Biosorption potential of two brown seaweeds in the removal of chromium
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ABSTRACT

The present work focused on the potential use of brown algae Cystoseira barbata and Cystoseira crinita from the Black Sea coast for removal and speciation analyses of Cr(III,VI) ions from aqueous and wastewater solutions. The biosorption process of Cr(III) and Cr(VI) was designed as a function of pH and contact time. Potentiometric titration and Fourier transform infrared spectroscopy (FT-IR) analysis techniques revealed the potential binding sites present at the surface of the algae for both oxidation states of Cr. Various chemical treatments have been used to indicate the mechanisms of binding Cr(III,VI) and bioreduction of Cr(VI) by the biosorbents. Acidic treatment was the most successful in removing and reducing total Cr(VI). Algae samples were subjected to methylation and esterification processes for modification of amino and carboxyl groups, respectively. The Langmuir model was applied to describe the biosorption of Cr(III,VI) by algae. Total Cr and Cr(VI) determinations were simultaneously made using the diphenyl carbazide spectrophotometric method and flame atomic absorption spectroscopy (FAAS). In conclusion, these algae can be used as a potentially cost-effective biosorbent for the uptake of two different oxidation states of Cr and subsequently for Cr speciation analysis.

Key words | biosorption, chemical treatment, Cr(III,VI), Cystoseira barbata, Cystoseira crinita, wastewater

INTRODUCTION

Chromium, which can be found at different oxidation stages notably as Cr(III) and Cr(VI), which has far more toxic effect in aqueous systems, is one of the most significant environmental problems that still cause a heavy metal pollution based on industrial development (Yalçın et al. 2001; Shanker & Venkateswarlu 2011). Industrial wastes and heavy metals that exist in the soil are dissolved by acid rain and pollute the underground and surface waters (i.e. rivers, lakes, and seas) (Gao & Liu 2017). Heavy metals that have been deposited as a result of the industrial process must be removed before given into water due to being highly toxic for aquatic environments and also the whole ecosystem. Chromium is widely used in iron and steel, leather, metal coating, textile industry, electrical power stations, coil coating, electrical, electronic, battery, and automotive battery manufacturing industries as well as other chemical industries and different industrial areas (Rihai Samani et al. 2016). Disposal of these widely used materials into the environment generates a high level of pollution. The Cr(VI) species depending on pH exist in the natural waters as [Cr(H2O)5(OH)]2+, [Cr(H2O)4(OH)2]+, HCrO4-, and CrO4^{2-}. It is much more toxic than trivalent Cr (Yalçın & Apak 2004). Conventional physicochemical methods such as chemical precipitation, adsorption, solvent extraction, ion exchange, and membrane separation technology are not economical enough to remove Cr from industrial wastewater. Since the Cr removal efficiencies are not sufficient in these methods, the use of biological materials (e.g. biosorbents) for removal of Cr is preferred as an alternative method (Cobas et al. 2014; Vendruscolo et al. 2017). In some studies, the high metal binding capacities of non-living biosorbents were generally observed in comparison to living biosorbents. It has been widely accepted that they have an excellent metal binding capacity as a result of numerous biosorption experiments with marine algae. The cell walls of brown algae are usually composed of cellulose in fibrous skeletal structure and alginic acid (1,4-β-D-mannuronic acid (M), α-L-guluronic acid (G)), which is a straight chain polysaccharide containing the carboxyl (-COOH) group, is major responsible for metal binding
and their sulphated polysaccharides (fucoidan matrix) with sodium, potassium, magnesium, calcium salts (alginate) (Davis et al. 2003). Due to having these features, algae (biosorbents) can selectively separate metal-ions from the complex environment in the aqueous solution in a short period of time and they can reduce the heavy metal concentration to ppb level (Wang & Chen 2006). Physicochemical analysis methods, such as Fourier transform infrared spectroscopy (FT-IR) and potentiometric titration used in the identification of the biosorbent structure and in the description of the sorbate-biosorbent interaction to determine the mechanism of the biosorption process have been routinely dealt with by some researchers playing a pioneering role in the biosorption field (Fourest & Volesky 1996; Andreada et al. 2005). Numerous studies have been carried out in the literature to examine only the adsorption processes extensively in the context of the intermittent contact tests without disclosing the biosorption mechanism (Lee et al. 2000; Bishnoi et al. 2007). However, studies to elucidate new and first-time screening of a biosorbent or sorbent structure have almost become a prerequisite to evaluate sorbate-biosorbent processes. The biosorption mechanism between biosorbent and heavy metals is thought to include one or more of these ion exchange, adsorption, complexation, micro-precipitation formation, chelation and electrostatic interaction balances (Veglio & Beolchini 1997). However, it has been determined that the most important mechanism in biosorption of heavy metal ions by algal biomass is ion exchange (Crist et al. 1990). Until today, numerous studies have taken part in the world literature to detect and remove chromium by using synthetic or natural sorbents from environments containing both oxidation steps together or alone. Only small portions of these were carried out using brown marine algae, which has a significant advantage of retaining Cr in its structure. Figuera and his colleagues (Figuera et al. 1999) attributed to the presence of sulfate groups as well as a large number of carboxyl groups in brown marine algae, which can uptake trivalent metal cations. Cr(III) biosorption study has been carried out by using microalgae Spirulina sp. and the green macroalgae Pithophora varia that grows in the botanical garden (Michalak et al. 2007). Park and his colleagues (2005) have also used a macrostructural brown marine algae Ecklonia sp. for Cr(VI) biosorption. The reduction capacity of the algae used in this study for Cr(VI) by comparing chemically processed algae with various reagents was investigated that the algae carried out through the mechanism to be able to reduce Cr(VI). The Cr(VI) reduction capacity of the same biosorbent and afterward pH-dependent retention ability for Cr(III) formed after the reduction which was also investigated in another study of the same authors using X-ray photoelectron spectroscopy (XPS) and X-ray absorption spectroscopy (XAS) techniques (Park et al. 2008). Focusing on the same subject particularly with different approaches of these researchers was derived from emphasizing the fact that Cr(VI), which is much more toxic than Cr(III), is biological, cheap and effectively reduced to Cr(III). Murphy and colleagues (2009) proposed various brown, green and red marine algae for the removal of Cr(III,VI) from wastewater environments as well as Cu(II). The reduction criterion of brown algae from Cr(VI) to Cr(III) was assessed with the results obtained from changes with a series of exposures to chemical treatments of the algae used. The methods of this research of selectively qualitative/quantitative determination, removal, as well as effective separation of Cr(III) and Cr(VI) maintain their importance currently in the field of analytical chemistry.

C. barbata and C. crinita marine algae, which are easily and biologically degradable and encountered in common on the seashores of Turkey, have been proposed for the first time as potential biosorbents to separate and remove Cr(III, VI) ions from the aqueous solution and electroplating wastewater. By using potentiometric titration method, functionalities of Cr(III) retention have been discussed by determining pKₐ values of biosorbents and the corresponding functional groups. The results have been supported by FT-IR spectroscopy findings.

**MATERIALS AND METHODS**

**Preparation of the raw algae**

The two brown macroalgae species C. crinita and C. barbata (sampling time: September 2017; location: Şile-Black Sea region) were obtained from the Institute of Marine Sciences of Istanbul University. These algae are generally found as heaps on shallow shores without being mixed with other species. The algae samples – without utilizing any isolation procedure (Strezov & Nonova 2003) – were washed several times with tap water and distilled water to remove any adherent particles, dried under sunlight and later in an oven for 24 hours at 60 °C until the constant weight was obtained. Dried algae samples were ground until homogenized in the range of 100–800 μm. These samples in polyethylene bottles were stored in a desiccator for further processing.
Preparation of algae using chemical treatment process

The use of various chemicals to activate ion trapping sites on algae and increase metal ion binding capacity of algae is a common practice. In this study, algae treated with CaCl₂ solution from the applied chemicals were converted to Ca-form and with 0.1 M HCl acid converted to H-form (Yalçın et al. 2012). In addition, algae were treated with acetone as organic solvent. This process is aimed at eliminating proteins and lipids that can be extracted with organic solvents, thereby increasing the more metal retention sites on algae and increasing the algae retention capacity. In addition, modifications of the carboxyl and amino groups on algae were carried out. The procedure of applying these reagents to algae is given below (Park et al. 2005).

CaCl₂ solution application: In order to remove accumulated metal ions in the structure of marine algae, it was treated with 0.2 M CaCl₂ solution for 2 hours. The excessive amount of calcium was removed from the marine algae by washing them with distilled water. The algae used in experiments were dried again until constant weight was stored in the desiccator for further use. Drying process was carried out in the same way in every other chemical process.

HCl solution application: Determined amounts of dried algae were converted into proton form by shaking in a rotary shaker with 0.1 M HCl solution for 3 hours. Washing and drying operations were carried out as mentioned above.

Treatment with acetone solution: 5 grams of raw dry algae were extracted with 200 ml 1 M acetone solution at room temperature (25 ± 1) for 6 hours by shaking. After filtration and washing with distilled water, they were dried and stored dry in polypropylene cups for subsequent applications.

Esterification of carboxyl groups: 5 grams of raw algae were treated with 500 ml of anhydrous methanol and 5 ml of concentrated HCl. The reaction mixture was shaken at room temperature for 6 hours at a speed of 150 rpm. The related reaction was performed as follows:

\[ \text{RCOOH} + \text{CH}_3 \xrightarrow{\text{H}^+} \text{RCOOCH}_3 + \text{H}_2\text{O} \]

Methylation of amino groups: 5 grams of raw algae were treated with 100 ml formaldehyde and 200 ml formic acid. The reaction mixture was treated with 0.1 M NaOH and 0.1 M HCl solutions. The related reaction takes place as follows:

\[ \text{RCH}_2\text{NH}_2 + \text{HCHO} \xrightarrow{\text{HCOOH}} \text{R(CH}_2\text{)N(CH}_3\text{)} \]

Carboxylation of the amino groups (succinic acid group): 5 grams of raw algae were shaken at room temperature with 200 ml of 0.1 M bromosuccinic acid solution at room temperature for 6 hours and then washed with distilled water and dried. All of the algae samples treated to chemical modification were used in aqueous solutions for retention of Cr(III) and Cr(VI) (Park et al. 2005). The related reaction takes place as follows:

\[ \text{RNH}_2 + \text{HOOC-CH}_2\text{-CHBr-COOH} \rightarrow \text{RNH}_2\text{-CH(COOH)-CH}_2\text{-COOH} + \text{HBr} \]

**Instrumentation and analysis of Cr(III)/Cr(VI)**

A Varian SpectraAAFS-220 atomic absorption spectrometer, equipped with a flame burner, hollow cathode lamps, and an air-acetylene burner was used for total Cr analyses. The measurements of Cr(VI) were carried out using the diphenylcarbazide spectrophotometric method by the aid of a Carry 1 E UV-Vis spectrophotometer at 540 nm. CrCl₃·6H₂O (Merck): a standard 1,000 mg/l Cr(III) solution was prepared by dissolving 0.5125 grams of the Cr(III) salt in 0.5 M HCl. K₂Cr₂O₇ (Merck): a standard 1,000 mg/l Cr(VI) solution was prepared by dissolving 2.8290 g K₂Cr₂O₇ in distilled water (by adding 5 ml concentrated HCl). Other Cr solutions were prepared daily with necessary dilution and pH adjustments from these solutions. The pH adjustments required for all solutions were performed using 0.1 M NaOH and 0.1 M HCl solutions.

**FT-IR analyses**

FT-IR analyses were carried out to examine the structural changes of algae after raw-form, proton (H-) form and chemical modification and additionally with the aim of determining the groups responsible for biosorption during the Cr(III,VI) retention process. For this purpose, FT-IR spectra of algae samples in KBr tablets were obtained by using a Thermo Fisher Scientific-2007 FT-IR device.

**Potentiometric titrations**

The titration processes were established under the following conditions to determine through which functional groups...
the Cr retention occurred on algae: Initial pHs of the all titrated solutions were made 2.5 (with 0.1 M HCl solution), the ionic strength was kept constant (containing 10⁻³ M NaCl). The titrations were carried out in three separate parts: containing algae sample + deionized water; algae sample + Cr(III) solution; only Cr(III) solution. For the first two titrations, ca. 0.2 g of protonated algae sample was dispersed in 50 ml (titration starting volume) of the total solution. The total chromium concentration was applied at 100 ppm. Titration was performed by the gradual addition of 0.1 ml of 0.1 M NaOH whilst the suspension was stirred under nitrogen atmosphere. After each addition of NaOH, it was ensured that the solution equilibrated and corresponding changes in pH were noted. All pH measurements were recorded using a Metrohm Herisau-E-512 pH meter equipped with a glass electrode. This was calibrated against buffer solutions of pH 4 and 7 prior to use.

**Batch biosorption procedure**

To find out the optimal metal ion retention capacity of algae samples depending on solution pH, an agitation process for 0.1 g of algae was performed at different pH values (2.0–4.5) in separate 50 ml solutions of Cr(III) and Cr(VI) ions at an initial concentration of 100 ppm. The results were evaluated using a graph drawn between the retention percentage of Cr(III) and pH. Due to the fact that maximum retention for Cr(VI) occurred at pH = 2.0, Cr(III)/Cr(VI) retention capacities of raw and other modified algae and reduction capacity from Cr(VI) to Cr(III) were shown as a percentage (%). Maximum Cr(III) retention capacities of marine algae were determined by varying initial Cr(III) concentrations (25–200 ppm) at pH = 4.5 and by a 2.0-hour intermittent contact (batch) test. After the agitation process, in other words, after the retention period acquired, biosorption of the metal ions in the sorption system were calculated using the following mass balance equation:

\[
q_e = \frac{V(C_i - C_e)}{W}
\]

where \(q_e\) is the amount of equilibrium metal concentration retained by the biomass, \(V\) is the solution volume, \(W\) is the amount of biomass, and \(C_i\) and \(C_e\) are the initial and equilibrium metal concentrations, respectively. In addition, the retention of Cr(III,VI) ions by the algae samples was examined as a function of time. The experiments were carried out for 30–180 minutes at pH = 4.5 for Cr(III), with 2–24 hours at pH = 2.0 for Cr(VI), respectively. Since Cr(VI) removal was very slow and at the same time significantly reduced as a result of the contact time, long working periods were applied for hexavalent chromium studies. All experiments were performed at room temperature due to the increased precipitation tendency of Cr(III) with increasing temperature.

**Electroplating wastewater application**

Authentic Cr plating-rinsing wastewater (at pH 4.0) collected from İkitelli Organized Industrial Region of Istanbul City was analyzed by DPC spectrophotometry and flame atomic absorption spectroscopy (FAAS) to contain 104 mg/L Cr(VI). The Cr(III) ion was not detected. Although plating bath waters are very acidic, the pH of wastewater solution was found to be 4.0, possibly caused by excessive dilution. Wastewater pH was adjusted to 2.0 using 1 M HCl. 0.5 grams of H-form sorbents and agitated with 50 ml of Cr plating-rinsing water for 24 hours at room temperature. Remaining Cr in solution was converted to Cr(III) by the biosorbents. The pH of the wastewater solution containing only Cr(III) was adjusted to 4.5 and Cr(III) in solution was removed by raw algae in 2 hours of batch time.

**RESULTS AND DISCUSSION**

**Fourier transform infrared spectroscopy**

FT-IR spectra of raw and modified forms of *C. barbata* and *C. crinita* algae are given in Figures 1 and 2. Protonated *C. barbata* has shown peaks belonging at 1,735 cm⁻¹ (free C = O), 1,637 cm⁻¹ (asymmetric C = O), 1,425 cm⁻¹ symmetrical (C = O) and the peak at 1,250 cm⁻¹ belonging to carboxyl groups (Yalçın *et al.* 2012). Protonated *C. crinita* similarly has shown peaks belonging to carboxyl groups at 1,244 cm⁻¹ and 1,731 cm⁻¹ (free C = O), 1,644 cm⁻¹ (asymmetric C = O). For both algae, the absorbance peaks observed at about 1,160 cm⁻¹ and 1,035 cm⁻¹, respectively, were attributed to the symmetric -OSO₃ group stretching in sulfonic acid and -C-O stretching of alcohol group. Peaks in the range of 1,525–1,545 cm⁻¹ are probably the -NH stretching band of amide groups showing the proteins of marine algae for both algae. In Figures 1 and 2, it was observed that significant changes occurred in the results of metal biosorbent interactions when compared with both protonated and raw algae form with its Cr(III) retained form. The disappearance of the peaks observed at 1,735 cm⁻¹ and 1,731 cm⁻¹ of the acidic forms of algae after Cr(III) adsorption (e) was most likely related to coordination bonds.
Figure 1  | FT-IR spectra of C. barbata algae before and after chemical treatment: (a) raw, (b) acid treated, (c) Ca-loaded, (d) esterification of carboxyl groups, (e) Cr(III) loaded, (f) Cr(VI) loaded, (g) succinization of amino groups, (h) methylation of amino groups.

Figure 2  | FT-IR spectra of C. crinita algae before and after chemical treatment: (a) raw, (b) acid treated, (c) Ca-loaded, (d) esterification of carboxyl groups, (e) Cr(III) loaded, (f) Cr(VI) loaded, (g) succinization of amino groups, (h) methylation of amino groups.
between Cr(III) ions and carbonyl groups. In addition to the carboxyl groups, the sulfonil groups detected at 1,153–1,167 cm\(^{-1}\) are the main groups that provide Cr(III) retention (Figueira et al. 1999). The spectrum obtained after Cr(VI) adsorption at pH 2.0 is quite similar to the spectrum obtained with protonated forms of algae. It is found that this retention occurring in the acidic environment is probably caused by the interaction between the amide groups (-NH) and Cr(VI) from attenuations and shifts at peaks observed at around 1,540 cm\(^{-1}\). It has been observed that the carboxyl groups at 1,730 cm\(^{-1}\) are converted to a shoulder rather than the characteristic peak when carboxyl groups are esterified with concentrated HCl and methanol solvent (d). However, the peak related to the amides at 1,540 cm\(^{-1}\) has become more definite. These attenuations observed for both algae are attributed to the esterification of carbonyl groups. No significant change was observed in FT-IR spectra of algae, which is thought to be succinic acid-linked with the modification of amine groups, given in Figures 1 and 2(g). Since the peaks belonging to possible amine groups corresponding to 3,400 cm\(^{-1}\) and around coincide with large OH- bands that peak in the same region, therefore possible changes cannot be observed. For methylation modification of the amine groups with formic acid and formaldehyde, no change was observed as mentioned for 3,400 cm\(^{-1}\) (g). However, it was observed that the peaks that appeared at 1,540 cm\(^{-1}\) representing amide groups were converted to small shoulders. No remarkable change has been observed in the spectra of algae treated with the acetone solvent matched up with its raw forms. Thus, the FT-IR spectra of acetone treated algae samples were not presented. However, acetone treated algae was used in the metal retention experiment because proteins and lipids existing in algae might be removed with acetone and metal binding sites of the biomass surface could probably be affected positively by this process. In addition, no significant changes were observed especially in Ca-forms in comparison with its raw forms. A molecular alteration related to calcium loading is not the expected result, because the marine algae are already loaded with abundant amounts of calcium and magnesium ions in the marine environment. However, it can be said that the sharpening of the peaks belonging to the carboxyl groups at 1,400, 2,200 and 1,600 cm\(^{-1}\) both in Cr(III) and Ca(II) retention, is carried out via carboxyl groups of related ions as expected. No significant changes were recorded in FT-IR spectra of raw and modified forms of the algae between the spectra of the region that remained at 2,000–4,000 cm\(^{-1}\). However, in general, the following evaluations have been made for this specific region: large peaks observed in the FT-IR spectrum of raw and modified forms of C. crinita and C. barbata algae at 3,421–3,442 cm\(^{-1}\) were attributed to -OH and -NH groups. The much weaker bands observed in the range of 2,912–2,929 cm\(^{-1}\) correspond to -CH\(_2\) groups.

### Potentiometric titration of algae samples

The negative logarithm of the ionization constants (pK\(_a\)) corresponding to functional groups of Cystoseira barbata (Yalçın et al. 2012) and Cystoseira crinita brown marine algae used for Cr(III) retention was determined by potentiometric titration (Figure 3(a) and 3(b)). The amounts of the various acidic groups in biosorbents and the corresponding pK\(_a\) values were calculated from the curve (bending points) drawn between the amount of NaOH added (x-axis) and pH (y-axis) (Fourest & Volesky 1996). More detailed information was obtained from the first derivative plots of potentiometric

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**Figure 3** | Potentiometric investigation of Cr(III) retention with C. barbata (a) and C. crinita (b) algae.
titration curve of protonated algae samples. The $pK_a$ values were found as 2.82, 3.33, 3.76, 4.10, 4.63, 5.09 for *C. barbata* and 2.86, 3.85, 4.15, 5.16 for *C. crinita* through the titration curve of algae in H-form. The aim of investigation of only the Cr(III) titration profile in our study is to see the different behavior of Cr in the same processes, determine the tendency with regard to neutralization of the Cr(III) ion and clarify the titration of algae in the presence of the Cr(III) ion. It can be seen that Cr is inclined to a weak acid titration curve profile from pH 3.0 in Cr(III) titration. The hydrolysis constant of Cr(III), a highly acidic salt, was given as $pK_a = 3.85$. The Cr in a solution environment with this pH value is approximately half and half of Cr(OH)$_2^{2+}$ and Cr$^{3+}$ forms. In addition, we can deduce it from the behavior with the tendency of extending parallel to the $x$-axis in the titration curve from pH 5.5 of Cr by forming [Cr(H$_2$O)$_2$(OH)$_2$]$^+$ and especially Cr(OH)$_3$ species. It was determined that the neutralization was slower and longer in the process depending on the functional groups present in the algae from the titration curves. In the titration curves carried out in the presence of Cr(III) ions, when Cr(III) ions retained by dissociated carboxylic acid groups of alginic acid existing on the algae surface at a pH range of 3.5–5, much more base was consumed as a result of titration of protons in the acid groups relocated with Cr, and the neutralization process was extended. As can be seen from Figure 3 in the titrations of (algae + Cr(III)) and only algae, retention of Cr(III) occurs in the buffer regions corresponding to the values of $pK_a$ (3.0–5.5), which is resistant to base addition. In this process, it was determined that Cr(III) converted to Cr(OH)$_2^{2+}$ according to the Cr$^{3+}$ + H$_2$O ⇌ Cr(OH)$_2^{2+}$ + H$^+$ reaction with increasing pH ($pK_a = 3.85$) and the retention occurred via this form along with Cr$^{3+}$. It was determined that the released proton from the algae consumed more base due to the selective relevance of the functional groups on the algae surface to Cr(III). The $pK_a$ values of the biosorbents calculated in the range of 2.82–5.16 refer to various carboxyl groups, which are weakly ionizable. On the other hand, considering the speciation of Cr(III) ions depending on the pH, the reactions related to chromium retention can be expressed as follows:

- in form Cr$_{3+}$ at pH $\leq$ 3.85:

\[ \text{RCOOH} + \text{Cr}^{3+} \leftrightarrow (\text{RCOO})_2\text{Cr} + 3\text{H}^+ \]

- in forms (Cr$^{3+}$(recessive species) + Cr(OH)$^{2+}$ (dominant species)) at pH < 4.5:

\[ \text{RCOOH} + \text{Cr(OH)}^{2+} \leftrightarrow (\text{RCOO})_2\text{Cr(OH)} + 2\text{H}^+ \]

- in forms (Cr(OH)$^{2+}$ (recessive species) + Cr(OH)$_2^{2+}$ (dominant species)) at pH $\geq$ 5.0:

\[ \text{RCOOH} + \text{Cr(OH)}_2^{2+} \leftrightarrow (\text{RCOO})_2\text{Cr(OH)}_2 + \text{H}^+ \]

R: Biopolymeric structure of seaweed.

In addition, the maximum retention of the Cr$^{3+}$/Cr(OH)$^{2+}$ buffer system was all together and it occurred substantially at pH = 4.5 through the Cr(OH)$_2^{2+}$ form (Yalçın et al. 2001). The region useful for Cr(III) retention is up to pH 5.0, as seen in Figure 3.

**Effect of solution pH on retention of Cr(III,VI) ions**

Figure 4 shows the effect of pH on the biosorption of Cr(III) onto *C. barbata* and *C. crinita* biomass from aqueous solution. The uptake of Cr(III) by both raw algae, which were also monitored by potentiometric titration, reached a maximum at pH 4.5. However, slightly above pH 4.5 is the area where Cr(III) hydroxides begin to form. Therefore, working at higher pH values was avoided due to surface precipitations resulting from the formation of the Cr(III) hydroxo form (Yalçın et al. 2001).

It was determined that no Cr(VI) ions retained at the maximum adsorption of pH 4.5 with *C. barbata* and *C. crinita* algae of Cr(III) ion in batch tests. It was indicated that the maximum retention of Cr(VI) ions at pH 2.0 occurred a much smaller rate in comparison to that of Cr(III), and it was reduced to Cr(III) at a large rate. On the other hand, it was determined that no Cr(III) ion was...
retained at pH 2.0. It was indicated that the speciation of Cr(III,VI) can be easily performed by using C. barbata and C. crinita algae with the determination of the two processes mentioned above. The same procedures were applied to all chemically treated algae samples. According to this, Cr(III)/Cr(VI) retention and Cr(VI) reduction processes of algae with its raw, Ca-, H-, acetone, amino methylation-, carboxyl esterification and succinic forms were investigated (Figure 5(a) and 5(b)). As can be seen in Figure 5, Cr(III) retention occurred in the raw forms with 70.7% and 73.34%, respectively at the top rate for C. barbata and C. crinita algae. Yields at lower rates were obtained for Cr(III) retention for both modified algae species. We had reported that Cr(III) retention occurred via carboxyl groups existing substantially in algae as a Cr(OH)2+ form of Cr at this pH. Cr(III) retention was detected to be 59.6% for C. barbata and 47.75% for C. crinita as a result of the modification by the esterification of carboxyl groups; a decrease in Cr(III) uptake by binding of carboxyl groups has been an expected result. Despite a decrease in Cr(III) retention, it was not completely eliminated with this modification. Therefore, we can deduce that Cr(III) ions can also bind through a phenolic structure and sulfate groups in algae (Figueira et al. 1999). A significant decrease (70.54%) was not recorded for C. crinita in H-form, while a reduction in Cr(III) up to 54.15% was observed for H-form C. barbata. No significant decrease is expected in the Cr retention when working with H-form algae because the pH is fixed at 4.5 in this experiment. If pH was not kept constant during the Cr retention, because of releasing 3 protons for each Cr(III) ion retained and so because of reducing the solution pH significantly, the amount of retained Cr would remain at minimum levels. However, it could be asserted that the proton forms of some sites remain more stable at 4.5 pH, weakly acidic, for the reduction of C. barbata and it affects the chromium retention negatively. When studied with protonated form algae in Cr(VI) solution at 100 ppm concentration, it was determined that about 45 ppm of Cr(VI) was retained by algae and about 50 ppm of Cr was reduced to Cr(III).

In the end, approximately 5 ppm Cr(VI) remained in the solution. Thus, 90% of the remaining chromium in the solution was reduced to chromium(III). The total chromium was removed by 95% by means of retention and reduction. Cr(VI) retention occurred at the average rate of 35% in the form in which the amino groups in both algae were modified by methylation. The maximum retention of algae at pretty acidic pH = 2.0 of Cr(VI) occurs via this form due to being protonically positive-loaded and that of Cr(VI) being in the form of HCr2O7-/HCrO4- (Park et al. 2005; Prabhakaran et al. 2009). It has been reported that the Cr(VI) ion is mostly retained in biosorbents via amino groups as well as carboxyl groups (Garza-González et al. 2017). The greater retention of the amino groups in the modification-bond

![Figure 5](https://iwaponline.com/wst/article-pdf/78/12/2564/525468/wst078122564.pdf)
form is due to the carboxyl groups liberating much more, especially during this process, and it could be asserted that Cr(VI) retention is likely to occur via these groups (Zhou et al. 2016). In addition to the active role of amino groups commonly known in the retention of Cr(VI), it is strongly possible that -OH groups in tannic structures found in abundant brown algae are also effective. A tannic sorbent was obtained by Lima et al. (1998) as a result of the treatment of tannic extracts with the cellulose sorbent. Cr(VI) retention was reported to have been performed at pH 2.0 over the hydroxyl groups present in the tannic structure. It was indicated that hydroxyl groups contributing to Cr(III) retention also played an important role in Cr(VI) retention (Lima et al. 1998). In our study, there was no significant decrease in the removal of Cr(VI) after the modification of amino groups. Thus, we can infer that hydroxyl groups, which are found abundantly through the tannic structures on both brown algae, play an active role in the retention of Cr(VI) (Zhou et al. 2016). The removal of Cr(VI) by using dried biosorbents occurred in three phases as: (i) direct reduction to Cr(III); (ii) indirect reduction by means of electron donor groups in the sorbent; (iii) transition to solution environment of reduced Cr(III) (mechanism: chain of events in the form of related redox have been explained by schemes and formulas below). The second phase is a slower one involving the binding of Cr(VI) with the result of interaction with positively charged groups on biomass and, subsequently, the partial reduction of the retained Cr(VI) to Cr(III) by electron transfer from neighboring electron donor groups and the release of Cr(III) by electronic repulsion effect between positively charged groups and Cr(III) ion (the transition to the solution environment). When pH is lowered as described in step II, the hydrogen ions are coordinated with amino and carboxyl groups so that the surface charge of protonized algae becomes more positive at lower pH and the anionic Cr(VI) ions become easier to retain. The rate of formation speed of -I and -II. phases and speed of the reduction process increase at low pH because protons participate in the reaction. The effect of the functional groups in the biosorbent (containing electron donor atom) and the retention of Cr(VI) via amine groups can be formulated as follows. B: Biosorbent

\[
\text{B-NH}_2(s) + \text{HCrO}_4^-(aq) + \text{H}^+(aq) \rightarrow \text{BNH}_3^+ \cdots \text{HCrO}_4^-(s)
\]

34.63% and 38.13% values of C. barbata in raw and ester form and 26.31% and 31.48% values of raw and ester form of C. crinita were detected in binding Cr(VI) and no decrease in Cr(VI) retention, with a slight increase occurring, with forms which carboxyl groups were modified by esterification in comparison with the raw form. On the other hand, Cr(VI) uptake was 29.86% for C. barbata and 16.3% for C. crinita in the modification of carboxylation of amino groups with succinic acid. In the light of these values, the lowest retention rate was obtained in the modification with succinic acid. It was detected that a significant portion of the Cr(VI) ions during the chromium retention period in the solution, by reducing, converted to Cr(III). Oxidation of organic materials by reaction with Cr(VI) in acidic medium and reduction of Cr(VI) to Cr(III) is an expected result (chemical oxygen demand). However, this phenomenon occurs at different rates for each biological substance. Therefore, the investigation of Cr(VI) reduction capacities of algae in the Cr removal studies done with marine algae is a frequently applied process. The fundamental upon which a reduction process is based on a redox reaction (Mandal et al. 2017):

\[
\text{Organic substance} + \text{oxidizing agent} \rightarrow \text{CO}_2 + \text{H}_2\text{O}
\]

it is as C6H12O6 + 4Cr2O72− + 32H+ → 6CO2 + 8Cr3+ + 22H2O

The groups responsible for the reduction of Cr(VI) by biosorbents are electron donor polyphenolic and tannic groups, existing especially in great quantity in brown algae (Elagonvan et al. 2008). Possible reactions related to the reduction of Cr(VI) to Cr(III) can be shown as follows (Prabhakaran et al. 2009):

\[
\text{HCrO}_4^- + 7\text{H}^+ + 5\text{e}^- \rightarrow \text{Cr}^{3+} + 4\text{H}_2\text{O}
\]

\[
\text{H}_2\text{CrO}_4 + 6\text{H}^+ + 3\text{e}^- \rightarrow \text{Cr}^{3+} + 4\text{H}_2\text{O}
\]

\[
\text{Cr}_2\text{O}_7^{2-} + 14\text{H}^+ + 6\text{e}^- \rightarrow 2\text{Cr}^{3+} + 7\text{H}_2\text{O}
\]

We can show these reactions of reduction processes are generally biomass + Cr(VI) → biomass (oxidized) + Cr(III).

As shown in Figure 5, the highest rate of reduction of Cr(VI) occurred at 90.38% and 89% rates for C. barbata and C. crinita algae in the form of a proton. Cr(III) reduction yields were obtained at 81.6% and 82.86%, respectively, for C. barbata and C. crinita algae in the raw form. Cr(III) reduction yields varying between 75–85% for barbata and 70–85% for crinita were obtained in other species of modified algae. In the light of these results, it was determined that there was no additional significant increase yield in
modifications of algae studied both in the uptake and reduction of Cr(VI) and Cr(III) uptake. However, the purpose of all these modifications is to detect functional groups in biosorbents and their functions in removing Cr(III,VI), and to evaluate the characterization of algae in a broader sense. In this context, we can say that the acidic forms of the algae that would be evaluated for Cr(VI) retention and reduction removal are very suitable. Chemical treatments applied to the biosorbents naturally cause some mass losses. In the current study, the mass losses of C. barbata and C. crinita algae after acidic (HCl) treatment were determined as 18 ± 3% and 21 ± 2%, respectively. Since Cr(VI)-containing wastewaters were acidic, use of protonated biosorbents was preferred for removing Cr(VI) from aqueous solutions and effluents. As compared to the literature (Murphy et al. 2009), lower mass losses occurred advantageously in the studied biosorbents after acidic treatment.

The time effect on retention of Cr(III,VI) ions

The retention of Cr(III) ions: As can be seen in Figure 6, it was found that retention of Cr(III) ions is fast within the first 30 minutes in the intermittent contact test experiments at pH = 4.5, optimum retention for both algae occurred and reached a saturation capacity, by slowing down, within 1 hour and 2 hours. It was observed that it reached a stable zone, with no significant increase, within 2 and 3 hours. Therefore, Cr(III) retention studies were performed in 2 hours.

Retention of Cr(VI) ions: Cr(VI) uptake by C. barbata and C. crinita algae was studied over a period of 4–24 hours. It was determined that 3% retention occurred for C. barbata algae and no retention occurred for C. crinita algae in a 4 hour intermittent contact test at pH = 2.0 of Cr(VI) (Figure 7). It was determined that Cr(VI) retention in intermittent contact tests occurred at 7.54 and 18.5% rates with C. barbata at 6 and 8 hours and 4.77 and 7.27 rates with C. crinita. Experiments carried out over a 24-hour period resulted in 34.63% and 26.1% retention rates with raw algae. In contrast to Cr(III), Cr(VI) is retained rather slowly, but its mechanism has been determined to reduce Cr(VI) to Cr(III) considerably, as previously presented experiments show in detail. The removal of Cr(VI), the phase (mechanism (II)) that occurs indirectly through the functional groups in the algae, is a fairly slow process and a 24-hour intermittent contact test has been found to be necessary for the success of these processes because both phases (retention and reduction) are evaluated together.

Batch experiments

The Langmuir isotherm model was applied to explain biosorption equilibrium data. The batch capacity was 36.63 and 37.31 mg Cr(III) per gram of biosorbents for C. barbata and C. crinita algae, as demonstrated by a Langmuir-type adsorption isotherm (Figure 8).

In order to avoid some precipitation of Cr(III) ions, the experiments were performed at pH 4.5 and at room temperature (25 ± 2 °C). Higher temperatures and higher pH values were not applied since the probability of Cr hydrolysis increases with increasing temperature and pH, and surface precipitations would occur as Cr(OH)₃. Following the biosorption process with 100 mg/L concentration for Cr(III), elution was performed using 20 ml of 0.1 M HCl.
for 25 min, with success of approximately 98.6%. After elution of bound metal ions, the biomass was washed with water repeatedly before metal loading. The Langmuir capacities for biosorption of Cr(III,VI) by different biosorbents are also listed in Table 1.

Electroplating chromium wastewater treatment

The results of characterisation of Cr-plating-rinsing wastewater were found as follows: Cr(VI):104 ppm, Cu: ppm, 3.1 ppm, Ni: 4.3 ppm, Zn: 2.6 ppm, Na: 190 ppm, K: 21 ppm, pH: 4.0, TDS: 68 ppm. In batch experiments, the removal of Cr(VI) from electroplating wastewater was applied for 24 h since the retention and reduction of Cr(VI) were very slow. The retention efficiency was achieved at 83.5 ppm for C. barbata and 79.6 ppm for C. crinita. It was determined that the remaining chromium in the wastewater solution was completely converted to Cr(III) by the reduction of biosorbents. Cr(III) was completely removed after two hours of the batch process (pH 4.5) of the biosorbents with waste solutions. All the results have been given in Table 2.

CONCLUSIONS

The characterization analysis of brown sea algae Cystoseira barbata and Cystoseira crinita was performed by using potentiometric titration and FT-IR spectroscopy. The values of pKₐ = 3.76, 3.33 (C. barbata) and 3.82 (C. crinita) obtained as a result of potentiometric titration were found to be very close to the pKₐ values (M = 3.38, G = 3.65) of mannuronic and guluronic acid groups, which are alginic acid carboxyl groups responsible for the retention of metal ions. This structure in both algae was also confirmed by FT-IR spectrum. In the result of our study, the separation and analysis of Cr(III)/Cr(VI) were achieved by brown seaweeds C. barbata and C. crinita through natural chelating functional groups such as carboxyl, amino, sulphate and hydroxyl. It was determined that no retention occurs for Cr(VI) ions at pH = 4.5 while it...
S. Yağcı & M. Özyürek | Cr(III,VI) biosorption with two brown algae

**REFERENCES**


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**Table 2** Removal efficiency of chromium from electroplating wastewater with *C. barbata* and *C. crinita* algae

<table>
<thead>
<tr>
<th>Biosorbent</th>
<th>Cr(VI) removal efficiency, %</th>
<th>Reducing rate of Cr(VI) to Cr(III), %</th>
<th>Removal efficiency of reduced Cr(III), %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. barbata</em></td>
<td>80.3</td>
<td>19.7</td>
<td>100</td>
</tr>
<tr>
<td><em>C. crinita</em></td>
<td>76.5</td>
<td>23.5</td>
<td>100</td>
</tr>
</tbody>
</table>

*Initial chromium (Cr(VI)) concentration of wastewater: 104 ppm, pH = 2.0, 0.5 g H-form algae.

is maximum for the Cr(III) ion, and besides no retention occurs for Cr(III) ion at pH = 2.0 while it is maximum for the Cr(VI) ion and the Cr(VI) species, which is extremely toxic, is reduced to the non-toxic Cr(III) form. In acidic conditions, extremely toxic Cr(VI) can be removed from various aquatic environments by using *C. barbata* and *C. crinita* algae in the proton form by taking advantage of Cr(VI) reduction features through electron transfer occurring in the acidic environment of electron-donor groups. Since chromium-containing wastewaters are highly acidic in general, this behavior of biosorbents here-with provides an important advantage for chromium removal from wastewater. It is necessary to review Cr(VI) removal as the total chromium removal together with the rate of retention and reduction due to the fact that Cr(VI) is highly toxic in comparison with Cr(III). The removal process by reducing Cr(VI) to Cr(III) is a self-purification process. When studied with H-form algae, approximately 45% of Cr(VI) (initial conc. 100 ppm) is retained with functional groups on the surface of the algae, and the unreduced remaining amount is about 5% at the removal phase as Cr(VI) in the solution. Therefore, Cr(VI) removal is considered as total chromium removal that is dealt with by both processes, since it involves the reduction processes that take place together with the retention. The use of *C. barbata*, and *C. crinita* algae makes it possible to put into practice an environmentally friendly, cost-effective project to be used for the separation, analysis and purification of Cr(III,VI) species from aqueous solution and also both marine algae are provided abundantly and easily on seashores. In addition, on the basis of our studies, the requisite parameters have been set at a laboratory scale to purify electroplating wastewater, removing Cr(VI).

**ACKNOWLEDGEMENTS**

This research was supported by Istanbul University Scientific Research Fund (Project grant no. UDP-45494-46279 and 46145).


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First received 16 April 2018; accepted in revised form 3 January 2019. Available online 8 January 2019