Effects of pH on chromate(VI) adsorption by *Spirulina platensis* biomass: batch tests and FT-IR studies

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**ABSTRACT**

Raw and methylated biomass of *Spirulina platensis* was employed in chromate batch adsorption tests at pH range 1–7. The acid conditions seemed to favour the removal of chromium (Cr) with a yield of 87.0 and 97.6% by using raw and methylated biomass, respectively. However, the chromate and total chromium determination, carried out in the same sample, evidenced that a fraction of the initial chromate present in solution was reduced to Cr(III). This was ascribed to the presence of reducing groups on the biomass surface, active in the acid medium. The data showed that the methylated biomass was able to operate an effective Cr(VI) removal only. In fact, the biomass treatment allowed a lowering of the amount of negative functional groups, making the biomass surface available to bind the anions. The real best efficiency of Cr(VI) removal (83.5%) was obtained by methylated biomass of *S. platensis* at pH about 7.0. The nature of the biomass/chromate interactions was investigated by Fourier transform infrared spectroscopy (FT-IR) analysis. The bands ascribing to the adsorbed Cr(VI) species were well evident in the spectra of the biomass after adsorption, confirming this experimental finding.

**Key words** | biosorption, chromium(VI) removal, FT-IR, pH

**INTRODUCTION**

Aqueous waste streams coming from various industries contain heavy metals which cause various diseases and disorders for living beings as well as deleterious ecological effects (Cimino *et al.* 2000; Afkhami & Conway 2002). After conventional treatments, the wastewaters can contain residual metals at toxic concentration so that, before discharge, they have to be submitted to a further treatment. For this purpose, several methods have been applied, such as adsorption onto activated carbon, or another appropriate adsorbent, post-precipitation reactions, ion-exchange, reverse osmosis and electrochemical treatments (Kurniawan *et al.* 2006). These conventional methods are either becoming inadequate to address current stringent regulatory effluent limits or are increasing in costs. The use of biological materials as biosorbent has gained popularity over the years due to a good performance, availability and low cost (Febrianto *et al.* 2009). In addition, the biosorption process may be an advantageous method for metals removal, avoiding the production of hazardous sludge; in fact, the metal-laden biomass system can be regenerated to obtain biomass for use again, as well as to recover the adsorbed ions.

Among the most common metals used in industrial applications, chromium is employed in various industrial processes, such as electroplating, glass, ceramics, fungicides, fertilizers, tanning and mining (Kumar *et al.* 2008). The most common forms of chromium are trivalent (Cr(III)) and hexavalent chromium (Cr(VI)); this latter form is highly mobile and acutely toxic, carcinogenic and mutagenic to living organisms, and hence more hazardous than other heavy metals. Therefore, in order to prevent deleterious impact on ecosystems and public health, the Cr(VI) removal is an urgent problem. The treatment technologies commonly adopted have many disadvantages such as incomplete metal removal, high energy and reagent requirements, producing toxic sludge and requiring proper disposal. Thus, the possibility of employing a treatment method that is simple, inexpensive and effective can offer an interesting alternative.

In recent years, various low-cost adsorbents, such as shells of lentil, wheat and rice (Aydin *et al.* 2008), lignite (Uçurum 2009), peanut husk, charcoal fly ash and natural zeolite (Abdel Salam *et al.* 2011), have been successfully
used in heavy metal adsorption. As discussed by Loukidou et al. (2004) few authors investigated the phenomenon of chromate adsorption, maybe due to a difficulty of finding substrates active for the anions removal. In fact, the substrates generally used for the heavy metals removal show low capacity for Cr(VI) adsorption. Prevalently, all different kinds of biological support have negatively charged chemical groups. Hence, the need to modify the characteristics of the sorbent surface arises, so increasing the removal potential of substrates for chromate (Alvarez et al. 2007; Gurgel et al. 2009).

In a previous work (Finocchio et al. 2010) the authors investigated the positive effect of *Spirulina platensis* methylated biomass in Cr(VI) adsorption, carrying out batch experiments in the pH range 7–8, focusing exclusively on the methylation influence on the biomass adsorption capacity in the absence of any hydrolysis reaction.

In this work, either raw or methylated biomass has been tested for estimating the Cr(VI) removal at pH range 1–7, aiming at: (i) comparing the adsorption capacity of raw and methylated biomass; (ii) evaluating the necessity of modifying the biomass; (iii) estimating the influence of acid pH on the chromate adsorption.

Fourier transform infrared spectroscopy (FT-IR) studies have been performed on the raw or methylated biomass before and after chromate adsorption, in order to gain further insight about the biosorption mechanisms.

**MATERIALS AND METHODS**

**Preparation of biosorbent**

*Spirulina platensis* (UTEX 1926) was obtained from the University of Texas Culture Collection. Cells were grown batch-wise in outdoor cultivation ponds, as described by Finocchio et al. (2010). After cultivation, cells were collected and dried. Then, the dry biomass was washed until reaching pH 7.0 in the washing water. A portion was methylated, according to the method reported by Fraenkel-Conrat & Olcott (1945). Cr(VI) adsorption tests were carried out by methylated and raw biomass in the pH range 1–7.

**Analytical procedures**

Adsorption tests were carried out in 200 mL Erlenmeyer flasks agitated on a rotary shaker (150 rpm) at room temperature. Methylated and raw biomass (2 g L⁻¹) were put in contact with a K₂Cr₂O₇ solution, containing 17.0 mg L⁻¹ Cr(VI) at pH range 1–7. In each test, the pH was adjusted to a desired value by means of HCl 0.1 N; during the experiments the pH was monitored and, if necessary, corrected by acid addition.

At fixed times, samples (5.0 mL) were withdrawn, filtered through membrane filters (Millipore, Vimodrone, Italy) with 0.45 μm pore diameter, and the filtrate was analyzed for determining the Cr(VI) content by an ion chromatograph, IC mod. 761 (Metrohm Italia, Varese, Italy), and for the total chromium content (Crₜot) by an atomic adsorption spectrophotometer (Varian, model AA240FS, Palo Alto, CA).

All tests were carried out in triplicate and the experimental results are presented as mean values. Relative standard deviations have been calculated for each run, and the results have been further averaged among different runs. So, the values, calculated as above, never exceeded 6%.

**Characterization study**

The point of zero charge (pzc) of the raw biomass was determined by potentiometric titrations of solutions containing 5.0 g L⁻¹ of dry biomass in 0.01, 0.1 and 1.0 M NaCl, using 0.1 M HCl as titrant (Romero-Gonzáles et al. 2005), whilst the methylated biomass was potentiometrically titrated by 0.1 N NaOH.

Samples of raw and methylated biomass were prepared for FT-IR analysis by dilution of pure powders in KBr disks (1% w/w) and analyzed using a Nicolet 6700 FT-IR instrument (Thermo Fisher, Waltham, MA) equipped with DTGS-KBr detector and OMNIC software. The acquisition was 100 scans for each spectrum and the resolution was 2 cm⁻¹.

**RESULTS AND DISCUSSION**

Cr(VI) removal by raw and methylated biomass at increasing pH values.

Several studies (Herrero et al. 2005; Ben Hamissa et al. 2010) highlighted the importance of the pH solution in the metal ion adsorption process. In fact, the pH affects the cell wall binding sites and the metal ion chemistry. Figure 1 shows the maximum Cr(VI) removal yield for raw and methylated biomass at increasing pH. Using raw biomass, the optimum adsorption pH range seems to be 1–3, while the worst results correspond to pH ≥ 4. This behaviour is confirmed by the pzc (pHₚzc = 4.0) of biosorbent (Ferreira et al. 2011), i.e. the pH value at which the sorbent surface does not carry...
charges. When the pH solution is higher than pH_{zpc}, the number of negatively charged sites on the biosorbent increases, and anion adsorption is not favoured due to the electrostatic repulsion between ions and functional groups. Oppositely, when solution pH < pH_{pzc}, the surface of the biosorbent gets positively charged, and the anion adsorption is favoured. The above considerations are consistent with the hypothesis that chromate ions were adsorbed by means of an ion-exchange mechanism. In fact, at low pH values the active sites on the cell surface are protonated and able to bind negative ions; increasing pH, and approaching the isoelectric point of biosorbent, the number of active sites decreases and, consequently, the adsorbing biomass ability decreases. At pH > 4, the S. platensis biomass, as well as most of the algal biomasses, has negative charges; thus it is not able to bind anions.

The methylation process aims to make inactive the negative sites traditionally available for the cation binding, as it performs the esterification of carboxylate groups, mainly. Moreover, this treatment favours the protonation of amino and amide groups of protein components, thus making them available to bind the anions. Then, the yield of Cr(VI) removal by methylated biomass is >90.0% in the pH range 1–4, decreasing to 71.6–83.5% in the pH range 5–7.

Figure 2 illustrates, for raw and methylated biomass, the residual chromium concentration in solution as Cr(VI), total Cr and Cr(III), the latter calculated as difference between total Cr and Cr(VI).

The employment of raw biomass evidenced that, at acid pH (1–3), a high chromium concentration (10.2–12.4 mg L\(^{-1}\)) was still detectable in solution, partly as Cr(VI) and partly as Cr(III). In the 4–7 pH interval, the Cr(VI) and total Cr residual concentrations are quite similar, whilst that of Cr(III) is very low. This is consistent with the assumption that at pH 4, corresponding to the biomass pzc, the adsorption capacity of substrate is very low. At pH higher than 4, the cell surface being negatively charged, the biomass is unable to bind anions. The presence, at pH 1–3, of Cr(VI) and Cr(III) could be ascribed to a partial reduction of Cr(VI) to Cr(III) caused by the presence of reducing agents on the biomass surface.
(Murphy et al. 2009), active in acid environment. In fact, at pH 1.0, the Cr(VI) exists in the form of HCrO$_4^-$, while in the pH range 2–4 different forms of chromium (HCrO$_4^-$, CrO$_2^{2-}$ and Cr$_2$O$_7^{2-}$) can be present, HCrO$_4^-$ being predominant. At increasing pH, this form shifts to CrO$_2^{2-}$ and Cr$_2$O$_7^{2-}$ (Gurgel et al. 2009). Then, at pH < 5.0, the dichromate and chromate ions can be reduced to Cr(III) in the presence of a reducing C-containing substrate (CxOH) (Cimino et al. 2000), as follows:

\[
3\text{CxOH} + \text{Cr}_2\text{O}_{7}^{2-} + 4\text{H}^+ \leftrightarrow 3\text{CxO} + \text{HCrO}_4^- + 3\text{Cr}^{3+} + 5\text{H}_2\text{O} \quad (1)
\]

\[
3\text{CxOH} + \text{HCrO}_4^- + 4\text{H}^+ \leftrightarrow 3\text{CxO} + \text{Cr}^{3+} + 4\text{H}_2\text{O} \quad (2)
\]

Equations (1) and (2) suggest that, lowering pH values, the reaction will be favoured to the right side, increasing the reduction of Cr(VI) to Cr(III). Therefore, Cr(VI) behaves as a strong oxidant, possibly leading to the partial cleavage of C–C bonds and moreover increasing the acidity of biomass, by exposing new acidic functional groups (Park et al. 2008). Consequently, it can be supposed that, in acid medium, the S. platensis biomass acts as reducing agent. This hypothesis has been proved by blank tests (results not shown), carried out at pH 1–3, without S. platensis biomass: the analytical determination of Cr(VI) and total Cr highlighted that chromium was present in solution as chromate only, confirming that any reducing process was present.

Considering the chromate adsorption carried out by methylated biomass in pH 1–3 (Figure 2), a marked difference between Cr(VI) and Cr(III) residual concentrations can be detected. As observed with raw biomass, the methylated biomass can act as reducing agent, thus allowing a partial chromate removal. In spite of what has been observed with raw biomass, at pH 4, the difference between Cr(VI) and Cr(III) residual concentrations is well evident, pointing out a good adsorption activity, which is ascribable to the methylation. At pH ≥ 5, the residual concentrations of Cr(VI) (4.8–2.8 mg L$^{-1}$) and total Cr (4.9–3.0 mg L$^{-1}$) are low, suggesting a progressive chromate adsorption.

The chromate specific adsorption, summarized in Figure 3, shows that the methylated biomass is able to remove 5.0–7.0 mg Cr(VI) g$^{-1}$ dry mass at pH interval 1–7, confirming that pH 7 assures the best conditions for chromate removal. In contrast, only 3.4 mg Cr(VI) g$^{-1}$ dry mass were removed by raw biomass, at the best operating conditions (pH 1–3).

The above considerations are strengthened by titration of methylated biomass, which showed two equivalence points at pH 4.5 and 7.0. The increase of chromate adsorption at pH 5–6 corresponds to the first equivalent point and the further increase observed at pH 7.0 can be ascribed to the second one.

**FT-IR studies**

The FT-IR technique has been applied in order to elucidate the possible interactions of chromium species with the biomass functional groups. Although biomass spectra are very complex, the identification of several diagnostic bands is indeed possible. Their shift in frequencies as well as the decrease in intensity following adsorption processes is an indication of interaction with chromium species. In this work, FT-IR studies have been carried out analyzing the raw biomass and the methylated biomass in comparison with both biomasses following chromate adsorption.

Figure 4 shows the FT-IR spectra of the biomass, recorded before and after chromate adsorption. In the spectrum of the raw biomass (spectrum a, high frequency region), the main bands are due to stretching vibrational modes of OH groups interacting through H bonds (3,420 cm$^{-1}$), NH groups (3,300 and 3,100 cm$^{-1}$) and CH groups (3,000–2,800 cm$^{-1}$ range).

In the low frequency region of the spectrum, the band due to C=O stretching mode of the ester groups is detected at 1,728 cm$^{-1}$, whereas the two complex bands at 1,650 and 1,520 cm$^{-1}$ are assigned to amide I and amide II modes, partially overlapping with asymmetric stretching mode of the –COO$^-$ group. The corresponding –COO$^-$ symmetric stretching mode is detected at 1,405 cm$^{-1}$. The complex band around 1,230 cm$^{-1}$ and several components in the range 1,150–1,040 cm$^{-1}$ are due to OCO stretching mode of ester groups and CC/CO stretching modes of the carbohydrate fraction.
After adsorption at acid pH (spectra b, c), a decrease in intensity of the band due to C=O stretching mode, a broadening of the amide bands at 1,650 and 1,520 cm\(^{-1}\) and a decreasing in intensity of the symmetric carboxylate stretching at 1,405 cm\(^{-1}\) can be noticed. Following these observations, some interaction of the Cr(III) cations, formed by chromate reduction, with these electron-rich groups at the biomass surface can be envisaged, particularly a coordination of cations at the residual carboxylate groups (Sheng et al. 2004). On the other side, protonation of the same groups at low pH values favors the adsorption of the residual negatively charged chromate species. As for the spectrum recorded following adsorption at pH 7, no significant differences can be observed with respect to the raw biomass spectrum, in agreement with the low adsorption activity reported.

Figure 5 shows the FT-IR spectra of methylated S. platensis biomass before and after chromate adsorption. The effect of methylation over the raw algae has been already discussed by the authors (Finocchio et al. 2010). In summary, methylation results in a decrease in exposed carboxylic groups and, possibly, formation of ether and substituted amine/amide groups (spectrum a). In the low frequency region of the spectrum, the band due to C=O stretching mode of the ester groups is still evident, as well as the two complex bands at 1,650 and 1,520 cm\(^{-1}\) assigned to amide I and amide II modes. The component at 1,409 cm\(^{-1}\), assigned to COO- symmetric stretching mode, is reduced in intensity, with respect to the raw biomass spectrum, as a result of esterification of carboxylate groups.

Following adsorption at pH 7 (spectrum e), the main bands of the biomass spectrum are almost the same as described above, although reduced in intensity. A small band shift (around 4 cm\(^{-1}\)) could be observed for the amide bands. On the other side, in the low frequency region new adsorption bands appear around 920, 895 and 880 cm\(^{-1}\) (inset), which can be assigned to Cr-O vibrational modes of the residual adsorbed chromate and/or dichromate groups (Nakamoto 1989), in agreement with the observation that chromium is mainly detected as chromate species at this pH and with the good values obtained for Cr(VI) removal tests described above. These bands decrease in relative intensity from pH 7 to 3 (spectra e–c), probably due to the increased formation of Cr(III) ions. It is worth noting that, in the previous section, the best chromate removal can be observed at pH 5–7. In this pH range, adsorption of chromate ions can be attributed mainly to an electrostatic interaction with positively charged groups, together with weak molecular interactions with biomass exposed groups, such as amino, amide and –OR groups of carbohydrate and protein fractions (Deng & Bai 2004).

Low pH values (spectra b, c) allow the following considerations. In the high frequency region of the spectra, a decrease in the intensity of bands due to NH groups can be observed, which are probably protonated in the
acidic environment and thus can interact with chromate species. Moreover, the band at 1,410 cm\(^{-1}\) almost disappears, further evidence that residual carboxylate groups convert to carboxylic acid groups, as expected at such a low pH. This effect, i.e. a reduced biomass negative charge and the protonation of functional groups at low pH values, can explain the small gain in the chromate adsorption capacity evidenced by the tests, due to an increased adsorption of anion species through electrostatic attraction. Bands due to the chromate adsorption in the low frequency region of the spectrum are very weak, due to reduction of chromate species to Cr(III).

The results highlighted that the methylated biomass of \textit{S. platensis} was able to remove Cr(VI) with a yield of 83.5\% at pH 7.0. This result could be applied with success in the Cr(VI)-rich wastewater treatment, where such conditions are needed to avoid an expensive preliminary step of neutralization before the activated sludge process.

FT-IR data provided direct evidence, confirming the good adsorption results obtained by the use of methylated biomass in a wide pH range. Bands due to adsorbed Cr(VI) species were well evident in spectra of biomass after adsorption in the pH 4–7 range. Protonation of the amine, amide and hydroxyl functional groups allowed chromate adsorption, whilst, at low pH values, Cr(III) ions interacted with negatively charged carboxylic groups.

**CONCLUSIONS**

Raw and methylated biomass of \textit{Spirulina platensis} was employed in chromate batch adsorption tests at pH range 1–7. The acid medium seemed to favour the removal of chromium with a yield of 87.0 and 97.6\% with raw and methylated biomass, respectively. The determination of Cr(VI) and total Cr evidenced that an aliquot of the initial chromate was reduced to Cr(III) by the reducing groups present on the biomass surface, active in acid medium.

The results highlighted that the methylated biomass of \textit{S. platensis} was able to remove Cr(VI) with a yield of 83.5\% at pH 7.0. This result could be applied with success in the Cr(VI)-rich wastewater treatment, where such conditions are needed to avoid an expensive preliminary step of neutralization before the activated sludge process.


due to chromate/dichromate species are evidenced.

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First received 9 February 2012; accepted in revised form 20 December 2012