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Chromium (VI) removal by methylated biomass of *Spirulina platensis*: The effect of methylation process

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ABSTRACT

Biosorption of heavy metals is an interesting approach to treat industrial wastewaters by an environmentally friendly system. *Spirulina platensis* biomass, an effective biosorbent for cations, cannot be used to adsorb chromate due to its negatively charged surface close to neutral conditions; therefore, methylation of biomass was performed to increase its adsorption capacity under these conditions. Batch adsorption tests carried out varying both Cr(VI) and methylated biomass concentrations showed that 2–4 g l^{-1} of biosorbent were able to remove Cr(VI) with efficiency ≥80%, while higher Cr(VI) levels (43–50 mg l^{-1}) showed low removal efficiency. The model of Langmuir was shown to describe the adsorption phenomenon better than the Freundlich one. The values of the overall adsorption capacity of methylated biomass suggested that increased biosorbent availability does not necessarily correspond to larger amount of adsorbed metal. FT-IR spectra of dried and methylated biomass of *S. platensis* allowed us monitoring the efficiency of the methylation process through the analysis of CH and COO^- vibrational stretching modes, taken as diagnostic of this process.

1. Introduction

A potential alternative to the existing methods to remove toxic metals from industrial wastewaters can be offered by the use of microorganisms as biosorbents. This technique has advantages over the conventional methods, among which no production of chemical sludge, high selectivity, easiness to operate and effectiveness in the treatment of large volumes of wastewaters containing pollutants in low concentrations. The metal sequestering ability of microorganisms such as yeast, bacteria and fungi that are generated in large quantities as waste by-products from fermentation industries has been investigated by several authors [1,2]. Some interesting methods are reported in the literature to modify biomass in order to improve its ability to remove cations, among others chemical pre-treatment with laundry detergent, sodium hydroxide and sodium bicarbonate [3] or extraction of alginates and carragenans [4].

Chromium (VI) is a common pollutant introduced into natural waters from a variety of industries, such as textile dyeing, leather tanning, electroplating and metal finishing industries [5,6]. In aqueous solutions, depending on pH, Cr(VI) can be present as chromate (CrO_4^{2-}), hydrogen chromate ($HCrO_4^-$) or dichromate ($Cr_2O_7^{2-}$) [7]. Since Cr(III) is insoluble at neutral and high pH, chemical reduction of Cr(VI) to Cr(III) followed by precipitation as $Cr(OH)_3$ has been long applied as a method for decontaminating waters from chromate [8]. However, there is evidence that $Cr(OH)_3$ may itself be oxidized to Cr(VI), leading to contamination of ground water [9]. Moreover, owing to the high toxicity of this element [10,11], possible industrial application of biomass as sorbent should only utilize dead or non-growing living cells, which would not require nutrients and allow desorbing and recovering metals by appropriate methods.

Microalgal biomass has been successfully employed as sorbing agent, because of its simplicity of cultivation and harvesting. In particular, *Spirulina platensis* can be cultivated in open ponds and collected by harvesting the layer of biomass formed on the surface [12].

S. platensis biomass as well as that of most other marine algae [13–15] presents onto its surface net negative charge; consequently, it has low affinity for anions. Hence, Cr(VI), which is present in solution as CrO_4^{2-}, $HCrO_4^-$ or $Cr_2O_7^{2-}$, cannot be adsorbed onto negatively charged biomass. Seki et al. [16,17] developed a new method to increase the adsorption capacity of yeast biomass, consisting in its methylation, and then they successfully applied it to the bioadsorption of chromium (VI) and arsenic (V). Following these appealing results, we applied methylation procedures over *S. platensis* biomass and studied the effect of

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biomass methylation on Cr(VI) adsorption at increasing chromium (8.0–50 mg l^{-1}) and biomass (2–4 g l^{-1}) concentrations.

In this work the adsorption experiments were carried out in the pH range 7–8, in order to focus exclusively the methylation influence on the biomass adsorption capacity in the absence of any hydrolysis reaction. In addition, since the final aim of this work will be the improvement of Cr(VI)-rich wastewater treatment, such conditions are needed to avoid an expensive preliminary step of neutralization before the activated sludge process. An adsorption isotherm study was also performed, and the adsorption data were processed by either the Langmuir or the Freundlich model.

In order to evaluate how functional groups modifications induced by methylation impact on the biosorption process and to which extent biomass is chemically modified [3,4,18], raw and methylated biomasses have been investigated by FT-IR spectroscopy.

2. Materials and methods

2.1. Spirulina platensis cultivation

S. platensis (UTEX 1926) was obtained from the University of Texas Culture Collection. Cells were grown batch-wise in four outdoor cultivation ponds filled with the medium of Schlösser [19], having the following composition (per liter): 13.61 g $NaHCO_3$, 4.03 g Na_2CO_3, 0.50 g K_2HPO_4, 2.50 g $NaNO_3$, 1.00 g K_2SO_4, 1.00 g NaCl, 0.20 g $MgSO_4{\cdot}7\,H_2O$, 0.04 g $CaCl_2{\cdot}2\,H_2O$, pH 9.6. All nutrients were dissolved in distilled water containing (per liter): 6 ml of metal solution (97 mg $FeCl_3{\cdot}6H_2O$, 41 mg $MnCl_2{\cdot}4H_2O$, 5 mg $ZnCl_2$, 2 mg $CoCl_2{\cdot}6H_2O$, 4 mg $Na_2MoO_4{\cdot}2H_2O$), 1 ml of micronutrient solution (50.0 mg Na_2EDTA, 618 mg H_3BO_3, 19.6 mg $CuSO_4{\cdot}5H_2O$, 44.0 mg $ZnSO_4{\cdot}7H_2O$, 20.0 mg $CoCl_2{\cdot}6H_2O$, 12.6 mg $MnCl_2{\cdot}4H_2O$, 12.6 mg $Na_2MoO_4{\cdot}2H_2O$) and 1 ml of 1.5 μg l^{-1} B12 vitamin solution.

Each pond, having surface area of 0.15 m^2 and depth of about 6–7 cm, was kept at 30 °C and continuously illuminated by two fluorescent lamps (40 W) located at about 40 cm over its surface, furnishing 8.0 klx light intensity, corresponding to a photosynthetic photon flux density (PPFD) of 96 μmol photons m^{-2} s^{-1}. After one-month cultivation, a layer of the cyanobacterium formed on the surface of the tanks; it was collected by centrifugation, dried in oven at 50 °C and stored in desiccator at room temperature (20 ± 1 °C).

2.2. Preparation of biosorbent

Dried biomass was washed until reaching pH 7 in the washing water; then it was dried and methylated, according to the method reported by Fraenkel-Conrat and Olcott [20]. A sample of 1.0 g was suspended in 100 ml of methyl alcohol containing 0.1 mol l^{-1} HCl as a catalyst for the esterification reaction and stirred at room temperature. After 24 h, biomass was separated by centrifugation at 3300 rpm, washed, dried and ground to a fine powder, which was sieved through a 120 mesh (0.125 mm) sieve. The undersized fraction was used for Cr(VI) adsorption tests.

2.3. Biosorption and analytical procedures

Before using, the methylated biomass employed in adsorption tests was rinsed by deionised water for about 60 min. Adsorption tests were carried out in 200-ml Erlenmeyer flasks agitated on a rotary shaker (150 rpm) at room temperature. Biomass at concentration of 1.0, 2.0 or 4.0 g l^{-1} was put in contact with $K_2Cr_2O_7$ solutions with Cr(VI) concentration increasing from 8.0 up to 50 mg l^{-1}.

Biomass was removed from samples (5.0 ml) withdrawn at fixed times by filtration through membrane filters (Millipore, Vimodrone, Italy) with 0.45 μm pore diameter, and the filtrate was analyzed for metal content using an ionic chromatograph, IC mod. 761 (Metrohm Italiana, Varese, Italy).

Chromium adsorption tests were carried out at starting pH 7–8; since no significant pH changes were observed during the experiments, they were considered to be performed at constant pH. In this pH range, the Cr(VI) species in equilibrium are mainly CrO_4^{2-}, $HCrO_4^-$ or $Cr_2O_7^{2-}$ [8] while the raw biomass is negatively charged [17]. All tests were carried out in quadruplicate, and the experimental results presented as mean values. Errors between experimental data and mean values never exceeded ±6%.

2.4. Characterization study

The isoelectric point of raw biomass was determined by potentiometric titrations of solutions containing 5 g l^{-1} of dry biomass in 0.01, 0.1 and 1.0 M NaCl, using 0.1 M HCl as titrant [21].

Samples of dry 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™ acquisition software. The acquisition was 100 scans for each spectrum and the resolution 2 cm^{-1}.

3. Results and discussion

3.1. Cr(VI) removal tests

One of the parameters that strongly affect the biosorption capacity is the concentration of the biosorbent. Tests carried out with raw biomass at pH around 7.0 (results not shown) pointed out a very low (about 10%) efficiency of Cr(VI) removal. This result is consistent with the acidic isoelectric point of biomass (p*I* = 4.3), which was calculated as the average of p*K*a values resulting by titration (4.6, 3.9 and 4.4), which suggests that, under neutral conditions, it has net negative charge. As expected, the above value is close to the p*I* range (3–4) reported by Tzesos [22] for biomass of different microalgae and cyanobacteria.

Therefore, according to Seki et al. [16,17] methylated biomass was used to remove chromate. Figs. 1 and 2 illustrate the trend of Cr(VI) removal yield vs. time at metal concentration increasing from 8.0 up to 50 mg l^{-1}, and using 1.0, 2.0 or 4.0 g l^{-1} of methylated biomass.

Except for the tests carried out at the two highest initial Cr(VI) concentrations (43 and 50 mg l^{-1}), the Cr(VI) removal efficiency was higher than 80% since the beginning of biosorption, and equilibrium was completely established after a contact time of about 2–4 h (Fig. 1). The yield of Cr(VI) biosorption increased linearly along the time during the first hour and then remained nearly constant after this period.

At the lowest Cr(VI) concentration (8.0 mg l^{-1}), this metal was satisfactorily removed by 1–2 g l^{-1} of biosorbent (Fig. 1a and b), whilst at intermediate initial metal concentrations (18–25 mg l^{-1}), 2–4 g l^{-1} of biosorbent were needed to assure a removal efficiency ≥80% (Fig. 1b and c). On the contrary, when biomass concentration (X_0 = 1.0, 2.0 and 4.0 g l^{-1}) was largely insufficient with respect to the metal availability (Cr_0 = 43 and 50 mg l^{-1}), the adsorption efficiency was very low, and sometimes, after metal uptake, a slow metal release occurred. The same trend was previously observed under similar conditions for other cations [23,24].

The decrease in the removal yield observed at high Cr_0 concentration suggests that the adsorption of Cr(VI) was a physical phenomenon, as expected by a non-living material. This suggestion is confirmed by the results of Fig. 2 that shows the influence of X_0 at three different Cr_0 values (18, 35 and 50 mg l^{-1}). At a given Cr_0, the removal efficiency did in fact increase with

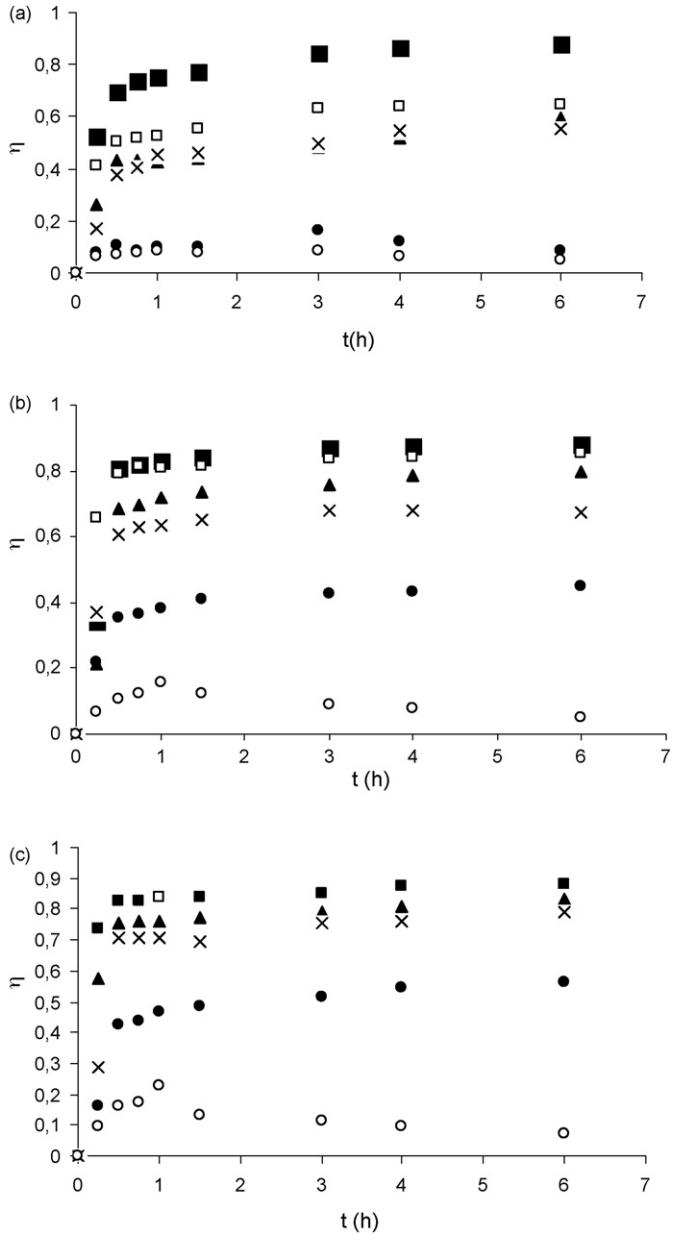

Fig. 1. Yield of *S. platensis* methylated biomass on Cr(VI) removal at different initial Cr(VI) concentrations (mg l^{-1}): (■) 8.0; (□) 18; (▲) 25; (×) 35; (●) 43; (○) 50. Biomass concentration (g l^{-1}): (a) 1.0; (b) 2.0; (c) 4.0.

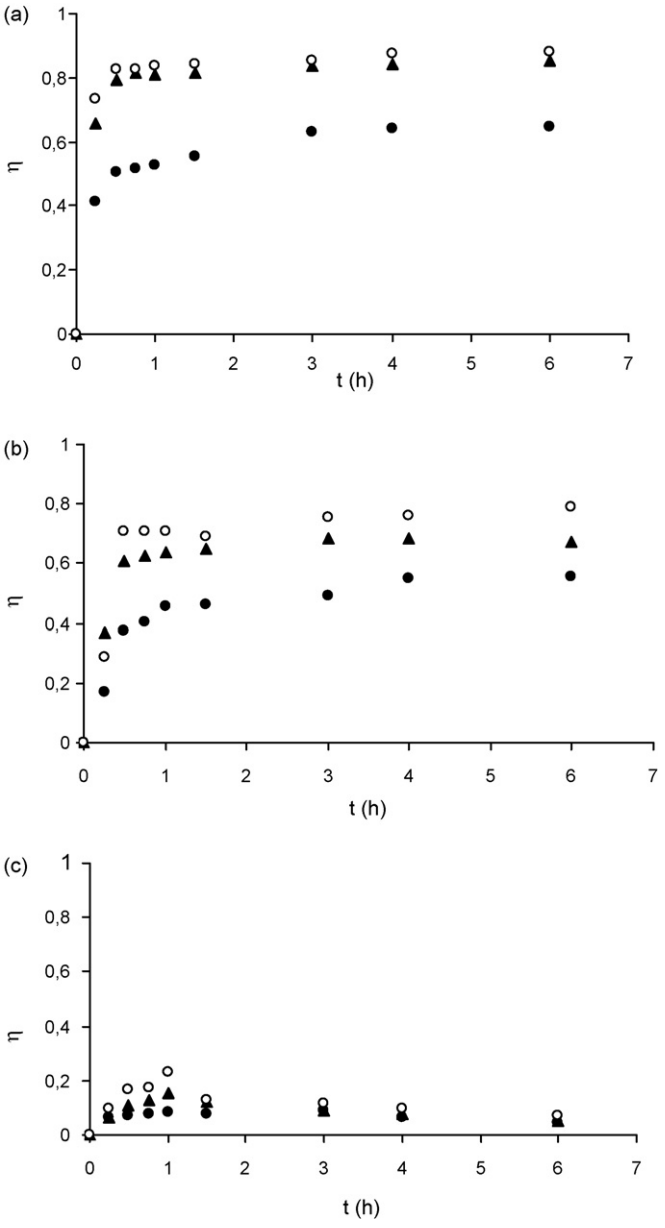

Fig. 2. Efficiency of *S. platensis* methylated biomass on Cr(VI) removal at different biomass concentrations (g l^{-1}): (●) 1.0; (▲) 2.0; (○) 4.0. Initial Cr(VI) concentration (mg l^{-1}): (a) 18; (b) 35; (c) 50.

X_0, as a result of the increased number of adsorption sites. At low Cr_0 (18 mg l^{-1}) the removal was very effective (85%) using 2.0 g l^{-1} of methylated biomass (Fig. 2a): an increase in the adsorbent level (4.0 g l^{-1}) allowed the removal efficiency to increase only to 88%, and about one half of the biosorbent kept unemployed. Doubling Cr_0 (35 mg l^{-1}), it was necessary to use a twice biosorbent concentration to achieve a removal efficiency of 78% (Fig. 2b). Finally, at high metal concentration (Cr_0 = 50 mg l^{-1}) a dynamic equilibrium between adsorbed and dissolved metal took place, so that the adsorbed metal was progressively released (Fig. 2c).

3.2. Adsorption isotherm study

Taking in mind that the adsorbing material was not biologically active because of drying and methylation treatments, the metal uptake can be regarded as a passive adsorption process. Therefore, it can be described by the isotherm models of Langmuir [25] or Freundlich [26], which are widely used to describe adsorption data in water and wastewater applications.

The Langmuir model is based on the assumption that maximum adsorption occurs when a saturated monolayer of solute molecules is present on the adsorbent surface, the energy of adsorption is constant, and there is no migration of adsorbate molecules in the surface plane [27]. The linear form of Langmuir model is:

$$\frac{C_e}{q_e} = \frac{1}{q_{\max} b} + \frac{C_e}{q_{\max}} \quad (1)$$

where C_e (mg l^{-1}) is the equilibrium concentration of metal, q_e (mg g^{-1}) is the adsorption capacity of the system, q_{max} (mg g^{-1}) is its overall adsorption capacity, and b (l mg^{-1}) is a parameter related to the affinity between the sorbent and the sorbate.

The Freundlich equation is an empirical relationship whereby it is assumed that the adsorption energy of a metal binding to a site

Table 1
Parameters of Langmuir and Freundlich models for the adsorption of Cr(VI) at variable concentration of methylated *S. platensis* biomass (X_0).

X_0 (g l^{-1})	Langmuir			Freundlich		
	q_{max} (mg g^{-1})	b (l mg^{-1})	r^2	K_f (mg$^{1-1/n}$ l$^{1/n}$ g^{-1})	n	r^2
1	16.7	0.72	0.91	6.74	2.90	0.95
2	13.7	0.38	0.96	4.09	2.05	0.93
4	7.42	0.55	0.87	3.71	4.80	0.61

on an adsorbent depends on whether or not the adjacent sites are already occupied [28]. The linear form of Freundlich model is:

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \quad (2)$$

where $1/n$ (dimensionless) represents the adsorption intensity and K_f is a parameter whose value is related to the adsorption capacity and whose unit varies with $1/n$ in the form mg$^{1-1/n}$ l$^{1/n}$ g^{-1} [29].

The values of the Langmuir and Frundlich adsorption parameters calculated from the adsorption isotherms are listed in Table 1 together with the corresponding determination coefficients. It should be noticed that the Langmuir model was able to describe the adsorption phenomenon better than the Freundlich one, exhibiting, in general, higher determination coefficients ($0.87 \leq r^2 \leq 0.96$), especially at the highest biomass concentration. The values of q_{max} of the Langmuir equation decreased with increasing X_0, which means that an increased biomass level does not necessarily correspond to larger amount of adsorbed metal. So, as it was previously observed by other authors [28], the adsorption process provided better results with little amounts of either biosorbent or metal. The values of the parameter b, representing the affinity of the binding sites for the adsorption, indicate a fairly ability of *S. platensis* biomass in the removal of Cr(VI).

However, the estimated values of q_{max} (7.42–16.7 mg g^{-1}) were lower than those previously observed for the removal of Cr(III) by non-methylated biomass of the same microorganism (30.1–36.8 mg g^{-1}) [23], but comparable with the ones (10.6–15.6 mg g^{-1}) that can be calculated from the data reported by Seki et al. [17] for Cr(VI) removal by methylated yeast biomass. These results suggest that, although the methylation was able to promote the removal of anionic compounds, the adsorption capacity of the resulting biosorbent was far to exhibit the same potential compared to the removal of cations by raw biomass. Nevertheless, the q_{max} values obtained in this study are not so different from the ones reported for the removal of Cr(VI) by different adsorbing raw and treated biomass (9.9–37.4 mg g^{-1}) [29,30] and are very close to those obtained using sun dried *Spirogyra* sp. (3.38–14.7 mg g^{-1}) [31].

3.3. Properties of raw and methylated dry biomass of S. platensis

Cell walls of algal biomass have a complex chemical composition, with different functional groups being responsible for ions removal. In order to evaluate the chemical modification caused by methylation, either native or treated biomass was analyzed by FT-IR spectroscopy.

Infrared spectroscopy of biomass films and powders can be applied as an effective way to characterize the carbohydrate (mainly chitin and chitosan), protein and lipid fractions of plant cell walls. To the best of our knowledge, the effect of methylation process on biomass was not extensively investigated by such a spectroscopic methodology. Sawalha et al. [32] only commented that spectra of methylated biomass did not show a clear trend; therefore, in the present work an attempt was made to evaluate the chemical effect of methylation in acidic environment through the study of functional groups chemical modifications by FT-IR spectroscopy. The main IR bands considered are summarized in Table 2 and are briefly discussed in the following, in relation to their variation after methylation.

The spectrum of native *S. platensis* biomass is illustrated in Fig. 3 (spectrum a). In the high frequency region, the broad and strong band centered at about 3450 cm^{-1} can be assigned to stretching vibrational modes of H-bonded OH groups, belonging to the dry material itself (likely –CH_2OH groups or OH groups of glycoside rings) superimposed to the broad band due to molecularly adsorbed water. A shoulder at 3300 cm^{-1} was likely associated to NH_2 stretching mode of primary amides/amine compounds, mainly belonging to alkaline aminoacids or terminal NH_2 protein groups, while a shoulder at lower frequencies (3080 cm^{-1}) can be assigned to NH stretching mode of a secondary amide/amine, likely related to protein proline and/or *N*-acetyl-D-glucosamine and *N*-acetylmuramic acid of peptidoglycan. These features are consistent with the presence of peptidoglycan in the cell walls, polyglucan granules and proteins [33]. Superimposed to the absorption in the region 3600–2800 cm^{-1}, several bands are clearly detectable at 2960, 2932, 2875 and 2859 cm^{-1}, all of them being assignable to aliphatic asymmetric and symmetric CH stretching modes. In particular, the band at 2960 cm^{-1} may be attributed to the ν_{as}CH of CH_3 groups, and the one at 2932 cm^{-1} to ν_{as}CH of CH_2 groups.

Following biomass methylation in acidic environment, the relative intensity of the broad band due to OH groups slightly decreased (Fig. 3, spectrum b), while the ratio of the intensities of the bands at 2960 cm^{-1} (νCH_3) and 2932 cm^{-1} (νCH_2) (i.e., $[I_{\nu CH_3}/I_{\nu CH_2}]$) increased, likely due to methylation of the OH groups; therefore, this ratio has been taken as an actual diagnostic of the methylation process.

The low frequency region of the IR spectra of native biomass (Fig. 3, spectrum a, 2000–400 cm^{-1}) highlighted the presence of several components partially superimposed. The band at 1730 cm^{-1} was likely due to C=O stretching mode of a carboxylic compound, mostly the lipid glycerol esters whose C–O stretch-

Table 2
Assignation of main IR bands in the native biomass spectrum.

Assignation	Position (cm^{-1})	Functional groups or compounds
νOH	3450	H-bonded hydroxy groups
νNH_2	3300	Amine/amide
νNH	3080	
$\nu_{as}CH_3$	2960	Alkyl chains
$\nu_{as}CH_2$	2932	
$\nu_s CH_3$	2875	
$\nu_s CH_2$	2859	
νC=O	1730	Ester
νC=O + νCN	1650	Amide I band of proteins
$\nu_{as}COO^-$	1620 sh	Carboxylate
δNH + νCN	1540	Amide II band
δCH/CH_2	1455	Alkyl chains
$\nu_s COO^-$	1410	Carboxylate
δCH_3	1380	Alkyl chains
Amide III, δC–O–C, δOH	1300–1230	Amide, ester, polysaccharides
νCC/CO	1150–1050	Polysaccharides

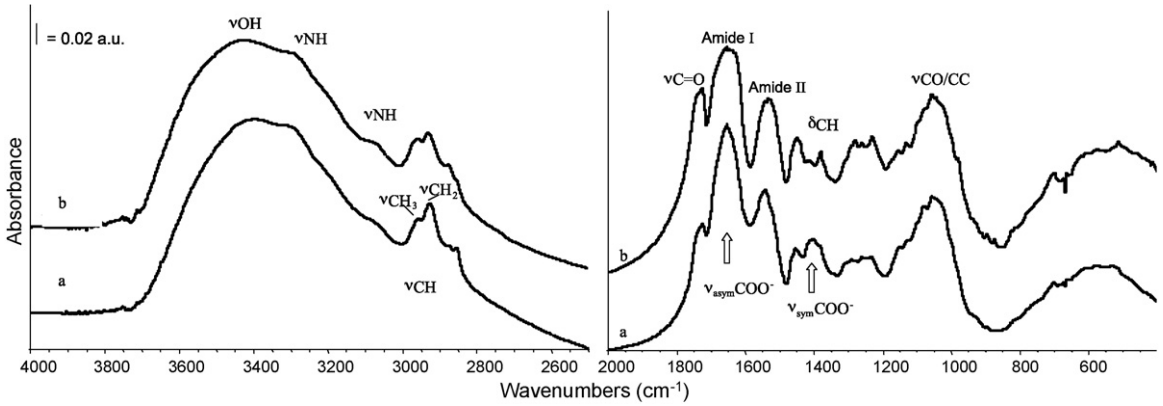

Fig. 3. FT-IR spectra of *S. platensis* (a) dry biomass, (b) methylated biomass.

ing mode lays around 1250 cm^{-1}, while the strong and complex absorption at 1650 cm^{-1} can be assigned to the so-called amide I band. The asymmetric component of carboxylate (COO^-) stretching modes lay in a similar frequency range (1620–1580 cm^{-1}), as reported for COO^- groups of several aminoacids [34], hence explaining the tailing of this band toward lower frequencies. The amide II mode could have been responsible for another strong peak centred at 1540 cm^{-1} [18,32]. The complex absorption centred at 1410 cm^{-1} can mainly be assigned to the symmetric stretching of a carboxylate group, completely consistent with the bands reported for the ionic form of aminoacids by Nakamoto [34], and the band at 1383 cm^{-1} to the bending mode of alcoholic C–O–H group [35]. At lower frequencies two complex absorptions were detected around 1250 and 1060 cm^{-1}. The former showed several weak maxima that can be associated with the amide III mode of proteins and to the C–O stretching mode characterizing an ester group [36]. Moreover a shoulder at higher frequency (1300 cm^{-1} ca.) can also be assigned to OH bending mode of alcohol compounds. Bands around 1100 cm^{-1} are typical of CO/CC vibrational stretching modes of –CHOH groups [37] and of bridging oxygen in a glycoside type structure.

After methylation, the relative intensities of the two complex amide I and II bands decreased with respect to the C=O stretching band (Fig. 3, spectrum b). Two explanations are possible: (a) the acidic catalysis could have led to a partial hydrolysis of the protein amide group, allowing the amide related peaks to decrease, and/or (b) the methylation could have reduced, as expected, the number of carboxylate groups, whose asymmetric stretching band was likely masked by one of these bands. This latter hypothesis is confirmed by the parallel decrease in intensity of the band at 1410 cm^{-1}, due to the symmetric COO^- stretching mode. However, this band did not disappear completely, suggesting that not all the carboxylate groups present could have been methylated under these conditions. A complex absorption growing at 1450 cm^{-1} and superimposed to the peak of biomass alkyl chains at 1455 cm^{-1} (as reported in Table 2) can be assigned to symmetric CH deformation mode of the $–O–CH_3$ group [36], and this is another evidence of the biomass methylation. The absorption around 1300 cm^{-1} was also affected by the methylation process: the shoulder at high frequency we assigned to OH deformation modes almost disappeared, pointing out the OH groups also reacted, likely giving rise to methyl ethers. As a consequence of the esterification, also the maximum at 1230 cm^{-1}, which can be related to ester C–O–C vibrational mode, increased. The broad band around 1060 cm^{-1}, due to CC/CO stretching modes, was still very strong and complex. The component at 980 cm^{-1}, detectable after methylation in acidic environment, can be assigned to the O–H out of plane deformation band of residual "free" carboxylic groups, also formed at low pH values.

Summarizing, methylation reduced the amount of carboxylate species, leading to the formation of methyl esters. Although the present IR study pointed out that not all the exposed carboxylic groups could be esterified, a decreased amount of carboxylate (i.e., negatively charged groups) was expected to significantly favor chromate adsorption. Moreover, the reaction was not selective, and methylation of biomass hydroxy groups to methylethers could also be envisaged. Both hydroxyl and ether functionalities can also be involved in the adsorption process, as recently reported by Murphy et al. [37] for Cr(VI) binding on red seaweeds. A likely explanation, as proposed also for amino groups, is that these groups may be protonated at low pH, thus allowing an electrostatic attraction with Cr(VI) species [37].

The acidic conditions required by methylation likely led also to a partial hydrolysis of the protein amide groups and the glycerol ester groups, allowing the detection of free carboxy groups claimed by Sawalha et al. [32] to be responsible for Cr(VI) adsorption at pH as low as 5 [34]. Thus, following methylation, more active groups were available for chromate species adsorption, whose further modification upon adsorption will be investigated in a second set of experiment to be performed at different pH values.

4. Conclusions

Methylated biomass of *S. platensis* was employed in batch tests for Cr(VI) adsorption at pH 7–8, yielding substantially better results than the untreated biomass. At the lowest Cr(VI) concentration (8.0 $mg\,l^{-1}$), this metal was satisfactorily removed by 1–2 $g\,l^{-1}$ of biosorbent, whilst at intermediate initial metal concentrations (18–25 $mg\,l^{-1}$), 2–4 $g\,l^{-1}$ of biosorbent were needed to assure a removal efficiency $\geq$80%. At the highest initial metal concentrations (43 and 50 $mg\,l^{-1}$) the adsorption efficiency was very low even at the highest biomass level (X_0 = 4.0 $g\,l^{-1}$).

The Langmuir model was used to describe the adsorption phenomenon, showing values of q_{max} decreasing with increasing biomass concentration, which means that increased biosorbent availability does not necessarily correspond to larger amount of adsorbed metal.

FT-IR spectra of dried and methylated biomass allowed us monitoring the efficiency of the methylation process through the relative intensity of diagnostic IR bands (CH_3/CH_2 and COO^- stretching modes), thus confirming the decreased number of carboxylate groups at the biomass surface ($–COO^- \rightarrow –COOCH_3$). Moreover, other functional groups, such as "free" carboxy groups (–COOH), amines (–NHR, $–NH_2$) and ether ($–C–OCH_3$), formed as consequence of methylation, could have been involved in the adsorption process.

Once defined in this study the effect of methylation, further investigation on chromate species adsorption will be carried out at lower pH values.

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