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Improved biosorption for Cr(VI) reduction and removal by *Arthrobacter viscosus* using zeolite

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ABSTRACT

The aim of the present work was to optimize the reduction and removal of chromium from aqueous solutions by a biosorption system consisting of a bacteria supported on a zeolite. The system proposed combines the biosorption properties of *Arthrobacter viscosus*, with the ion exchange capacity of NaY zeolite. Experiments were also performed without the zeolite for comparison purposes. Experimental parameters such as solution pH, biomass concentration and initial Cr(VI) concentration were investigated in order to assess their influence on the biosorption system. The results revealed that chromium biosorption was highly pH dependent. The lower pH values favored Cr(VI) reduction, while higher solution pH enhanced total chromium removal. After the optimization of the parameters in study, the highest content of chromium in the zeolite (0.9%) and best uptake (13.0 $mg_{Cr}/g_{zeolite}$) were obtained for the experiment at pH 4, biomass concentration of 5 g L^{-1} and initial Cr(VI) concentration of 100 mg L^{-1}. After the biosorption process, the samples were characterized by chemical analyses (ICP-AES) and X-ray photoelectron spectroscopy (XPS). The XPS spectra of bacteria revealed that the chromium loaded on the biomass surface was in the trivalent form.

1. Introduction

Nowadays, increasing awareness of water pollution with heavy metals and its long term effects has encouraged intensive efforts toward pollution abatement. Chromium is one of the most harmful toxic metals discharged into the environment and has become a serious health concern. It occurs most frequently as Cr(VI) or Cr(III) in aqueous solutions, with quite different characteristics in respect of toxicity, mobility and solubility. Hexavalent chromium is very toxic and carcinogenic, while trivalent chromium species are less harmful to living organisms (Kalabegishvili et al., 2003; Tsibakhashvili et al., 2004; Mohan and Pittman, 2006).

The utilization of several biological materials, such as microbial cells, for the removal and recovery of heavy metal pollutants from wastewater, called biosorption, has been reported in numerous studies (Brady et al., 1994; Matheickal et al., 1999; Pagnanelli et al., 2000; Loukidou et al., 2004; Hsu et al., 2010). Although the mechanisms of biosorption are defined as metabolic independent, some authors (Matheickal et al., 1999) have defined biosorption as the ability of biological materials to accumulate heavy metals from waste streams by either metabolically mediation or by purely physico-chemical uptake pathways. In fact, cell walls, consisting mainly of polysaccharides, proteins and lipids, offer many functional groups which can bind ions, such as metal ions (Brady et al., 1994; Pagnanelli et al., 2000). Numerous studies have demonstrated a reduction of toxic Cr(VI) to non-toxic Cr(III) by various types of biomaterials, such as bacteria (Cheung and Gu, 2003, 2007; Asatiani et al., 2004; Tsibakhashvili et al., 2004; Park et al., 2004, 2005, 2007, 2008; Dermou et al., 2005; Cheung et al., 2006). Recently it has been shown that several species of *Arthrobacter* can reduce hexavalent chromium into its less toxic trivalent form (Tsibakhashvili et al., 2004; Lin et al., 2006).

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). It had been demonstrated that the bacterium *Arthrobacter viscosus* produces a significant amount of exopolysaccharides, which is an advantage for the capture of metal ions (Quintelas and Tavares, 2001; Silva et al., 2008a).

In this research work, a system for the removal of hexavalent chromium from water, that combines the biosorption properties of the microorganism with the ion exchange capacity of the zeolite, was optimized. Zeolites are low cost ion exchangers and have peculiar properties that result from the presence of positively charged exchangeable ions. Those ions can be replaced by heavy

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metals (Santen and Kramer, 1995). Due to this net negative charge, zeolites have a strong affinity for transition metal cations, but much lower affinity for anions, such as dichromate or chromate (Vaca Mier et al., 2001). However, this lack of affinity of the zeolite for Cr(VI) species can be overcame by the use of bacteria, such as *A. viscosus*, that have the capacity of transforming toxic Cr(VI) compounds through reduction into cationic Cr(III) species that are much less harmful (Silva et al., 2008a,b; Figueiredo et al., 2010a). An advantage of using this system for the treatment of wastewater contaminated with metals is the reutilization of the obtained metal-loaded zeolites as catalysts in oxidation reactions of organic compounds, as reported in our previous work (Figueiredo et al., 2006, 2010b; Silva et al., 2012).

For the practical use of biosorption systems it is important to identify and study extensively the factors that affect their performance. The main operational conditions that significantly influence the biosorption of heavy metals are the solution pH, the ionic strength, the biomass and the metal concentrations, the temperature and the presence of other metals in solution. The aim of the present work is to evaluate the effects of various operational parameters such as solution pH, biomass concentration and initial Cr(VI) concentration, in order to optimize the reduction and removal of chromium by the combined system bacteria/zeolite. So far, most of the research done on Cr(VI) biosorption did not take in account the reduction of Cr(VI) to Cr(III) and stated that Cr(VI) is removed from aqueous systems through anionic adsorption. In those studies, several kinetic evaluations have been made not taking into account the reduction process, which led to incorrect conclusions. In this work, both Cr(VI) and total Cr concentrations were monitored in order to elucidate the Cr(VI) removal mechanism.

2. Experimental

2.1. Materials and reagents

A. viscosus was obtained from the Spanish Type Culture Collection of the University of Valencia. Aqueous potassium dichromate solution was prepared by diluting $K_2Cr_2O_7$ (Panreac) in deionized water. All glassware used for experimental purposes was washed in 10% nitric acid to remove any possible interference by other metals. The faujasite zeolite NaY in powder form (Si/Al = 2.83) with specific surface area of 787 $m^2\ g^{-1}$, was obtained from Zeolyst International.

2.2. Methods

2.2.1. Preparation of the biomass

A medium with 10 g L^{-1} of glucose, 5 g L^{-1} of peptone, 3 g L^{-1} of malt extract and 3 g L^{-1} of yeast extract was used for the microorganism growth. The medium was sterilized at 121 °C for 20 min, cooled to room temperature, inoculated with bacteria and kept at 28 °C for 24 h with moderate stirring in an incubator. The cells were then harvested by centrifugation at 7000 rpm for 15 min and re-suspended in a pre-established volume of residual culture medium (obtained after the microorganism growth) in order to obtain the desired concentrated suspension to be used in the biosorption assays.

2.2.2. Biosorption assays

The biosorption assays were performed under different operating conditions of solution pH (1, 2, 3 and 4) and biomass concentration (1, 2, 4 and 5 g L^{-1} – dry weight), using Cr(VI) solutions prepared with initial concentration of 10, 25, 50, 75, 100 mg L^{-1}. Batch experiments were conducted in 250 ml Erlenmeyer flasks using 1.0 g of NaY zeolite, 150 ml of each potassium dichromate solution and 15 ml of the *A. viscosus* suspension previously prepared. The concentration of Cr(VI) in each Erlenmeyer flask after adding 15 ml of biomass suspension (9.1, 22.7, 45.5, 68.2 or 90.9 mg L^{-1}) was calculated taking into account the dilution effect caused by the addition of this extra volume. Additional assays were performed with bacteria in suspension, without the zeolite, for comparison purposes. The solution pH was regularly maintained at the desired pH value using concentrated solutions of sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH), in order to minimize the variation of volume (<1%) and consequently the concentration of Cr. After the experiments at different pH values, pH 4 was selected for the remaining tests. The Erlenmeyer flasks were kept at 28 °C, with moderate stirring. Samples of 1 ml were taken, centrifuged and analyzed for chromium quantification.

In order to investigate separately the role of each component of the bacterial suspension in the reduction of Cr(VI), several control assays were made. These assays were conducted with the same methodology above described, although under different experimental conditions. In the control assay A, the residual culture medium, obtained by the centrifugation of the cells after the bacterial growth during 24 h, was added to the zeolite and to a Cr(VI) solution of 100 mg L^{-1}. In the control assay B, deionized water was used to suspend the fresh cells instead of the residual culture medium. For the control assay C, the biomass obtained after the microorganism growth was autoclaved at 121 °C, during 20 min and re-suspended in residual culture medium. These control assays were compared with the standard suspension consisting of fresh cells suspended on residual culture medium.

2.2.3. Analysis of chromium ions in solution

Samples taken for chromium quantification were previously centrifuged in order to remove any biomass and/or zeolite present in solution. Hexavalent chromium was quantified by measuring absorbance at 540 nm of the purple complex of Cr(VI) with 1,5-diphenylcarbazide, in acidic solution (Eaton et al., 1995). For total Cr quantification, the Cr(III) was first oxidized to Cr(VI) at high temperature by the addition of potassium permanganate previous to the reaction with 1,5-diphenylcarbazide. The Cr(III) concentration was calculated by the difference between the total Cr and Cr(VI) concentration.

2.3. Characterization procedures

Elemental chemical analyses (Si, Al and Cr) of zeolite samples were performed by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) using a Philips ICP PU 7000 Spectrometer. Previously to elemental chemical analyses, the zeolite samples were calcined at 500 °C during 8 h under a dry air stream, in order to remove the organic matter from bacteria.

X-ray photoelectron spectroscopy (XPS) analysis of the Cr-loaded biomass was obtained at the C.A.C.T.I. from Vigo University (Spain) in a VG Scientific ESCALAB 250iXL spectrometer using monochromatic Al-Kα radiation (1486.92 eV). In order to correct possible deviations caused by electric change of the samples, the C 1s line at 285.0 eV was taken as internal standard (Xie and Sherwood, 1990; Moulder et al., 1992).

3. Results and discussion

3.1. Control assays

These preliminary assays were conducted in order to establish the effects of the inoculum components, such as bacteria and the compounds dissolved in the residual culture medium, in the reduction rate of hexavalent chromium. In Fig. 1, the variation of

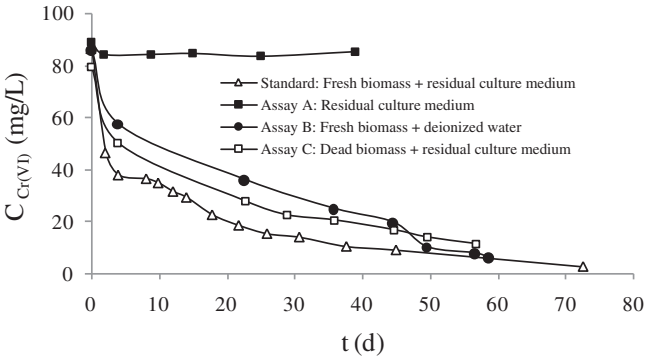

Fig. 1. Concentration of Cr(VI) as a function of contact time, for the standard experiment and the control assays A, B and C. Experimental conditions: initial Cr(VI) concentration of 100 mg L^{-1}, biomass concentration of 5 g L^{-1} and pH 4.

Cr(VI) concentration during the contact time is shown for the control assays A, B, C and the standard experiment, performed at pH 4 and biomass concentration of 5 g L^{-1}.

As it can be observed, the reduction of hexavalent chromium by the residual culture medium (assay A) was very slight, which is an evidence that the sugars present in this medium were not able to reduce Cr(VI) at pH 4. In order to evaluate the role of the cells metabolism on the reduction of Cr(VI), the performances of fresh cells (standard experiment) and dead cells (assay C) were compared. However, it was not possible to establish an accurate comparison between the control experiment and assay C due to different values of biomass concentration. Although it was expected a biomass concentration of 5 g L^{-1} for all experiments, the maximum biomass concentration achieved for assay C was 3.2 g L^{-1}. During the sterilization process of the cells, a loss of mass was observed, probably due to cell lysis and consequent dilution of the intracellular components in the external medium. However, despite of this difference in biomass concentration, a quite similar behavior for the living and for the dead biomass was observed. These results are probably an indication that the cell metabolism does not interfere significantly in the reduction process of Cr(VI), at these operation conditions. As cellular metabolism can be neglected, all the other assays were performed with living cells even at extremely acid condition (pH < 4), since the use of dead biomass would require the consumption of energy in the inactivation step.

Analyzing the results obtained from the assay B, it is clear that the polysaccharides present in the residual culture medium played an important role in the reduction of Cr(VI), in the presence of biomass, once the reduction was significantly faster. However, as mentioned previously, the residual culture medium itself was not capable of a significant reduction of Cr(VI). Thus, this behavior can be probably related to a synergism between the bacterium and the sugars present in the residual culture medium.

3.2. Effect of the solution pH

The pH of aqueous solution is an important factor that influences not only the dissociation of the cell wall functional groups, but also the solution chemistry of metals. Processes such as hydrolysis, complexation by organic and/or inorganic ligands, redox reactions and precipitation are strongly influenced by pH and, on the other hand, strongly affect speciation and biosorption availability of the metals (Esposito et al., 2002). Chromium exhibits different types of pH dependent equilibria in aqueous solutions. As pH is shifted, the equilibrium will also shift; at lower pH (pH < 2.0), $Cr_3O_{10}^{2-}$ and $Cr_4O_{13}^{2-}$ species are formed; at a pH range of 2–6, $HCrO_4^-$ and $Cr_2O_7^{2-}$ ions are in equilibrium. For pH values above 8, CrO_4^{2-} is the predominant species in the solution (Rollinson, 1973; Bai and Abraham, 2001; Mor et al., 2007). It was reported that at lower pH protonation of the cell wall components increases and the negatively charged chromium species bind through electrostatic attraction to positively charged functional groups on the surface of biomass. At increasing pH values, the negative charge density increases, due to the deprotonation of the metal binding sites and therefore adsorption of Cr(VI) species decreases (Deng et al., 2009). On the other hand, the reduction process of hexavalent to trivalent chromium requires a large amount of protons (Barrera et al., 2006). As a result of these trends, an optimum pH for hexavalent chromium removal was selected in the range 1–4, as similar values of optimum pH were reported for Cr(VI) removal using various biosorbents (Park et al., 2004; Ziagova et al., 2007; Yin et al., 2008; Deng et al., 2009).

3.2.1. Biosorption by A. viscosus in suspension

Fig. 2a and b show the time-dependent concentration of Cr(VI) and total Cr, at various solution pH values in the range of 1–4. These experiments were made just with bacteria in suspension, without the zeolite, for an initial Cr(VI) concentration of 100 mg L^{-1}.

As it can be seen in Fig. 2a, the depletion rate of Cr(VI) was strongly pH dependent, increasing with a decrease in pH. According to the reduction reactions of Cr(VI) species, it is essential to supply a large amount of protons for promoting the rate of the whole process.

$$Cr_2O_7^{2-} + 14H^+ + 6e^- \leftrightarrow 2Cr^{3+} + 7H_2O \quad (1)$$

$$CrO_4^{2-} + 8H^+ + 3e^- \leftrightarrow Cr^{3+} + 4H_2O \quad (2)$$

$$HCrO_4^- + 7H^+ + 3e^- \leftrightarrow Cr^{3+} + 4H_2O \quad (3)$$

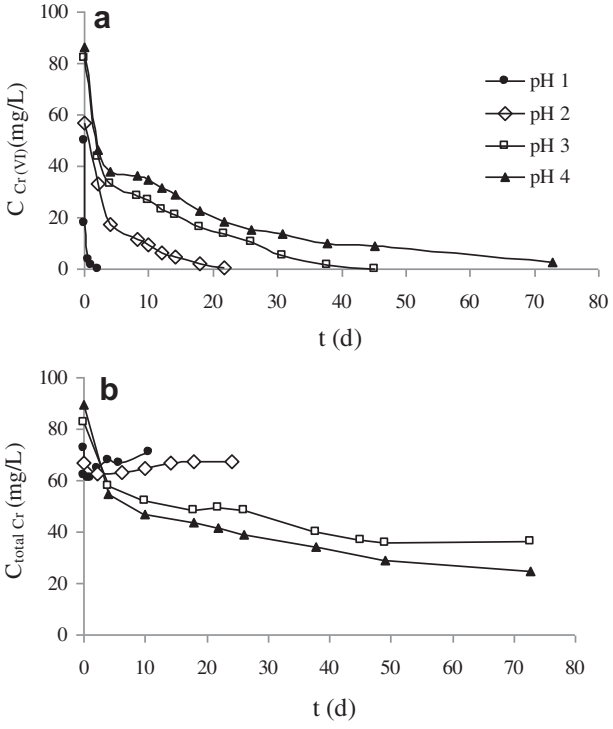

Fig. 2. Concentration of Cr(VI) **(a)** and total Cr **(b)** as a function of contact time, for solution pH values of 1, 2, 3 and 4. Experiments with *A. viscosus* in suspension with an initial Cr(VI) concentration of 100 mg L^{-1} and biomass concentration of 5 g L^{-1}.

Hexavalent chromium was completely removed from solution for pH values of 1, 2 and 3. The contact time necessary for complete Cr(VI) removal was 52 h, 22 days and 45 days for pH 1, pH 2 and pH 3, respectively. Total depletion of Cr(VI) was not achieved at pH 4, for 73 days, remaining in solution 2.5 mg L^{-1} of Cr(VI). Although Cr(VI) reduction was favored by very acidic conditions, higher pH values enhanced total Cr removal with the increase in contact time as shown in Fig. 2b. As a result of these opposite trends, the optimum pH for total chromium removal was found to be pH 4.

These results may be explained by the multiple phenomena involved in this system such as reduction of Cr(VI), adsorption/desorption of chromium ions and protonation/deprotonation of the cell wall functional groups which depend on the solution pH. Recent reports of Park et al. (2004, 2005, 2007, 2008) demonstrate that the biosorption mechanism of Cr(VI) by biomaterials is not "anionic adsorption" but "adsorption-coupled reduction". When Cr(VI) comes in contact with biomaterials, especially in an acidic solution, it can spontaneously be reduced to Cr(III) by electron-donor groups of the biomass, because Cr(VI) has a high *redox* potential value (above +1.3 V at standard conditions). These authors stated that Cr(VI) can be removed through both direct and indirect reduction mechanisms. In the first mechanism Cr(VI) is directly reduced to Cr(III) in the aqueous phase by contact with the electron-donor groups of the biomass surface which have lower reduction potential than that of Cr(VI). The indirect reduction involves the binding of Cr(VI) anions to the positively charged groups of the biomass surface and the reduction of Cr(VI) to Cr(III) by adjacent electron-donors groups. After the reduction, Cr(III) can be released into the aqueous solution due to the electronic repulsion between the positively charged groups of the biomass and Cr(III) cations, or can form complexes with adjacent groups capable of Cr-binding. Currently, this "adsorption-coupled reduction" mechanism has been widely reported and accepted as the mechanism of Cr(VI) biosorption (Deng et al., 2009; Janos et al., 2009; Murphy et al., 2009; Wu et al., 2010; Cui et al., 2011).

Analyzing Fig. 2b, for the lowest pH values (pH 1 and pH 2) it is possible to observe a high spontaneous removal of total chromium when the biomass was brought into contact with the solution. This was due to the strong protonation of functional groups, thus making the biomass more positively charged and hence creating an electrostatic attraction with Cr(VI) species. However, an increase of total Cr concentration was observed at pH 1 and pH 2, after 1 day and 6 days of contact time, respectively. This release of chromium to the solution is related to the electronic repulsion between the positively charged groups of the cell wall and the cationic Cr(III) species that result from the reduction of hexavalent chromium on the bacterium surface. In opposition, a decrease of total chromium was observed, as the contact time increases, for the highest pH values (pH 3 and 4). So, in this case, as the solution was less acidic, some of the metal binding sites of the biomass surface were not protonated, being negatively charged. Therefore, these sites were able to attract Cr(III) cations that result from the direct reduction of Cr(VI) in the solution. These results are in agreement with the assumptions of Park et al. (2004, 2005, 2007, 2008) above mentioned. More detailed information is given in Fig. 3 that presents the time-dependent concentration of Cr(VI), Cr(III) and total Cr in solution, during the biosorption of Cr(VI) by *A. viscosus* in suspension at pH 2 and pH 4.

Analyzing Fig. 3a, it can be observed that Cr(III), which was not initially present, appeared in the aqueous solution as a result of the reduction of Cr(VI). At pH 2, the significant depletion of total Cr that was observed at the initial instant of time can be attributed to two processes that occur simultaneously: the retention of Cr(VI) on the positively charged biomass surface and the direct reduction of Cr(VI) to Cr(III) due to the numerous protons available in the solution. After the initial instant of time, the concentration of Cr(III) increased proportionally to the Cr(VI) depletion, which means that Cr(VI) was only reduced to Cr(III) and was not retained on the biomass surface, as it can be seen by the total Cr concentration profile. This is probably due to the saturation of the biomass surface after the high initial retention of Cr(VI). At pH 4 (Fig. 3b), the concentration profiles of the chromium species were significantly different. It can be observed that at the initial instant of time, a lower amount of Cr(VI) was retained on the biomass surface in comparison with that retained at pH 2. This is probably due to the bacterial surface that was less protonated and therefore less positively charged at pH 4. It should be noted that after the initial instant of time, the rate of Cr(VI) depletion was higher than the increasing rate of Cr(III) concentration, which means that the difference between these rates can be attributed to the chromium bound to the biomass surface. These results are an indication that, at pH 4, the direct reduction of Cr(VI) to Cr(III), in the aqueous solution, and the adsorption of Cr(III) cations by the available negatively charged groups of the biomass surface occurs simultaneously.

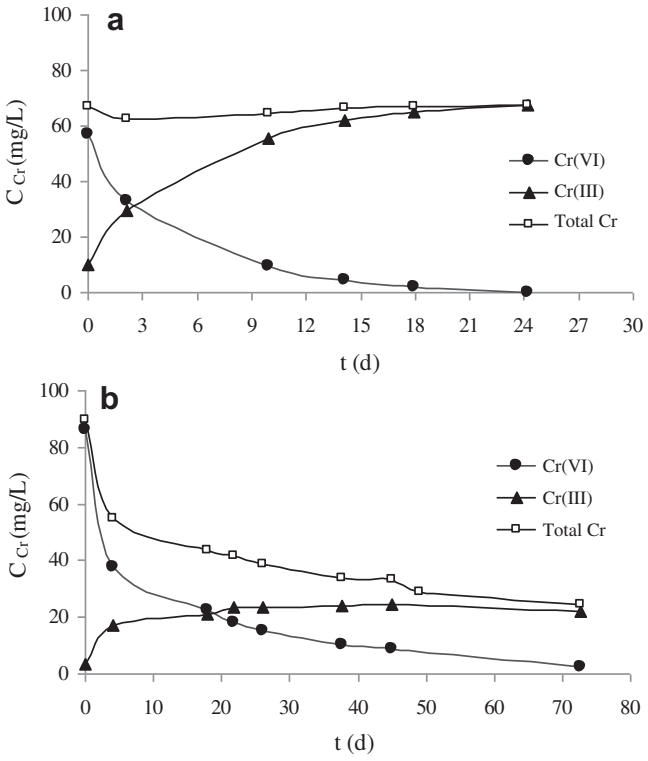

Fig. 3. Concentrations of different chromium species as a function of contact time, during Cr(VI) biosorption by *A. viscosus* in suspension at pH 2 (**a**) and pH 4 (**b**). Experiments with an initial Cr(VI) concentration of 100 mg L^{-1} and biomass concentration of 5 g L^{-1}.

The oxidation state of the Cr bound to the biomass was characterized using XPS. Fig. 4 shows the XPS wide scan spectrum and the high-resolution spectrum, collected from the Cr 2p core region, of the chromium-loaded biomass obtained after Cr biosorption at pH 4. The wide scan spectrum was obtained to identify the surface elements present on the biomass surface and to carry out a quantitative analysis. This spectrum revealed the presence of carbon (72.9%), oxygen (18.6%), nitrogen (6.9%), phosphorous (0.9%) and chromium (0.8%). The high content of chromium on the biomass surface is in agreement with the good removal efficiency performed by the bacterium at this pH value. The peaks in the Cr 2p region were fitted with a mixed Gaussian–Lorentzian function,

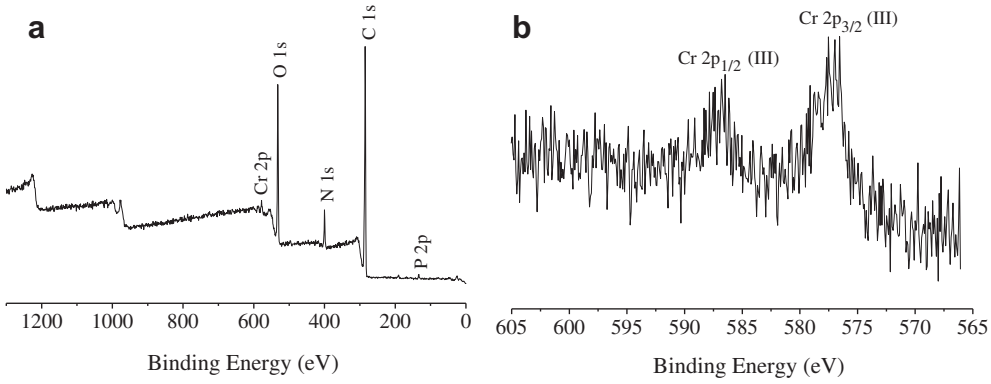

Fig. 4. XPS survey scanning spectrum **(a)** and high resolution Cr 2p spectrum **(b)** of chromium-loaded biomass: initial Cr(VI) concentration of 100 mg L^{-1}, biomass concentration of 5 g L^{-1} and pH 4.

using XPSPEAK 4.1 software. The spectrum showed two bands at binding energies of 577.3 eV and 586.9 eV, the first corresponds to a Cr $2p_{3/2}$ orbital and the second to a Cr $2p_{1/2}$ orbital. In reference compounds the Cr $2p_{3/2}$ orbitals are assigned at 577.2 eV ($CrCl_3$) and 576.2–576.5 eV (Cr_2O_3) for Cr(III) compounds, while Cr(VI) forms are characterized by higher binding energies such as 578.1 eV (CrO_3) or 579.2 eV ($K_2Cr_2O_7$) (Dambies et al., 2000). Boucetta et al. (2009) demonstrated that Cr $2p_{1/2}$ orbitals for Cr(III) species are assigned to the range 587.0–587.5. Accordingly the XPS results obtained, it can be clearly concluded that chromium loaded on the biomass surface is in the trivalent form. Several studies reported the same conclusions as in here, i.e., the chromium bounded to biomass is in the trivalent state (Lytle et al., 1998; Gardea-Torresdey et al., 2000; Park et al., 2004).

3.2.2. Biosorption by A. viscosus supported on NaY zeolite

Fig. 5a and b show the concentration of Cr(VI) and total Cr, as a function of contact time, at various solution pH values in the range of 1–4. These experiments were made with the combined system bacteria/zeolite, for an initial Cr(VI) concentration of 100 mg L^{-1}.

It is important to establish a comparison between the performances of the combined system (Fig. 5) and the bacteria in suspension (Fig. 2) in the reduction of Cr(VI) and removal of total Cr. It was expected that the addition of the zeolite would improve the removal of total Cr due to the ability of the zeolite to exchange Cr(III) cations resulting from the reduction of Cr(VI). However, at very low pH, such as pH 1 and pH 2, the exchange procedure can be difficult for the high concentration of H^+ ions in solution, which compete with Cr^{3+} ions for the exchange sites of the zeolite. The exchange of H^+ is easier when compared with Cr^{3+}, because the compensating cation of NaY zeolite is monovalent (Na^+). In addition, at pH values extremely acidic, the structure of the zeolite was probably damaged and therefore the cation exchange capacity of the zeolite decreased substantially. In order to confirm if the morphology of zeolite was affected by very acidic pH values, SEM images (not shown) were taken for the starting NaY zeolite and for the samples obtained at pH 1 and pH 2. It was observed that the biosorption process performed at very acid pH values led to changes in the morphology of the zeolite, that became more irregular and rougher, and to the decrease of the particle size. Therefore, for very low pH values the presence of the zeolite did not improve the removal of total Cr. However, for the less acidic pH values (pH 3 and pH 4) the combined system performed a higher removal of total Cr than bacteria in suspension, which is an indication that the zeolite was able to capture the Cr^{3+} ions at these pH values. In addition, the combined system also performed a faster depletion of Cr(VI), since it was required a shorter contact time in order to achieve the complete removal in comparison with bacteria in suspension. The reduction of Cr(VI) was enhanced by the presence of the zeolite since it allowed the removal of Cr(III) cations from the solution while these species were formed during the reduction process. Therefore, the concentration of Cr^{3+} ions in the aqueous medium decreased, allowing the formation of new cations through the reduction of Cr(VI).

The removal efficiencies of total Cr for both systems in study and the uptake of total Cr by *A. viscosus* supported on zeolite, for pH values between 1 and 4 are presented in Fig. 6a and b. For the lowest pH values, pH 1 and pH 2, the removal efficiency of the combined system bacteria/zeolite was lower than the one obtained with bacteria in suspension. As it was discussed above, the extremely acidic conditions of the medium affected negatively the ion

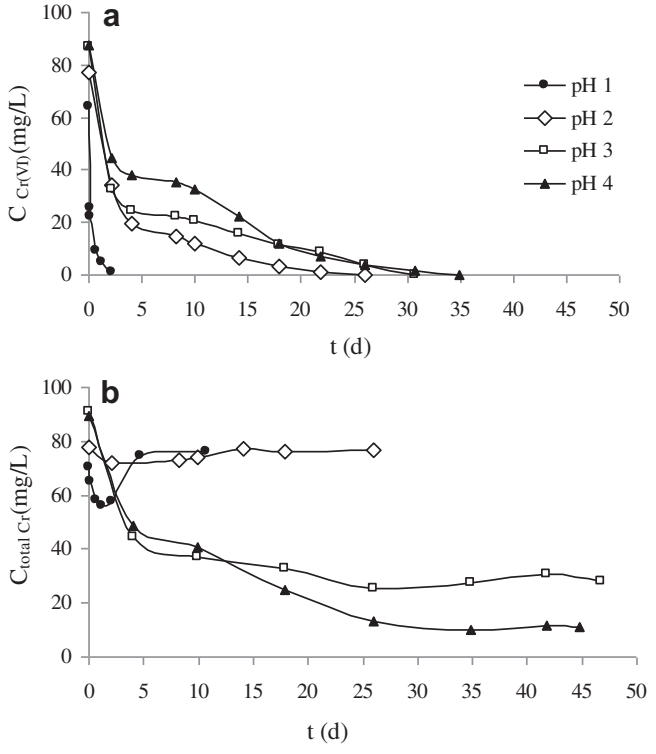

Fig. 5. Concentration of Cr(VI) **(a)** and total Cr **(b)** as a function of contact time, for solution pH values of 1, 2, 3 and 4. Experiments with *A. viscosus* supported on NaY zeolite, with an initial Cr(VI) concentration of 100 mg L^{-1} and biomass concentration of 5 g L^{-1}.

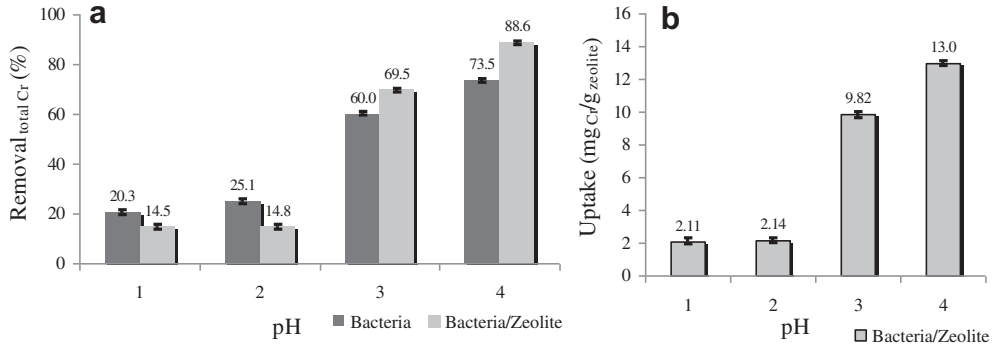

Fig. 6. Removal efficiencies of total Cr for both systems **(a)** and uptake of total Cr by *A. viscosus* supported on NaY zeolite **(b)** at pH values in the range of 1–4, initial Cr(VI) concentration of 100 mg L^{-1} and biomass concentration of 5 g L^{-1}.

exchange properties of the zeolite due to the damages provoked on its surface structure. In contrast, for pH 3 and pH 4, the combined system had higher removal efficiencies of total Cr than bacteria in suspension. The best removal efficiency (88.6%) and uptake of total Cr (13 $mg_{Cr}/g_{zeolite}$) were achieved at pH 4, remaining in solution 11.4 mg L^{-1} of Cr(III) at the end of the experiment. It should be noted that the best uptake value obtained in this work is more than four times higher than that the one achieved in our previous work (Silva et al., 2008b) that was only 3 $mg_{Cr}/g_{zeolite}$. This significant improvement is a result of the optimization of one of the most important experimental parameter in biosorption studies, the solution pH. In addition, a considerably higher concentration of biomass was used in this work compared to the one used in the previous work. The effect of the biomass concentration on chromium removal will be discussed further in Section 3.3.

Bulk chemical analyses were performed at the zeolite samples obtained after the experiments at pH 3 and pH 4. The results confirmed a high content of chromium in the zeolite, 0.76% and 0.90%, for pH 3 and pH 4, respectively. Chemical analyses also showed that the molar Si/Al ratio of the samples increased with the decreasing of pH. For assays performed at pH 3 and pH 4, Si/Al ratios of 2.95 and 3.37 were obtained, respectively, with the parent NaY zeolite showing a ratio of 2.80. These results are an indication of a slight dealumination of the zeolite structure that was promoted by the acidic medium of the solution.

3.3. Effect of the biomass concentration

To investigate the effect of biomass concentration on the removal efficiency of Cr(VI), several assays were performed at pH 4, with an initial Cr(VI) concentration of 100 mg L^{-1} and biomass concentration in the range of 1–5 g L^{-1}. These results are shown in Fig. 7, for the experiments with bacteria in suspension and supported on NaY zeolite.

The results show that an increase of biomass concentration in the range of 1–5 g L^{-1} led to an improvement of the removal efficiency of Cr(VI). As it can be observed, the removal efficiency of Cr(VI) was higher when bacteria were used in suspension, instead of bacteria supported on zeolite, particularly for the lower biomass concentration values. The difference between the efficiency of the two systems in study became less significant as the biomass concentration increased. In fact, for the experiment at the maximum biomass concentration, 5 g L^{-1}, the complete removal of Cr(VI) for both systems was observed. When bacteria and zeolite were simultaneously present in solution, the reduction and the removal of chromium were probably affected by mass transfer limitations. As the biomass concentration increases, the transfer of chromium becomes more limited. In opposition, the increase of bacteria mass enhances considerably the reduction of Cr(VI), being this effect predominant over the mass transfer limitations as the concentration of biomass increases.

In terms of total Cr removal, it is possible to observe that the system consisting of bacteria supported on the zeolite presented higher removal efficiency for each biomass concentration in study. The presence of the zeolite allowed the retention of chromium by ion exchange that was previously reduced by the bacterium. Thus, the combined system performed the maximum removal of total chromium, 88.6%, for a biomass concentration of 5 g L^{-1}. As it was mentioned, the content of chromium determined by the chemical analysis in this sample was 0.90% (w/w). For the zeolite sample obtained with a biomass concentration of 2 g L^{-1}, the chemical analysis revealed a percentage of chromium of 0.62% (w/w).

In Table 1 the uptake values of total chromium are presented as a function of initial bacteria mass. These values were obtained for the experiments with biomass in suspension. A comparison of the uptake of total chromium achieved by different biosorbents is also given in Table 1. In this work, the maximum uptake, 17 $mg/g_{biomass}$, was attained at a biomass concentration of 2 g L^{-1}, and the lowest uptake value, 11.2 $mg/g_{biomass}$, was achieved at 1 g L^{-1} of biomass concentration. The present study showed that *A. viscosus* demonstrated a good removal capacity for chromium as compared to the reports of other biosorbents.

3.4. Effect of initial chromium concentration

Several experiments were performed with different initial Cr(VI) concentrations, in order to study the effect of this parameter in the removal of chromium. These experiments were made with

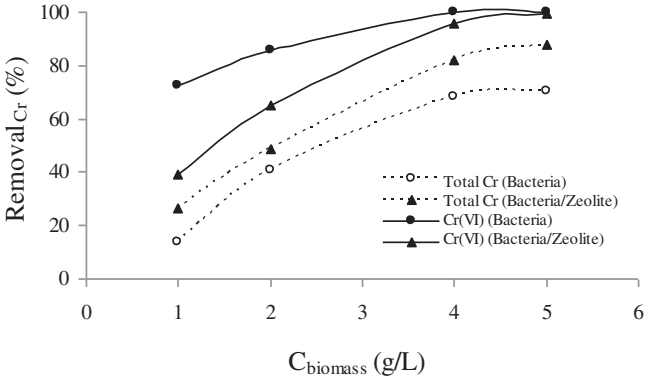

Fig. 7. Removal efficiency of Cr(VI) and total Cr as a function of biomass concentration, for an initial Cr(VI) concentration of 100 mg L^{-1} and pH 4. Comparison between bacteria in suspension and supported on NaY zeolite.

Table 1
Uptake capacity of hexavalent chromium for different biosorbents.

Biosorbent	$C_{Cr(VI)}$ (mg L^{-1})	$C_{biomass}$ (g L^{-1})	pH	Uptake (mg g^{-1})	Reference
A. viscosus	100	2	4	17.0	This work
		5		12.8	
C. albida	50	2	2	5.0	(Deng et al., 2009)
C. utilis CR-001	20	25	4	0.76	(Yin et al., 2008)
	100	25	2	1.0	
	20	5	2	1.1	
Sunflower waste	50	4	2	6.6	(Jain et al., 2009)
CTAB treated *A. niger*	10	2	3	3.8	(Mungasavalli et al., 2007)
Mangrove leaves	25	10	5	1.6	(Elangovan et al., 2008)
Silver impregnated groundnut husk carbon	50	10	3	11.3	(Dubey and Gopal, 2007)
Brown seaweed *Ecklonia* biomass	100	5	4	14.4	(Park et al., 2004)
Modified coir pith (CPHDTMA)	20	1	2	18.0	(Namasivayam and Sureshkumar, 2008)
	100			65.2	

Table 2
Removal efficiency of total chromium and content of chromium in the zeolite, for the experiments with initial Cr(VI) concentration in the range of 10–100 mg.

Sample[a]	pH	$C_{biomass}$ (g L^{-1})	$C_{Cr(VI)}$ (mg L^{-1})	$Removal_{total\ Cr}$ (%)	Cr (%)[b]
Cr-NaY_{10}			10	98.4 ± 0.6	–
Cr-NaY_{25}			25	78.7 ± 1.0	0.21
Cr-NaY_{50}	4	5	50	84.6 ± 0.9	0.45
Cr-NaY_{75}			75	91.9 ± 0.7	–
Cr-NaY_{100}			100	88.6 ± 0.9	0.90

[a] In the sample designation Cr-NaY_X, X corresponds to the initial concentration of Cr(VI).
[b] Results obtained by bulk chemical analyses (ICP – AES).

the combined system bacteria/zeolite at pH 4 and initial biomass concentration of 5 g L^{-1}. In Table 2 the removal efficiencies of total Cr and the amount of Cr in the zeolite, obtained for the different initial concentration of Cr(VI) in study, are presented.

These results indicate that the complete removal of total chromium was not achieved for any initial concentration in test. The highest removal percentage (98.4%) was attained for the lowest initial concentration, 10 mg L^{-1}, and the lowest removal efficiency (78.7%) for the concentration of 25 mg L^{-1}. As it was predictable, the content of chromium in the zeolite is enhanced with the increase of the initial Cr(VI) concentration.

4. Conclusions

In practical use of biosorption systems it is essential to identify the main parameters that have a significant effect on the process. The biosorption of Cr(VI) by *A. viscosus* in suspension or supported on NaY zeolite was affected by several parameters such as solution pH, biomass concentration and initial Cr(VI) concentration. The solution pH was the most important parameter that affected the biosorption performance. The removal of total Cr by the combined system bacteria/zeolite was favored by higher solution pH. The experiments performed with *A. viscosus* in suspension demonstrated good removal capacities of chromium compared with those reported in literature for other biosorbents. The use of the combined system bacteria/zeolite allowed an improvement of total Cr removal efficiencies for the higher pH values, in comparison with the experiments with bacteria in suspension. Therefore, this work demonstrated the ability of the combined biosorption system for an effective detoxification of contaminated Cr(VI) wastewater under optimized and controlled conditions.

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References

Asatiani, N.V., Abuladze, M.K., Kartvelishvili, T.M., Bakradze, N.G., Sapojnikova, N.A., Tsibakhashvili, N.Y., Tabatadze, L.V., Lejava, L.V., Asanishvili, L.L., Holman, H.-Y., 2004. Effect of Chromium(VI) action on *Arthrobacter oxydans*. Current Microbiology 49, 321–326.
Bai, R.S., Abraham, T.E., 2001. Biosorption of Cr (VI) from aqueous solution by *Rhizopus nigricans*. Bioresource Technology 79, 73–81.
Barrera, H., Ureña-Núñez, F., Bilyeu, B., Barrera-Díaz, C., 2006. Removal of chromium and toxic ions present in mine drainage by *Ectodermis* of *Opuntia*. Journal of Hazardous Materials 136, 846–853.
Boucetta, C., Kacimi, M., Ensuque, A., Piquemal, J.-Y., Bozon-Verduraz, F., Ziyad, M., 2009. Oxidative dehydrogenation of propane over chromium-loaded calcium-hydroxyapatite. Applied Catalysis A: General 356, 201–210.
Brady, D., Stoll, A., Duncan, J.R., 1994. Biosorption of heavy metal cations by non-viable yeast biomass. Environmental Technology 15, 429–438.
Cheung, K.H., Gu, J.-D., 2003. Reduction of chromate ($CrO4^{2-}$) by an enrichment consortium and an isolate of marine sulfate-reducing bacteria. Chemosphere 52, 1523–1529.
Cheung, K.H., Lai, H.Y., Gu, J.-D., 2006. Membrane-associated hexavalent chromium reductase of *Bacillus megaterium* TKW3 with induced expression. Journal of Microbiology and Biotechnology 16, 855–862.
Cheung, K.H., Gu, J.-D., 2007. Mechanisms of hexavalent chromium detoxification by bacteria and bioremediation applications. International Biodeterioration and Biodegradation 59, 8–15.
Cui, H., Fu, M., Yu, S., Wang, M.K., 2011. Reduction and removal of Cr(VI) from aqueous solutions using modified byproducts of beer production. Journal of Hazardous Materials 186, 1625–1631.
Dambies, L., Guimon, C., Yiacoumi, S., Guibal, E., 2000. Characterization of metal ion interactions with chitosan by X-ray photoelectron spectroscopy. Colloids and Surfaces A: Physicochemical and Engineering Aspects 177, 203–214.
Deng, L., Zhang, Y., Qin, J., Wang, X., Zhu, X., 2009. Biosorption of Cr(VI) from aqueous solutions by nonliving green algae *Cladophora albida*. Minerals Engineering 22, 372–377.
Dermou, E., Velissariou, A., Xenos, D., Vayenas, D.V., 2005. Biological chromium(VI) reduction using a trickling filter. Journal of Hazardous Materials 126, 78–85.
Dubey, S.P., Gopal, K., 2007. Adsorption of chromium(VI) on low cost adsorbents derived from agricultural waste material: a comparative study. Journal of Hazardous Materials 145, 465–470.
Eaton, D., Clesceri, L.S., Greenberg, A.E., 1995. Standard Methods for the Examination of Water and Wastewater. American Public Health Association (APHA), Washington.
Elangovan, R., Philip, L., Chandraraj, K., 2008. Biosorption of chromium species by aquatic weeds: kinetics and mechanism studies. Journal of Hazardous Materials 152, 100–112.
Esposito, A., Pagnanelli, F., Vegliò, F., 2002. pH-related equilibria models for biosorption in single metal systems. Chemical Engineering Science 57, 307–313.
Figueiredo, H., Neves, I.C., Quintelas, C., Tavares, T., Taralunga, M., Mijoin, J., Magnoux, P., 2006. Oxidation catalysts prepared from biosorbents supported on zeolites. Applied Catalysis B: Environmental 66, 274–280.
Figueiredo, H., Silva, B., Quintelas, C., Neves, I.C., Tavares, T., 2010a. Effect of the supporting zeolite structure on Cr biosorption: performance of a single-step

reactor and of a sequential batch reactor – a comparison study. Chemical Engineering Journal 163, 22–27.
Figueiredo, H., Silva, B., Quintelas, C., Raposo, M.M.M., Parpot, P., Fonseca, A.M., Lewandowska, A.E., Bañares, M.A., Neves, I.C., Tavares, T., 2010b. Immobilization of chromium complexes in zeolite Y obtained from biosorbents: synthesis, characterization and catalytic behaviour. Applied Catalysis B: Environmental 94, 1–7.
Gardea-Torresdey, J.L., Tiemann, K.J., Armendariz, V., Bess-Oberto, L., Chianelli, R.R., Rios, J., Parsons, J.G., Gamez, G., 2000. Characterization of Cr(VI) binding and reduction to Cr(III) by the agricultural byproducts of *Avena monida* (Oat) biomass. Journal of Hazardous Materials 80, 175–188.
Hsu, L.C., Wang, S.L., Lin, Y.C., Wang, M.K., Chiang, P.N., Liu, J.C., Kuan, W.H., Chen, C.C., Tzou, Y.M., 2010. Cr(VI) removal on fungal biomass of Neurospora crassa: the importance of dissolved organic carbons derived from the biomass to Cr(VI) reduction. Environmental Science and Technology 44, 6202–6208.
Jain, M., Garg, V.K., Kadirvelu, K., 2009. Equilibrium and kinetic studies for sequestration of Cr(VI) from simulated wastewater using sunflower waste biomass. Journal of Hazardous Materials 171, 328–334.
Janos, P., Hula, V., Bradnová, P., Pilarová, V., Sedlbauer, J., 2009. Reduction and immobilization of hexavalent chromium with coal- and humate-based sorbents. Chemosphere 75, 732–738.
Kalabegishvili, T.L., Tsibakhashvili, N.Y., Holman, H.-Y.N., 2003. Electron spin resonance study of chromium(V) formation and decomposition by basalt-inhabiting bacteria. Environmental Science and Technology 37, 4678–4684.
Lin, Z., Zhu, Y., Kalabegishvili, T.L., Tsibakhashvili, N.Y., Holman, H.-Y., 2006. Effect of chromate action on morphology of basalt-inhabiting bacteria. Materials Science and Engineering: C 26, 610–612.
Loukidou, M.X., Zouboulis, A.I., Karapantsios, T.D., Matis, K.A., 2004. Equilibrium and kinetic modeling of chromium(VI) biosorption by *Aeromonas caviae*. Colloids and Surfaces A: Physicochemical and Engineering Aspects 242, 93–104.
Lytle, C.M., Lytle, F.W., Yang, N., Qian, J.-H., Hansen, D., Zayed, A., Terry, N., 1998. Reduction of Cr(VI) to Cr(III) by wetland plants: potential for in situ heavy metal detoxification. Environmental Science and Technology 32, 3087–3093.
Matheickal, J.T., Yu, Q., Woodburn, G.M., 1999. Biosorption of cadmium(II) from aqueous solutions by pre-treated biomass of marine alga *Durvillaea potatorum*. Water Research 33, 335–342.
Mohan, D., Pittman Jr., C.U., 2006. Activated carbons and low cost adsorbents for remediation of tri- and hexavalent chromium from water. Journal of Hazardous Materials 137, 762–811.
Mor, S., Ravindra, K., Bishnoi, N.R., 2007. Adsorption of chromium from aqueous solution by activated alumina and activated charcoal. Bioresource Technology 98, 954–957.
Moulder, J.F., Strickle, W.F., Sobol, P.E., Bomben, K.D., 1992. Handbook of X-ray Photoelectron Spectroscopy. Perkin-Elmer, Minnesota.
Mungasavalli, D.P., Viraraghavan, T., Jin, Y.-C., 2007. Biosorption of chromium from aqueous solutions by pretreated Aspergillus niger: batch and column studies. Colloids and Surfaces A: Physicochemical and Engineering Aspects 301, 214–223.
Murphy, V., Tofail, S.A.M., Hughes, H., McLoughlin, P., 2009. A novel study of hexavalent chromium detoxification by selected seaweed species using SEM–EDX and XPS analysis. Chemical Engineering Journal 148, 425–433.
Namasivayam, C., Sureshkumar, M.V., 2008. Removal of chromium(VI) from water and wastewater using surfactant modified coconut coir pith as a biosorbent. Bioresource Technology 99, 2218–2225.
Pagnanelli, F., Petrangeli Papini, M., Trifoni, M., Veglió, F., 2000. Biosorption of metal ions on *Arthrobacter* sp.: biomass characterization and biosorption modeling. Environmental Science and Technology 34, 2773–2778.
Park, D., Yun, Y.-S., Park, J.M., 2004. Reduction of hexavalent chromium with the brown seaweed Ecklonia biomass. Environmental Science and Technology 38, 4860–4864.
Park, D., Yun, Y.-S., Hye Jo, J., Park, J.M., 2005. Mechanism of hexavalent chromium removal by dead fungal biomass of *Aspergillus niger*. Water Research 39, 533–540.
Park, D., Yun, Y.-S., Ahn, C.K., Park, J.M., 2007. Kinetics of the reduction of hexavalent chromium with the brown seaweed *Ecklonia* biomass. Chemosphere 66, 939–946.
Park, D., Yun, Y.-S., Kim, J.Y., Park, J.M., 2008. How to study Cr(VI) biosorption: use of fermentation waste for detoxifying Cr(VI) in aqueous solution. Chemical Engineering Journal 136, 173–179.
Quintelas, C., Tavares, T., 2001. Removal of chromium(VI) and cadmium(II) from aqueous solution by a bacterial biofilm supported on granular activated carbon. Biotechnology Letters 23, 1349–1353.
Rollinson, C.L., 1973. Chromium, Molybdenum and Tungsten, third ed. Pergamon Press, Oxford.
Santen, R.A.V., Kramer, G.J., 1995. Reactivity theory of zeolitic Bronsted acidic sites. Chemical Reviews 95, 637–660.
Silva, B., Figueiredo, H., Quintelas, C., Neves, I.C., Tavares, T., 2008a. Iron and chromium removal from binary solutions of Fe(III)/Cr(III) and Fe(III)/Cr(VI) by biosorbents supported on zeolites. Materials Science Forum 587–588, 463–467.
Silva, B., Figueiredo, H., Quintelas, C., Neves, I.C., Tavares, T., 2008b. Zeolites as supports for the biorecovery of hexavalent and trivalent chromium. Microporous and Mesoporous Materials 116, 555–560.
Silva, B., Figueiredo, H., Soares, O.S.G.P., Pereira, M.F.R., Figueiredo, J.L., Lewandowska, A.E., Bañares, M.A., Neves, I.C., Tavares, T., 2012. Evaluation of ion exchange-modified Y and ZSM5 zeolites in Cr(VI) biosorption and catalytic oxidation of ethyl acetate. Applied Catalysis B – Environmental 117–118, 406–413.
Tsibakhashvili, N., Mosulishvili, L., Kalabegishvili, T., Kirkesali, E., Frontasyeva, M., Pomyakushina, E., Pavlov, S., Holman, H.Y., 2004. ENAA studies of chromium uptake by *Arthrobacter oxydans*. Journal of Radioanalytical and Nuclear Chemistry 259, 527–531.
Vaca Mier, M., López Callejas, R., Gehr, R., Jiménez Cisneros, B.E., Alvarez, P.J.J., 2001. Heavy metal removal with Mexican clinoptilolite: multi-component ionic exchange. Water Research 35, 373–378.
Wu, J., Zhang, H., He, P.-J., Yao, Q., Shao, L.-M., 2010. Cr(VI) removal from aqueous solution by dried activated sludge biomass. Journal of Hazardous Materials 176, 697–703.
Xie, Y., Sherwood, P.M.A., 1990. X-ray photoelectron-spectroscopic studies of carbon fiber surfaces. 11. Differences in the surface chemistry and bulk structure of different carbon fibers based on poly(acrylonitrile) and pitch and comparison with various graphite samples. Chemistry of Materials 2, 293–299.
Yin, H., He, B., Lu, X., Peng, H., Ye, J., Yang, F., 2008. Improvement of chromium biosorption by UV-HNO2 cooperative mutagenesis in *Candida utilis*. Water Research 42, 3981–3989.
Ziagova, M., Dimitriadis, G., Aslanidou, D., Papaioannou, X., Litopoulou Tzannetaki, E., Liakopoulou-Kyriakides, M., 2007. Comparative study of Cd(II) and Cr(VI) biosorption on *Staphylococcus xylosus* and *Pseudomonas* sp. in single and binary mixtures. Bioresource Technology 98, 2859–2865.