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RESEARCH ARTICLE

Removal of chromium from wastewater by swine hair residues applied as a putative biofilter

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Abstract

Swine production chain generates residues with potential application in environmental processes. This study aimed at the use of swine hair as a potential biofilter for hexavalent chromium (Cr(VI)) removal from wastewater of tannery industry. The hair was pretreated using H_2O_2 in alkaline medium, and statistical analysis was carried out to evaluate the hair degradation, as well the Cr(VI) removal by the potential pretreated biofilter. The results showed 99% of Cr(VI) removal in 105 min of treatment in large pH range (1–10). Treated and untreated effluents were submitted to cytotoxicity study using vegetable and animal cells, demonstrating a significant reduction on toxicity to both cells. Therefore, swine hair demonstrated to be a promising residue for heavy metal removal on the perspective of an environmentally friendly technique.

Keywords Waste valuation · Biomaterial · Hexavalent chromium · Cytotoxicity

Introduction

Demand for swine meat consumption is growing fast worldwide (USDA 2015; Calayag et al. 2017). In Brazil, exports of swine meat are increasing, and Brazil occupies the fourth position among the largest producers and global exports (USDA 2015; Ali et al. 2017).

The production process of swine meat generates several residues. These residues, mainly liquid effluents, are considered to have a high polluting potential, including swine manure from the creation and concentration of these animals in the same area (Rizzoni et al. 2012; Cherubini et al. 2015). The environmentally adequate destination of most of these wastes is the sanitary or industrial landfills. However, these residues have the potential to be used as products in other processes, since they present high added value, which consequently ends up positively valuing these agroindustrial wastes (Segat et al. 2015).

A by-product from swine production is swine hair, which does not have its potential being exploited, possibly due to its high degradation time due to the presence of high keratin content (Mabrouk 2008). The keratin present in swine hair is hardly degraded, as it presents disulfide-type bonds, hydrogen bonds, and hydrophobic interactions in its structure (Feghelman and Haly 1960; Thankaswamy et al. 2018). Because of this complex structure, hair is a by-product that requires treatment to break the disulfide bonds, before exploring its potential applications (Cai and Zheng 2009).

Complex material treatment has the purpose to facilitate the subsequent processes of use of the material under study, several types of treatment can be applied, such as reactions in the presence of H_2O_2, use of acid, or high temperatures and pressures (Croce et al. 2016; Paudel et al. 2017; Venturin et al. 2018). The treatment is considered adequate when the final

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material presents a simple and bioavailable structure (Zhao et al. 2018; Venturin et al. 2018).

A relevant alternative for the application of the pretreated swine hair would be in the treatment of wastewaters containing heavy metals. Some studies have reported the ability of these metals to form complexes between the effluent and the organic compound (Wan et al. 2010; Jiang et al. 2018).

In this context, the objective of this study was to pretreat the swine hair of the food agroindustry aiming at its application in Cr(VI) removal from wastewater, developing a possible alternative to solve an environmental problem that affects many industries, and to make this by-product a promising technology in the treatment of effluents with a heavy metal load.

Material and methods

Swine hair and tannery effluents

Swine hairs were obtained from a food agroindustry, located in the state of Rio Grande do Sul, Brazil. The tannery wastewater was collected from a local industry at the discharge point of the leather processing tanks. The wastewater has an initial concentration of Cr(VI) of 7.22 ± 1.78 mg L^{-1} and pH 3.49.

Swine hair pretreatment using hydrogen peroxide in alkaline medium

Swine hair was washed with water for removal of the main residual impurities (skin and blood). Thereafter, the pretreatment by alkaline hydrogen peroxide (H_2O_2) was studied in an orbital shaker. The experiments were designed with a central composite design 2^3 (CCD) with 8 tries and more than 3 repetitions at the central point (Table 1). The factors investigated were exposure time (1.3–9.7 h), swine hair mass (0.8–9.2 g), and H_2O_2 concentration (2.0–12.0%). The H_2O_2 concentration was adjusted according to Rabelo et al. (2011), Sun et al. (2015), and Venturin et al. (2018). The pH was adjusted to 11.5 ± 0.2 (Sun et al. 2015).

The test runs were incubated in an orbital shaker at 28 °C and 150 rpm and maintained for the period determined in the CCD. The samples were maintained at 60 °C during 24 h (Reddy et al. 2017) and weighed in analytical balance, for determining the dry mass after pretreatment.

Results were evaluated based on mass loss of the swine hairs (Reddy et al. 2017). Swine hair degradation was calculated from Eq. 1, where M_o is the dry mass of biomaterial initially added and M_f is the dry mass after the pretreatment process (Cheong et al. 2018).

$$\text{Degradation}\ (\%) = \frac{M_o - M_f}{M_o} \times 100 \quad (1)$$

Application of pretreated swine hair for Cr(VI) removal in a synthetic effluent

Firstly, the potential of the pretreated biomaterial for removal of Cr(VI) was tested using a central composite design (CCD 2^3). Therefore, synthetic effluents were prepared with 20 mg Cr(VI) L^{-1}, added in the form of potassium dichromate solution ($K_2Cr_2O_7$). The samples were prepared with 50 mL of Cr(VI) solution, 1.0% (m v^{-1}) of the pretreated swine hair, and pH 2.0 (Khelaifia et al. 2016), and incubated in an orbital shaker at 40 °C, 200 rpm for 3 h (Khelaifia et al. 2016).

Then, a central composite rotational design with two factors (CCRD 2^2) was carried out for evaluation of the effects of concentrations of Cr(VI) (2.0–20.0 mg Cr^{6+} L^{-1}) and pretreated swine hair (1.0–10% m v^{-1}) on Cr(VI) removal. The pH was maintained at 2.0 (Table 2) and the incubation conditions were the same as in the previous assay.

Table 1 Central composite delineation 2^3 (real and coded values) evaluating the degradation of swine hairs using hydrogen peroxide

Assay	Time (h)	Mass (g)	H_2O_2 (%)	Degradation (%)
1	3 (− 1)	2.5 (− 1)	4 (− 1)	31.46
2	8 (+ 1)	2.5 (− 1)	4 (− 1)	35.48
3	3 (− 1)	7.5 (+ 1)	4 (− 1)	36.08
4	8 (+ 1)	7.5 (+ 1)	4 (− 1)	33.96
5	3 (− 1)	2.5 (− 1)	10 (+ 1)	37.38
6	8 (+ 1)	2.5 (− 1)	10 (+ 1)	79.58
7	3 (− 1)	7.5 (+ 1)	10 (+ 1)	48.62
8	8 (+ 1)	7.5 (+ 1)	10 (+ 1)	37.82
9	5.5 (0)	5 (0)	7 (0)	61.04
10	5.5 (0)	5 (0)	7 (0)	63.28
11	5.5 (0)	5 (0)	7 (0)	61.45

Table 2 Central composite rotational design 2^2 design (real and coded values) for removal of hexavalent chromium

Assay	Cr(VI) (mg L^{-1})	Mass (%)	Cr(VI) removal (%)
1	4.6 (− 1)	2.3 (− 1)	81.30
2	17.4 (+ 1)	2.3 (− 1)	50.85
3	4.6 (− 1)	8.7 (+ 1)	99.41
4	17.4 (+ 1)	8.7 (+ 1)	82.42
5	2.0 (− 1.41)	5.5 (0)	97.62
6	20.0 (+ 1.41)	5.5 (0)	55.47
7	11.0 (0)	1.0 (− 1.41)	66.38
8	11.0 (0)	10.0 (+ 1.41)	83.03
9	11.0 (0)	5.5 (0)	70.17
10	11.0 (0)	5.5 (0)	76.25
11	11.0 (0)	5.5 (0)	72.71

After the incubation period, the samples were filtered and the Cr(VI) concentration was quantified by colorimetric method described in *Standard Methods* (APHA 1998), using 1.5 diphenylcarbazide ($C_{13}H_{14}N_4O$). Removal of Cr(VI) of the medium was evaluated by the difference between the concentration at time zero and the concentration after incubation.

The evaluation of contact time was carried out to determine the shortest possible time for Cr(VI) removal by the pretreated biomaterial, with equal efficiency as the 3 h used in the CCRD 2^2. Thus, destructive samples were prepared and Cr(VI) concentration was evaluated at 0, 15, 30, 45, 60, 90, 105, and 120 min. The best relation of concentration of Cr(VI) and pretreated swine hair was obtained by optimization, as described in the "Application of pretreated swine hair for Cr(VI) removal in a synthetic effluent" section. The samples had the pH set to 2.0 and were maintained in an orbital shaker at 200 rpm and 40 °C.

Subsequently, the effect of sample pH was evaluated at 1.0, 2.0, 3.0, 4.0, 7.0, and 10.0. The Cr (VI) concentration and mass of pretreated swine hair used were the same optimized in the "Application of pretreated swine hair for Cr(VI) removal in a synthetic effluent" section, and the contact time determined in previous assays. The samples were incubated an orbital shaker at 200 rpm and 40 °C.

The concentration of Cr(VI) was determined by the method of 1.5 diphenylcarbazide ($C_{13}H_{14}N_4O$). The Cr(VI) removal was evaluated by the difference between the final and initial concentrations of the medium.

Evaluation of the biomaterial for Cr(VI) removal in real tannery wastewater

The assays in tannery effluent were carried out with 8.7% (m v^{-1}) of pretreated swine hair in 50 mL of wastewater. The samples were placed in orbital shaker at 200 rpm, 40 °C for 105 min. The pH of the effluent was not altered.

Hair morphology and qualitative composition by electronic scanning microscopy and X-ray dispersive energy spectroscopy

Electronic scanning microscopy (SEM) and X-ray dispersive energy spectroscopy (EDX) analyses were performed in a scanning electron microscope (SEM/EDX, TM-3030, Hitachi) in four samples: crude swine hair (control), assays 3 and 6 of the CCD 2^3, and assay 3 of CCRD 2^2. Samples were fixed with carbon tape on aluminum holders and then coated by gold sputtering (Ernest Fullam) for 30 s. All the SEM analyses were carried out at 15 kV.

Cytotoxicity assay in a real tannery wastewater

The evaluation of the real tannery wastewater cytotoxicity was carried in vitro, using vegetal meristem of *Allium cepa*, and the assays were performed as described by Dusman et al. (2014).

The meristems of *A. cepa* were exposed to real effluent before and after pretreatment with swine hairs, at a dilution of 1:8. The cytotoxicity analysis in the cells war performed by mitotic index (MI) analysis, for which the relation between the total number of cells in the division process (NCM) and the total number of cells observed (NTC) was used.

Statistical analysis of data

The data obtained from the tests were analyzed by analysis of variance (ANOVA) using the software Statistica 8.0 (StatSoft, Tulsa, USA) and online software Protimiza Experimental Design (http://experimental-design.protimiza.com.br/).

Results and discussion

Pretreatment of swine hairs by means of CCD 2^3 using hydrogen peroxide

The results for the degradation of swine hairs as a function of hair amount, concentration of peroxide, and time of degradation are shown in Table 1. Assay 6 yielded the greatest mass loss (79.58%), with maximum contact time (8 h) and hydrogen peroxide (10 mL), and lower mass (2.5 g of swine hair). The lowest degradation (31.46%) was observed in assay 1, with lower conditions (3 h; 2.5 g of swine hair and 4 mL of hydrogen peroxide).

The hair is composed basically of keratin, which is a biopolymer formed by polypeptide bonds of aminoacids, which give their hard and strong appearance (Pienpinijtham et al. 2018). Keratin fibers are incorporated into a matrix by means of many chemical bonds, such as disulfide bonds. The H_2O_2 has a strong interaction capacity with the keratin chain groups, which can cause chemical changes in the keratin bonds (Zhang et al. 2015). Structural alterations occur by disrupting the disulfide bonds of the keratin structure and oxidation to cysteic acid, causing disintegration of the structure (Baias et al. 2009). The reduction of the disulfide bonds in the keratinolytic material causes the loss of mechanical resistance and can be observed by increasing the alkalization of the medium (Millington 2006).

The mechanism of degradation using H_2O_2 is strongly dependent on the pH of the reaction, which is reported as pH 11.5–11.6. Under these conditions, the dissociation of H_2O_2 and the formation of hydroperoxide anions, which will react with other H_2O_2 molecules, occur, and this reaction will result in the formation of hydroxyl radicals and superoxide radicals (Gould 1985; Venturin et al. 2018).

Considering these factors, the results obtained could be explained due to the rapid decomposition of H_2O_2. So, in the

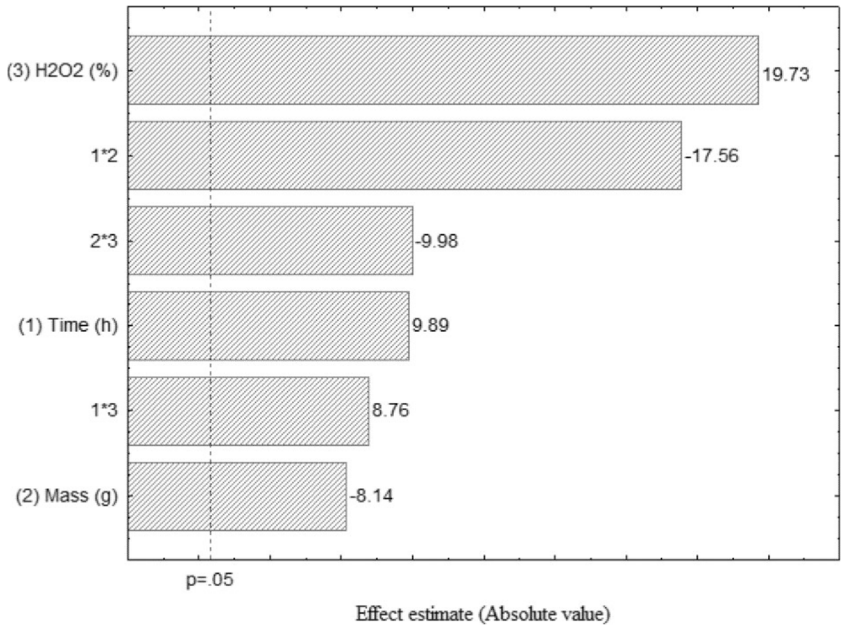

Fig. 1 Pareto graph generated to indicate the effects of the variables investigated on the swine hair degradation

presence of lower mass, longer reaction time, and higher concentration of the oxidizing agent (H_2O_2), the greater deformation of keratin occurred. Additionally, the increase of the hair mass in lower concentrations of H_2O_2 yielded a smaller degradation, considering the smaller amount of hydroxyl radicals to break the chemical bonds of the swine hair. The scanning electron microscopy (SEM) micrographies are presented in Fig. 5a, in which it is possible to observe the difference in structures of swine hair in the control sample compared with the sample of greater degradation (run 6 of the CCD 2^3), with 79.58% degradation and run 3 of the CCD 2^3, with 36.08% degradation. The Pareto chart presented in Fig. 1 was generated to identify significant effects.

All analyzed parameters were statistically significant ($p < 0.05$), being positive for H_2O_2 concentration, time, and interaction of the two respective variables, and negative for swine hairs weight and interaction between swine hair weight and tempo, and swine hair weight and concentration of H_2O_2.

Removal of hexavalent chromium in synthetic effluent using pretreated swine hair

Based on the degradation results obtained in the CCD 2^3, the biomaterials were tested in Cr(VI) removal assays of a synthetic medium with 20 mg Cr(VI) L^{-1}, aiming at the evaluation of the behavior of the biomaterial pretreated for detoxification of the solution containing the heavy metal. The results obtained are shown in Fig. 2.

In the assays where the biomaterial presented degradation higher than 60%, the lowest percentages of Cr(VI) removal were observed (tests 6, 9, 10, and 11). Also, a sample was treated using crude swine hairs (without H_2O_2 treatment),

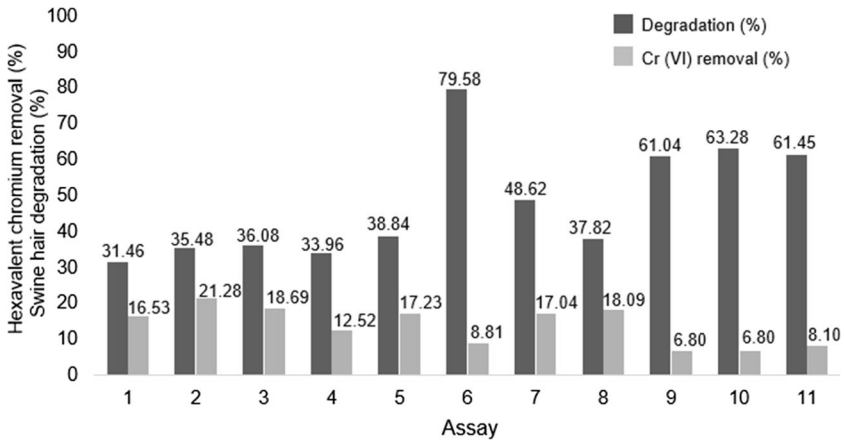

Fig. 2 Comparison of the results of hair degradation in CCD 2^3 and removal of hexavalent chromium in the respective assays

confirming that the non-treated hair was not able to remove the metal.

The affinity between the Cr(VI) ions and the surface layer of the pretreated biomaterial showed satisfactory results after 3 h of contact of the heavy metal solution with the hair. The oxidative process with hydrogen peroxide possibly acted upon disruption of the disulfide bonds. This oxidative process released the amino acid (cysteine and cystine) residues, which have affinity with heavy metals (Sanri and Pant 2006; Baias et al. 2009). Therefore, the variation in Cr(VI) removal capacity between the tests may be closely related to the chemical groups of the hair surface, responsible for the interaction with the metallic ions, such as the metal-cysteine interaction.

Animal hair is rich in cysteine, an amino acid that can form inter- and intra-chain disulfide bonds, resulting in cystine, which plays an important role in the protein structure. The protein keratin is the major component in human and animal hair (Baker and Czarnecki-Maulden 1987; Sato et al. 2019). Cysteine has the ability to compose free radicals, acting as a binder, mainly with metals (Baker and Czarnecki-Maulden 1987). Because of this characteristic, this amino acid is used for the removal of heavy metals from the environment and in

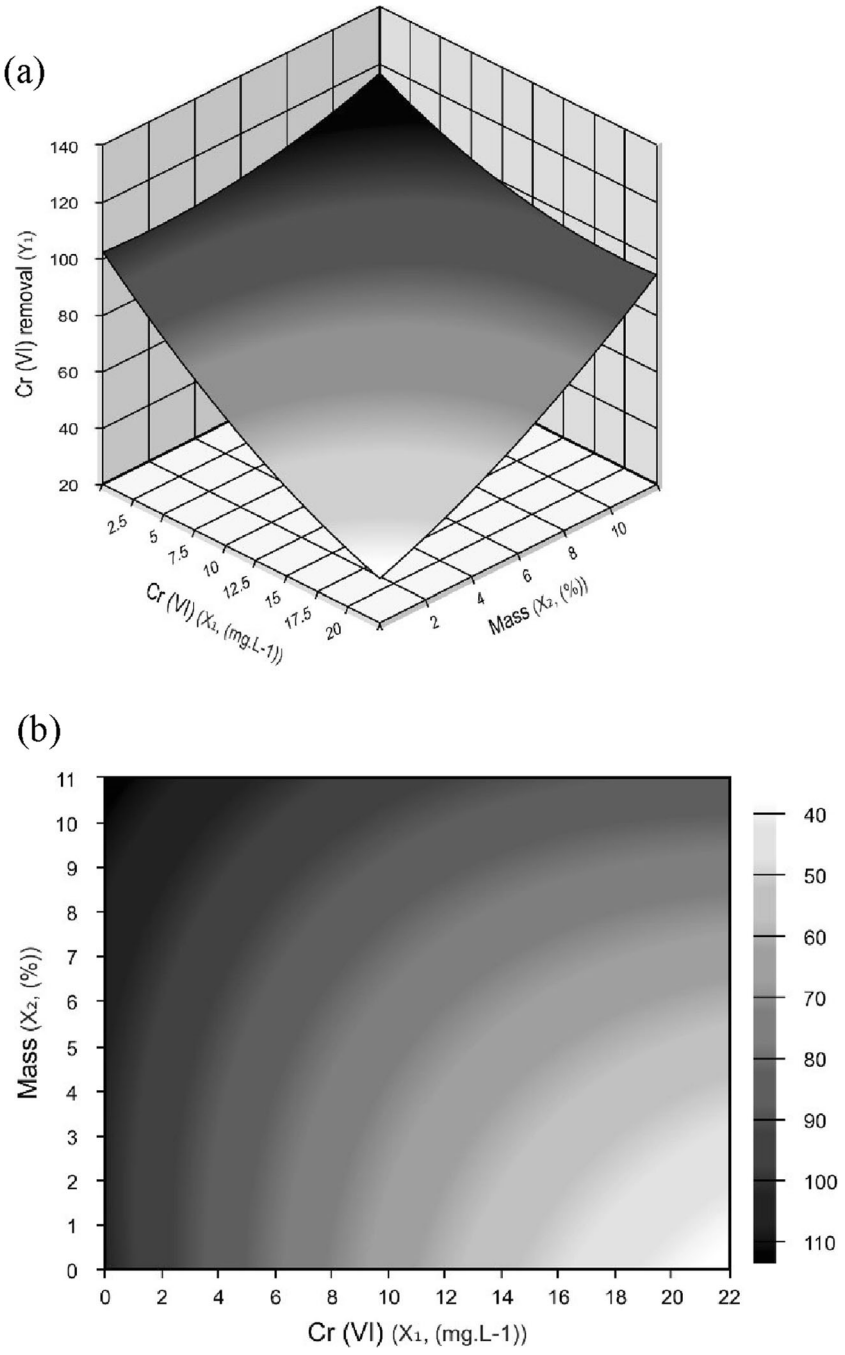

Fig. 3 Response surface (**a**) and contour curve (**b**) for the Cr(VI) removal using swine hair pretreated

drugs and supplementation for cases of intoxication, such as copper, arsenic, and selenium, among others (Albert 1961; Robbins and Baker 1980). This process involves the sequestration of metal ions in the presence of phytochelatins (Panda and Chouldhury 2005).

In higher percentages of degradation, there were smaller removals of the metal. This factor may be associated with the oxidation process by H_2O_2, which causes loss of hair resistance. This happens since the structure of the hair is formed by three fibrous layers: medulla (internal); cortex (medium); cuticle (external) (Pienpinijtham et al. 2018). The high degradation that resulted in the smaller Cr(VI) removals may have occurred due to the excessive removal of the fibrous layers of keratin.

The removal of Cr(VI) was similar for swine hair degradations of less than 50% of the material, so the hypothesis that the oxidative process results in a similar disruption of disulfide bridges between samples. That is, there are a number of disulfide bonds possible to be ruptured before irreversible degradations of the chemical bonds of keratin occur, oxidizing the molecule to compounds with no affinity for the metal. Furthermore, degradation of swine hairs evaluated by loss of mass results in the quantification of degradation of other compounds, including residues from the skin of the animals that are present in the medium. Thus, this explains why pig hair with degradations between 31.46 and 48.62% removes similar concentrations of Cr(VI).

In the sequence, three factors were considered for the selection of experimental conditions: the lower concentration of the chemical, because the high consumption of H_2O_2 increases the economic cost of the process (Alvarez-Vasco and Zhang 2017); the largest swine hair weight; and the shortest processing time. Therefore, the experimental conditions of assay 3 (3 h, 7.5 g of swine hair and 4% (w/v) of H_2O_2) were used in the subsequent experiments.

Central composite rotational design 2^2 for evaluation of Cr(VI) removal

Using the contact time of 3 h, a CCRD 2^2 was performed to analyze the efficiency of the pretreated pig hair in the removal of hexavalent chromium from a synthetic effluent. The tests and the respective percent removal of the metal concentration are shown in Table 2. The higher metal removal was obtained in assay 3 with the conditions 4.6 mg Cr^{6+} L^{-1} and 8.7 % (m v^{-1}) of hair. The highest efficiencies were obtained in the tests with larger swine hair masses and lower concentrations of Cr(VI).

The results were statistically analyzed and an optimized mathematical model was obtained (Eq. 2) for the Cr(VI) removal as a function of the Cr(VI) concentration (mg Cr^{6+} L^{-1}) [Cr] and swine hair pretreated (% m v^{-1}) [W].

$$\begin{aligned}\text{Cr(VI) removal} = {} & (73.0287) - (26.7966 \times [\text{Cr}]) \\ & + (4.9744 \times [\text{Cr}])^2 \\ & + (18.3437 \times [\text{W}]) \\ & + (3.1230 \times [\text{W}])^2 \\ & + (6.7301 \times [\text{W}] \times [\text{Cr}]) \end{aligned} \quad (2)$$

Model validation was performed by variance analysis (ANOVA), and the correlation coefficient (0.94) and the value of F (calculated F greater than the tabulated F) validated the model ($p < 0.05$) and allowed the construction of response surface and contour curve (Fig. 3).

The response surface shows that the highest Cr(VI) removals are obtained at the lower concentrations of Cr(VI) and higher mass quantities, since in this case there is a higher availability of chemical bonds with the metal in the presence of Cr(VI) ions in the solution, that is, more binding sites than metal ions in the medium (Fig. 4). Thus, the result that showed the highest removal of the metal was assay 3 (Fig. 5b), where the biomaterial mass was added to the medium in a ratio of 8.7% (m v^{-1}) and the metal concentration was 4.6 mg Cr^{6+} L^{-1}, resulting in a removal of 99.41% Cr(VI). This result is promising considering the detoxification of Cr(VI) wastewaters by pig hair.

Evaluation of contact time and pH for Cr(VI) removal by biomaterial

The contact time for chromium removal, previously fixed at 3 h, was further investigated at lower adsorption times. The conditions previously established in assay 3 of CCRD 2^2 were used and the results are shown in Fig. 4.

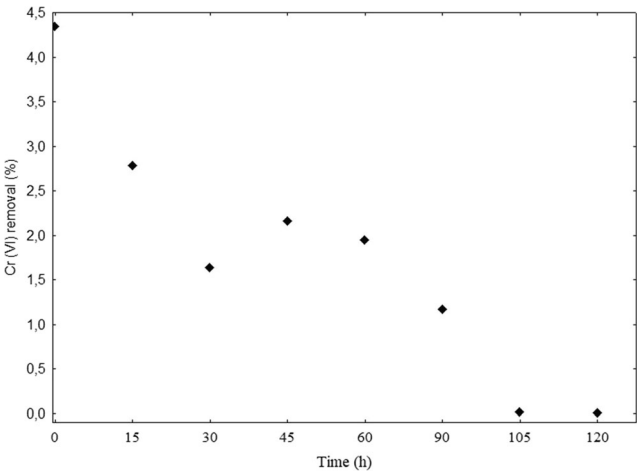

Fig. 4 Graph of Cr(VI) removal in medium containing 8.7% (m v^{-1}) of pretreated swine hairs and an initial metal concentration of 4.34 mg Cr^{6+} L^{-1}

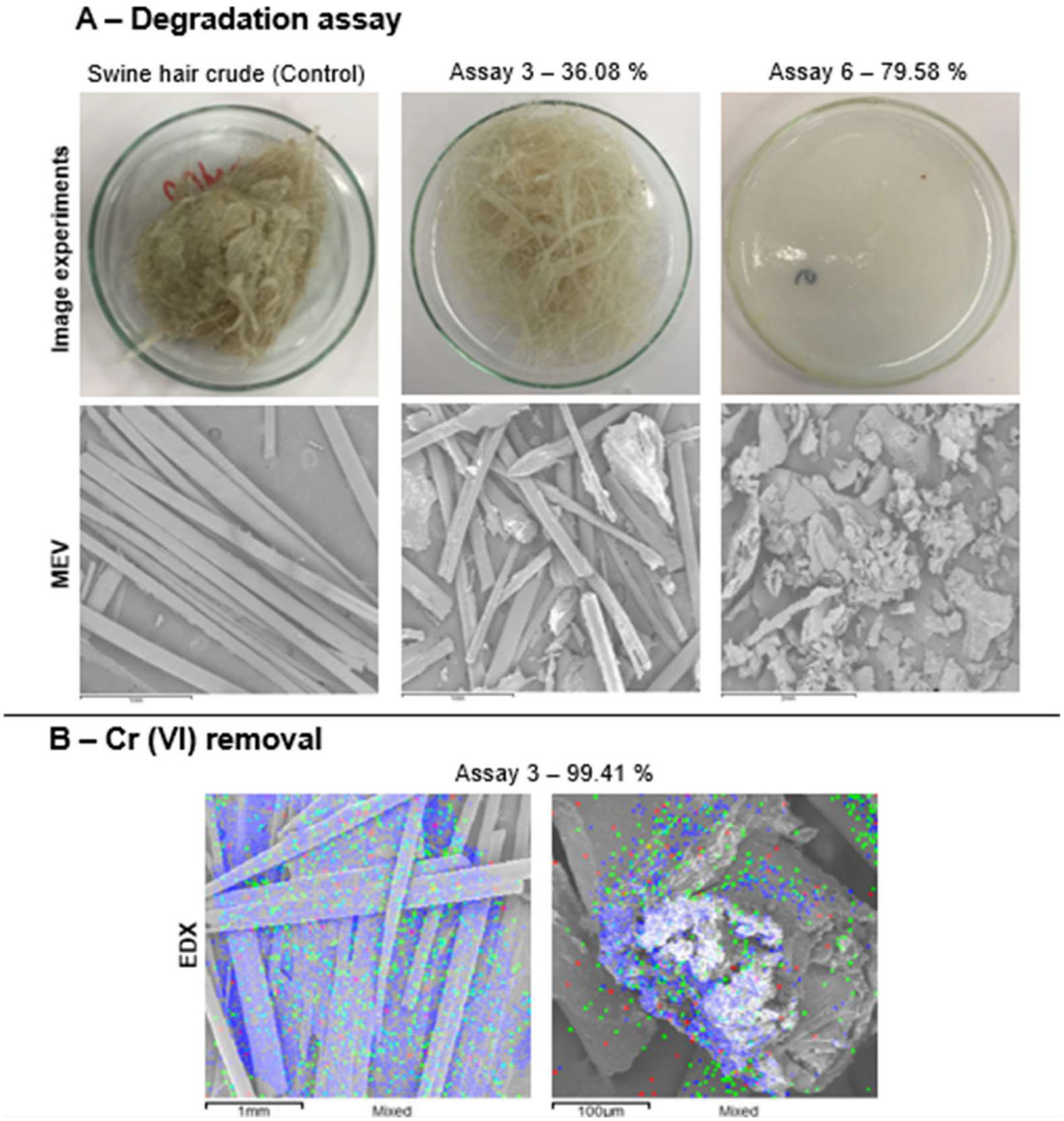

Fig. 5 Swine hair evaluated in scanning electron microscopy (SEM) and X-ray dispersive energy spectroscopy (EDX). **a** Degradation assay. **b** Cr(IV) removal

In 1 h 45 min (105 min), the removal of chromium was equivalent to that observed in 3 h. Thus, it was possible to reduce the process time by 75 min.

From the pH variation assay and Cr(VI) removal, swine hairs pretreated showed affinity with all forms of Cr(VI) ions in the pH range of 1.0 to 10.0, with removals above 99%. This may result from the affinity of metal ions for functional groups on the surface of the biomaterial (Ullah et al. 2013), causing an attraction and consequent removal of the toxic metal from the medium.

The hexavalent chromium can take the form of chromate (CrO_4^{2-}), dichromate ($Cr_2O_7^{2-}$), and hydrogen chromate ($HCrO_4^-$), and these oxidation states are strongly dependent on the pH of the solution and the total concentration of Cr(VI). Considering the solubility equilibrium of Cr(VI), the dominant species at pH lower than 1 is H_2CrO_4, the dominant species at pH between 1 and 6.5 is $HCrO_4^-$, and at pH greater than 6.5, the dominant form is CrO_4^2 (Sanri and Pant 2006; Moussavi and Barikbin 2010; Ullah et al. 2013; Yang et al. 2014; Kan et al. 2017).

Several studies have monitored the influence of pH on metal removal. Sanri and Pant (2006) reported that increasing the pH from 1.5 to 9.0 reduced Cr(VI) removal capacity by eucalyptus peel. In that study, the authors pointed out that with increasing pH, there was a gradual decrease in the percentage of Cr(VI) removal, and from 1.5 to 5.0, the removal efficiency

of the metal fell from 99 to 93%, and the maximum removal occurred in pH 2.0 with a percentage near 99%. The same was observed by Al-Homaidan et al. (2018) who evaluated the Cr(VI) removal using three algae. The removal efficiency of the metal was observed at pH 2.0, with a decline when increasing pH from 2.0 to 6.0. Jiang et al. (2018) used bimetallic Fe/Cu nanoparticles stabilized by chitosan to remove Cr(VI) in wastewater. The same pH relationship was observed, with maximum efficiency obtained at pH 3 of 80.3%, declining to 50.2% at pH 9.

In this context, the results obtained in this study are relevant considering the efficiency of Cr(VI) removal by swine hair pretreated in a wide range of pH, and can be applied for several purposes without the need for pH control to increase efficiency.

Application of pretreated swine hair in Cr(VI) removal from real tannery effluent

Pretreated swine hair was tested in the Cr(VI) removal in a real wastewater from a tannery industry, to evaluate its efficiency in the presence of interferers. The results obtained were promising, yielding more than 99% removal of heavy metal from real effluent, using 8.7% (m v^{-1}) of the biomaterial, in 1 h 45 min of contact time, for an initial concentration of 7.22 ± 1.78 mg Cr^{6+} L^{-1}. The pH of the wastewater was maintained at 3.49.

The presence of secondary compounds of the tannery effluent did not reduce the efficiency of the biomaterial as a potential biofilter in the removal of Cr(VI) from wastewater. Ullah et al. (2013) evaluated the Cr(VI) removal in sugarcane bagasse residue in the tannery effluent, obtaining a maximum removal of 80.6% in optimized conditions (pH 2 and 24 h of contact). A biomaterial composed of the mixture of tea leaf ash and pumpkin seeds studied in the removal of hexavalent chromium from contaminated wastewater resulted in removal values similar to this study, approximately 98% in 60 min of treatment (Maingi et al. 2016; Jobby et al. 2018). Thus, it is possible to observe the process advantages related to the use of only one component biomaterial and in terms of contact time (1 h 45 min), Cr(VI) removal percentage (> 99%), and irrelevance of pH adjustment.

Table 3 Determination of mitotic index (MI) from meristematic cells of *Allium cepa*, being represented by mean ± standard error (SE)

Sample	Mitotic index (MI) ± SE
Celular control	4.75 ± 0.25[a]
Meristem in untreated effluent	5.26 ± 0.35[b]
Meristem effluent treated with biofilter	4.95 ± 0.30[a]

Different superscript lowercase letters present significant difference with 95% confidence according to Tukey's test

Evaluation of the cytotoxicity of the tannery effluent

Table 3 shows the results for mitotic index from *Allium cepa* meristem cells. The MI that was determined for the crude effluent presented induction in the cell division, that is, it portrayed a larger number of nuclei, a result of accelerated DNA transcription. This data is consistent with reports in the literature, since the Cr(VI) heavy metal has characteristics such as high solubility, non-biodegradation, and biomagnification, as well as having a high carcinogenic potential that, through the modification of the DNA transcription process, generates aberrations in chromosomes (Dai et al. 2015; Luo et al. 2017).

When analyzing the results obtained for MI of the meristem in the treated effluent, where removal occurred with application of the biofilter, it is possible to perceive that the data are statistically equal to those of the cellular control. This result is of extreme relevance, demonstrating that the treatment in the biofilter actually made the solution with lower cytotoxicity.

Conclusions

Pretreatment of swine hair using H_2O_2 caused changes in the keratin conformation, propitiating until 99% of Cr(VI) removal using 8.7% of swine hair in a large pH range (1–10), demonstrating its potential as sustainable biofilter for tannery wastewater treatment.

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