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Evaluation of the bioremoval of Cr(VI) and TOC in biofilters under continuous operation using response surface methodology

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Abstract In the present study, the bioremoval of Cr(VI) and the removal of total organic carbon (TOC) were achieved with a system composed by an anaerobic filter and a submerged biofilter with intermittent aeration using a mixed culture of microorganisms originating from contaminated sludge. In the aforementioned biofilters, the concentrations of chromium, carbon, and nitrogen were optimized according to response surface methodology. The initial concentration of Cr(VI) was 137.35 mg l^{-1}, and a bioremoval of 85.23% was attained. The optimal conditions for the removal of TOC were 4 to 8 g l^{-1} of sodium acetate, >0.8 g l^{-1} of ammonium chloride and 60 to 100 mg l^{-1} of Cr(VI). The results revealed that ammonium chloride had the strongest effect on the TOC removal, and 120 mg l^{-1} of Cr(VI) could be removed after 156 h of operation. Moreover, 100% of the Cr(VI) and the total chromium content of the aerobic reactor output were removed, and TOC removals of 80 and 87% were attained after operating the anaerobic and aerobic reactors for 130 and 142 h, respectively. The concentrations of cells in both reactors remained nearly constant over time. The residence time distribution was obtained to evaluate the flow through the bioreactors.

Keywords Cr(VI) bioremoval · Biofilters · Anaerobic and aerobic processes · Kinetic study · Hydrodynamic study

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Introduction

Chromium is a highly toxic metal that is discharged into the environment through various industrial effluents, causing significant health problems (Stearns et al. 1995a; Rengaraj et al. 2003; Thacker et al. 2006). Tanneries, electroplating plants and catalytic industrial processes discharge large amounts of chromium every year. According to the US EPA (Baral and Engelken 2002) and the European Union (EC 1998), the concentration of Cr(VI) in water must be less than 0.05 mg l^{-1}, and the total Cr concentration, including Cr(III) and Cr(VI), must be less than 2 mg l^{-1} (Baral and Engelken 2002). In Brazil its concentration is limited to 0.1 mg l^{-1} for hexavalent chromium and 1 mg l^{-1} to trivalent chromium for discharge in waters, in agreement with the legislation of CONAMA (Resolution CONAMA no. 397, April 3, 2008-Brazil).

Currently, the most commonly used technology for the treatment of heavy metals in wastewater is chemical precipitation after the reductant addition to reduce Cr(VI) to Cr(III). The Cr(III) is the ionic species predominant at low pHs ($pH < 3.6$) (Francoise and Bourg 1991).

In slightly acidic to alkaline solutions, ionic Cr(III) species precipitate as hydroxides (Rai et al. 1987; Francoise and Bourg 1991; Wang and Shen 1997, Chirwa and Wang 1997; Patterson 1985; Chen and Hao 1998). Studies have shown that certain species of bacteria, mixed bacterial cultures, yeast, algae, and fungi are able to transform Cr(VI) to the trivalent form Cr(III), which is less toxic and mobile (Dermou and Vayenas 2008; Desai et al. 2008; Lee et al. 2008; Molokwane et al. 2008; Okeke et al. 2008; Bankar et al. 2009; Kong et al. 2009; Quintelas et al. 2009; Sanghi and Sankararamakrishnan 2009; Ahmad et al. 2010; Elangovan et al. 2010; Fernández et al. 2010; Orozco et al. 2010; Dalcin et al. 2011; Ilias et al. 2011; Orozco et al. 2011).

The processes by which microorganisms interact with toxic metals and enable their removal or recovery include bioaccumulation, biosorption, and enzymatic reduction (Srinath et al. 2002). A wide range of microorganisms reduce the ion by chromate reductase activity that included the following stages: (1) the binding of chromium to cell surfaces; (2) translocation of chromium into the cell; (3) reduction of Cr(VI) to Cr(III). Intracellular reduction of Cr(VI) to Cr(III) is known to be the main detoxification mechanism (Dönmez and Koçberber 2005). Stearns et al. (1995b) shows that Cr(VI) reduction by ascorbate under physiological conditions produced Cr(V) and carbon-based radicals as intermediates that reacted with DNA to produce Cr-DNA adducts and DNA single-stand breaks.

The enzymatic reduction of Cr(VI) to Cr(III) is a defense mechanism used by microorganisms living in environments contaminated with Cr(VI). Using a mixed culture of microorganisms originating from sludge, the bioremoval of Cr(VI) and the removal of TOC were achieved with an anaerobic filter followed by a submerged biofilter with intermittent aeration. The optimal chromium, carbon, and nitrogen contents were determined by applying response surface methodology. In addition, a hydrodynamic study of the biofilters was performed.

Materials and methods

Source of microorganisms and Cr(VI) and culture medium

A mixed culture obtained from tannery sludge was acquired from the AMCO (the Association of Manufacturers of Leather and Allied of Industrial district of Franca, Brazil), and potassium dichromate ($K_2Cr_2O_7$, PA Vetec mark) was used as a source of hexavalent chromium. According to the methodology described by APHA (American Public Health Association), potassium dichromate was dried in an oven at 120 ± 1°C for 24 h, weighed and diluted in distilled water (APHA 2005).

The culture medium was prepared according to the procedure described by Dermou et al. (2007). Namely, 1 g of NH_4Cl (acclimation experiments), 0.2 g of $MgSO_4{\cdot}7H_2O$, 0.001 g of $CaCl_2{\cdot}2H_2O$, 2.5 g of $CH_3COONa{\cdot}3H_2O$ (acclimation experiments) and 0.5 g of K_2HPO_4 were dissolved in 1 l of tap water. In the experimental design the concentrations of acetate and ammonium chloride were used in agreement with each experiment.

The culture medium pH was initially set to six and microorganisms used in anaerobic biofilters were acclimated in anaerobic conditions and micro-organisms used in the aerobic biofilter were acclimated under aerobic conditions.

Acclimation of micro-organisms

To acclimate the micro-organisms, 60 ml of sludge was added to 200 ml of the culture medium, which was supplemented with 0.5 mg l^{-1} of Cr(VI). The flasks were agitated in a shaker at 27 ± 1°C for 72 h and centrifuged at 12,500 rpm and 25 ± 1°C for 10 min. Subsequently, the reduction of Cr(VI) was determined from the supernatant, and the solid was transferred to fresh medium. Using the aforementioned procedure, the adaptation of the microorganisms to the culture medium was determined with 50 and 150 mg l^{-1} Cr(VI), respectively. After acclimation, the bacteria obtained from the sludge were used as inoculum for the filters.

Analytical procedures

The concentration of hexavalent chromium was determined using the colorimetric method 3,500-Cr D. The reaction was conducted in acidic medium with 1,5-diphenylcarbazide, as described in by APHA (2005). The total organic carbon (TOC) was determined by high temperature catalytic combustion using a TOC-VCPH-ASI+TNM-1 Shimadzu analyzer. All samples were diluted to meet the DOC concentrations

in the range of the equipment use and then were filtered through membranes of 0.2 μ. The concentration of total chromium was determined by atomic absorption spectrometry on a Varian spectrae 220 spectrometer. The VSS was determined by the fixed and volatile solids method at a temperature of 550°C, according to (APHA 2005). Supports of silicone rings were periodically withdrawn to determine biofilm accumulation with the exposure time. During sampling the peristaltic pump was stopped and the supports were removed from the biofilter. The sampled support of silicone rings were replaced by new ones so that the flow conditions through the pile were maintained (Cammarota and Sant'Anna 1998).

Experimental setup

Figure 1 shows a schematic depiction of the experimental system. As shown in the figure, an anaerobic biofilter (descending flow), a submerged aerated biofilter (ascendant flow), and peristaltic pump were used. The tank used to store the synthetic effluent was maintained at 70°C to reduce the risk of contamination from the environment. The system was fed with a peristaltic Watson Marlow model 520S pump, and the effluent was pumped directly from the tank. The anaerobic biofilter consisted of an acrylic cylinder, 1.0 m in height and 0.095 m in diameter, with descending flow. After fill with the material support this filters showed capacity of 3.2 l. The aerobic biofilter consisted of an acrylic cylinder, 1.0 m in height and 0.11 m in diameter with ascendant flow. After fill with the material support this filters showed capacity of 5.4 l. A system for supplying compressed air was placed at the base of the submerged aerated biofilter, and air was supplied four times a day for 30 min at a flow rate of 100 l/h. The selection of intermittent aeration mode was primarily for operational maintenance of the biofilm adhered to the support. The good results of Vieira et al. (2009) using intermittent aeration led us to work with this type of aeration.

Dissolved oxygen data were measured daily and in the case of anaerobic reactor ranged from 0.5 to 1.0 mg l^{-1} and on aerobic reactor of 2 to 5 mg l^{-1}.

A flow rate of 3.0 ml/min was applied, corresponding to a hydraulic residence time of 48 h. Preliminary tests revealed that the aforementioned residence time was sufficient to stabilize the reduction of chromium in the bioreactors. The pH was initially set to six to enhance the growth and maintenance of the mixed culture. Preliminary tests indicated that the abiotic reduction of Cr(VI) occurs when the pH is less than four. Initially, the tests were conducted in bioreactors to check for abiotic losses. The acclimated sludge was allowed to rest for 20 days inside the biofilters to ensure attachment prior to filling. Subsequently, the nutrient medium was fed into the reactor at the aforementioned flow rate for 40 days to achieve a constant concentration of biomass inside the bioreactors.

The use of combined systems of filters (anaerobic–aerobic) had as purpose increasing the removal of organic load and simultaneously assesses the capacity reduction/removal of Cr(VI) in combined effluent treatment (Chernicharo, 1997).

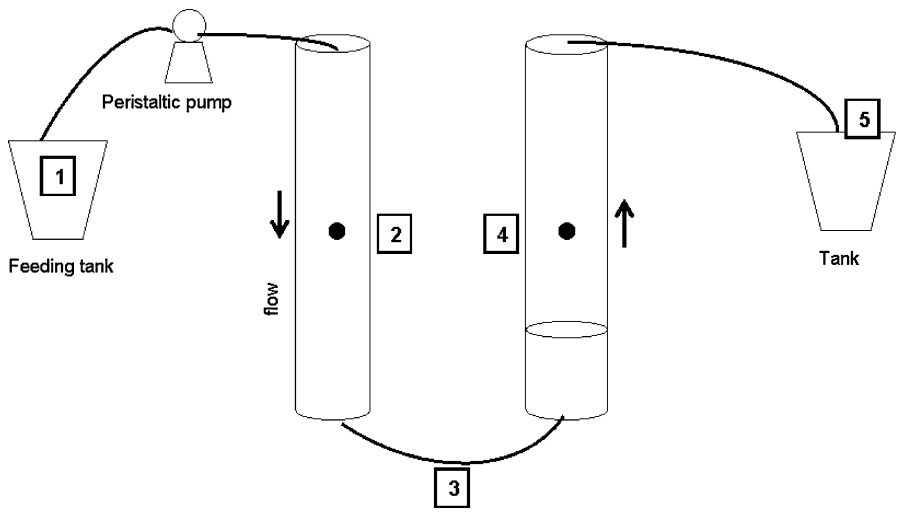

Fig. 1 Experimental setup. Numbers *1*, *2*, *3*, *4*, and *5* indicate the points of sample collection

Support material used in the filters was silicone rings. In the anaerobic biofilter the support had 0.32 cm internal diameter, 0.65 cm external diameter and 6.25 ± 0.79 cm length while in the aerobic biofilter the silicone rings had 0.48 cm internal diameter, 1.12 cm external diameter and 7.82 ± 1.07 cm length, respectively.

Preliminary tests with industrial support media (Dalcin et al. 2011) were not very efficient. Then we started a search for a support media that: (i) has the ability to remove high loads of organic and of Cr(VI) per unit volume; (ii) has a structure that was widely open, to avoid clogging by biomass growth and to ensure an adequate supply of oxygen, without the use of forced aeration; (iii) provides structural strength enough to support its own weight and the weight of the biomass that grows attached to its surface; (iv) was light enough to allow significant reductions in the cost of construction; (v) was biologically inert; so it would not be attacked by micro-organisms or toxic to the process; (vi) was chemically stable and presented the lowest possible cost per unit of organic matter removed. Thus, the silicone rings were chosen. These fillings were also chosen because they are lightweight. This property would allow the filters to be much higher without causing structural problems, thereby decreasing the area required for their installation. Another factor that led us to choose this filling was to meet the design criteria for the diameter of the tube/particle diameter ratio, that has to be larger than 10.

Design of the experiments

As shown in Table 2, a central composite design (CCD) was applied to verify the effect of the chromium, sodium acetate (carbon source), and ammonium chloride (nitrogen source) concentration on the bioremoval of Cr(VI) and TOC. Potassium dichromate ($K_2Cr_2O_7$) was used as a source of chromium, and the concentration of chromium, sodium acetate, and ammonium chloride were varied from 2.34 to 137.35 mg l^{-1}, 0.6 to 11.412 g l^{-1} and 0.06 to 1.141 g l^{-1}, respectively. The initial hexavalent chromium concentrations were selected according to the concentration of total chromium present in the tannery effluents. The selected concentrations of sodium acetate and ammonium chloride were based on data obtained from the literature to be representative of tannery effluents (Dermou et al. 2005; Dermou et al. 2007; Chen and Gu 2005). The CCD consisted of 2^3 factorial points, six axial points and three replicates at the central point. All of experiments were realized in duplicate (totaling 34 experiments), and Statistica 7.0 software was employed.

Kinetic study

Kinetic study was conducted on anaerobic and aerobic biofilters operating in a continuous flow mode and the culture medium with 6 g l^{-1} of $CH_3COONa{\cdot}3H_2O$, 1 g l^{-1} of NH_4Cl and 120 mg l^{-1} of Cr(VI). The other nutrients concentrations were defined at culture medium item.

Feed pH was adjusted to six and the aerobic biofilter was airing four times daily for 30 min.Samples were collected at pre-determined times and the kinetics of the removal Cr(VI), TOC and the growth of the microorganisms on the initial concentration of hexavalent chromium (120 mg l^{-1}) were determined.

Hydrodynamic study: characterization of the experimental unit

The axial dispersion model more appropriately represented the flow of the effluent in the biological reduction of hexavalent chromium in the biofilters. To monitor the behavior of the fluid in the reactor, response stimulus techniques were applied, and NaCl was used as a tracer to determine the distribution of residence times. Degree input of NaCl followed by monitoring conductivity of effluent was applied to biofilters fed with culture medium in the presence of biomass. To monitor the behavior of the fluid in the reactor, a solution of sodium chloride P.A. (0.1 M) was used. The amount of reagent added to the reactor was based on the biofilter volume and the residence time. The total duration of the assay was determined such that samples were collected at least twice during the theoretical hydraulic retention time.

The first biofilter in the series was the anaerobic reactor, and the second filter was the submerged aerated biofilter. The selected flow rate corresponded to a hydraulic retention time of 17.78 h in the anaerobic reactor and 30.17 h in the aerated biofilter, resulting in a total hydraulic retention time of 47.95 h. The ABNT NBR 7229:1993 Corrected version: 1997 (1997) recommends a minimum residence time of 12 h for the operation of biological filters; however,

the ideal operation time is 24 h. This standard describes the conditions required for design, construction and operation of septic tank systems, including treatment and disposal of affluent and settled sludge. The same has aimed to preserve the environmental and public health, hygiene, comfort, and safety of people in areas served by these systems.

Table 1 presents the equations used to calculate the dimensionless function F, average theoretical residence time, variance, Peclet number and axial dispersion number (Levenspiel 1974). The mean residence time (τ) was calculated by determining the dimensionless function F, which was obtained by calculating the area between $F(t)$ and the y-axis (the area above $F(t)$ versus time) using *Origin 7.0*® software.

After adjusting $F(\theta)$ with a sigmoidal function and a coefficient of determination of 0.995, $E(\theta)$ was calculated as the derivative of $F(\theta)$ in Origin® software to avoid the appearance of errors. The mean residence time was calculated from $E(\theta)$ to determine if errors were obtained during the derivation of $F(\theta)$.

Results and discussion

Acclimation of microorganisms

The mixed culture used in the initial tests provided promising results in the flasks. After acclimation with 0.5, 50, and 150 mg l^{-1} of chromium, the reduction levels of Cr(VI) were 100, 99.92, and 76%, respectively, after 60 days.

Before introducing the microorganisms into the reactor, abiotic losses were determined under the experimental conditions. The results revealed that abiotic losses ranged from 0.5 to 1.5%, and the data were adjusted to account for these losses.

After 20 days of rest, the concentration of microorganisms in the biofilter fillings was 4.6 ± 0.7 g l^{-1}. However, after 40 days at a flow rate of 3.0 ml/min, the concentration stabilized at 7.0 ± 0.5 g l^{-1}.

Table 1 Equations used in the hydrodynamic study

Dimensionless function F	$F(\theta) = \frac{\lambda_t - \lambda_0}{\lambda_\infty - \lambda_0}$	(1)
Residence time theoretical	$\tau_{teo} = \frac{V_{util}}{\upsilon}$	(2)
Variance	$\sigma^2 = \int_0^\infty (t-\tau)^2 E(t)\mathrm{d}t$	(3)
Peclet number	$\sigma_\theta^2 = \frac{\sigma^2}{\tau^2} = 2\frac{1}{Pe} - 2\left(\frac{1}{Pe}\right)^2(1 - e^{-Pe})$	(4)
Axial dispersion number	$ND = \frac{D_{ax}}{uL} = 1/Pe$	(5)

Where

θ normalized time [−]; λt electrical conductivity at the outlet of the reactor at each time $[(\Omega - m)^{-1}]$; λo electrical conductivity at the outlet of the reactor at the initial time $[(\Omega - m)^{-1}]$; λ_∞ electrical conductivity at the outlet of the reactor at the end time $[(\Omega - m)^{-1}]$; V_{util} volume of liquid in the reactor and piping (L^3); υ flow used ($L^3\ T^{-1}$) and σ^2 the variance

Experimental design

Table 2 shows the original and coded values of the variables and the corresponding Cr(VI) and TOC removals. Prior to each experiment, the biomass concentration was measured, and the values remained stable between 6.5 and 7.5 g l^{-1}. As shown in the Table, the removal of Cr(VI) ranged from 77.28 (experiment 6) to 100% (experiment 13), and the TOC removal ranged from 37 (experiment 1) to 86.05% (experiment 12). The central points presented minor variations for all of the responses, indicating that the proposed procedure was repeatable. The chromium removal results were promising. Namely, at concentrations less than 20 mg l^{-1}, the chromium concentration was reduced by more than 90%. In experiment 14, the concentration of chromium was 137.35 mg l^{-1}, and the reduction was relatively high (85.23%). Dermou et al. (2005) obtained removals of 100% at initial hexavalent chromium concentrations of 30 mg l^{-1} in 40 min using biological filters in stirred batch mode. The same removal was found by Chirwa and Wang (1997) with 50 mg l^{-1} of chromium in a fixed film bioreactor using a *Bacillus* sp. strain and operated in continuous mode at a high recycling ratio.

The removal rates obtained by the works of Alam (2004), Middleton et al. (2003), Dermou and Vayenas (2008), Elangovan and Philip (2009), Brunet et al. (2006); Ekenberg et al. (2005); Tziotzios et al. (2008), and Ahmad et al. (2010) were close to 100%. The removals obtained by this work were slightly lower except for experiment 13 (Table 2) in which the removal result was the same. It is noteworthy that the cited works used low initial chromium (VI) concentrations varying of 0.3 to 81 mg l^{-1} and did not consider the joint variation of

Table 2 Variables used in the CCD and the corresponding responses

Experiment	X_1 Sodium acetate (g l^{-1})	X_2 Ammonium chloride (g l^{-1})	X_3 Chromium (mg l^{-1})	Cr(VI) (%) removal ± (SD)	TOC (%) removal ± (SD)
1	2 (−1)	0.2 (−1)	20 (−1)	95.8 ± 3.95	37.0 ± 1.19
2	2 (−1)	0.2 (−1)	120 (1)	85.1 ± 5.31	50.0 ± 2.52
3	2 (−1)	1 (1)	20 (−1)	98.5 ± 4.19	64.0 ± 3.13
4	2 (−1)	1 (1)	120 (1)	85.3 ± 6.86	80.5 ± 1.85
5	10 (1)	0.2 (−1)	20 (−1)	89.6 ± 3.88	40.6 ± 1.68
6	10 (1)	0.2 (−1)	120 (1)	77.3 ± 6.26	55.2 ± 3.84
7	10 (1)	1 (1)	20 (−1)	94.1 ± 5.05	65.6 ± 3.15
8	10 (1)	1 (1)	120 (1)	80.8 ± 3.77	74.1 ± 3.05
9	0.6 (−α)	0.6 (0)	70 (0)	84.2 ± 6.04	54.1 ± 1.16
10	11.412 (α)	0.6 (0)	70 (0)	79.9 ± 4.92	57.9 ± 2.93
11	6 (0)	0.06 (−α)	70 (0)	79.8 ± 2.99	62.1 ± 2.39
12	6 (0)	1.141 (α)	70 (0)	83.7 ± 5.44	86.1 ± 4.55
13	6 (0)	0.6 (0)	2.34 (−α)	100. ± 2.98	40.0 ± 0.980
14	6 (0)	0.6 (0)	137.35 (α)	85.2 ± 4.95	55. 5 ± 2.62
15 (C)	6 (0)	0.6 (0)	70 (0)	89.4 ± 1.13	82.6 ± 1.89
16 (C)	6 (0)	0.6 (0)	70 (0)	88.8 ± 1.14	79.9 ± 1.89
17 (C)	6 (0)	0.6 (0)	70 (0)	87.4 ± 1.16	83.5 ± 1.89

other variables as was done in the experimental design proposed in this article. Thus, the proposed system has an advantage of the joint evaluation and removal of organic load removal of chromium (VI) at high initial concentrations of Cr(VI) simulating industrial conditions.

Cr(VI) bioremoval

Equation 6 shows the significant variables for the reduction process after the elimination of insignificant parameters. A significance level (p) of 10% was employed. After adjustment, a correlation coefficient (R^2) of 0.94 was obtained, indicating that the proposed empirical equation accounted for 94% of the variability in the data.

$$\text{Removal } (\%) = 87.08 - 2.46X_1 - 1.75X_1^2 + 1.38X_2 - 1.89X_2^2 - 5.97X_3 + 4.03X_3 \quad (6)$$

Because the model was significant, response surface diagrams were constructed, and the regions of interest were defined. Figure 2 illustrates the response surface and contour curve as a function of 1× and 2× for Cr(VI) bioremoval.

In the model equation (Eq. 6), the concentration of chromium had the strongest effect on Cr(VI) bioremoval, followed by the concentration of sodium acetate and ammonium chloride. The response surface methodology and the results presented in Table 2 indicated that high concentrations of chromium resulted in minimal reduction of Cr(VI). As shown in Fig. 2, lower concentrations of sodium acetate (0–6 g l^{-1}) and higher concentrations of ammonium chloride (0.5 to 1 g l^{-1}) promoted the removal of Cr(VI). Dermou and Vayenas (2008) used three different organic carbon concentrations of about 150, 175, and 200 mg l^{-1}, to verify the effect of the organic carbon (electron donor) concentration for constant Cr(VI) influent concentration at about 5.5 mg l^{-1} and the carbon concentration of 200 mg.l^{-1} proved results more efficient as Cr(VI) was reduced completely at the reactor's effluent, resulting in values below the maximum permitted limit of 0.05 mg l^{-1}.The presence of the organic substrate is a critical parameter for biological Cr(VI) extracellular reduction. This difference is related to the fact that the authors did not consider the joint influence of the nitrogen concentration, carbon concentration, and initial Cr(VI) concentration. Another important fact is due to the needs of the mixed culture used. Dermou et al. (2005) and Dermou et al. (2007) used 1 g l^{-1} of NH_4Cl

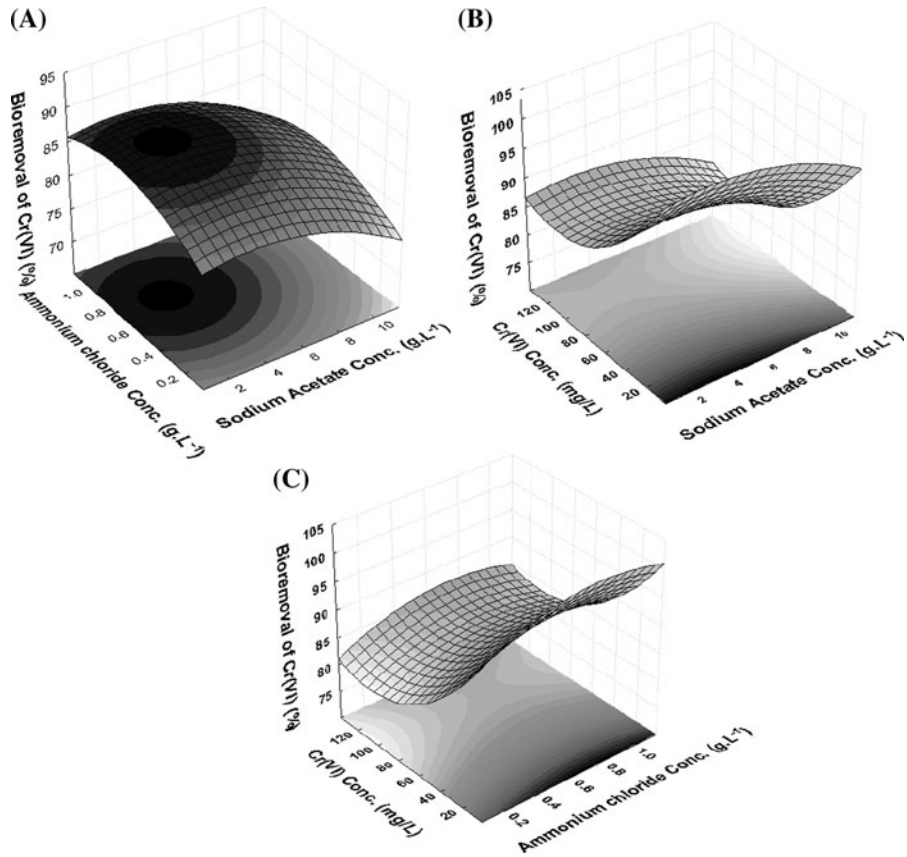

Fig. 2 Response surfaces: **a** Cr(VI) bioremoval versus the concentration of sodium acetate and ammonium chloride; **b** Cr(VI) bioremoval versus the concentration of sodium acetate and initial Cr(VI) concentration; and **c** Cr(VI) bioremoval versus the concentration of ammonium chloride and initial Cr(VI) concentration

as a nitrogen source and obtained significant Cr(VI) removal rates. Chen and Gu (2005) used 100 mg l^{-1} of NH_4SO_4, and good results for the removal of Cr(VI) were obtained. Dermou et al. (2005) analyzed the concentration of carbon during the reduction of 33 mg l^{-1} of hexavalent chromium, and found that 265, 150, and 20 mg l^{-1} of carbon were obtained after 40, 30, and 340 min of operation, respectively, in the complete reduction of 33 mg l^{-1} of Cr(VI). Dalcin et al. (2011) studied the Cr(VI) removal in a continuous biological filter using CCD methodology with successfully. The analysis of the CCD showed that the initial hexavalent chromium concentration was the most significant variable in the process and the pH value also contributed to the rate of hexavalent chromium removal but to a lesser extent than the initial concentration of Cr(VI).

TOC removal

Equation 7 shows the significant variables for the reduction of TOC after the elimination of insignificant parameters. A significance level (p) of 10% was applied. After adjustment, the regression coefficient (R^2) was equal to 0.91, indicating that the proposed empirical equation reproduced the experimental results.

$$\text{TOC Removal}\,(\%) = 78.43 - 9.10X_1^2 + 11.47X_2 + 60.30X_3 - 1362_3^2 \quad (7)$$

In Equation 7, the concentration of ammonium chloride had the strongest effect on the TOC removals, followed by the concentration of Cr(VI). Figure 3 illustrates the response surface and contour curve of the TOC removal as a function of the concentration of sodium acetate (X_1) and the concentration of Cr(VI) (X_3).

During the analysis of the contour curves shown in Fig. 3, the range of sodium acetate and chromium concentrations that maximized the TOC removal were determined. To maximize the response, a sodium acetate concentration of 4–8 g l^{-1} and a Cr(VI) concentration of 60 to 100 mg l^{-1} should be employed. Due to the combined effects of the sodium

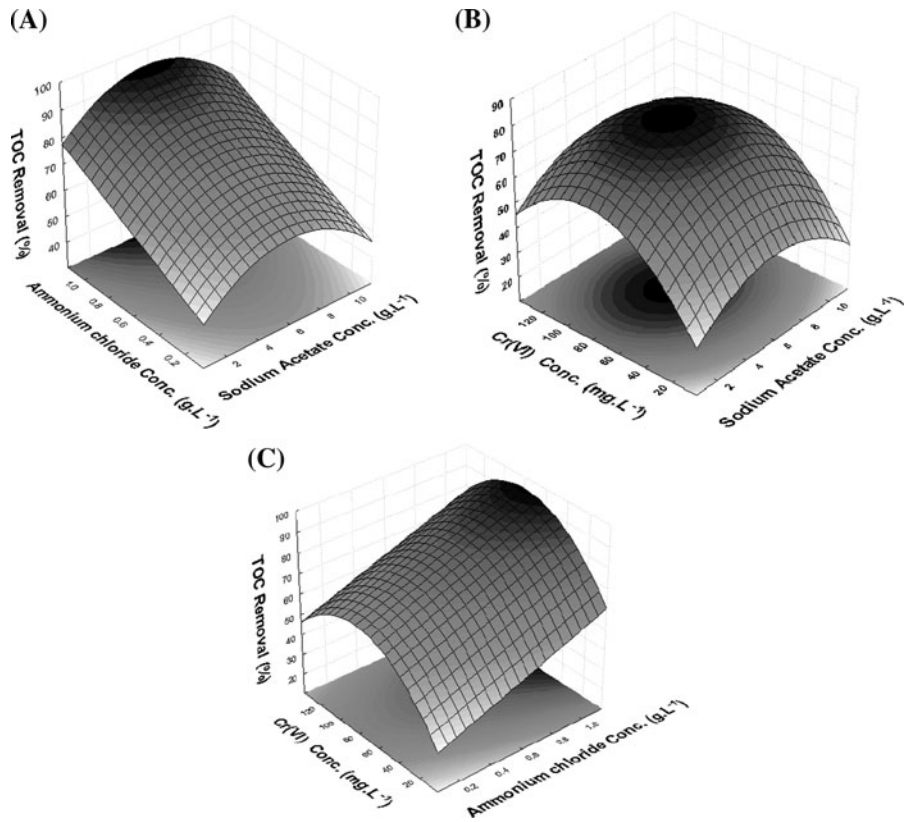

Fig. 3 Response surfaces: **a** removal of TOC as a function of the sodium acetate and Cr(VI) concentration; **b** removal of TOC as a function of the concentration of sodium acetate and ammonium chloride and **c** removal of TOC as a function of the ammonium chloride and Cr(VI) concentration

acetate and Cr(VI) concentrations, the concentration of sodium acetate should be approximately 6 g l^{-1}.

Likewise, to maximize the TOC removal, the optimal initial concentration of ammonium chloride must be greater than 0.8 g l^{-1}.

Upon jointly analyzing the removal of Cr(VI) and TOC, it was found that the optimal sodium acetate concentration was 4–6 g l^{-1}, and the optimal ammonium chloride concentration was 0.8 to 1 g l^{-1}. Thus, to determine the reproducibility of the results, the following conditions were selected: 6 g l^{-1} of sodium acetate and 1 g l^{-1} of ammonium chloride. These conditions were applied to avoid limiting the concentration of nutrients to cells, which may hinder the removal of Cr(VI). Moreover, in industrial effluents, TOCs greater than 1,000 mg l^{-1} are often observed, and TOCs of 1,000 mg l^{-1} can be obtained when the sodium acetate concentration is optimal (6 g l^{-1}). The hexavalent chromium concentration was set to 120 mg l^{-1} to obtain reproducible results under conditions that are similar to those of industrial effluents. The experimental results revealed that the Cr(VI) removal was 100% and the TOC removal was 79% after 96 h of operation. These results also contributed to the choice of the aforementioned concentrations in the kinetic study. The experimental TOC removal results were similar to those obtained from the model; however, the actual Cr(VI) removal was greater than the theoretical results due to the adaptation of the microbiota in the reactors. Thus, the results indicated that the study of optimal responses (removal of Cr(VI) and TOC) using response surface methodology and a CCD was satisfactory.

In agreement with Dermou and Vayenas (2008) the determination of the organic carbon concentration is of great importance, since carbon addition greatly increases the operational cost of the plant unit during industrial applications. They studied the Cr(VI) concentration of triplicate profiles along the filter depth for three different organic carbon concentrations of about 150, 175, and 200 mgC l^{-1} for constant Cr(VI) influent concentration at about 5.5 mg l^{-1} and found the presence of the organic substrate is a critical parameter for biological Cr(VI) extracellular reduction. According to the experimental results, the 200 mgC l^{-1} concentration proved more efficient as

Cr(VI) was reduced completely at the reactor's effluent. Tekerlekopoulou et al. (2010) found that the carbon source is a key parameter for microbial community structure and Cr(VI) reduction. A change of carbon source completely altered the structure of the microbial community from bacteria-dominated to fungus-dominated. Fungi were associated with a higher Cr(VI) reduction rates than those exhibited by bacteria.

Nitrogen is one of the main constituents of the biomass and several studies showed that the nitrogen content for most activated sludges is a constant (Atkinson and Mavituna 1991). For this reason, by modifying the initial nitrogen source concentration in a batch assay, the amount of biomass produced can be controlled; this strategy was used by Orozco et al. (2010) in their studies of the Cr(VI) reduction capacity of activated sludge affected by nitrogen and carbon sources. They studied the relationship between biomass growth and the amount of Cr(VI) removed a low initial biomass concentration and suggested that the Cr(VI) reduction could be associated with the cell multiplication stage. Maximum Cr(VI) removal rates were observed during the exponential growth phase, where no carbon or nitrogen limitation occurs. They confirms that is necessary the presence of both carbon and nitrogen sources to obtain high Cr(VI) removal rates. However, it must be pointed out that when one of both substrates is depleted, Cr(VI) removal continues but at a lower rate. They observed that the biological Cr(VI) removal was the consequence of two processes: (1) a fast Cr(VI) removal process, associated to the biomass growth, and (2) a slow removal process that is independent of the presence of the carbon and nitrogen sources. The second process may be important under certain conditions, such as in continuous cultures in which substrate concentrations are normally low. Biomass production and Cr(VI) consumption increased as a function of the ratio between initial nitrogen and carbon source concentrations, demonstrating the relationship between biomass growth and Cr(VI) removal process. The obtained results demonstrate that biological Cr(VI) removal is associated to the cell multiplication phase, as a result, maximum Cr(VI) removal rates occur when there is no limitation in carbon or nitrogen sources.

Elangovan and Philip (2009) evaluated the biokinetic parameters of *A. rhombi*-RE under aerobic and anoxic conditions. In the aerobic suspended growth system, 95% Cr(VI) reduction (initial concentration: 20 mg l^{-1}) was achieved, and a 90–95% COD removal (initial concentration: 3,000 mg l^{-1}; carbon source: molasses) was attained at a hydraulic retention time of 24 h. However, under the same operating conditions, the anoxic growth system achieved a Cr(VI) reduction efficiency of 95–98% and a COD removal efficiency of only 45–50%. These authors demonstrated that a number of viable process arrangements and microbial technologies can reduce Cr(VI) to Cr(III), including aerobic suspended growth systems, aerobic attached growth systems, and anoxic attached growth systems. Hence, most studies are directed towards the evaluation of the feasibility of the process at the pilot-scale level (Brunet et al. 2006; Ekenberg et al. 2005; Tziotzios et al. 2008; Ahmad et al. 2010). Reductions of ~100% have been obtained from initial Cr(VI) concentrations ranging from 5.5 to 81 mg l^{-1} in anaerobic and aerobic bioreactors.

Kinetics of the removal of chromium, TOC and volatile soluble solids (VSS)

Figure 4 shows the removal of Cr(VI) and total chromium by the anaerobic and aerobic reactor as a function of the operating time at a sodium acetate concentration of 6 g l^{-1}, an ammonium chloride concentration of 1 g l^{-1} and a Cr(VI) content of 120 mg l^{-1}.

Removal of Cr(VI) and total Cr remained ~100% at the output of the aerobic reactor up to 156 h of operation. Thus, the choice of operating conditions was satisfactory. Similar removals were obtained by Han et al. (2007) using bioreactors kind conical flask that removed 100% of Cr(VI) from initial Cr(VI) of 100 mg l^{-1} in 150 h. Dermou et al. (2005) obtained a Cr(VI) reduction of 100% at an initial hexavalent chromium concentration of 30 mg l^{-1} using chromium-resistant bacterial populations, indicating that the biological removal of hexavalent chromium from aqueous effluents is an economical and efficient technique for the treatment of industrial effluents. Other authors who have also achieved 100% of removal from 30 mg l^{-1} of Cr(VI) were Córdoba et al. (2008).

As shown in Fig. 4 for about 200 and 314 h occurred chromium removals in the anaerobic and aerobic reactors, respectively. After these mentioned

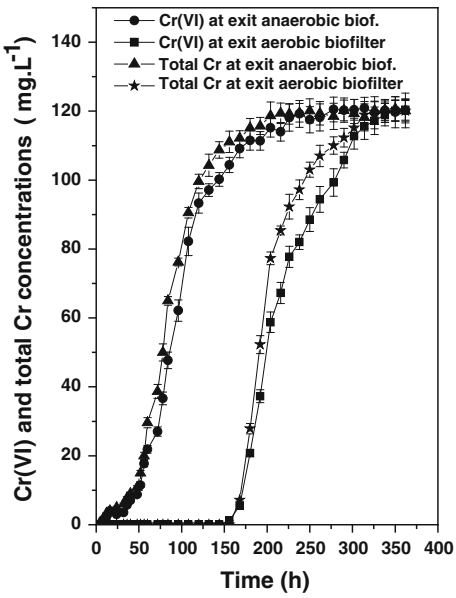

Fig. 4 The concentrations of Cr(VI) and total chromium as a function of time in the anaerobic and aerobic reactors. (Conditions: sodium acetate concentration = 6 g l^{-1}; ammonium chloride concentration = 1 g l^{-1}; Cr(VI) concentration = 120 mg l^{-1})

times for both reactors no Cr(VI) removal was observed. This delay in the chromium exit was expected because the concentration of chromium at the entrance of the aerobic reactor was lower than chromium at the entrance of the anaerobic reactor. Thus, the results revealed that chromium was retained within the bioreactors until stabilization was attained.

Kinetic studies was realized by Alam (2004) for the bioreduction of Cr(VI) by *Shewanella oneidensis* MR-1 in continuous, laboratory-scale flow through sand columns to Cr(VI) initial concentrations of 0.31 to 2.85 mg l^{-1}. At several retention hydraulic time, MR-1 completely reduced Cr(VI); however, at feed concentrations greater than 1.32 mg l^{-1}, the concentration of reduced Cr(VI) was inversely related to the Cr(VI) concentration of the feed. Batch kinetic experiments revealed that the growth of MR-1 was completely inhibited by the presence of Cr(VI). However, Cr(VI) reduction was still observed despite inhibition (Viamajala et al. 2004). Middleton et al. (2003) reported that MR-1 cells have a finite reduction capacity and demonstrated that the rate of reduction of resting MR-1 cells receiving sequential spikes of Cr(VI) ranging from 2.6–3.9 mg l^{-1} decreased gradually over time.

The process by which microorganisms interact with toxic metals and enable their removal and recovery includes bioaccumulation, biosorption and enzymatic reduction (Wang and Xiao 1995; Srinath et al. 2002). The accumulation of heavy metals by microorganisms frequently includes two phases. The initial phase is rapid and includes chemical adsorption or ion exchange on the cell surface. Subsequently, a slow phase involving active metabolism-dependent transport of metal inside the bacterial cells is observed (Gadd 1990; Dönmez and Koçberber 2005). After Cr(VI) reduction, Cr(III) may become bound to organic ligands, producing a soluble organo-Cr(III) complex. Cr(III) can also form complexes with organic compounds (James and Bartlett 1983; Nieboer and Jusys 1988). This observation demonstrated that a bacterial flavin reductase system reduced chromate to a soluble Cr(III)-NAD^+ complex (Puzon et al. 2002).

As shown in Fig. 4, the concentrations of Cr(VI) and total chromium in the output of the anaerobic reactor increased after 24 h. In the aerobic reactor, increases in Cr(VI) and total chromium contents were observed after 156 h. Moreover, the concentrations of Cr(VI) and total chromium in the aerobic reactor increased when the output of the anaerobic reactor reached 110 mg l^{-1} (when the anaerobic reactor was near stabilization).

After stabilization, we evaluated the regeneration of the beds in which feeding was used without chromium (only culture medium) with flow rate of 3 ml/min and intermittent aeration four times a day with a flow rate of 100 l/h. In this experiment the anaerobic reactor not liberate more chromium (VI) after 50 h of feeding medium without chromium and total chromium after 62 h of operation. As for the aerobic reactor the time was higher than anaerobic reactor time, the output of Cr(VI) ends after 85 h and the total chromium after 108 h. After 98 h of feeding of medium without chromium the concentration of biomass was 7.29 g l^{-1} in anaerobic reactor and 7.52 g l^{-1} at aerobic reactor. Thus, the chromium that could be removed from the biofilter by feeding of a substrate without chromium was removed in this regeneration process.

Figure 5 and Table 3 show the TOC and volatile suspended solids (VSS) concentration versus time, respectively. The aforementioned reactor and initial conditions were employed.

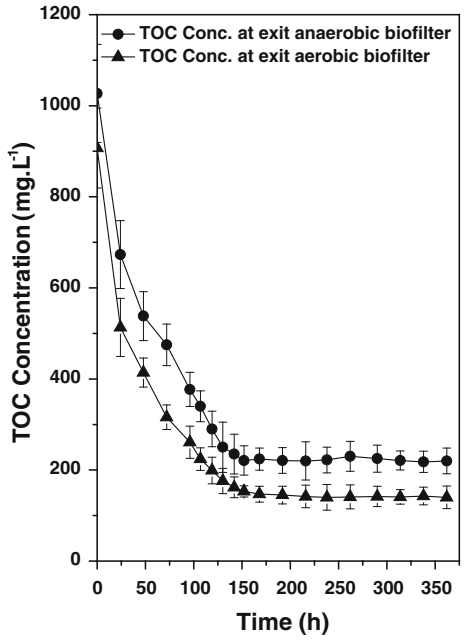

Fig. 5 The TOC concentration versus time in the outputs of the anaerobic and aerobic reactors (conditions: sodium acetate concentration = 6 g l^{-1}; ammonium chloride concentration = 1 g l^{-1}; Cr(VI) concentration = 120 mg l^{-1})

As shown in Fig. 5, the TOC removal stabilized at 80% after 130 h in the anaerobic reactor and 87% after 142 h in the aerobic reactor. Compared to the inlet concentration of each reactor, the reduction of the organic load was greater in the anaerobic reactor due to the relatively high load of chromium received by the reactor, which resulted in high nutritional needs. The TOC removal percentage of the anaerobic biofilter was 78.6% and of aerobic biofilter was 84.6%. This small difference may be related to the long period without aeration (approximately 6 h). Shortest periods entailed a great deal of detachment of biofilm support. However, this difference of removals shows that the biomass is active in both biofilters (high initial concentration of TOC). Moreover, as shown in Table 3, the concentration of cells in the reactor remained approximately constant, indicating that nutrients were not used for cell growth. The organic load removal stabilized before chromium saturation was observed in both biofilters; thus, after organic matter remained constant, cellular activity was focused on the removal of metal, not on an increase in the biomass content. Probably, strains work in a consortium, and the product metabolite of one strain could be the substrate for another species. This behavior may have contributed to the maintenance metabolism of the strain, as the amount of biomass did not decrease over time.

Table 3 VSS of anaerobic and aerobic reactors as a function of time

Hours	Anaerobic (g l^{-1}) ± (SD)	Aerobic (g l^{-1}) ± (SD)
0	7.58 ± 0.26	7.52 ± 0.036
12	7.53 ± 0.19	7.53 ± 0.035
24	7.54 ± 0.27	7.54 ± 0.046
36	7.63 ± 0.23	7.53 ± 0.026
48	7.41 ± 0.2	7.52 ± 0.043
72	7.23 ± 0.23	7.53 ± 0.033
84	7.3 ± 0.29	7.52 ± 0.044
96	7.21 ± 0.16	7.51 ± 0.037
107	6.98 ± 0.24	7.58 ± 0.042
119	7.22 ± 0.26	7.52 ± 0.025
130	6.98 ± 0.2	7.48 ± 0.040
142	7.27 ± 0.28	7.47 ± 0.036
152	7.07 ± 0.22	7.42 ± 0.029
168	7.17 ± 0.21	7.57 ± 0.032

Conditions: sodium acetate concentration = 6 g l^{-1}; ammonium chloride concentration = 1 g l^{-1}; Cr(VI) concentration = 120 mg l^{-1}

Growth was not observed in the bioreactors when the Cr(VI) removal was equal to 100%, indicating that Cr(VI) is inhibiting growth via a toxic response. Moreover, the aerobic reactor was receiving intermittent aeration, which may have contributed to the constant concentration of microbiota. Despite no substantial increase in biomass, biomass remained active up through stabilization. The eventual loss of Cr(VI) reduction capability suggest Cr(VI) is toxic or cells have a finite Cr(VI) reduction capacity (Alam 2004).

Kong et al. (2009) studied the kinetics of Cr(VI) reduction by *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli (E. coli)* under both pure and mixed cultures. Initially, the study of kinetics was performed in pure culture. It was observed that the growth of the two bacteria was both inhibited in the presence of Cr(VI). The results showed that *P. aeruginosa* had obvious inhibition effect on *E. coli* in the mixed culture. On the other hand, *P. aeruginosa* played primary role in Cr(VI) reduction. Molokwane et al. (2008) collected a mixed culture of bacteria from a wastewater treatment plant in Brits, North–West

Province (South Africa) and found that experiments run on purified individual species did not achieve the same level of Cr(VI) reduction as observed in the original consortium from sludge indicating possible existence of interspecies interactions necessary for optimum Cr(VI) reduction.

Two chromium-resistant bacteria (IFR-2 and IFR-3) capable of reducing/transforming Cr(VI) to Cr(III) were isolated from tannery effluents by Ilias et al. (2011). Isolates IFR-2 and IFR-3 were identified as *Staphylococcus aureus* and *Pediococcus pentosaceus*, respectively by 16S rRNA gene sequence analyzes. Reduction of Cr(VI) was found to be growth associated in both isolates IFR-2 and IFR-3 indicating that the two bacterial isolates can be good candidates for detoxification of Cr(VI) in industrial effluents.

Distribution of residence times

The tracer study was accomplished to show the characterization of biofilters used and mainly shows the good condition of packaging obtained with low formation of: preferential flow, short circuit, dead zones, stagnant areas, and recycling.

The cumulative distribution curve for the average residence time in the biofilters is shown in Fig. 6a. The cumulative distribution curve was used to obtain $E(\Theta)$ [the first derivative of $F(\Theta)$], which was used to calculate the variance and axial dispersion coefficient. The $E(\Theta)$ curve for the biofilters under the studied conditions is shown in Fig. 6b. From the cumulative distribution curve, the average residence time was calculated by determining the area above $F(\Theta)$ as a function of Θ. The values of this parameter were compared to the theoretical residence time, which was defined by the ratio of the volume and flow according to Eq. 2 (Fogler 1999). The variance of each experiment was calculated using Eq. 3, and the axial dispersion coefficient was calculated according to Eq. 5. The estimated values of the residence time (τ), variance (σ_θ^2), Peclet number (Pe) and dispersion number (DN) are shown in Table 4.

As shown in Table 4, the deviation between the ideal conditions and the residence time calculated by the DRT was 2.21% for the anaerobic and aerated biofilter. Thus, the behaviors of both filters were close to ideal, and the deviations were characteristic of the formation of by-pass in the reactor. Therefore, as shown in the cumulative distribution curves (Fig. 6), little formation of short by-pass was observed. Due to the sequence of biofilters (anaerobic reactor + submerged aerated biofilter), dispersed flow was observed, and a dispersion number of 7.472×10^{-2} was attained.

Conclusions

From the results obtained in the present study, the sequential use of two biological filters (the first reactor was anaerobic, and the second reactor was aerobic) with intermittent aeration and the application of mixed cultures is a promising method for the removal of

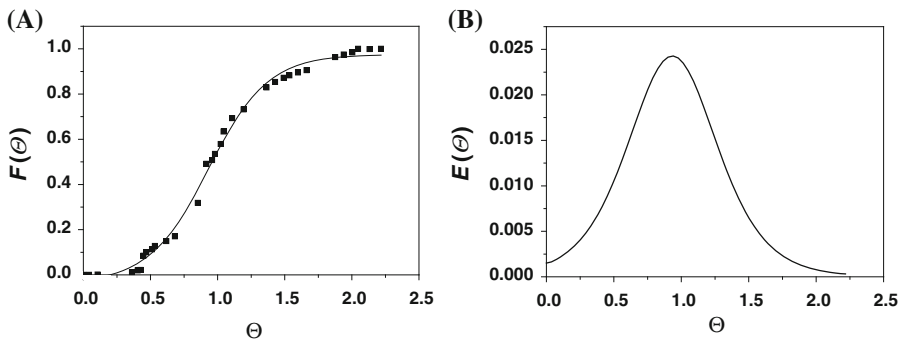

Fig. 6 **a** Dimensionless cumulative distribution curves: the points represent experimental data, and the line was adjusted to the sigmoid model of E(Θ). **b** Residence time distribution curve (E(Θ)) for the set of biofilters operating in series

Table 4 Estimated parameters for the average residence time, variance, Peclet number and axial dispersion

τ_{teo} (h)	τ (h)	Deviation (%)	σ_θ^2	Pe	DN
47.95	46.89	2.21	1.383×10^{-1}	13.38	7.472×10^{-2}

Cr(VI). The variables were evaluated by applying response surface techniques, and Cr(VI) and TOC removals of 100 and 79%, respectively, were obtained after 96 h of operation at an initial chromium concentration of 120 mg l^{-1}. The results revealed that 100% of the Cr(VI) and total chromium content could be removed from the output of the aerobic reactor after 156 h of operation when the initial concentration of Cr(VI) was set to 120 mg l^{-1}. Alternatively, the removal of TOC stabilized at 80% after 130 h of operation in the anaerobic reactor and 87% after 142 h of operation of the aerobic reactor. At the aforementioned operation times, the output of total chromium was lower than the input, indicating that chromium accumulated in the reactors. The cell concentration of both reactors remained nearly constant, even after the bioreactor was saturated and the organic matter removal remained constant. The difference between the ideal and calculated residence times was 2.21% for the anaerobic and aerated biofilters, indicating that the behaviors of the biofilters were close to ideal. Moreover, the deviations were characteristic of the formation of by-pass in the reactor.

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