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Research Article

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

The aim of this study was to investigate the feasibility of a bioreactor system and its scale-up to remove Cr(VI) from solution. The bioreactor is based on an innovative process that combines bioreduction of Cr(VI) to Cr(III) by the bacterium *Arthrobacter viscosus* and Cr(III) sorption by a specific zeolite. Batch studies were conducted in a laboratory-scale bioreactor, taking into account different operating conditions. Several variables, such as biomass concentration, pH and zeolite pre-treatment, were evaluated to increase removal efficiency. The obtained results suggest that the Cr removal efficiency is improved when the initial biomass concentration is approximately 5 g L^{-1} and the pH in the system is maintained at an acidic level. Under the optimised conditions, approximately 100 % of the Cr(VI) was removed. The scale-up of the developed biofilm process operating under the optimised conditions was satisfactorily tested in a 150-L bioreactor.

Keywords: 13X zeolite, *Arthrobacter viscosus*, Bioreactor, Cr removal, Scale-up

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1 Introduction

In recent years, the presence of heavy metals in aqueous streams from the discharge of untreated wastewater into natural water bodies has become an important environmental issue. The disposal of industrial effluents into freshwater resources has caused the deterioration of several aquatic ecosystems due to the accumulation of metals in biota and flora [1, 2].

Chromium (Cr) has been introduced into the environment as a result of different industrial activities, such as iron and steel manufacturing, Cr leather processing, Cr plating, wood preservation and other anthropogenic sources [3]. The most prevalent forms of Cr found in the environment are Cr(VI) and Cr(III). The toxicity of this contaminant is highly dependent on its oxidation state. Cr(VI) is generally soluble and toxic to many plants, animals and microorganisms in the aquatic environment, and Cr(VI) is considered to be mutagenic and carcinogenic. By contrast, Cr(III) is less toxic and less soluble than Cr(VI) and is an essential micronutrient for most biota, including humans [4].

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The removal and recovery of Cr(VI) from wastewater are of great importance in protecting the environment and human health as well as in reducing heavy metal toxicity, a potential threat for aquatic animals and plants. Limits on the total Cr discharge in effluent vary widely between 0.05 and 10 mg L^{-1} for direct discharges into water bodies and 1–50 mg L^{-1} for indirect discharges into sewage systems [5].

Traditional treatment of Cr(VI)-contaminated industrial effluents generally involves the chemical reduction to insoluble Cr(III) compounds [3]. Conventional methods, such as precipitation, oxidation/reduction, ion exchange, filtration, membranes and evaporation, are extremely expensive or inefficient for metal removal [6].

Recently, biosorption of heavy metals by microbial cells has been recognised as a potential alternative to the traditional treatment technologies for waste streams and natural waters [7, 8]. The ability of Cr(VI) anions to overcome the permeability barrier of a prokaryotic cell, such as a bacterium, can be attributed to the chemical similarity between CrO_4^{2-} and SO_4^{2-} ions [9]. The applicability of bacteria as biosorbents has some advantages due to these microbes' small size, ubiquity, ability to grow under controlled conditions and resilience to a wide range of environmental conditions [10].

Çetin et al. [11] reported that studies on Cr(VI)-bioaccumulating microorganisms have shown that microbial Cr(VI) removal from solutions typically include the following stages:

(i) binding of Cr(VI) to cell surfaces, (ii) translocation of Cr(VI) into the cell and (iii) reduction of Cr(VI) to Cr(III). The intracellular reduction of Cr(VI) to Cr(III) is known to be the main detoxification mechanism. A wide range of microorganisms, including *Arthrobacter viscosus*, reduce the ion Cr(VI) by chromate reductase activity [12–15].

A. viscosus is a non-pathogenic soil bacterium that produces a high amount of viscous extracellular polysaccharides [16, 17]. This characteristic favours its adhesion to different support structures and increases its ability to remove heavy metals from liquid streams [18, 19]. Until now, publications have demonstrated that pH is one of the most important variables in Cr(VI) bioremoval. Blázquez et al. [20] reported that the removal rate was enhanced by very acidic pH conditions (pH lower than 3).

Inversely, removal of heavy metals from industrial waste streams by the use of natural zeolites has been applied to medium- and large-scale treatment facilities, with variable success [21]. Zeolites are aluminosilicate minerals containing exchangeable alkali or alkaline-earth metal cations (usually Na, K, Ca and Mg) and water in their structural framework. Their physical structure is porous, and alkali or alkaline-earth cations reversibly fixed in the cavities can easily be exchanged by surrounding positive ions. Zeolites have little affinity for anions and non-polar organic molecules [22] and can be changed by surface pre-treatment or surface coverage by a specific biofilm [23].

Figueiredo et al. [24] reported an innovative process that combines the use of a zeolite and an *A. viscosus* biofilm to remove Cr(VI) in solution. They found that the *A. viscosus* bacteria supported on the zeolite reduce Cr(VI) to Cr(III), and the Cr(III) is retained in the zeolite by ion exchange. Furthermore, these authors demonstrated that the resulting metal-zeolite can be applied as catalyst in the oxidation of persistent organic compounds.

This study focuses on the scale-up of a new technology developed by Figueiredo et al. [24] to remove low concentrations of heavy metals from industrial effluents. To complete the previous study, the bioreduction of Cr(VI) to Cr(III) using *A. viscosus* combined with the Cr(III) removal using a specific zeolite was conducted in bioreactors. A detailed study was initially carried out on a smaller scale in a laboratory to determine the influence of biomass concentration, pH and zeolite pre-treatment on the efficiency of the process. Under the optimised conditions, the scale-up of the process was performed to explore the feasibility of a cost-effective process for industrial application.

2 Materials and Methods

2.1 Microorganism and Growing

The bacterium used was *A. viscosus* (CECT 908), which was obtained from the Spanish Type Culture Collection of the University of Valencia. The microorganism was grown on a basal medium containing per litre: 10 g glucose, 5 g peptone, 3 g malt extract and 3 g yeast extract. The culture was grown in Erlenmeyer flasks (1000 mL) containing 250 mL of the basal medium, previously sterilised at 121 °C for 20 min. The flasks were inoculated and incubated in an orbital shaker at 180 rpm, 28 °C and capped with cotton stoppers that permitted passive aeration. The growth of the bacterial community was evaluated by optical density measurements at 620 nm (T60 UV Visible, PG Instruments).

2.2 Zeolite

Zeolite 13X 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 was 5–8 mm, and the normal pore diameter was 13 Å.

Zeolite increases the pH in the medium to basic values [25]. Thus, different pre-treatments were used during 24 h to improve the zeolite efficiency: 0.1 M H_2SO_4 and basal medium. To determine the amount of Cr retained in the zeolite, an acid digestion following the EPA 3010 protocol was performed at the end of the industrial-scale assay.

2.3 Bioreactors

2.3.1 Laboratory-Scale Bioreactor

The experiments to optimise the operational conditions were performed in a laboratory-scale bioreactor consisting of two Plexiglas columns with 30 cm height and 4 cm internal diameter (Fig. 1). Two-thirds of each column was filled with zeolite 13X. The ratio between zeolite and treated solution was 0.14 kg L^{-1}. The final working volume of each column was ap-

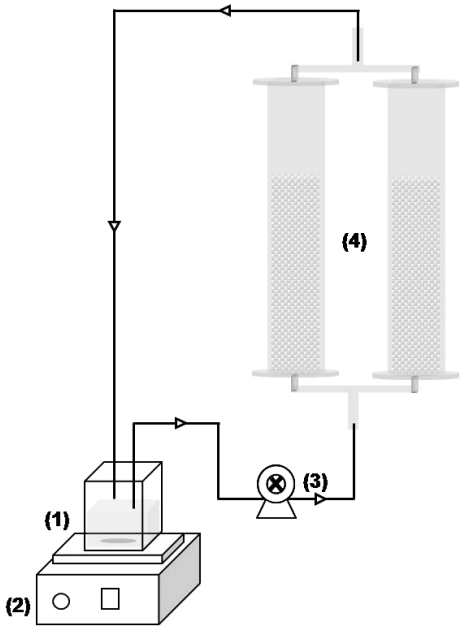

Figure 1. Experimental set-up: (1) reservoir tank, (2) magnetic stirrer, (3) peristaltic pump, and (4) Plexiglas columns.

proximately 150 mL, and the total volume of metallic solution treated was 2.5 L per batch. A reservoir tank was used to take samples and to control the pH. A peristaltic pump ($3\,L\,h^{-1}$) was used to avoid concentration gradients.

2.3.2 Industrial-Scale Bioreactor

The assays to evaluate a possible scale-up of the process were performed in an industrial-scale bioreactor consisting of three stainless-steel columns with 1.5 m height and 30 cm internal diameter [26]. Two-thirds of each column was filled with zeolite 13X. The ratio between zeolite and treated solution was $0.3\,kg\,L^{-1}$. A reservoir tank was used to take samples and to control the pH. The final working volume of the bioreactor was approximately 150 L per batch. A membrane pump ($5\,L\,h^{-1}$) was used to avoid concentration gradients.

2.4 Biofilm Formation

The inoculum culture was transferred to the bioreactor when the biomass concentration was $3\,g\,L^{-1}$. This medium was kept static for 24 h. Afterward, fresh basal medium was pumped through the bioreactor at a flow rate of $50\,mL\,min^{-1}$ during 24 h. Following this, a diluted medium containing 3.3 g glucose, 0.167 g peptone, 1 g malt extract and 1 g yeast extract per litre was pumped in over 72 h at the same flow rate to favour the hydrodynamics of the biofilm [27]. Operating under stress conditions with the diluted medium, the biofilm formation increases the production of polysaccharides and this allows for the formation of a coherent biofilm with a strong adhesion to the support surface [23].

2.5 Experimental Procedure and Cr Removal

Once the biofilm formation was created, a solution of $100\,mg\,L^{-1}$ Cr(VI) was pumped through the bioreactor. Although this process was designed to remove trace amounts of Cr(VI), the assays were performed with a $100\text{-}mg\,L^{-1}$ solution to validate the system in extreme conditions. Metal solutions were prepared with K_2CrO_4 provided by Riedel de Haën.

Different assays were carried out inside a laboratory-scale bioreactor in order to optimise the operating variables and, subsequently, the industrial-scale assay was realised under the optimised conditions (Tab. 1). In each assay, new biofilm formation was done and new zeolite was used. According to the results obtained by Silva et al. [15], batch assays were performed at fixed acid pH to assess the Cr removal efficiency.

Samples of Cr solution were taken from the bioreactor during the treatment and centrifuged at 5000 rpm for 15 min. The supernatant was analysed for pH and metal concentration. The Cr concentrations were determined by the 1,5-diphenylcarbazide method using a spectrophotometer (T60 UV Visible, PG Instruments) at 540 nm [28]. In the experimental conditions, the colorimetric determination is a selective method that distinguishes between Cr(VI) and total Cr in the solution. The applicable concentration limits are $100–1000\,\mu g\,L^{-1}$ using this diphenylcarbazide method. The reaction is very sensitive, with the molar absorptivity based on Cr being about $40\,000\,L\,g^{-1}cm^{-1}$ at 540 nm. The reaction with diphenylcarbazide is nearly specific to Cr.

3 Results and Discussion

3.1 Optimisation of Operating Conditions

Fig. 2 shows the profiles of the normalised concentration of Cr(VI) and Cr(III) in solution during the batch assay (E1). After 10 days of treatment, a reduction of 50 mg of Cr(VI) was reached (Tab. 2). The concentration of Cr(III) in solution was negligible during the first 4 days, although at the end of the treatment around 16 % of the reduced Cr(VI) was in solution as Cr(III). This outcome demonstrated that most of the Cr(VI) reduced by the biofilm was retained by the system biomass/zeolite. The results are in accordance with those reported by Figueiredo et al. [24]. These authors reported that a biofilm of *A. viscosus* supported on Y and X zeolites was able to remove chromium from dilute solutions (50–250 mg Cr(VI) per litre) with a maximum removal efficiency of 20 %. Although the pH was maintained at an acidic level, the obtained removal amount was low. The limiting factor in this system would be the low amount of biomass present in the bioreactor. Cr(VI) must be reduced by the bacteria biofilm to be retained by zeolite. Therefore, it is necessary to increase the biomass concentration in the system so that the Cr(VI) removal can be efficient.

A second assay (E2) was performed with the intention of increasing the biomass concentration inside the bioreactor. A similar biofilm formation protocol as used in the previous assay was conducted, with the exception that the amount of biomass in the inoculum culture was increased from 3 to 5 g biomass per litre in the system. Greater concentrations were not used because greater biomass concentrations in the system

Table 1. Experimental conditions of assays E1–E4.

Assay	Scale	Volume [L]	Zeolite pre-teatment	Inoculum [$g\,L^{-1}$]	pH	Batch
E1	laboratory	2.5	0.1 M H_2SO_4	3	3	1
E2	laboratory	2.5	0.1 M H_2SO_4	5	3/5	2
E3	laboratory	2.5	basal medium	3	4–4.5	2
E4	industrial	150	0.1 M H_2SO_4	5	3/5	1

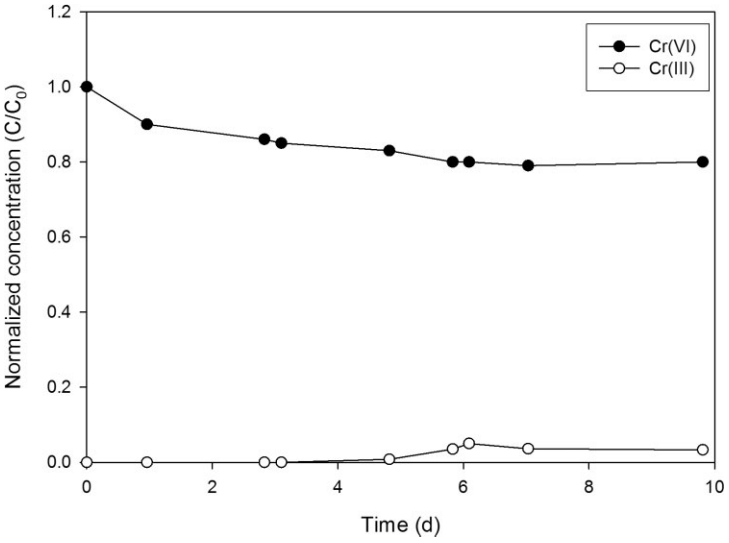

Figure 2. Cr concentration evolution during the laboratory-scale batch assay at pH 3 (E1).

Table 2. Chromium distribution for the different assays.

Assay	Initial Cr in solution [mg]		Final Cr in solution [mg]		Final Cr in zeolite [mg]
	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	
E1	250	0	200	8.25	n.m.
E2[a)]	250	0	0	67.5	n.m.
E3[a)]	250	0	65	0	n.m.
E4	15 000	0	507	3646.5	10 861

a) Reported data correspond to first batch.
n.m., Not measured.

provoke operational problems, such as clogging of the bioreactor. Furthermore, the diluted medium was substituted by a fresh basal medium. These changes increased the biofilm formation on the zeolite surface.

As seen in Tab. 2, the metal removed from this batch (E2) was greater than in the previous assay (E1). Approximately 80 % of the Cr(VI) was removed over the same period of time (10 days) (Fig. 3). This outcome confirmed the effectiveness of this enhanced procedure. Thus, the Cr(III) concentration increased over time at a value of approximately 50 %, which was nearly constant, probably as a result of the low pH of the system. Another improvement was discovered given that the aim of the bioreactor was the metal removal from the liquid stream, independently of its ionic form. In fact, Uluozlu et al. [29] reported that one of the most important factors affecting biosorption of metal cations is the surface charge of the sorbent. Their results showed that maximum biosorption was found to be at 95 % for Cr(III) ions at a pH of 5. Thus, after 10 days of treatment, the pH in the bioreactor was increased to a value of 5. As is shown in Fig. 3, the Cr(III) concentration decreased. At the end of the assay, 27 % of the Cr(III) were kept in solution and a negligible amount of Cr(VI) was detected. These results are in accordance with Chojnacka [30] and Yan and Viraraghavan [31]. These two research groups reported that a pH of 5 is the optimum pH to increase Cr(III) retention by a biomass system. The assay demonstrated a clear relationship between the pH of the system and the biomass behaviour in metal removal.

To investigate the possible use of the bioreactor in continuous mode, a fresh feeding of Cr(VI) at 100 mg L^{-1} was conducted, and the results are displayed in Fig. 3 (Batch 2). Initially, the pH of the system was controlled at a value of 3, and when the Cr(III) concentration reached a constant value, the pH was changed to 5. Although the removal was lower than the values observed during the first feeding, a higher elimination was obtained. A total of 85 % Cr(VI) removal was reached at the end of the treatment. Although a slight reduction of the Cr(III) concentration was detected when the bioreactor pH was raised to 5, the Cr(III) concentration remained nearly constant (30 %) throughout the experiment.

In these experiments, it was postulated that biomass retained in the support is one of the most important parameters in the bioremoval of Cr(VI). Thus, these tests were carried out in an assay (E3) with the intention of enhancing the biofilm formation on the zeolite 13X. Furthermore, this outcome would favour the contact between zeolite and biofilm, thus increasing the retention of Cr(III) inside the zeolite. A similar biofilm formation protocol as used in the E1 assay was conducted, although a different pre-treatment of zeolite was performed. The zeolite was pre-treated by immersion in the basal medium for 24 h to increase biofilm formation on the overall zeolite surface.

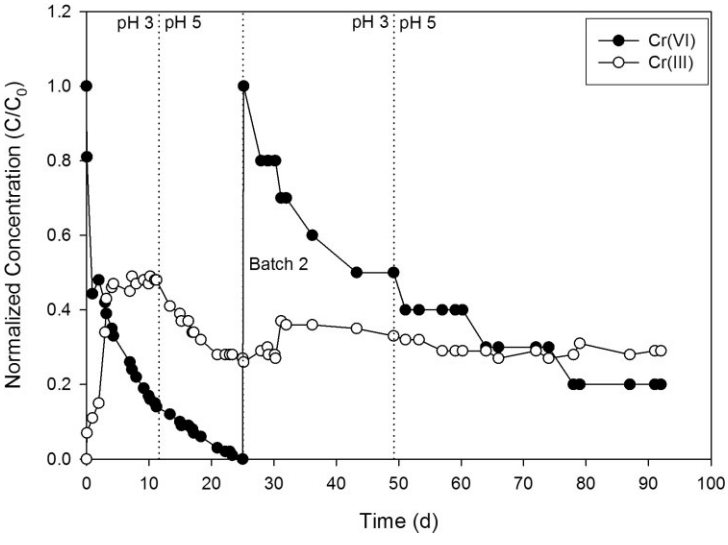

Figure 3. Cr concentration evolution during the laboratory-scale batch assay at pH 3 and 5 with biomass concentrations of 5 g L^{-1} in the two batches (E2).

The experimental data of this batch (E3) are shown in Fig. 4. In the bioreactor, no pH control was conducted, and the pH was stable between 4 and 4.5. At the beginning of the experiment, high reduction of Cr(VI) was obtained as a result of the high amount of biomass in the system. This removal was maintained over time, reaching 75 % after 14 days of the assay. A similar value was obtained in the previous assay (E2) at the same treatment time; however, the amount of Cr(III) retained in the system was higher in the E3 assay (Tab. 2).

A fresh feeding was added to evaluate the continuous operation of the bioreactor. The evolution of Cr over time is displayed in Fig. 4 (Batch 2). In this second batch, the Cr(VI) removal was lower compared to that obtained in the E2 assay. Initially, a decrease of Cr(VI) was observed, and after this, the amount of Cr(VI) in solution was constant over time. These results indicate that, although the amount of biofilm in the system is a limiting factor, pH control should be performed to favour the Cr(VI) reduction and Cr(III) retention.

3.2 Kinetic Studies

The Cr(VI) concentration profiles allow evaluation of the kinetic behaviour of the reduction reaction of Cr(VI) to Cr(III). In the assays with the higher Cr removals (E2 and E3), the reaction kinetics were evaluated during the first batch, and the regression coefficients for the zero-, first- and second-order reactions were calculated. The rate constants were determined using the Sigma Plot 8.00 software. The Sigma Plot curve fitter uses an iterative procedure based on the Marquardt-Levenberg algorithm that determines the values of the parameters that minimise the sum of the squared differences between the observed and the predicted values of the dependent variable. The results indicated that the reduction of Cr(VI) to Cr(III) in the bioreactor system could be quantitatively described by a second-order kinetic equation (Eq. 1) with respect to the Cr(VI) concentration:

$$\frac{dC}{dt} = -k\,C^2 \tag{1}$$

where C is the concentration of Cr(VI) (mg/L), t is the reaction time (days) and k is the kinetic coefficient for the second-order reaction (L mg^{-1}day^{-1}).

These results confirm that the reduction rate is favoured when the Cr(VI) concentration in the bioreactor is high. The rate constant values and the statistical correlation parameters are shown in Tab. 3. The constant reduction rate of Cr(VI) in the E2 assay is more than two-fold that obtained in E3. Based on these results, it was determined that there is no linear dependency between the kinetic coefficients and the inoculated biomass concentration. Thus, in this system, the behaviour of the pseudo-second-order reaction was defined and the kinetic equation for the system can be rewritten as:

$$\frac{dC}{dt} = -k^*\,C^2 \tag{2}$$

where C is the concentration of Cr(VI) (mg L^{-1}), t is the reaction time (days) and k^* is the kinetic coefficient for the pseudo-second-order reaction (L mg^{-1}day^{-1})

$$k^* = k\,C_b \tag{3}$$

where C_b is the concentration of biomass inoculated (mg L^{-1}) and k is the kinetic coefficient (L^{2}mg^{-2}day^{-1}).

Table 3. Kinetic coefficients for a pseudo-second-order reduction reaction of Cr(VI), Eq. (2), obtained for the different assays.

Assay	k^* [L mg^{-1}day^{-1}]	k [L^2 mg^{-2}day^{-1}]	R^2
E2	0.0048	0.00096	0.982
E3	0.0020	0.00066	0.987
E4	0.0034	0.00068	0.991

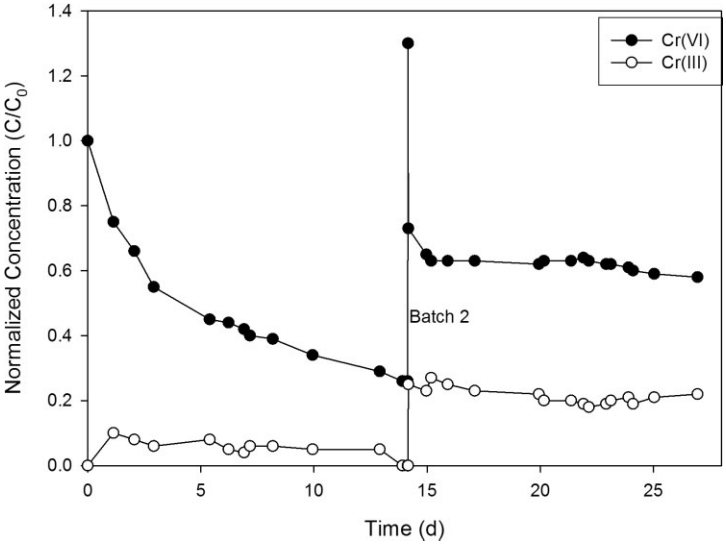

Figure 4. Cr concentration evolution during the laboratory-scale batch with previous treatment of the support by immersion in the basal medium for 24 h (E3).

Consequently, a moderate amount of biomass and pH control in the bioreactor are necessary to conduct an effective treatment. Based on these results, it can be concluded that the operating conditions applied during the E2 assay are the most appropriate to use in an industrial-scale bioreactor.

3.3 Industrial-Scale Bioreactor

Based on the previous results, a new assay was carried out in the industrial-scale bioreactor of 150 L. The bioreactor configuration was described in the Materials and Methods section and the experimental procedure to create the biofilm formation was the same as that used in the E2 assay. Similar to the E2 assay, pH control was performed during the experiment. Initially, the pH was fixed at a value of 3, and when the Cr(III) concentration reached a constant value, the pH was changed to 5. The results obtained (Fig. 5) indicate that the scale-up had been successful. The Cr(VI) removal pro-

file was similar to that obtained in the E2 experiment. Comparable kinetic behaviours were observed at the laboratory and the industrial scale. The pseudo-second-order kinetics fit well to the reduction reaction of Cr(VI) (Tab. 3). Similar to the E2 assay, the removal rate was influenced by the pH and the total Cr(VI) removal that can be achieved. The Cr(III) concentration in solution was nearly constant throughout the treatment, and a slight reduction was observed when the pH was changed to 5. In comparison with experiment E2, high retention of Cr(III) was shown. This fact can be due to the scale up of the process because the amount of zeolite utilized in the E4 assay per volume of Cr solution is almost double (0.3 kg L^{-1} in E4 and 0.14 kg L^{-1} in E2).

The Cr retention on the sorbent was evaluated after the assay (Tab. 2). Acid digestion of the zeolite determined that the Cr removed from the liquid phase was stored in the zeolite. The uptake was 0.24 mg Cr per g zeolite 13X. After the assay, the microorganisms stayed alive without the addition of any kind of nutrients. According to Quintelas et al. [32], it is postulated that the bacteria incorporated chromium in their metabolism, and this is probably the reason why the bacteria were metabolically active after several months without nutritional supplements. 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 metal. To our knowledge, no previous research is available regarding the use of industrial-scale bioreactors for the removal of Cr(VI) in solution. The outcomes discussed indicate the relevance and novelty of the results obtained in the present work.

4 Conclusions

According to the results obtained in this work, this new technology is adequate for the treatment of liquid streams with low Cr(VI) concentration. These assays confirmed that the designed bioreactor containing a biofilm supported on zeolite 13X can be effectively used in Cr removal. The key variables for metal removal were the biomass concentration and the pH of the system. This study demonstrated that a very acidic pH increased the Cr(VI) removal. However, the acidic pH also decreased the Cr(III) retention. Under the optimised conditions, approximately 100 % Cr(VI) removal was obtained. The assay conducted in the industrial-scale bioreactor showed a similar behaviour to the laboratory-scale assay. Furthermore, a great amount of reduced Cr(VI) was retained in the bioreactor, suggesting that this bioreactor has the potential of application in the treatment of liquid streams with low Cr(VI) concentrations.

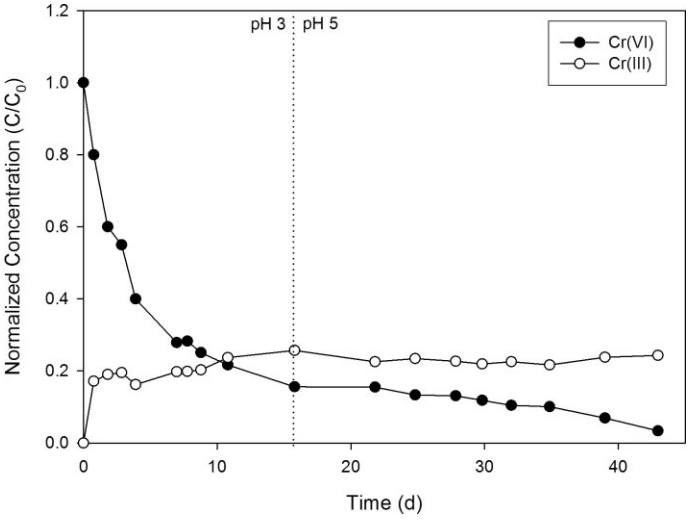

Figure 5. Cr concentration evolution during the industrial-scale batch assay under the optimised conditions (E4); initially, the system pH was fixed at a value of 3, and when the Cr(III) concentration reached a constant value, the pH was changed to 5.

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Symbols used

C	[mg L^{-1}]	concentration of Cr(VI)
C_b	[mg L^{-1}]	concentration of biomass inoculated
k	[L $mg^{-1}day^{-1}$]	kinetic coefficient for the second-order reaction
k^*	[L $mg^{-1}day^{-1}$]	kinetic coefficient for the pseudo-second-order reaction
R^2	[–]	coefficient of determination for linear regression
t	[day]	reaction time

References

[1] M. Gavrilescu, *Eng. Life. Sci.* **2004**, *4*, 219.
[2] L. Deng, Y. Su, H. Su, X. Wang, X. Zhu, *Adsorption* **2006**, *12*, 267.
[3] EPA (Environmental Protection Agency), EPA 625/R-00/004, **2000**.
[4] IPCS (International Programme on Chemical Safety), WHO, Geneva **1988**.
[5] United Nations Industrial Development Organization, *Regional Programme for Pollution Control in the Tanning Industry in South-East Asia*, (US/RAS/92/120/11-51) **2000**.
[6] S. Srivastava, I. S. Thakur, *Biodegradation* **2007**, *18*, 637.
[7] A. Özer, D. Özer, H. İ. Ekíz, *Adsorption* **2004**, *10*, 317.
[8] C. Quintelas, B. Fernandes, J. Castro, H. Figueiredo, T. Tavares, *Chem. Eng. J.* **2008**, *136*, 195.
[9] A. N. Mabbett, L. E. Macaskie, *Biotechnol. Lett.* **2001**, *23*, 683.
[10] *General Bacterial Sorption Processes* (Ed: M. M. Urrutia), Taylor and Francis Publishers, London **1997**.

[11] D. Çetin, S. Dönmez, G. Dönmez, *J. Environ. Manage.* **2008**, *88*, 76.
[12] E. M. N. Chirwa, Y.-T. Wang, *Environ. Sci. Technol.* **1997**, *31*, 1446.
[13] B. B. Nepple, J. Kessi, B. Bachofen, *J. Ind. Microbiol. Biotechnol.* **2000**, *25*, 198.
[14] T. Srinath, T. Verma, P. W. Ramteke, S. K. Garg, *Chemosphere* **2002**, *48*, 427.
[15] B. Silva, H. Figueiredo, I. C. Neves, T. Tavares, in *Proc of the World Academy of Science, Engineering and Technology* **2008**, *43*, 59.
[16] J. S. Novak, S. W. Tanenbaum, J. P. Nakas, *Appl. Environ. Microbiol.* **1992**, *58*, 3501.
[17] E. López, I. Ramos, M. A. Sanromán, *J. Food Eng.* **2003**, *60*, 463.
[18] A. Grappelli, J. S. Hard, W. Pietrosanti, U. Tomati, L. Campanella, E. Cardarelli, M. Cordatore, *Water Sci. Technol.* **1989**, *21*, 1759.
[19] J. A. Scott, G. K. Sage, S. J. Palmer, *Biorecovery* **1988**, *1*, 51.
[20] G. Blázquez, F. Hernáinz, M. Calero, M. A. Martín-Lara, G. Tenorio, *Chem. Eng. J.* **2009**, *148*, 473.
[21] M. J. Zamzow, J. E. Murphy, *Sep. Sci. Technol.* **1992**, *27*, 1969.
[22] M. Vaca Mier, R. Lopez Callejas, R. Gehr, B. E. Jimenez Cisneros, P. J. J. Alvarez, *Water Res.* **2001**, *35*, 373.
[23] S. Lameiras, C. Quintelas, T. Tavares, *Bioresour. Technol.* **2008**, *99*, 801.
[24] H. Figueiredo, I. C. Neves, C. Quintelas, T. Tavares, M. Taralunga, J. Mijoin, P. Magnoux, *Appl. Catal., B* **2006**, *66*, 276.
[25] C.-F. Lin, S.-S. Lo, H.-Y. Lin, Y. Lee, *J. Hazard. Mater.* **1998**, *60*, 217.
[26] T. Tavares, I. C. Neves, *PPI 35292/06*, **2006**.
[27] C. Quintelas, T. Tavares, *J. Resour. Environ. Biotechnol.* **2002**, *3*, 193.
[28] *Standard Methods for the Examination of Water and Wastewater* (Eds: D. Eaton, L. S. Clesceri, A. E. Greenberg), American Public Health Association (APHA), Washington **1995.**
[29] O. D. Uluozlu, A. Sari, M. Tuzen, M. Soylak, *Bioresour. Technol.* **2008**, *99*, 2972.
[30] K. Chojnacka, *J. Hazard. Mater.* **2005**, *121*, 167.
[31] G. Yan, T. Viraraghavan, *Water Res.* **2003**, *37*, 4486.
[32] C. Quintelas, B. Fonseca, B. Silva, H. Figueiredo, T. Tavares, *Bioresour. Technol.* **2009**, *100*, 220.

