Treatment of chromium solutions in a 15 dm^3 pilot bioreactor

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Abstract
Chromium is a heavy metal with large industrial applications such as in textile dying, chemicals and pigments production, wood preservation, tanning activity and electroplating for surface treatment. The treatment of wastewater containing this metal with biological methods is strongly recommended, especially when in the form of Cr (VI) due its high toxicity. The biosorption system consists of a bacterial biofilm supported on granular activated carbon (GAC), placed in contact with the polluted solutions. The bacterium used for the formation of the biofilm was *Arthrobacter viscosus*. Two concentrations of chromium were used: 10 mg/l and 100 mg/l, with a flow rate of 25 mg/l. The data obtained in a pilot-scale reactor showed an average removal percentage of 99.9%, during the first 30 days, for the initial concentration of 10 mg/l and average removal percentage of 72%, for the same period and for the initial concentration of 100 mg/l. Uptake values of 11.35 mg/g_{GAC} and 14.55 mg/g_{GAC} were obtained, respectively, for the initial concentration of 10 and 100 mg/l. The presence of functional groups on the cell wall surface of the biomass that may interact with the metal ion was confirmed by FTIR. The results obtained are very promising and encourage the utilization of this biofilm in environmental applications.

Keywords
Arthrobacter viscosus; bioreactor; biosorption; chromium

INTRODUCTION

The pollution caused by chromium is a very serious problem that needs to be solved. The conventional methods for the treatment of chromium solutions like chemical precipitation, coagulation or membrane separation are quite expensive mainly for small and medium size industries operating with tight budgets (Tavares *et al.*, 2006). Biosorption is an alternative to these conventional methods and is presented as one of the most promising technologies for the removal of heavy metals. The use of non-expensive waste biomass, the low cost of biomass immobilization and the possibility of biomass regeneration are some of the most important key factors that should be considered in the application of biosorption in the removal of toxic metals from industrial waste solution (Quintelas *et al.*, 2006).
The usage of bacteria as biosorbents has some advantages due to their small size, their ubiquity, their ability to grow under controlled conditions and their resilience to a wide range of environmental situations (Urrutia, 1997). *Arthrobacter* species is of particular interest because of its high potential for bioremediation. Bacteria can detoxify chromium wastewater, by either reduction or accumulation inside the cells and/or adsorption of the ion on their surface (Asatiani *et al*., 2004).

MATERIALS AND METHODS

Materials

The bacterium *Arthrobacter viscosus* (CECT 908) was obtained from the Spanish Type Culture

Collection of the University of Valência. Aqueous chromium solutions were prepared by diluting $K_2Cr_2O_7$ (Riedel) in distillated water. All glassware used for experimental purposes was washed in 60% nitric acid and subsequently rinsed with deionised water to remove any possible interference by other metals. Atomic absorption spectrometric standards were prepared from 1000 mg_{Cr}/L solution.
The support was granular activated carbon (GAC) from MERCK with an average particle size of 2.5 mm, characterised by N_2 adsorption (77K) with an ASAP Micromeritics 2001, which indicated a Langmuir area of 1270 m^2g^{-1} and an average pore diameter of 2 nm.

Methods

Pilot bioreactor studies

The bioreactor was a 15 dm^3 cylindrical tank (inner diameter 14.2 cm, total height 100 cm), with a maximum packing fraction of 1/3. The biofilm formation was prepared accordingly to previous studies (Quintelas and Tavares, 2002, 2001), adjusted to the bioreactor dimensions. 2 Kg of GAC were placed in an Erlenmeyer flask of 5 l with distilled water. It was heated at 120^0C for 20 min to release the air inside the pores. Then, it was placed in the bioreactor for open system studies. 18 l of a rich nutrient broth was prepared, sterilized at 120^0C for 30 min, inoculate with the bacteria and were pumped through at a flow rate of 250 ml/min, during 24 h, with total recirculation. During the next 48 h, 45 l of a different nutrient broth were used to grow the biofilm also at a flow rate of 250 ml/min, with total recirculation. The composition of the two different nutrient broths was well described in Quintelas and Tavares (2002). After biofilm formation, the bed was washed out and the metal solutions with Cr concentrations of 10 and 100 mg/l (prepared on laboratory) were passed through the column with a flow rate of 25 ml/min. At the end, the column was washed out and samples of the effluent were seeded in Petri plates with nutrient agar to assess the metabolic activity of the microorganism. Cr concentration at the inlet and at the outlet of the columns was measured by Atomic Absorption Spectroscopy, Varian Spectra AA-250 Plus, by acetylene flame emission and at wavelengths of 357.9 nm, 425.4 nm and 520.8 nm. The pH values were also measured during the experimental essays (Jenway 350 pH Meter).

Scanning Electron Microscopy (SEM)

Samples of the supported biofilm were taken and analysed (after dehydration with different concentrations of ethanol) by SEM (Leica Cambridge S360). Samples were gold coated prior to SEM observation. The images presented intend to show that the biofilm uniformly covered the GAC surface and each of them is an example of many pictures taken at various zoomed areas.

Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectra of the unloaded biomass and of the chromium loaded biomass, both in suspension, were obtained using a Fourier transform infrared spectrometer (FTIR BOMEM MB 104). For the FTIR study, biomass is centrifuged and dried, followed by weighting. Then 20 mg of finely ground biomass was encapsulated in 200 mg of KBr (Riedel) in order to prepare translucent sample disks. Background correction for atmospheric air was used for each spectrum. The analysis resolution was 4 cm^{-1} and a minimum of 5 scans for each spectrum were performed within the range 500 - 4000 wavenumbers.

RESULTS AND DISCUSSION

The biofilm of *A. viscosus* supported on GAC was tested for the initial Cr (VI) concentrations of 10 and 100 mg/l. The essay for the initial concentration of 10 mg/l lasted 226 days (approximately 7.5 months) and the essay for the initial concentration of 100 mg/l lasted 104 days (approximately 3.5 months). The volume of chromium solution treated was of 8140 litres for the essay with the initial concentration of 10 mg/l and of 3732 litres for the most concentrated solution. The results in terms of breakthrough curves, for both initial

concentrations, are represented in Figure 1.

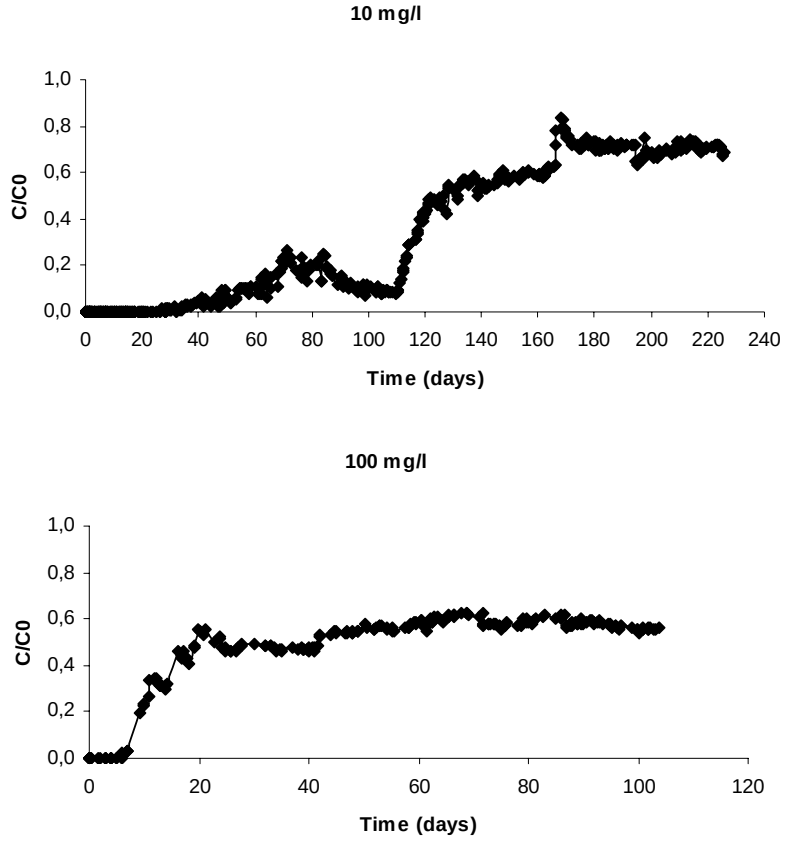

Figure 1. Breakthrough curves for the biosorption of Cr (VI) using an *A. viscosus* biofilm supported on GAC, for the initial Cr (VI) concentrations of 10 and 100 mg/l (Quintelas *et al.*, submitted).

It is important to refer that at the end of each run, the column was washed out and samples of the effluent were seeded in Petri plates with nutrient agar to assess the metabolic activity of the microorganism and even concentrations of 100 mg/l did not seem to be toxic for the bacterial culture used, indicating that this specific bacteria appears to be resistant in an actual industrial environment. After the biofilm formation, the microorganism survived without any kind of nutrients. It is possible to conclude that the bacteria incorporate chromium on their metabolism and this is probably the reason why the bacteria were metabolically active after several months without nutritional supplements. This conclusion is extremely important because the addiction of a pollutant to the food supply of a microorganism is a fundamental step for the success of a biosorption process (Quintelas *et al.*, submitted).
The removal percentage for the experimental essay with an initial concentration of 10 mg/l was of 100 % during the first 26 days of the experimental run. At the 27th day, the removal percentage started to decrease and, at the end of the experiment, after 226 days, the removal percentage was 32%. For the experimental essay with initial concentration of 100 mg/l, the removal percentage was 100 % during the first 6 days of the experimental run. At the end of the experiment, the removal percentage was 38%.
At the end of the experiments the biofilm was analysed by SEM (Scanning Electron Microscopy) (Figures 2a) and b)). On Figure 2a) it is possible to confirm the large surface coverage by the bacteria. The more relevant evidence that can be observed on these figures is the degradation of the surface of the activated carbon. This state of degradation is a consequence of erosion

motivated by the hydrodynamic effects suffered by carbon during the 226 days of experimental essay. Figure 2b), corresponding to the biofilm from the bioreactor operating with the initial concentration of 100 mg/l, shows that the biofilm covered uniformly the carbon surface. The activated carbon remains less degraded than the carbon used for the experimental essay at an initial concentration of 10 mg/l and this is justified by the lower erosion suffered during a shorter essay period (Quintelas *et al.*, submitted).

a) b)

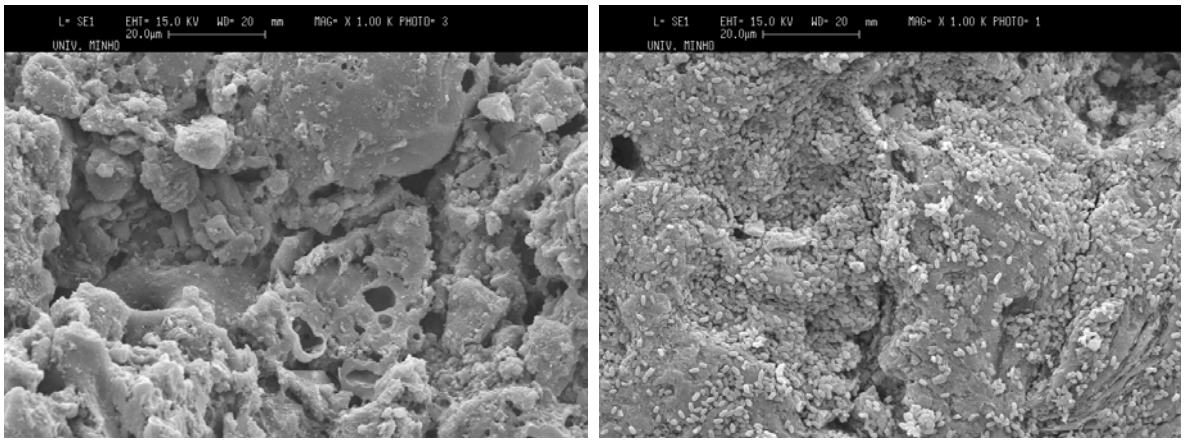

Figure 2. SEM images of the biofilm taken after 226 days of experimental essay, for the initial concentration of 10 mg/l (a) and after 104 days of experimental essay, for the initial concentration of 100 mg/l (b)(Quintelas *et al.*, submitted).

Uptake values are presented on Table 1 and compared with previous results obtained using minicolumns (Quintelas *et al.*, 2006). Uptake values obtained with the pilot bioreactor are much higher than those obtained with the minicolumns (internal diameter=2 cm, height=30 cm, flow rate= 10 ml/min). The higher amount of carbon and consequent amount of biomass and the increase of the retention time, from 9.4 min (minicolumns) to 200 min (bioreactor), are possible reasons for the increase on the uptake values.

Table 1. Comparison between the uptake values obtained for the pilot reactor and for minicolumns essays.

	Uptake (mg/g)	
Conc. of Cr (VI)	**Pilot reactor**	**Minicolumns**
10 mg/l	11.35	0.72
100 mg/l	14.55	5.30

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

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of unloaded and metal loaded *Arthrobacter viscosus* biomass in the range of 500–4000 cm^{-1} were taken to find out which functional groups may be responsible for the biosorption process and presented in Table 2. The unloaded biomass displays a number of absorption peaks, reflecting the complex nature of the biomass. Pradhan *et al.* (2007) and Volesky (2007) affirm that the main functional groups responsible for a biosorption process are the hydroxyl, carbonyl, carboxyl, sulfonate, amide, imidazole, phosphonate and

phosphodiester groups. Some of these groups are present on the *Arthrobacter viscosus* biomass and may interact with the metal ions.

Table 2. Frequencies (cm^{-1}) and assignments of FTIR bands.

Frequencies (cm^{-1})	**Assignments of FTIR bands**
3400	Bonded hydroxyl group and –NH stretching peak
1546	C-N stretching and N-H deformation
1398	COO^- anions
1238	$-SO_3$ groups
861	Aromatic –CH stretching peak

CONCLUSIONS

Arthrobacter species are known due to their high potential for bioremediation. The behaviour of a biosorption system consisting of an *Arthrobacter viscosus* biofilm supported on GAC was investigated in a pilot scale bioreactor. The results showed average removal percentage of 99.9%, during the first 30 days, for the initial concentration of 10 mg/l and average removal percentage of 72%, for the same period and for the initial concentration of 100 mg/l. Uptakes values of 11.35 mg/g_{GAC} and 14.55 mg/g_{GAC} were obtained respectively, for the initial concentration of 10 and 100 mg/l. These values are much higher than the obtained in previous studies developed with minicolumns. The presence of functional groups on the cell wall surface of the biomass that may interact with the metal ion, was confirmed by FTIR.

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