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Statistical design of experiments as a tool for optimizing the batch conditions to Cr(VI) biosorption on *Araucaria angustifolia* wastes

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Abstract

In order to reduce the total number of experiments for achieving the best conditions for Cr(VI) uptake using *Araucaria angustifolia* (named pinhão) wastes as a biosorbent, three statistical design of experiments were carried out. A full 2^4 factorial design with two blocks and two central points (20 experiments) was experimented (pH, initial metallic ion concentration—C_o, biosorbent concentration—X and time of contact—t), showing that all the factors were significant; besides, several interactions among the factors were also significant. These results led to the performance of a Box–Behnken surface analysis design with three factors (X, C_o and t) and three central points and just one block (15 experiments). The performance of these two statistical designs of experiments led to the best conditions for Cr(VI) biosorption on the pinhão wastes using a batch system, where: pH 2.0; $C_o = 1200$ mg l^{-1} Cr(VI); $X = 1.5$ g l^{-1} of biosorbent; $t = 8$ h. The maximum Cr(VI) uptake in these conditions was 125 mg g^{-1}. After evaluating the best Cr(VI) biosorption conditions on pinhão wastes, a new Box–Behnken surface analysis design was employed in order to verify the effects of three concomitant ions (Cl$^-$, NO$_3^-$ and PO$_4^{3-}$) on the biosorption of Cr(VI) as a dichromate on the biosorbent (15 experiments). These results showed that the tested anions did not show any significant effect on the Cr(VI) uptake by pinhão wastes. In order to evaluate the pinhão wastes as a biosorbent in dynamic system, a glass column was fulfilled with pinhão wastes (4.00 g) as biosorbent, and it was fed with 25.0 mg l^{-1} Cr(VI) at pH 2.0 and 2.5 ml min^{-1}. The breakpoint was attained when concentrations of effluent of the column attained the value of 0.05 mg l^{-1} Cr(VI) using 5550 ml of the metallic ion solution. In these conditions, the biosorbent was able to remove completely Cr(VI) from aqueous solution with a ratio of Cr(VI) effluent volume/biosorbent volume of 252.3.

Keywords: Aracauria angustifolia wastes; Biosorption; Chromium(VI); Factorial design; Box–Behnken surface analysis

1. Introduction

Chromium(VI) is largely employed in the chemical industry for chrome plating, the manufacture of dyes and pigments, leather tanning, wood preserving, battery, rust and corrosion inhibitors, textiles, rubbers, toner for copying machines and cement-producing plants [1]. Waste streams from all these industries can discharge Cr(VI) into waterways [1] causing severe impact to the environment [2]. In addition, chromium(VI) has

been reported to be carcinogen to humans [1]. The maximum allowed amount of Cr(VI) in natural waters is only 0.05 mg l^{-1} [1]. In this way, Cr(VI) must be removed from industrial effluents, before being delivered into the environment.

The common procedures for Cr(VI) removal from industrial effluents include chemical precipitation [3], ion-exchange resins [4], organic groups grafted on textiles [5] and separation by specific membranes [6]. The chemical precipitation is usually employed at the beginning of effluent treatment; however, to attain the toxic specie residual concentration in the effluent with the acceptable contents according with the legislation, other operations are required. Notwithstanding the ion-exchange resins, modified textiles and specific membrane provide to be

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suitable for removal of residual concentrations of toxic elements from industrial effluents; however, these procedures are very expensive, hindering their large application in industrial effluent treatments [4–6].

Activated carbon has been widely employed as the most popular adsorbent for the effluent treatment [7,8]. Besides its extensive use, activated carbon of high quality remains costly [7,8]. Besides that, the activated carbon requires the use of chelating agents for the removal of inorganic species, making this procedure even more expensive.

Due to the high costs of commercial adsorbents, biosorption is an economic feasible way to perform the treatment of potentially toxic species containing effluents. The cellular walls of biomass contain carboxylic acids, phenols, amine and amide groups which are able to complex heavy metals. The most studied biosorbents for Cr(VI) removal from aqueous solutions were the microorganisms [2,9–13]. However, the limitation of these biosorbents for industrial effluent treatment is its low available biomass. On the other hand, agro-industrial, marine and botanic residues are usually available in large amounts, allowing its application as biosorbents in industrial effluent treatment plants. These biosorbents such as hazelnut shell [14], cone biomass of tree [15], sugar beet pulp [16], rice husks [17,18], coirpith [17], sawdust [17,18], nut shell [18], peat moss [18,19], waste tea and exhausted coffee [18], chitosan [18–20], waste crab shell [21], bagasse fly ash [19], mucilaginous seeds [22], grape stalks [23], cork [23], olive stones [23], tree bark [18,23] and charcoal [17,24] have been employed for Cr(VI) removal from aqueous solutions.

The *Araucaria angustifolia* syn. *Araucaria brasiliensis* is a tall tree (30–52 m) of the genus *Araucaria* [25] which occupies large areas in the south and southeast of Brazil and east of Argentina [25]. The flowering of the *A. angustifolia* produces a bunch of seeds, which is named pinha. Each one of these presents a diameter of 10–25 cm and contains about 700–1200 scales with about 150 seeds weighing up to 4.7 kg [25]. The seeds are named pinhão, which contains a resistant coat that involves it [26]. The pinhão is cooked in water and it is largely consumed in Brazil. The coat of the pinhão seed is usually discarded on the garbage, and it takes so much time to being decomposed. Other foodstuffs from pinhão have been developed in Brazil generating about 10 tonnes of pinhão coat per year. Due to the need to find new and inexpensive adsorbents for metal removal, and also to diminish the large amounts of pinhão wastes, in this work is presented the first application of the use of pinhão wastes as a biosorbent for successful removal of chromium(VI) from aqueous samples.

2. Experimental

2.1. Biosorbent preparation

Pinhão was acquired at local market in Porto Alegre-RS, Brazil. The weight of each seed varied between 7 and 9 g. The seed coats correspond to approximately 22% of the seed. About 2 kg of pinhão was cooked in a 10 l glass beaker for 2 h, and then the seed was separated from the coat. The brown aqueous solution resulted from the pinhão cooking containing oxidized phenols [26] was discarded. Afterwards, the pinhão wastes were again heated to boiling for 2 more hours, in order to remove the water soluble phenolic compounds [26], in order to avoid releases of these compounds to the water solutions. Subsequently, the pinhão wastes were washed with distilled water, dried at 70 °C in an air-supplied oven for 8 h. After that, the pinhão wastes were grounded in a disk-mill and subsequently sieved. The fraction of biosorbent with diameter of particles lower than 250 μm was used. The apparent density of the powder pinhão wastes was 0.18 g cm^{-3}, and it presented an intense brown coloration that was assigned to polyphenols [26] which must be the main organic compounds responsible for biosorption of chromium(VI).

2.2. Reagents and solutions

Doubly distilled water was throughout employed.

A 5000 mg l^{-1} chromium(VI) stock solution was prepared dissolving 14.144 g of $K_2Cr_2O_7$ (Vetec, Rio de Janeiro-RJ, Brazil) in water and completing the final volume to 1.00 l. Working Cr(VI) solutions from 5.00 to 1200.0 mg l^{-1} were prepared from suitable serial dilution of the stock solution. The pH adjustments of the solutions were made with aliquots of 1.0 mol l^{-1} of H_2SO_4 and NaOH, utilizing a pH/mV hand-held meter handy-lab 1 Schott (Mainz, Germany) provided with combined glass electrode model Blue-Line 23.

The 1.0 g l^{-1} of 1,5-diphenylcarbazide (DPC) solution was made dissolving 250 mg of DPC in 100.0 ml of ethanol and completing the final volume to 250.0 ml with water.

For the interference studies on the chromium(VI) uptake by the biosorbent the following salts of elements were employed: NaCl (Merck), KNO_3 (Merck) and NaH_2PO_4 (Merck).

For recovery of Cr(VI) loaded pinhão wastes, a 3.00 mol l^{-1} of H_2O_2 (Merck) was employed.

2.3. Chromium(VI) batch biosorption procedure

An aliquot of 20.00 ml of 5.00–1200.0 mg l^{-1} Cr(VI) was added to a conical plastic tube (117 mm height and 30 mm diameter) containing 30.0–150.0 mg of pinhão wastes. The flasks were capped, poured horizontally in a horizontal shaker and agitated for 5 min–12 h. Afterwards, the flasks were filtered, using glass filter provided with Whatman filter paper, in order to separate the biosorbent from the aqueous solution, and aliquots of 1–5 ml of the supernatant were properly diluted to 50–100 ml in calibrated flasks using water. The chromium final concentrations were spectrophotometrically determined using DPC at 540 nm [27], after multiplying the measured concentration value found in the solution after the biosorption procedure, by the proper dilution factor.

The removal of Cr(VI) from aqueous solution was determined by Eq. (1), and the amount of Cr(VI) uptaken by the biosorbent is given by Eq. (2).

$$\% \text{ removal} = \frac{C_o - C_f}{C_o} \cdot 100 \qquad (1)$$

$$q = \frac{C_o - C_f}{X} \quad (2)$$

where q is the amount of metallic ion uptaken by the biosorbent ($mg\,g^{-1}$), C_o the initial Cr(VI) concentration put in contact with the biosorbent ($mg\,l^{-1}$), C_f the Cr(VI) concentrations ($mg\,l^{-1}$) after the batch biosorption procedure and X is the biomass concentration ($g\,l^{-1}$).

2.4. Statistical design of experiments

2.4.1. Full factorial design

Factorial design is employed to reduce the total number of experiments in order to achieve the best overall optimization of the system [28–30]. The design determines which factors have important effects on a response as well as how the effect of one factor varies with the level of the other factors. The determination of factor interactions could only be attained using statistical designs of experiments [28,30], since it cannot be shown when the system optimization is carried out by varying just one factor at the time and fixing the others.

For studying the chromium(VI) biosorption on pinhão wastes, the amount of biosorbed metallic ion (q) could depend on the acidity of the medium (pH), initial chromium(VI) concentration (C_o), biosorbent concentration (X) and the time of contact (t). Other variables such as speed of agitation was kept at 120 strikes/min and temperature was kept at $25\,°C$. A full 2^4 factorial design employed is given in Table 1. The factor levels were coded as -1 (low), 0 (central point) and 1 (high) [28,29]. For treatment of datum, the Minitab Statistical Software release 14.1 was employed throughout in order to obtain the effects, coefficients, standard deviation of coefficients and other statistical parameters of the fitted models.

2.4.2. Box–Behnken response surface design

After performing a screening of the factors with the factorial design, a response surface analysis statistical procedure [29] was employed in order to achieve the highest metal uptake. The experimental set was carried out according to Table 2. After optimizing the chromium(VI) uptake by pinhão wastes, the interference effects of Cl^-, NO_3^- and PO_4^{3-} on dichromate uptake were also evaluated using a Box–Behnken response surface design (Table 3). The percent of interference on chromium(VI) adsorption was obtained by using the following equation:

$$\%\text{interference} = \left(\frac{q\text{Cr(VI)} + \text{concomitants}}{q\text{Cr(VI) alone}} - 1 \right) \cdot 100 \quad (3)$$

where qCr(VI) + concomitants is the chromium(VI) uptake by the biosorbent in the presence of concomitant species and qCr(VI) alone is the chromium(VI) uptake by the biosorbent in the absence of concomitant species.

2.5. Column adsorption studies

A glass column of 250 ml containing a porous sintered glass disk at the bottom and a Teflon stopcock was packed with 4.00 g

Table 1
Optimization of chromium(VI) biosorption on pinhão wastes

Experiments	Blocks	pH	C_o	X	t	q ($mg\,g^{-1}$)
1	1	1	−1	−1	−1	1.61
2	1	−1	1	−1	−1	71.30
3	1	−1	−1	1	−1	42.35
4	1	1	1	1	−1	12.82
5	1	−1	−1	−1	1	90.01
6	1	1	1	−1	1	37.64
7	1	1	−1	1	1	20.77
8	1	−1	1	1	1	64.74
9	1	0	0	0	0	26.56
10	1	0	0	0	0	26.82
11	2	−1	−1	−1	−1	58.01
12	2	1	1	−1	−1	21.54
13	2	1	−1	1	−1	9.90
14	2	−1	1	1	−1	48.28
15	2	1	−1	−1	1	17.42
16	2	−1	1	−1	1	104.76
17	2	−1	−1	1	1	50.15
18	2	1	1	1	1	23.90
19	2	0	0	0	0	26.19
20	2	0	0	0	0	24.55

Factors	Levels		
	−1	0	1
pH	2.0	3.0	4.0
C_o ($mg\,l^{-1}$)	400.0	500.0	600.0
X ($g\,l^{-1}$)	2.50	5.00	7.50
t (h)	1.0	4.5	8.0

A full 2^4 factorial design divided in two blocks containing two central points per block.

Table 2
Optimization of chromium(VI) biosorption on pinhão wastes

Experiments	Blocks	X	C_o	t	q ($mg\,g^{-1}$)
1	1	−1	−1	0	90.66
2	1	1	−1	0	101.02
3	1	−1	1	0	125.20
4	1	1	1	0	121.51
5	1	−1	0	−1	112.31
6	1	1	0	−1	100.24
7	1	−1	0	1	116.63
8	1	1	0	1	110.86
9	1	0	−1	−1	88.99
10	1	0	1	−1	120.31
11	1	0	−1	1	76.75
12	1	0	1	1	101.67
13	1	0	0	0	89.65
14	1	0	0	0	89.65
15	1	0	0	0	87.48

Factors	Levels		
	−1	0	1
C_o ($mg\,l^{-1}$)	600.0	900.0	1200.0
X ($g\,l^{-1}$)	1.50	2.00	2.50
t (h)	8.0	10.0	12.0

Box–Behnken design containing 15 experiments, and 3 factors (X, C_o and t). The pH was adjusted to 2.0.

Table 3
Interference studies on the chromium(VI) uptake by pinhão wastes, using Box–Behnken design, containing 15 experiments, and 3 factors (Cl^-, NO_3^- and PO_4^{3-})

Experiments	Blocks	Cl^-	NO_3^-	PO_4^{3-}	Interference (%)
1	1	−1	−1	0	1.75
2	1	1	−1	0	−4.50
3	1	−1	1	0	−7.66
4	1	1	1	0	−5.31
5	1	−1	0	−1	−6.16
6	1	1	0	−1	3.67
7	1	−1	0	1	1.09
8	1	1	0	1	0.17
9	1	0	−1	−1	−4.22
10	1	0	1	−1	−2.76
11	1	0	−1	1	−4.97
12	1	0	1	1	6.51
13	1	0	0	0	5.50
14	1	0	0	0	−6.48
15	1	0	0	0	0.02

Factors	Levels		
	−1	0	1
Cl^- ($mg\,l^{-1}$)	1000.0	1500.0	2000.0
NO_3^- ($mg\,l^{-1}$)	1000.0	1500.0	2000.0
PO_4^{3-} ($mg\,l^{-1}$)	1000.0	1500.0	2000.0

The conditions employed were: pH 2.0, $C_o = 1000.0\,mg\,l^{-1}$ Cr(VI), $t = 8\,h$ and concentration of pinhão wastes $1.50\,g\,l^{-1}$.

of pinhão wastes, which occupied 22 ml of this column. Above the packing material, the column was filled with $25.0\,mg\,l^{-1}$ Cr(VI) solution. The effluent column flow-rate was adjusted to $2.5\,ml\,min^{-1}$ and the height of the Cr(VI) above the biosorbent was kept constant by feeding the column with a peristaltic pump (Milan, Colombo-PR, Brazil). The column effluents were collected at each 50.0 ml and Cr(VI) was spectrophotometrically determined using DPC at 540 nm [27].

3. Results and discussion

3.1. Preliminary experiments

The best performance of a factorial design depends on some knowledge about the system to being optimized [30]. If the values of all system parameters to be studied are unknown, the projection of the results obtained with the factorial design could not attain the maximum optimization of overall system [30]; besides, the total number of experiments required will be excessively increased, making the factorial design very complex [30]. Based on this, preliminary experiments about the optimization of the biosorption of Cr(VI) on pinhão wastes were carried out using $5.00–100.0\,mg\,l^{-1}$ Cr(VI), the acidity was adjusted at pH 2.0 according to literature [15], biosorbent concentration of $5\,g\,l^{-1}$, speed of agitation of 120 strikes/min and contact time of 5–360 min. It was observed that for solutions $5.00\,mg\,l^{-1}$ of Cr(VI) after shaking for 5 min, the removal of Cr(VI) from solution was 100.0% (see Fig. 1). For a $100.0\,mg\,l^{-1}$ of Cr(VI) solution a similar behavior was observed; how-
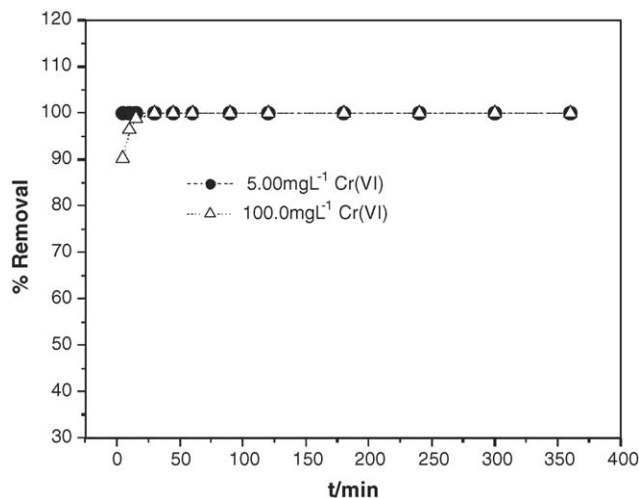

Fig. 1. Removal of Cr(VI) from aqueous solution by the pinhão wastes biosorbent. Agitation speed 120 strikes/min, biosorbent concentration (X) $5.00\,g\,l^{-1}$ and pH 2.0.

ever, at 5 min the removal of the element was 90.2% and attained 100.0% after 30 min of contact with the biosorbent (Fig. 1).

The total number of experiments carried out in this investigation was 24 (12 h), and the only valuable information is that the pinhão wastes presented to be a powerful biosorbent for Cr(VI) removal from aqueous solution at pH 2.0, since a $100.0\,mg\,l^{-1}$ of Cr(VI) was completely removed after just 30 min of contact with pinhão wastes. This concentration value is about 5–10 times higher than that found in real industrial effluents [17], which confirms the potential application of this biosorbent for Cr(VI) removal from aqueous solutions.

In order to continue the investigation of Cr(VI) uptake by pinhão wastes, the maximum amount of the metallic ion was optimized using statistical design of experiments.

3.2. Screening of factors for dichromate uptake by pinhão wastes

Metallic ion uptake by a biosorbent in a batch system usually depends on several factors, such as acidity of medium (pH), initial metallic ion concentration (C_o), concentration of the biosorbent (X) and time of contact between the metallic ion and the biosorbent (t), speed of shaking, etc. The optimization of all those variables using the univariate procedure is very tedious, because any variable (factor) is optimized, by varying just one factor by the time and fixing the others. Then, the best value achieved by this procedure is fixed and other factors will be varied by the time. The disadvantage of this univariate procedure is that the best condition could not be attained, because the interactions among all the factors are neglected, and also it is not known if the set of other fixed variables were kept at other levels, the results would lead to the same optimization. In addition, the total number of experiments to be carried out in the univariate procedure is much higher when compared with statistical design of experiments.

In this work, the factors screened were pH, initial Cr(VI) concentration (C_o), biosorbent concentration (X) and time of contact between the biosorbent and Cr(VI) (t), for best metallic ion uptake using a batch adsorption system. The experiments of Table 1 ($n = 20$, performed in two blocks of 8 h) were carried out, based on the preliminary experiments described above, obtaining the metallic ion uptake (q) in milligram of Cr(VI) per gram of pinhão wastes. The definitions of the factors and the levels used in the complete design are presented in Table 1. Main, interaction effect, coefficients of the model, and standard deviation of each coefficient, and probability for the full 2^4 factorial design are presented in Table 4. As can be seen, almost all main factors and their interactions were significant at 5% of probability level ($p < 0.05$). All the effects and interactions that presented probability lower than 0.05 were significant. With the exception of block ($p = 0.052$), pH $\cdot$ C_o ($p = 0.467$) and $C_o \cdot X \cdot t$ ($p = 0.087$) all other effects were significant at 95% of confidence level. The block was performed on two different working days making the two central points measurements in each block. The information that the block was not significant was very important, since the days to perform the measurements would not influence the metallic ion uptake by the biosorbent, at 5% level, which confirmed the robustness of the biosorption procedure. In addition, it should be highlighted that the four member interaction, pH $\cdot$ $C_o \cdot X \cdot t$, was aliased with the block term, which meant that its value was confounded with the block value, and did not appear in Table 4. In addition, the fit model presented an adjusted square correlation coefficient (R^2 adjusted) of 99.93%, fitting very well the statistical model. In this way, the Cr(VI) uptake by pinhão wastes could be expressed as the following

equation:

$$q = 42.20 - 24.00\text{pH} + 5.92C_o - 8.08X + 8.97t$$
$$+ 6.73\text{pH} \cdot X - 2.24\text{pH} \cdot t - 2.60C_o \cdot X + 0.66C_o \cdot t$$
$$- 3.20X \cdot t - 1.66\text{pH} \cdot C_o \cdot X - 0.60\text{pH} \cdot C_o \cdot t$$
$$+ 1.95\text{pH} \cdot X \cdot t + 0.45C_o \cdot X \cdot t \tag{4}$$

The values of the factors being coded, its levels are valid only to the levels described in Table 1. The positive values of effects meant that an increase in their levels led to an increase in the metallic ion uptake by the biosorbent (q); on the other hand, the negative values of the effects led to a diminution of the response (q), when their levels were increased.

In order to better evaluate each factor and its interaction, in Fig. 2A is presented the normal probability plot of standardized effects. This graph could be divided in two regions: the region with percent below 50%, where the factors and their interactions presented negative coefficients (pH, X, $X \cdot t$, $C_o \cdot X$, pH $\cdot t$, pH $\cdot$ $C_o \cdot X$ and pH $\cdot$ $C_o \cdot t$), and the region with percent above 50%, where the factors presented positive coefficients (t, C_o, pH $\cdot X$, pH $\cdot X \cdot t$ and $C_o \cdot t$). All these factors and interactions which were represented as a square were significant figures, and they were out of the central line that crosses the zero value at the abscissa at the 50%. The effects positioned in this line were represented by a circle and correspond to the estimate of errors of the effects, being not significant (pH $\cdot$ C_o and $C_o \cdot X \cdot t$).

In Fig. 2B is presented the Pareto Chart of standardized effects at $p = 0.05$. All the standardized effects were in absolute values (to verify which were positives and negatives, see Fig. 2A). All the values that presented an absolute value higher than 3.2 ($p = 0.05$), which were located at right of the dash line, were significant. The absolute standardized value of the effect of each factor and its interaction appeared at the right of each bar.

Analyzing the graphs of Fig. 2 and the values of Table 4, it can be inferred that the pH was the most important variable of the overall biosorption procedure. The negative value of its coefficient meant that the chromium(VI) uptake by pinhão wastes was favored at low pH values (pH 2.0). The increase in the pH led to a remarkable decrease of Cr(VI) uptake by the pinhão wastes, as reported earlier by Ucun et al. [15].

In order to avoid a disruption of the pinhão wastes at pH lower than 2.0, this value was fixed for continuing the optimization of this work. The second important factor for overall optimization of the batch system was the time of contact between the biosorbent and the biosorbate. An increase of the time of contact increased remarkably the chromium(VI) uptake by pinhão wastes. This factor was further optimized in the surface response analysis design, by increasing this level to higher values. The third factor important to the overall optimization of the biosorption process was the biosorbent concentration (X). As these biosorption experiments were carried out in a batch system using conical plastic tubes (117 mm height and 30 mm diameter), the amount of biosorbent was limited to the conical plastic tube dimension for performing the experiments. This justifies that low biosorbent concentration led to highest metallic ion

Table 4
Factorial fit: q vs. block; pH; C_o; X; t

Term	Effect	Coefficient	S.E. of coefficient	p
Constant		42.20	0.1774	0.000
Block		0.50	0.1587	0.052
Main factors				
pH	−48.00	−24.00	0.1774	0.000
C_o	11.84	5.92	0.1774	0.000
X	−16.17	−8.08	0.1774	0.000
t	17.95	8.97	0.1774	0.000
Interaction of two factors				
pH $\cdot$ C_o	−0.29	−0.15	0.1774	0.467
pH $\cdot X$	13.46	6.73	0.1774	0.000
pH $\cdot t$	−4.48	−2.24	0.1774	0.001
$C_o \cdot X$	−5.20	−2.60	0.1774	0.001
$C_o \cdot t$	1.33	0.66	0.1774	0.033
$X \cdot t$	−6.39	−3.20	0.1774	0.000
Interaction of three factors				
pH $\cdot C_o \cdot X$	−3.32	−1.66	0.1774	0.003
pH $\cdot C_o \cdot t$	−1.20	−0.60	0.1774	0.043
pH $\cdot X \cdot t$	3.91	1.95	0.1774	0.002
$C_o \cdot X \cdot t$	0.89	0.45	0.1774	0.087
Central point (cp)		−16.17	0.3967	0.000

Estimated effects and coefficients for q (coded units). Full 2^4 factorial design. The effects and coefficients are given in coded units. Block is aliased with pH $\cdot$ $C_o \cdot X \cdot t$. $S = 0.709667$. $R^2 = 99.99\%$. R^2 (adjusted) = 99.93%.
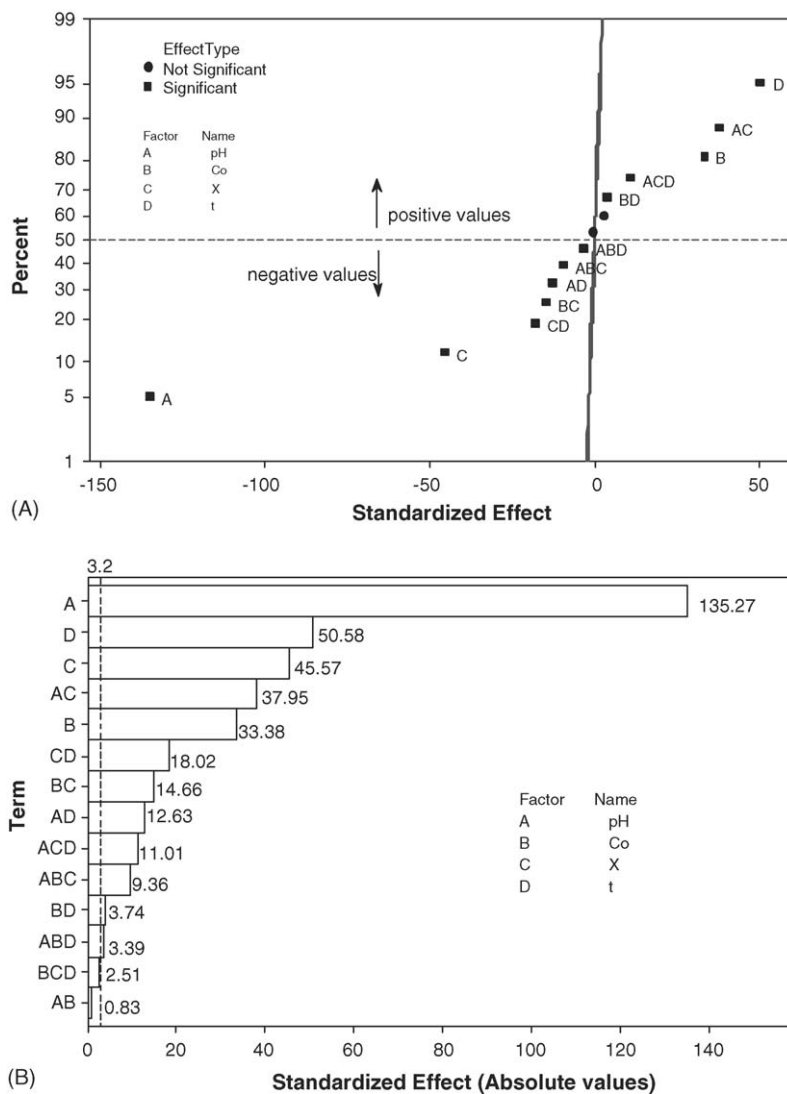
Fig. 2. (A) Normal probability plot of standardized effects at $p = 0.05$. The dotted line at 50% divides the negative effects from the positive ones. (B) Pareto plot of standardized effects at $p = 0.05$.

uptake (negative coefficient value). In the further experiments of surface response analysis design, biosorbent concentration was decreased. The fourth important factor for overall optimization of the biosorption system was the interaction of two factors pH · X which was more significant than the main factor C_o. Only the achievement of this result justifies the use of the statistical design of experiments over the conventional univariate process of optimization of the system. This information would not be acquired in a univariate optimization of the biosorption system. Otherwise, if the system were being optimized by using univariate procedure, a small diminution of the pH of the solution associated with a small diminution of biosorbent concentration could lead to a misinterpretation of the results achieved with the univariate procedure. Probably, the experimenter would remake all the measurements, because a small negative variation of the pH of the solution associated with a small decrease in the biosorbent concentration would lead to an unexplained increase in the metallic ion uptake, that could not be explained using the univariate procedure of optimization

of the system. The fifth important factor to overall optimization was the initial concentration of chromium(VI), C_o. This result showed that the initial concentration of the metallic ion should be increased in order to achieve the highest response (q), and this factor was further studied in the surface response analysis design, by increasing its levels. The decreasing order from the 6th to the 12th, in the ranking of the overall optimization of the biosorption system, in absolute values was: $X \cdot t > C_o \cdot X > \text{pH} \cdot t > \text{pH} \cdot X \cdot t > \text{pH} \cdot C_o \cdot X > C_o \cdot t > \text{pH} \cdot C_o \cdot t$.

In Table 5 is presented the analysis of variance for the full 2^4 factorial design with two blocks and two center points per block. As can be seen, the main factors, two-way interactions and three-way interactions were significant at 5% of probability level ($p < 0.05$), as discussed above. In addition to this, this analysis of variance has shown that this model presented a curvature, since its probability was lower than 5.0×10^{-4}, which meant that the curvature of the model should exist. In order to verify this fact, in Fig. 3 are shown the mean changes that occurred in the response (q) when the levels of the factors were changed from the lower

Table 5
Analysis of variance for q of the full 2^4 factorial design (coded units)

Source	d.f.	Seq SS	Adj SS	Adj MS	F	p
Blocks	1	4.9	4.9	4.94	9.80	0.052
Main effects	4	12110.5	12110.5	3027.63	6011.65	0.000
2-Way interaction	6	1084.7	1084.7	180.79	358.97	0.000
3-Way interaction	4	114.2	114.2	28.54	56.67	0.004
Curvature	1	836.8	836.8	836.78	1661.50	0.000
Residual error	3	1.5	1.5	0.50		
Lack of fit	1	0.1	0.1	0.14	0.20	0.700
Pure error	2	1.4	1.4	0.69		
Total	19	14152.6				

d.f.: degree of freedom, Seq SS: sequential sum of squares, Adj SS: adjusted sum of squares, F: factor F and p: probability.

level (−) going to higher level (+), passing the central point (0). Each lower (−) and higher level (+) was the average of eight measurements and the central point (0) was the average of four measurements. As can be seen, the response value at the central point did not correspond to the average of the response value for all the main factors studied. There should be a curvature of the responses, and a surface analysis design should be carried out in order to achieve a better comprehension of the biosorption system.

3.3. Surface analysis

After performing a screening of factors using a full 2^4 factorial design, a Box–Behnken response surface design was carried out according to the experiments described in Table 2 ($n = 15$ and 12 h), in order to achieve the highest chromium uptake (q) by the pinhão wastes. The levels of the chosen factors were set based on the previous factorial analysis described above, using increasing contact time between the Cr(VI) and pinhão wastes up to 12 h, increasing initial Cr(VI) concentration up to 1200 mg l^{-1} and diminishing the biosorbent concentration as low as 1.5 g l^{-1}.

In Fig. 4 are shown the contour plots of the response (q) for initial concentration versus biosorbent concentration (Fig. 4a),

time of contact versus biosorbent concentration (Fig. 4b) and time of contact versus initial concentration (Fig. 4c). As can be seen, the highest metallic uptake by the pinhão wastes occurred at higher initial Cr(VI) concentration (1200 mg l^{-1} Cr(VI)), lower time of contact (8 h) and lower pinhão wastes concentration (1.5 g l^{-1}). Under these conditions, the maximum Cr(VI) uptake was 125 mg g^{-1}.

The effect of time was not so significant when the contact time between the metallic ion and the biosorbent ranged from 8 to 12 h. In order to confirm these results, in Table 6 is presented the response surface regression of q as function of X, C_o and t. As can be seen, the time of contact between the Cr(VI) and pinhão wastes was not significant from 8 to 12 h ($p > 0.05$). Therefore, it is better to choose a lower contact time to perform the experiments, in order to save time.

Also, analyzing Table 6, it can be verified that at the levels chosen of the factors in Table 2, any interaction between any factor was not verified. In addition, at 5% of probability level, it can be seen that the full quadratic model does not fit properly (R^2 adjusted lower than 70.0%), because just the initial metallic ion concentration (C_o) and the square mass of biosorbent (m^2) were relevant in the model ($p < 0.05$). By this reason, the full quadratic model should be reduced to the relevant factors, and these results are shown in Table 7.

The analysis of variance of the reduced quadratic model is presented in Table 8. As can be seen, both the linear and square
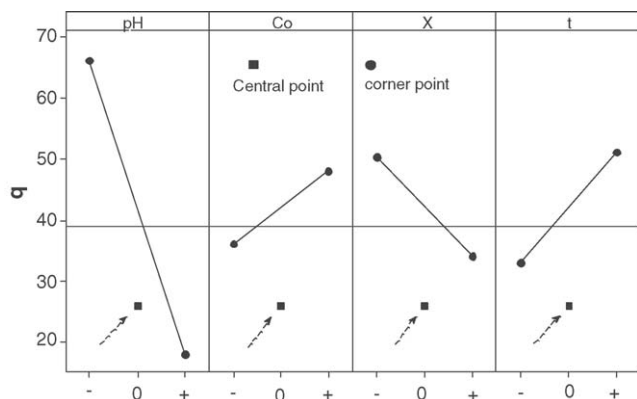

Fig. 3. Main effects plot (data means) for metallic ion uptake by biosorbent (q). Each low (−) and high level (+) was the average of eight measurements and the central point (0) was the average of four measurements. The arrow indicates the curvature, when the factor is increased from their low value (−) to the higher level (+) passing through the center point (0).

Table 6
Response surface regression for Cr(VI) uptake in pinhão wastes

Term	Coefficient	S.E. of coefficient	p
Constant	88.823	4.767	0.000
X	−1.398	2.919	0.652
C_o	13.907	2.919	0.005
t	−1.995	2.919	0.525
X^2	16.878	4.297	0.011
C_o^2	3.795	4.297	0.418
t^2	4.210	4.297	0.372
$X \cdot C_o$	−3.512	4.129	0.434
$X \cdot t$	1.574	4.129	0.719
$C_o \cdot t$	−1.598	4.129	0.715

Full quadratic model. p: probability and S.E.: standard error of coefficient. The coefficient values are given in coded units. $S = 8.257$. $R^2 = 89.0\%$. R^2 (adjusted) = 69.3%.

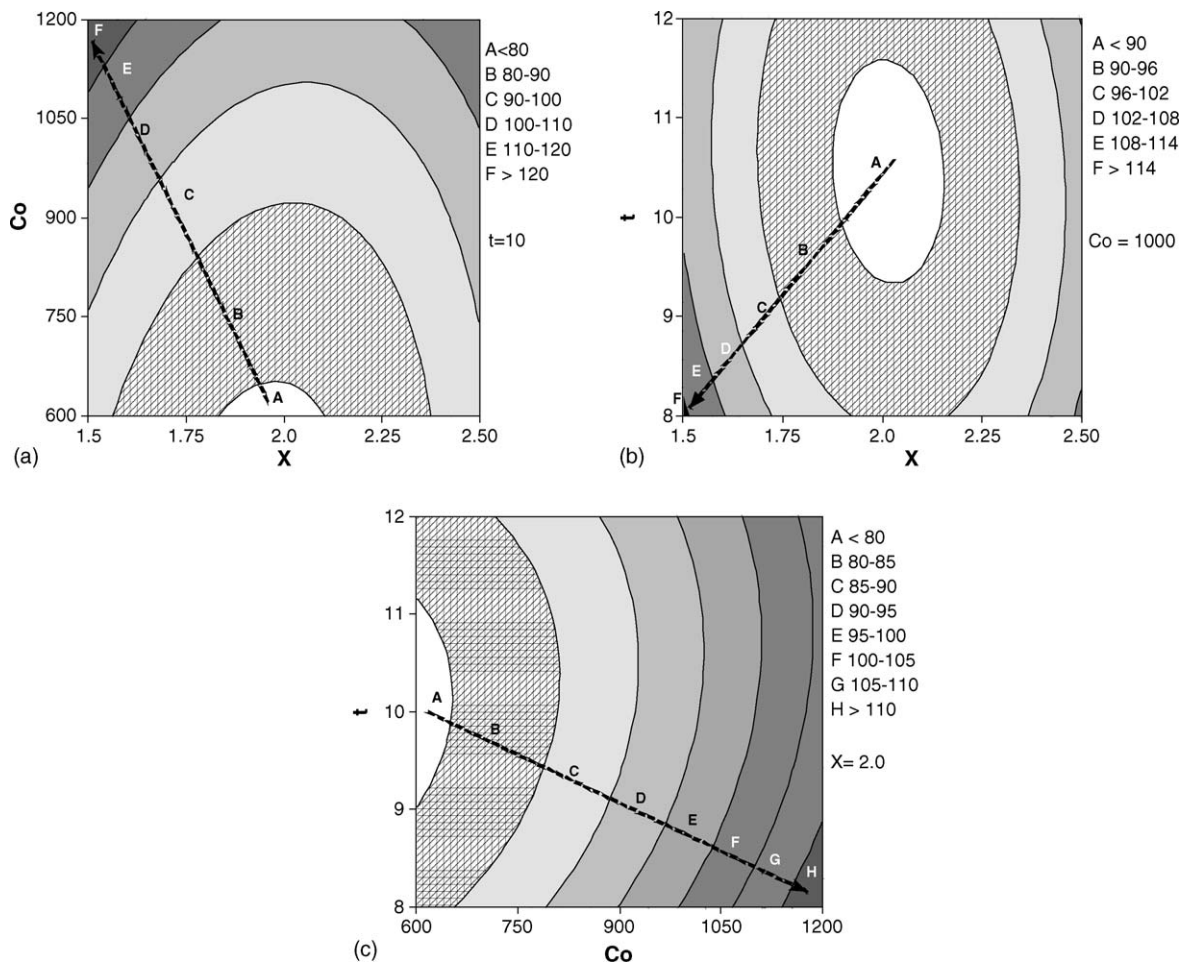Fig. 4. Contour plot of q: (a) C_o vs. X; (b) t vs. X; (c) t vs. C_o. The regions of the contour plot are divided in several regions, where the response q is expressed in mg g^{-1}. The arrows indicate the increase in the q at several regions. The time (t) is expressed in h, C_o is expressed in mg l^{-1} and X is expressed in g l^{-1}.

regressions were significant ($p < 0.05$) at 5% of probability level. In addition, the lack of fit was not significant at 5% of probability level. Therefore, the equation for the surface analysis can be written as: $q = 93.498 + 13.907C_o + 16.307X^2$ (coded units).

After optimizing the chromium(VI) biosorption conditions (pH 2.0, $C_o = 1200$ mg l^{-1}, $X = 1.5$ g l^{-1} and $t = 8$ h), a study of potential species that could interfere on the chromium uptake by the pinhão wastes was carried out. The choice of the concomitant species and their concentrations was based on species found in tannery effluents [17]. As the working solutions were adjusted to pH 2.0 using sulphuric acid, the anion sulphate was not investigated in this study.

The experiments of Table 3 were carried out, and the percent of interference was calculated according to Eq. (3). Table 9 presents the response surface regression for interference study on Cr(VI) uptake. As can be seen, there was no significant interference at 5% of probability level, of all tested concomitant species and either any interaction between these concomitant species. All the coefficients present $p > 0.05$, and also the constant was not significant. In addition, the model presented an adjusted square correlation coefficient of 0.00%. Based on these

Table 7
Response surface regression for metallic ion uptake in pinhão wastes

Term	Coefficient	S.E. of coefficient	p
Constant	93.498	2.678	0.000
X	−1.398	2.505	0.588
C_o	13.907	2.505	0.000
X^2	16.307	3.668	0.001

Reduced quadratic model. p: probability and S.E.: standard error of coefficient. The coefficient values are given in coded units. $S = 7.086$. $R^2 = 82.8\%$. R^2 (adjusted) $= 77.4\%$.

Table 8
Analysis of variance for q for reduced quadratic model of surface design (coded units)

Source	d.f.	Seq SS	Adj SS	Adj MS	F	p
Regression	3	2555.52	2555.52	851.840	16.96	0.000
Linear	2	1562.81	1562.81	781.403	15.56	0.001
Square	1	992.72	992.72	992.715	19.77	0.001
Residual error	11	552.39	552.39	50.217		
Lack of fit	5	234.84	234.84	46.968	0.89	0.542
Pure error	6	317.55	317.55	52.924		
Total	14	3107.91				

d.f.: degree of freedom, Seq SS: sequential sum of squares, Adj SS: adjusted sum of squares, F: factor F and p: probability.

Table 9
Response surface regression for interference study on Cr(VI) uptake by pinhão wastes

Term	Coefficient	S.E. of coefficient	p
Constant	−0.3200	3.290	0.926
Cl^-	0.6263	2.015	0.768
NO_3^-	0.3400	2.015	0.873
PO_4^{3-}	1.5337	2.015	0.481
$(Cl^-)^2$	−1.2788	2.965	0.684
$(NO_3^-)^2$	−2.3313	2.965	0.467
$(PO_4^{3-})^2$	1.2912	2.965	0.681
$(Cl^-) \cdot (NO_3^-)$	2.1500	2.849	0.484
$(Cl^-) \cdot (PO_4^{3-})$	2.6875	2.849	0.389
$(NO_3^-) \cdot (PO_4^{3-})$	2.5050	2.849	0.420

Full quadratic model. p: probability and S.E.: standard error of coefficient. The coefficient values are given in coded units. $S = 5.698$. $R^2 = 44.3\%$. R^2 (adjusted) $= 0.0\%$.

results, there was no significant interference on chromium(VI) uptake by pinhão wastes promoted by Cl^-, NO_3^- and PO_4^{3-}.

3.4. Destination of the pinhão wastes loaded with Cr(VI) and mechanism of biosorption of chromium(VI) on the biosorbent

After performing several experiments of biosorption of Cr(VI) on pinhão wastes, it was necessary to try to regenerate the wastes. The first trial was to pack 1.00 g of loaded Cr(VI) on pinhão wastes in a glass minicolumn. Several solutions of NaCl with pH adjusted to 8.0–9.0 were percolated by the column and the effluent was retained to analysis. No chromium was desorbed from the column. This result ruled out the hypothesis of the electrostatic attraction between the positively charged pinhão wastes by the anion $Cr_2O_7^{2-}$. If the electrostatic attraction were in fact the correct mechanism of biosorption, it would be possible to regenerate the pinhão wastes just by increasing the ionic strength of the medium associated with an increase in the pH of the solution. And also to reinforce that this mechanism was not correct for Cr(VI) biosorption, the interference study of Cl^-, NO_3^- and PO_4^{3-}, described above, did not show any significant effect of all those anions to Cr(VI) uptake by the pinhão wastes.

A second trial was carried out by passing a 3.0 mol l^{-1} of H_2O_2 in basic medium (pH 10.0) by the biosorbent column [31]. It was observed that the column effluent was a yellowish solution that is characteristic of chromate solution [31]. Afterwards, the effluent solution was measured with DPC, which confirmed the presence of Cr(VI).

This result confirmed several mechanisms of biosorption of Cr(VI) employing different biosorbents, which was attributed to the reduction of Cr(VI) to Cr(III) followed by the chelation of this last specie with the oxidized organic compound [8,14,16].

As it is known, pinhão wastes are rich in polyphenols [26]. Polyphenols could be oxidized to polyquinones by dichromate [32], according to the proposed mechanism given in Scheme 1.

The biosorption process occurred at low pH value (pH 2.0) because in the oxidation step of polyphenols, H^+ ions were consumed [32]. The polyquinone formed in the first step formed a complex with the Cr^{3+}.
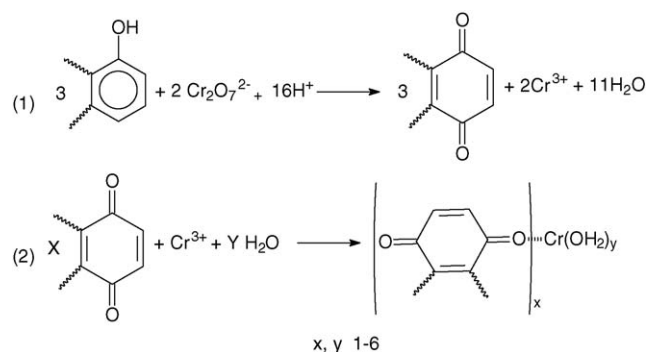
Scheme 1. Chromium(VI) uptake by pinhão wastes.

The chromium uptaken by the pinhão wastes could only be destroyed by the presence of H_2O_2 in basic medium [31]. However, large amounts of 3.0 mol l^{-1} H_2O_2 at pH 10.0 passing through the loaded Cr(VI) pinhão wastes present in the column provoked a disruption of the material.

In order to find a new applications to Cr(VI) loaded pinhão wastes, experiments of adsorption of textile-dyes on this new waste material are in progress in the authors' laboratory.

3.5. Comparison of pinhão wastes as biosorbent for Cr(VI) uptake with other biosorbents

In Table 10 is presented a comparison of several biosorbents employed for Cr(VI) uptaken. As can be seen, pinhão wastes is a very good biosorbent for Cr(VI) removal from aqueous solution, presenting adsorption capacity compared with activated carbon and modified chitosan.

3.6. Breakthrough curve of Cr(VI) using pinhão wastes as biosorbent

In order to evaluate pinhão wastes as a biosorbent for wastewater treatment of Cr(VI) containing effluents, a breakthrough curve of Cr(VI) using pinhão wastes as biosorbent was obtained (Fig. 5). As can be seen, the breakthrough point, determined when the Cr(VI) effluent from column attained the level of 0.050 mg l^{-1} (which corresponds to the maximum allowed Cr concentration in natural waters [1]), corresponds to a bed volume of 252.3 (bed volume is the ratio of effluent volume/biosorbent volume). This was a very good value of attained breakpoint for a biosorbent in a dynamic system, since 1 volume of biosorbent (22.0 ml) was able to remove Cr(VI) completely from a 252.3 higher volume (5550 ml) of aqueous solution. It should be mentioned that the amount of Cr(VI) removed (5550 ml of 25.0 mg l^{-1} Cr(VI) solution) completely from aqueous solution at the breakpoint corresponded to an amount of 138.75 mg of the metallic ion. Considering that this amount was uptaken by 4.00 g of biosorbent, the breakpoint capacity of pinhão wastes to remove Cr(VI) completely from aqueous solution was 34.7 mg metallic ion/g pinhão wastes using a flow-rate of 2.5 ml min^{-1}. Of course, at other flow-rates this breakpoint capacity achieved in a dynamic system would change.

Table 10
Comparison of different biosorbents for Cr(VI) uptaken

Biosorbent	Characteristic	q_{max} (mg g^{-1})	Reference
Coconut shell charcoal	Biosorbent treated with HNO$_3$	10.88	[8]
Coconut shell charcoal	Biosorbent treated with H$_2$SO$_4$	4.05	[8]
Coconut shell charcoal	Without treatment	2.18	[8]
Neurospora crassa—fungal biomass	Biomass treated with acetic acid	15.85	[9]
Chryseomonas luteola bacteria	Bacteria isolated from wastewater treatment of petrochemical industry	3.0	[10]
Hazelnut shell	Biosorbent treated with H$_2$SO$_4$	17.7	[14]
Pinus sylvestris cone biomass	400 mesh powder	201.8	[15]
Sugar beet pulp	Composite of biosorbent with FeOH$_3$	5.12	[16]
Sawdust	Adsorption capacity in column	0.893	[17]
Rice husks	Adsorption capacity in column	0.634	[17]
Coirpith	Adsorption capacity in column	1.204	[17]
Turkish coffee	–	1.63	[18]
Walnut shell	–	1.33	[18]
Waste tea	–	1.55	[18]
Chitosan	–	27.3	[18]
Rice hulls	–	164.31	[18]
Irish sphagnum moss peat	–	43.9	[18]
Bagasse fly ash	–	260	[19]
Sawdust	–	2.29	[19]
Activated carbon (GAC) Filtrasorb 400	–	145	[19]
Chitosan	Modified chitosan	153.8	[20]
Waste crab shell	Treated with HCl	28.1	[21]
Mucilaginous seeds	Seeds treated with water	205.0	[22]
Grape stalks	Particle size 1.0–1.5 mm	59.8	[23]
Yohimbe bark	Particle size 1.0–1.5 mm	42.5	[23]
Cork	Particle size 1.0–1.5 mm	0.022	[23]
Olive stones	Particle size 1.0–1.5 mm	0.012	[23]
Auracaria angustifolia wastes	Treatment with water boiling	125.0	This work

It should be stressed that the maximum amount of metallic ion uptaken by the biosorbent (q_{max}) obtained in the batch experiments is different from breakpoint capacity of the biosorbent obtained under dynamic situation. The first parameter was always measured with non-retained metallic ion by the biosorbent (see Eq. (2)); on the other hand, the breakpoint was measured when the first amount of the metallic ion attains its
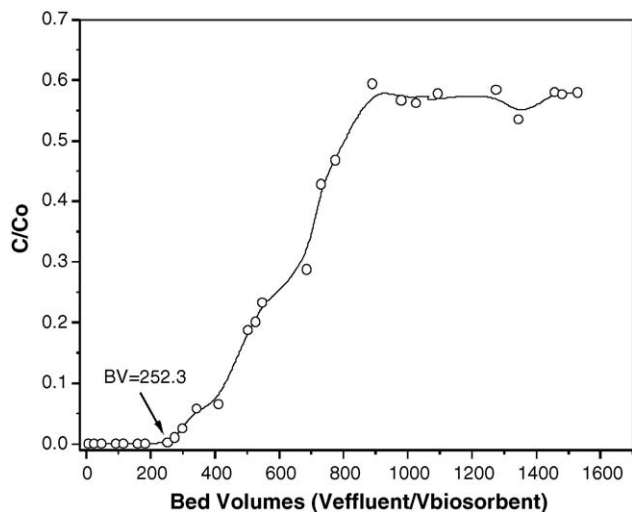
maximum allowed concentration that could be disposed into the environment.

In addition, observing the breakthrough curve for Cr(VI) removal from aqueous solution (Fig. 5), it was observed that at a bed volume of 888.64 (effluent volume of 19,550 ml), the ratio C/C_o was leveled to an average of 0.57. Considering a volume of 19,550 ml of 25.0 mg l^{-1} Cr it corresponds to an amount of 488.75 mg Cr(VI) uptaken by 4.00 g of pinhão wastes which corresponds to 122.2 mg Cr(VI) uptaken/g of the biosorbent. This value is close to the maximum amount of Cr(VI) uptaken in the batch system ($q_{max} = 125.0$ mg g^{-1}).

4. Conclusion

A 100.0 mg l^{-1} solution of Cr(VI) was completely removed from aqueous solution by pinhão wastes (5.0 g l^{-1}) in just 30 min.

In order to achieve the best conditions for Cr(VI) uptake by pinhão wastes, a full 2^4 factorial design was employed for screening the factors that would influence on the overall optimization of a batch procedure of biosorption. This optimization has shown that the best initial conditions were: pH 2.0, initial concentration (C_o) Cr(VI) of at least 600.0 mg l^{-1}, biosorbent concentration (X) of at least 2.50 g l^{-1} and agitation time (t) of at least 8.0 h. Based on these results, a Box–Behnken surface response design was carried out, investigating other limits of the previous values attained with the full factorial design, and

Fig. 5. Breakthrough curves for Cr(VI) removal from aqueous solution using pinhão wastes as biosorbent. Bed volume 22.0 ml, flow-rate 2.5 ml min^{-1} and column volume 250 ml.

the best conditions for Cr(VI) uptake by pinhão wastes were: pH 2.0, $C_o = 1200.0\,mg\,l^{-1}$, $X = 1.50\,g\,l^{-1}$ and $t = 8\,h$. In order to verify if anions such as Cl^-, NO_3^- and PO_4^{3-} would interfere on Cr(VI) uptake by pinhão wastes, a new Box–Behnken surface response design was carried out, where different levels of these anions were tested, using the best conditions for Cr(VI) uptake by the pinhão wastes. The results showed that there is no interference of either of these anions on dichromate uptake by the biosorbent.

In order to regenerate Cr(VI) loaded pinhão wastes, experiments were firstly carried out with NaCl solutions (pH 8.0). These media were improper for Cr(VI) removal from the metallic ion loaded biosorbent. A new trial was performed utilizing $H_2O_2 + NaOH$ which is a known media to oxidize Cr^{3+} to CrO_4^{2-}. This experiment confirmed that Cr(VI) was reduced to Cr(III) by the biosorbent and this element was retained in the pinhão wastes.

In addition, the dynamic studies of Cr(VI) removal by pinhão wastes showed that this biosorbent was a powerful and low-cost biosorbent for this metallic ion removal from aqueous solution, opening the possibility of this biosorbent to be employed in effluent treatment of tanneries or metallurgical industries that need to treat their effluents before being delivered into the environment.

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