrium data and were applied to the experimental results herein reported. The kinetics was also analyzed using pseudo-first and pseudo-second order models. The behavior of the biosorbent was studied in continuous mode with total recirculation, at laboratory scale. The scale-up of the process was performed using a pilot bioreactor fulfilling the operational parameters optimized at lab scale, in order to explore the feasibility of a cost-effective process for industrial application. The modeling of the pilot breakthrough curves was established using Adams–Bohart, Thomas and Yoon–Nelson models. The uptake, defined as mass of nickel retained per mass of biosorbent used, and removal percentages were determined for all the different modes of operation.

The presence of functional groups in the system zeolite-biomass that may have a role in biosorption process was confirmed by FTIR. The sorption mechanism was investigated in detail through FTIR, SEM and EDX analyses.

2. Methods

2.1. Microorganisms, growth media and zeolites

The bacterium used was *A. viscosus* (CECT 908), which was obtained from the Spanish Type Culture Collection of the University of Valencia. Two different media were used for the biofilm growth: a rich medium containing, per liter, 10 g glucose, 5 g peptone, 3 g malt extract and 3 g yeast extract and a diluted medium containing, per liter, 3.3 g glucose, 0.167 g peptone, 1 g malt extract and 1 g yeast extract. The media were sterilized at 121 °C for 20 min.

Zeolite 13 X supplied by Xiamen Zhongzhao Imp. & Exp. Co. was used as the biosorbent support in the shape of spherical pellets. This support has been conventionally produced using mainly clay minerals as the binder for the pellets. The diameter of the pellets ranged from 5 to 8 mm and the average pore diameter was 13 Å.

2.2. Bioreactors and experimental set up

2.2.1. Batch bioreactor-equilibrium and kinetic behaviour

A rich medium was used for the microorganism growth. The culture was grown in Erlenmeyer flasks (1000 mL) containing 500 mL of the rich medium, previously sterilized at 121 °C for 20 min. The flasks were inoculated and incubated in an orbital shaker at 150 rpm, 28 °C and capped with cotton stoppers that permitted passive aeration during 24 h. The growth of the bacterial community was evaluated by optical density measurements at 620 nm (T60 UV Visible, PG Instruments). Then, batch experiments (Erlenmeyer flasks of 250 mL) were conducted using 0.1, 0.5, 1, 1.5 and 2 g of 13 X zeolite with 15 mL of *A. viscosus* culture media and 150 mL of nickel solution (100 mg/L). Nickel stock solution was prepared by diluting $NiCl_2{\cdot}(6H_2O)$ (Riedel, pure), in distilled water. Control assays using bacteria and zeolite, separately, were also performed. The pH of the initial solutions was measured (pH meter ORION 720A) and they presented a pH value around 6. The Erlenmeyer flasks were kept at 28 °C, with moderate stirring to promote the contact between the biofilm and the metal solutions, for about 21 days (time required to reach the equilibrium, according to previous assays).

Samples of 1 mL were taken, centrifuged (13400 rpm for 10 min) and the aqueous phase was stored at −4 °C. Prior to analysis, the liquid samples were thawed and homogenized by vortexing. The metal ions concentrations were determined using a Varian Spectra AA-400, an atomic absorption spectrophotometer (AAS). All experimental work was conducted in duplicate. The results presented are an average of both assays. The relative standard deviation and relative error of the experimental measurements were <1% and 3%, respectively.

Four isotherm equations have been tested in the present study: Langmuir, Freundlich, Sips and Dubinin–Raduskevich. The simplest method to determine constants of isotherms with two parameters (Langmuir, Freundlich and Dubinin–Raduskevich) is the application of a linear regression to those equations. For Sips equation, the model parameters were estimated by non-linear regression using MATLAB and EXCEL software.

The linearized Langmuir isotherm applied for Ni(II) sorption is expressed by the equation (Langmuir, 1918):

$$1/Q_e = 1/(Q_{máx} \cdot b \cdot C_e) + 1/Q_{máx} \quad (1)$$

where C_e is the metal residual concentration at equilibrium, Q_e is the amount of nickel adsorbed per mass unity of sorbent (mg/g) at equilibrium, $Q_{máx}$ is maximum specific uptake of metal and b is the Langmuir constant related to the affinity of binding sites to the metal ion.

The linear form of Freundlich isotherm is represented by the following equation (Freundlich, 1906).

$$\mathrm{Ln}(Q_e) = \mathrm{Ln}(K_F) + Ln(C_e)/n \quad (2)$$

where Q_e is the amount of metal adsorbed per mass unity of sorbent (mg/g) at equilibrium, K_F is the Freundlich constant, C_e is the residual metal concentration in solution and n stands for adsorption intensity. K_F and n values are obtained from plots of Ln (Q_e) versus Ln (C_e). Large K_F and n values imply high sorption capacity.

Sorption data were also subjected to Dubinin and Radushkevich (1947) modeling and this is represented by the following linearized equation.

$$\mathrm{Ln}Q_e = \mathrm{Ln}Q_D - 2B_D\mathrm{RTLn}(1 + 1/C_e) \quad (3)$$

where Q_D is the theoretical saturation capacity (mg/g), B_D is a constant related to adsorption energy (mol^2/kJ^2), R is the gas constant (kJ/mol.K) and T is the temperature (K). The apparent energy (E_D) of adsorption from Dubinin–Radushkevich isotherm model can be calculated by the following equation.

$$E_D = 1/\sqrt{(2B_D)} \quad (4)$$

If the value of E_D is <8 kJ/mol, the adsorption process is physical in nature, if it ranges between 8 and 16 kJ/mol, it consists mainly of ion-exchange and higher values indicate strong chemisorption between the biosorbent and the sorbate (Sivakumar and Palanisamy, 2009).

Sips model (Sips, 1948) was also used in the present form:

$$Q_e = (K_S C_e^{1/bS})/(1 + a_S C_e^{1/bS}) \quad (5)$$

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 a monolayer sorption capacity characteristic of the Langmuir isotherm. K_S (L^{bS} mg^{1-bS}/g), a_S $(L/mg)^{bS}$ and b_S are the Sips isotherm parameters.

The kinetic behavior was also evaluated by pseudo-first and pseudo-second order models. The linearized forms of both models are shown below as Eqs. (6) and (7), respectively:

$$\log(Q_e - q_t) = \log(Q_e) - k_1 \cdot t \quad (6)$$

$$t/q_t = 1/(k_2 \cdot Q_e^2) + t/Q_e \quad (7)$$

where Q_e is the amount of nickel sorbed at equilibrium per mass unity of sorbent (mg/g) as previous defined, q_t the amount of nickel sorbed at time t per mass unity of sorbent (mg/g), k_1 the pseudo-first order rate constant (h^{-1}) and k_2 is the pseudo-second order rate constant (g/mg.h).

2.2.2. Laboratory scale bioreactor

A set of experiments was performed in a laboratory scale setup consisting of two acrylic (Plexiglas) columns with 30 cm height and 4 cm internal diameter, working in parallel mode, with total recirculation. Two-thirds of each column was filled with zeolite 13 X (258 g).

For the biofilm formation, a bacterial culture was grown in an Erlenmeyer flask (1000 mL) containing 500 mL of the rich medium, previously sterilized at 121 °C for 20 min. The flasks were inoculated and incubated in an orbital shaker at 150 rpm, 28 °C and capped with cotton stoppers that permitted passive aeration during 24 h. The inoculum culture was transferred to columns setup and was pumped upwards at a flow rate of 25 mL/min during 24 h with total recirculation in order to support the biofilm onto the zeolite. Following this, the diluted medium was pumped during 72 h at the same previous flow rate to favour the hydrodynamics of the biofilm (Quintelas and Tavares, 2001). After this period of time the beds were washed out and the metal solutions were passed through the columns with a flow rate of 10 mL/min. The ratio between zeolite and treated solution was 0.11 kg/L. The final working volume of each column was ca. 150 mL, and the total volume of metal solution treated was 1.6 L per run. A peristaltic pump was used to avoid concentration gradients. Samples of the effluent (5 ml) were taken, centrifuged and the aqueous phase was analyzed for nickel using atomic absorption spectrophotometry, AAS. As in point Section 2.2.1 all experimental work was conducted in duplicate and the results presented are an average of both assays.

2.2.3. Pilot scale bioreactor

These assays were performed in a pilot-scale bioreactor consisting of three stainless-steel columns in series with 1.5 m height and 30 cm internal diameter each. Two-thirds of each column was filled with zeolite 13 X (5 kg per column). The biofilm formation occurred as described in point Section 2.2.2 but using longer periods of media flow, 24 h for rich medium and 10 days for diluted medium. Once the biofilm was created, a solution of 50 mg/L Ni(II) was pumped upwards through the series of columns at a flow rate of 5 L/h. The ratio between zeolite and treated solution was 0.10 kg/L. The final working volume of the bioreactor was approximately 150 L per run. A membrane pump (5 L/h) was used to avoid concentration gradients. At pre-established time intervals, samples of the effluent were taken, centrifuged and analyzed for nickel concentration. The pH was also measured.

Three breakthrough modeling equations have been tested in the present study: Adams–Bohart, Thomas and Yoon–Nelson models. The breakthrough is defined as the pre-established saturation concentration accepted at the outflow of the system. The linearized forms of all models are presented herein.

Adams–Bohart model (Bohart and Adams, 1920) is based on the surface reaction theory that established the relationship between C/C_0 and t in a continuous system. This model assumes that equilibrium is not instantaneous. It is used for describing the initial part of the breakthrough curve. The expression is as follows:

$$\mathrm{Ln}(C/C_0) = K_{AB}C_0 t - K_{AB}N_0(Z/U_0) \quad (8)$$

where C_0 and C are the influent and effluent nickel concentration (mg/L), respectively; K_{AB} is the kinetic constant (L/mg h), N_0 is the saturation concentration (mg/L), Z is the bed depth of the packed bed reactor (m), U_0 is the superficial velocity (L/h) defined as the ratio of the volumetric flow rate Q (L/h) to the cross-sectional area of the bed A (m^2) and t ranges from the beginning of the assay to the breakthrough. The parameters K_{AB} and N_0 can be calculated from the linear plot of $\mathrm{Ln}(C/C_0)$ against t.

Thomas model (Thomas, 1944) is one of the most common equation used to describe the performance of the sorption process in a packed bed reactor. The linearized form of this model can be described by the following expression:

$$\mathrm{Ln}(C_0/C - 1) = K_{TH}q_0 m/Q) - K_{TH}C_0 t \quad (9)$$

where is the adsorption capacity (mg/g), and t stands for total flow time (h). The values of K_{TH} and q_0 can be determined from the linear plot of ln $[(C_0/C_t)-1]$ against t.

Yoon and Nelson (1984) developed a model to describe the breakthrough behavior of adsorbate gases on activated charcoal. Later, the model was adjusted to liquid systems using different adsorbents. The Yoon–Nelson model is based on the assumption that the rate of decrease in the probability of adsorption for each adsorbate molecule is proportional to the probability of adsorbate adsorption and the probability of adsorbate breakthrough on the adsorbent. The linearized Yoon–Nelson model for a single component system can be expressed as:

$$\mathrm{Ln}(C/C_0 - C) = K_{YN}t - \tau K_{YN} \quad (10)$$

where K_{YN} is the rate constant (min^{-1}) and τ is the time required for 50% adsorbate breakthrough (min). A linear plot of Ln $[C_t/(C_0 - C_t)]$ against t determined the values of K_{YN} and s from the intercept and slope of the plot.

2.2.4. SEM- EDX and FTIR

Samples of the biofilm (from pilot-scale reactor) were taken and analyzed (after dehydration with different concentrations of etha-

nol) by SEM (Leica Cambridge S360). Samples were gold coated prior to SEM observation. The surface elemental analyses of a Ni(II) interacted 13 X zeolite were carried out by Energy dispersive X-ray spectroscopy (EDX) (Link eXL II Oxford). Infrared spectrum of the unloaded and metal loaded zeolite was obtained using a Fourier transform infrared spectrometer (FTIR BOMEM MB 104). For the FTIR study, the system zeolite-biomass was smashed. Then, 10 mg of finely grounded zeolite was encapsulated in 100 mg of KBr in order to prepare translucent sample disks. FTIR was performed in the range 4000–500 cm^{-1} by averaging 20 scans at a maximum resolution of 4 cm^{-1}.

3. Results and discussion

The removal of hazardous elements or compounds by microorganisms is a very complex process. Accordingly to Reddy et al. (2012), heavy metal binding onto a biomass involves several mechanisms like adsorption, complexation, chelation and entrapment in capillaries and spaces within the polysaccharide network due to concentration gradients causing diffusion through the cell walls and membrane. Previous studies (Quintelas et al., 2011) used *A. viscosus* in their research as it is a very good producer of exopolyssacharides which allows good properties for the entrapment of metals and it presents on its surface several functional groups that might contribute to the biosorption process (Quintelas et al., 2010). The main bands present on the spectrum include bonded hydroxyl groups at 3400 cm^{-1}, 1398 cm^{-1} (indicative of COO^{-} anions), 1200 cm^{-1} (C–O stretching of COOH) and at 860 cm^{-1} (aromatic –CH stretching peak).

Different assays were done using the bacteria *A. viscosus* supported on 13 X zeolite to retain Ni ions from liquid solutions and the results will be discussed.

3.1. Batch bioreactor- equilibrium and kinetic behaviour

It was observed that as the amount of zeolite increases, the uptake decreases from 28.37 to 6.76 mg/g, and the removal percentage increases from 20.62% to 98.20% (Table 1). The explanation is obvious as at higher zeolite amounts the ratio of the initial moles of nickel to the available surface area is low and subsequently sorption is independent of the initial concentrations. On the other hand, at lower zeolite amounts the available sites become fewer compared to the number of moles of nickel present and hence the removal percentage of nickel is dependent of the initial concentration. Comparing with previous studies using NaY zeolite covered by a biofilm of *E. coli*, for the concentration of 100 mg/L and 1 g of zeolite, is possible to conclude that the current biofilm is more efficient because it allows an increment in the removal percentage, from 85.5%, (previous work) to 94.1% (present work). Sari et al. (2007) also studied biosorption of Pb(II) and Ni(II) from aqueous solution by lichen (*Cladonia furcata*) biomass and found, for an initial concentration of 10 mg/L of nickel, a maximum of removal percentage of 70% and Amini et al. (2009) analyzed the biosorption of nickel (II) from aqueous solution by *Aspergillus niger* and found removal percentages also about 70% for an initial concentration of 30 mg/L. The present work describes a much better performance.

Table 1
Equilibrium adsorbed quantities and removal percentages of Ni(II) ion obtained with different amounts of zeolite (28 °C, 150 rpm, C_0 = 100 mg/L).

Zeolite amount (g)	Uptake (mg/g)	Rp (%)
0.1	28.37	20.62
0.5	22.34	81.17
1.0	12.95	94.09
1.5	8.87	96.69
2.0	6.76	98.20

Table 2
Isotherm constants for all the equilibrium models considered for nickel adsorption onto a biofilm supported on 13 X zeolite.

Langmuir parameters			
$Q_{máx}$	b		R^2
26.81	0.19		0.97
Freundlich parameters			
K_f	n		R^2
6.08	2.56		0.95
Dubinin–Radushkevich parameters			
Q_D	B_D		R^2
20.03	0.0093		0.74
Sips parameters			
K_S	a_S	b_S	R^2
4.58	0.14	0.96	0.99

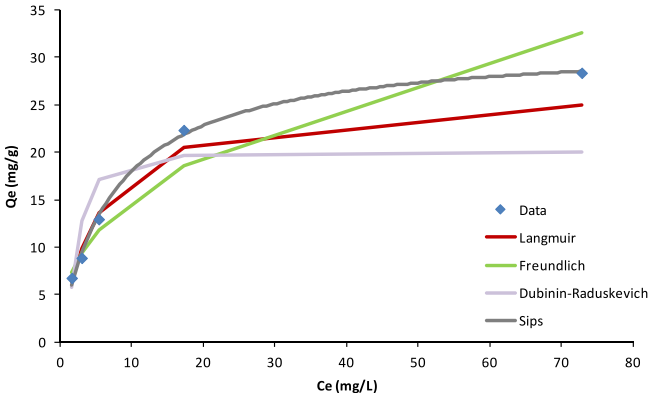

Fig. 1. Isotherm equilibrium data for nickel sorption and attempted fitting by four different models.

Assays using only zeolite and only bacteria were also performed (data not shown) and the results evidence that the bacteria is able to remove nickel but the removal is slow. The removal using only zeolite is faster than that using only bacteria and it is presumed that the main contribution for the removal of nickel is given by the zeolite and bacteria increases the overall performance.

The equilibrium of adsorption is a very important issue that should be analyzed. The adsorption isotherms give information about the capability of the biosorbents to interact with some hazardous compounds and about the mechanisms of adsorption. For the biosorbent used (biofilm + 13 X zeolite), equilibrium data were experimentally determined. Four different models- Langmuir, Freundlich, Dubinin–Radushkevich and Sips were fitted and calculated constants are presented in Table 2. The fit of the data is also represented in Fig. 1 and the best fit, with no doubt, was obtained using the Sips equation. The fact that Sips equation incorporates the features of Langmuir and Freundlich equations contributes to its quality. The worst fit was obtained for the Dubinin–Raduskevich. No matter this fact, the energy of adsorption was calculated and its value is 7.33 kJ/mol. The value indicates that the adsorption process is physical in nature. Nevertheless, it is strongly believed that the adsorption process described herein is an ion-exchange process and should the Dubinin–Raduskevich had a better adjustment to experimental data, probably the energy of adsorption would range between 8 and 16 kJ/mol.

Adsorption kinetics was investigated to understand the dynamics of sorption of metal ions onto the sorbents. According to Pandey

Table 3
Constant parameters of pseudo-first and pseudo-second order kinetic models for different zeolite amounts for the initial nickel concentration of 100 mg/L.

Zeolite amount (g)	Pseudo-first order model		Pseudo-second order model	
	k_1 (h)	R^2	k_2 (g/mg.h)	R^2
0.1	0.0060	0.86	0.00029	0.82
0.5	0.0067	0.83	0.00031	0.90
1.0	0.0069	0.97	0.00247	0.99
1.5	0.0075	0.98	0.00571	1.00
2.0	0.0051	0.89	0.01305	1.00

et al. (2010), adsorption kinetics is expressed as the solute removal rate is controlled by the residence time of the sorbate in the solid–solution interface. The popular kinetic models, pseudo-first and pseudo-second order were applied to the kinetic data and the rate constant values obtained, k_1 and k_2 are presented in Table 3. The correlation coefficient (R^2) values suggest that pseudo second-order model fits better than the pseudo-first order model for *A. viscosus* biofilm supported on 13 X zeolite, with the eventual exception verified for the smallest amount of zeolite (0.1 g). The pseudo-second order rate assumes that the adsorption process is controlled by surface reaction, with chemisorption involving valence forces, through sharing or exchange of electrons between bacteria + zeolite and Ni(II) species (Zhang et al., 2010). The pseudo-first order model assumes that the reaction rate is limited only by one process or mechanism on a single class of sorbing sites and that all sites are of the time dependent type (Fonseca et al., 2009). The better fit obtained using this equation for the smaller amount of zeolite could be explained by the fact that, in the presence of a large excess of Ni(II), the rate of sorption highly depends on the capacity of sorbent which in turn depends on the available sites for binding or ion-exchange, indicating that the rate limiting step for sorption is imposed by the sorbent capacity. Fig. 2 presents the results for the fit obtained using both models for all the different amounts of zeolite used.

3.2. Laboratory scale bioreactor

A new assay was carried out in the laboratory-scale bioreactor. The biofilm of *A. viscosus* supported on 13 X zeolite was tested for a solution with the initial Ni(II) concentration of 50 mg/l. For each batch, 1.6 L of nickel solution were treated in total recirculation mode (10 mL/min) meaning that 100.3 L were passed through the setup. The assay, with three renewal of the original solution, was followed during 16 days. Fig. 3 shows the profiles of the normalized concentration of Ni(II) in solution during the close loop assay. After 7 days of treatment (1 batch), almost all the nickel was

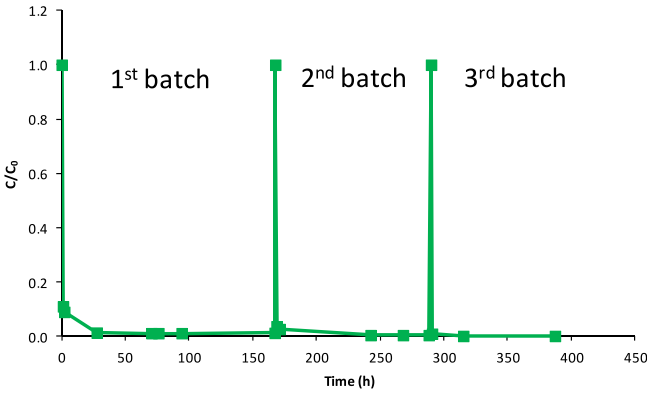

Fig. 3. Normalized nickel concentration evolution during the laboratory-scale assay, for fed-batch.

removed (98.8%) with an uptake of 20.21 mg/g. Identical removal percentages were found for the 2nd and 3rd batches, following the substitution of the feeding solution. These two extra batches were performed to investigate the possible use of the bioreactor in continuous mode. This outcome demonstrated that most of the Ni(II) was retained by the system biomass/zeolite and that the bioreactor could be used in a continuous mode.

Can et al. (2010) studied the removal of Ni(II) and obtained an uptake of 6.6 mg/g, with an initial concentration of 150 mg/L, using a zeolitic tuff. Keshtkar et al. (2012) analyzed the biosorption of Ni(II) by Ca-pretreated *Cystoseira indica* in a fixed-bed column, for an initial concentration of 30 mg/L, and obtained a maximum removal percentage of 57%. Khan et al. (2012) tried the biosorption of the same metal using mustard oil cake and obtained a removal percentage of 69% (C_I = 10 mg/L). The biofilm described in this report presents better uptakes and can be applied to an industrial environment.

3.3. Pilot scale bioreactor

Based on the results from the batch and minicolumns studies a new assay was carried out in the pilot-scale bioreactor of 150 L. The biofilm of *A. viscosus* supported on 13 X zeolite was tested for the initial Ni(II) concentration of 50 mg/L. The assay was followed during 35 days and the volume of nickel treated was of 4200 L. During the whole experiment pH was measured and the value was around 6 (from 5.4 to 6.5). According to the literature, the optimal pH for the biosorption of nickel is 6 (Quintelas et al., 2009).

The results obtained (Fig. 4) indicate that the scale-up had been successful and the system is far from the saturation (C/C_0 = 1).

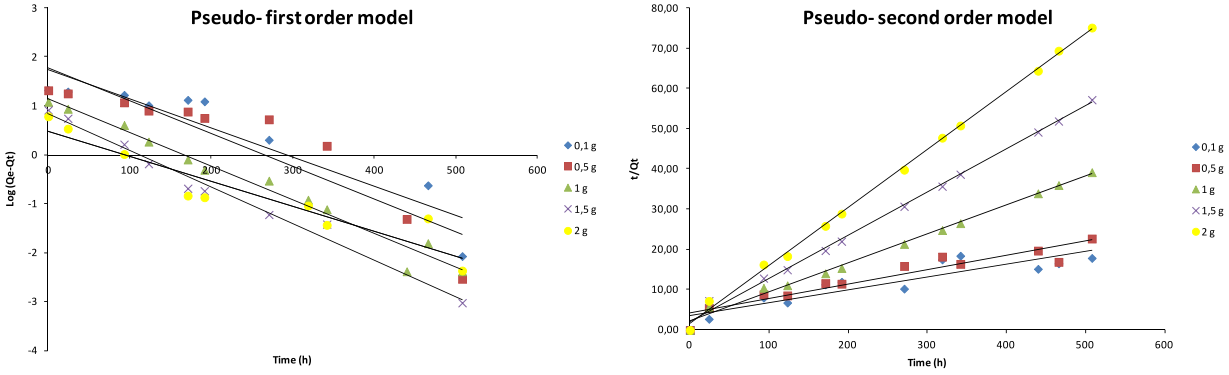

Fig. 2. Kinetic data for nickel sorption and attempted fitting by two different models.

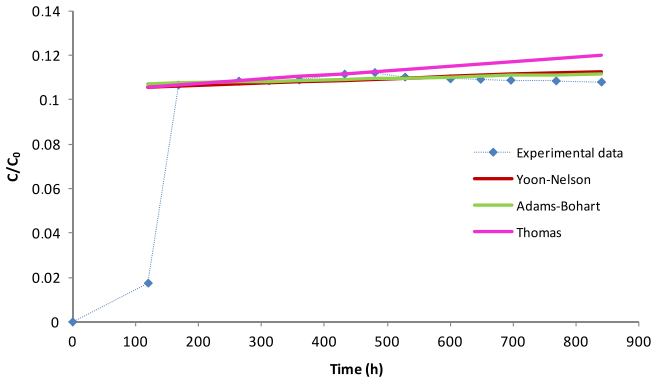

Fig. 4. Breakthrough data and attempted fitting by three different models, for the assay performed in the pilot-scale bioreactor.

Comparable behavior was observed between lab and pilot scale as nickel was removed from solution with high removal percentage in both cases. For pilot-scale bioreactor, the uptake was 11.13 mg Ni per g zeolite 13 X and the removal percentage is 89%. The removal percentages suffer a decrease, from 98% to 89%, in the scale-up from minicolumns to pilot-scale, probably due to the limiting amount of biomass present in the pilot-scale bioreactor and to the volume of nickel solution treated. No matter this fact, the behavior of the pilot-scale bioreactor is very good and could be optimized in terms of biofilm formation. A pilot scale reactor test is a very important step for the industrial implementation of a biosorption system and it is a remarkable finding that the system developed in this study was able to operate at a large scale without incurring operational problems while successfully removing nickel.

It is also important to refer that 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 and the concentration of Ni used did not seem to be toxic for the bacterial culture, indicating that this specific microorganism appears to be resilient in an actual industrial environment.

The Adams–Bohart, Thomas and Yoon–Nelson 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) and kinetic constant (K_{AB}) from Adams–Bohart model, Thomas constant (K_{TH}) and adsorption capacity q_0m from Thomas model and rate constant (K_{YN}) and time required for 50% adsorbate breakthrough (τ) from Yoon and Nelson model. After applying Eqs. (8)–(10) to the experimental data for different inlet nickel concentrations, a linear relationship between Ln C/C_0 and t was obtained. All the parameters were calculated from the Ln C/C_0 versus t plots and are presented in Table 4 together with the correlation coefficients.

Predicted and experimental breakthrough curves are shown in Fig. 4. It is clear from this graph that there is a good agreement between the experimental and predicted values during almost the whole experiment. Discrepancies where found between the experimental and the predicted curves for the first two points. Adams–Bohart model assumes that the rate of adsorption is limited by external mass transfer. Thomas model assumes Langmuir kinetics of adsorption–desorption and no axial dispersion is derived with the assumption that the rate driving force obeys second-order reversible reaction kinetics. Yoon–Nelson model assumes that the rate of decrease in the probability of adsorption for each adsorbate molecule is proportional to the probability of adsorbate adsorption and the probability of adsorbate breakthrough on the adsorbent

Table 4
Fitting parameters of Adams–Bohart, Thomas and Yoon–Nelson models (C_0 = 50 mg/L).

Adams–Bohart model		
N_0 (mg/L)	K_{AB} (L/(mg.h))	R^2
5.30E6	1.35E-6	0.76
Thomas model		
K_{TH} (L/h mg)	q_0m (mg/g)	R^2
4.49E-6	1.60E5	0.94
Yoon–Nelson model		
K_{YN} (1/min)	τ (min)	R^2
1E-4	2.15	0.77

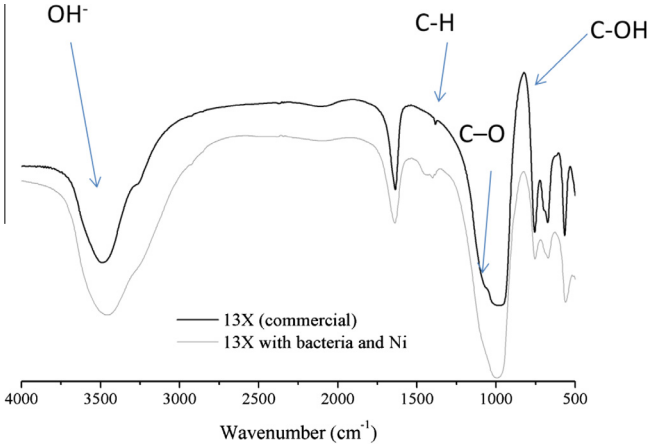

Fig. 5. FTIR spectrum of Ni(II) un-loaded and loaded zeolite.

(Ghribi and Chlendi, 2011). Looking at the correlation coefficient (R^2) it seems that Thomas model provided a better fitting than the Adams–Bohart and Yoon–Nelson models, but from Fig. 4 it is easy to conclude that Adams–Bohart and Yoon–Nelson models were more suitable for the adsorption process, indicating that the external mass transfer may be the limiting step.

At the end of the experiments, the biofilm was analyzed by SEM-EDX (scanning electron microscopy) and it was possible to confirm the presence of a large number of bacteria (Supplementary Figure as material). The images also show the porous nature of 13 X zeolite along with depressions and grooves on the surface, indicating availability of a large number of binding sites for fixation of metal ions. Several white incrustations are visible on pictures. Sharma et al. (2008) affirm that these incrustations represent the binding of metal ions on the surface of biomass and are an evidence of the biosorption of metal by the biofilm. Similar evidences were found in previous works (Quintelas et al., 2011).

EDX spectrum obtained for 13 X zeolite after loaded with nickel did not confirm the presence of nickel ions on the sorbent surface. The results in terms of removal percentage show that 89% of nickel was removed so, the fact that the EDX spectrum does not evidence the presence of nickel probably means that the energy used (10 keV) is not enough to reach the deepest porous. The EDX spectrum confirms the presence of O, Si, Al, Na and Mg (vestigial) and a significant amount of Au. The intensity for gold in the EDX data is due to sputtered gold on the surface of sorbent studied.

The biofilm was also analyzed by FTIR (Fig. 5). After interaction of bacteria supported on zeolite with Ni(II) solution, bands at 3400, 1300 and 1030 cm^{-1} corresponding to free and intermolecular bonded hydroxyl groups (–OH), to the C–H bending ($-CH_3$) (Bansal et al., 2009) and to C–OH stretching vibrations, suffer changes on the intensity or on the shape and a new peak appear (1300 cm^{-1}). Moreover, considerable changes in the intensity of

the peaks in fingerprint region 550–800 cm^{-1} (bending vibrations) were also observed after interaction with Ni(II). All these changes may be due to the involvement of these functional groups in the adsorption of Ni(II). Similar results were described in previous publications (Quintelas et al., 2009) for C–H and C–O groups which re-enforce the idea of the involvement of these groups. Accordingly to Volesky (2007) 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 *A. viscosus* biomass.

These results are very promising. The high removal rates of nickel that were achieved indicate a feasible, economical and efficient process for biological nickel removal from industrial wastewater effluents.

4. Conclusions

According to the results obtained, this new eco-friendly technology is adequate for the treatment of aqueous systems contaminated with Ni(II). These assays confirmed that the designed bioreactor containing a biofilm supported on zeolite 13 X can be effectively used in Ni removal. For the batch and minicolumns assays a maximum of 98% was reached. The assay conducted in the pilot-scale bioreactor showed a similar behaviour to the laboratory-scale assay, although a slight decrease on the removal percentage was verified (89%). Furthermore, the good results suggest that this bioreactor has the potential for application in nickel contaminated water streams.

Acknowledgements

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 and co- funding by FSE (programme QREN – POPH). Cristina Quintelas thanks FCT for a Post-Doc grant (SFRH/ BPD/ 32113/ 2006).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.05.059.

References

Amini, M., Younesi, H., Bahramifar, N., 2009. Biosorption of nickel(II) from aqueous solution by *Aspergillus niger*: response surface methodology and isotherm study. Chemosphere 75, 1483–1491.

Bansal, M., Singh, D., Garg, V.K., Rose, P., 2009. Use of agricultural waste for the removal of nickel ions from aqueous solutions: equilibrium and kinetics studies. Int. J. Civil Environ. Eng. 1 (2), 108–114.

Bohart, G., Adams, E.Q., 1920. Some aspects of the behaviour of charcoal with respect to chlorine. J. Am. Chem. Soc. 42, 523–544.

Can, Ö., Balköse, D., Ülkü, S., 2010. Batch and column studies on heavy metal removal using a local zeolitic tuff. Desalination 259, 17–21.

Dubinin, M.M., Radushkevich, L.V., 1947. Equation of the characteristic curve of activated charcoal. Chem. Zentr. 1, 875.

Fonseca, B., Maio, H., Quintelas, C., Teixeira, A., Tavares, T., 2009. Retention of Cr(VI) and Pb(II) on a loamy sand soil: kinetics, equilibria and breakthrough. Chem. Eng. J. 152, 212–219.

Freundlich, H., 1906. Adsorption in solutions. Phys. Chem. 57, 384–410.

Ghribi, A., Chlendi, M., 2011. Modeling of fixed bed adsorption: application to the adsorption of an organic dye. Asian J. Text. 1, 161–171.

Keshtkar, A.R., Kafshgari, F., Mousavian, M.A., 2012. Binary biosorption of uranium(VI) and nickel(II) from aqueous solution by Ca-pretreated *Cystoseira indica* in a fixed-bed column. J. Radioanal. Nucl. Chem. 292, 501–512.

Khan, M.A., Ngabura, M., Choong, T.S.Y., Masood, H., Chuah, L.A., 2012. Biosorption and desorption of nickel on oil cake: batch and column studies. Bioresource Technol. 103, 35–42.

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

Macomber, L., Hausinger, R.P., 2011. Mechanisms of nickel toxicity in microorganisms. Metallomics 3, 1153–1162.

Pandey, P.K., Sharma, S.K., Sambi, S.S., 2010. Kinetics and equilibrium study of chromium adsorption on zeolite NaX. Int. J. Environ. Sci. Tech. 7, 395–404.

Pazos, M., Branco, M., Neves, I.C., Sanromán, M.A., Tavares, T., 2010. Removal of Cr(VI) from aqueous solutions by a bacterial biofilm supported on zeolite: optimisation of the operational conditions and scale-up of the bioreactor. Chem. Eng. Technol. 33, 2008–2014.

Qian, J., Li, D., Zhan, G., Zhang, L., Su, W., Gao, P., 2012. Simultaneous biodegradation of Ni–citrate complexes and removal of nickel from solutions by *Pseudomonas alcaliphila*. Bioresource Technol. 116, 66–73.

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

Quintelas, C., Rocha, Z., Silva, B., Fonseca, B., Figueiredo, H., Tavares, T., 2009. Biosorptive performance of an *Escherichia coli* biofilm supported on zeolite NaY for the removal of Cr(VI), Cd(II), Fe(III) and Ni(II). Chem. Eng. J. 152, 110–115.

Quintelas, C., Silva, B., Figueiredo, H., Tavares, T., 2010. Removal of organic compounds by a biofilm supported on GAC: modelling of batch and column data. Biodegradation 21, 379–392.

Quintelas, C., da Silva, V.B., Silva, B., Figueiredo, H., Tavares, T., 2011. Optimization of production of extracellular polymeric substances by *Arthrobacter viscosus* and their interaction with a 13X zeolite for the biosorption of Cr(VI). Environ. Technol. 32, 1541–1549.

Ramana, D., Reddy, D., Kumar, B., Harinath, Y., Seshaiah, K., 2012. Removal of nickel from aqueous solutions by citric acid modified *Ceiba Pentandra* hulls: Equilibrium and Kinetic Studies. Can. J. Chem. Eng. 90, 111–119.

Reddy, D., Seshaiah, K., Reddy, A.V.R., Lee, S.M., 2012. Optimization of Cd(II), Cu(II) and Ni(II) biosorption by chemically modified *Moringa oleifera* leaves powder. Carbohydr. Polym. 88, 1077–1086.

Rodrigues, M., Ferreira, L., de Carvalho, J., Lodi, A., Finocchio, E., Converti, A., 2012. Metal biosorption onto dry biomass of *Arthrospira (Spirulina) platensis* and *Chlorella vulgaris*: multi-metal systems. J. Hazard. Mater. 217–218, 246–255.

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

Schumann, K., Unger, B., Brandt, A., Scheffler, F., 2012. Investigation on the pore structure of binderless zeolite 13X shapes. Micropor. Mesopor. Mat. 154, 119–123.

Sharma, M., Kaushik, A., Somvir, B.K., Kamra, A., 2008. Sequestration of chromium by exopolysaccharides of *Nostoc* and *Gloeocapsa* from dilute aqueous solutions. J. Hazard. Mater. 157, 315–318.

Shinde, N.R., Bankar, A.V., Kumar, A.R., Zinjarde, S.S., 2012. Removal of Ni (II) ions from aqueous solutions by biosorption onto two strains of *Yarrowia lipolytica*. J. Environ. Manag. 102, 115–124.

Sips, R., 1948. Combined form of Langmuir and Freundlich equations. J. Chem. Phys. 16, 490–495.

Sivakumar, P., Palanisamy, P.N., 2009. Adsorption studies of basic red 29 by a non convencional activated carbon prepared from *Euphorbia Antiquorum L.* Int. J. ChemTech Res. 1, 502–510.

Thomas, H.C., 1944. Heterogeneous ion exchange in a flowing system. J. Am. Chem. Soc. 66, 1664–1666.

Turp, S.M., Eren, B., Ateş, A., 2011. Prediction of adsorption efficiency for the removal of nickel (II) ions by zeolite using artificial neural network (ANN) approach. Fresenius Environ. Bull. 12, 3158–3165.

Volesky, B., 2007. Biosorption and me. Water Res. 41, 4017–4029.

Yoon, Y.H., Nelson, J.H., 1984. Application of gas adsorption kinetics- II. A theoretical model for respirator cartridge service life and its practical applications. Am. Ind. Hyg. Assoc. J. 45, 509–516.

Zhang, H., Tang, Y., Cai, D., Liu, X., Wang, X., Huang, Q., Yu, Z., 2010. Hexavalent chromium removal from aqueous solution by algal bloom residue derived activated carbon: equilibrium and kinetic studies. J. Hazard. Mater. 181, 801–808.