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Enhancement strategies for Cu(II), Cr(III) and Cr(VI) remediation by a variety of seaweed species

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ABSTRACT

Various chemical treatments have been applied to six brown, red and green seaweed species with a view to enhancing their metal removal for Cu(II), Cr(III) and Cr(VI). Treatment with acetone resulted in the greatest enhancement for both cationic and anionic species with relatively low mass losses (15–35%), indicating its low risk to biomass operational stability. Cation binding was increased by 69%, while the total Cr removal was augmented by 15%. Cr(VI) binding was shown to be an adsorption-coupled reduction, whereby Cr(VI) was bound to the biomass surface at pH 2 and subsequently reduced to Cr(III). Acetone treatment also resulted in biomasses that were capable of converting up to 83% of Cr(VI) in solution to Cr(III). Blocking of carboxyl and amino functionalities had significant negative effects both on total Cr removal as well as percentage conversion of Cr(VI) to Cr(III). Results therefore indicated the significant role played by these moieties in metal binding to these seaweeds. Potentiometric titrations displayed agreement between the degree of esterification and the decrease in Cu(II) removal for *Ulva* spp. and *Polysiphonia lanosa*. FTIR analysis identified changes in biomass functionality and availability after chemical modification, the results of which were in agreement with metal removal studies. In conclusion, these biosorbents represent suitable candidates to replace conventional removal technologies for metal bearing wastewaters, in particular for the detoxification of hazardous Cr(VI) waste streams.

1. Introduction

Heavy metal contamination of aquatic environments is a widespread phenomenon. This is especially true in developing countries where high-cost remediation technology is not affordable [1]. Conventional metal removal processes such as ion-exchange, activated carbon adsorption, reverse osmosis, and membrane filtration can be expensive or ineffective at low concentrations (<100 mg L^{-1}) and may cause additional issues for waste disposal [2–6].

Thus, the development of alternative metal removal technologies, including biosorption, is essential. Seaweed biomass represents a viable solution for metal removal as these materials are benign, cost-effective, renewable and extremely effective in binding metal ions [7–9].

Biosorption may be based on one or more of a number of mechanisms including ion-exchange, physical adsorption, complexation and precipitation [7,10]. Numerous functionalities including –COOH, $-OSO_3$, $-NH_2$ and –OH are responsible for metal biosorption by seaweeds [10], with their relative importance depending the quantity of sites, their accessibility and affinity between site and metal [11]. Extensive studies have reported that biosorption of cations by seaweeds occurs predominately via an ion-exchange mechanism with some complexation also taking place [3,7,10], while anion binding is primarily by an adsorption-coupled reduction mechanism [12–15].

While cationic Cu(II) is an essential element, prolonged exposure to the metal causes adverse health effects and large acute doses can potentially produce fatal effects [16]. Chromium is commonly used in tanning, electroplating, pigmentation and as a catalyst for corrosion inhibitors and wood preservatives [6,17,18]. While both hexavalent ($HCrO_4^-$ and $Cr_2O_7^{2-}$) and trivalent (Cr^{3+} and $CrOH^{2+}$) species are present in industrial waste solutions, Cr(VI) is considered more hazardous to public health due to its mutagenic and carcinogenic properties [18]. Thus, the maximum allowable level for Cr(VI) in domestic water supplies is 0.05 mg L^{-1}, while total Cr, including Cr(III), Cr(VI), and its other forms, is regulated to below 2 mg L^{-1} [5].

The present study compares Cu(II), Cr(III) and Cr(VI) removal by six raw and chemically modified seaweeds in order to identify the most effective treatment methodology for enhancement of

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binding. The primary incentives for biomass modification include the reduced quantities of raw material required for operation and increased removal leading to lower process costs as well as producing industrial discharges which meet the safe and allowable limits set by various agencies. The seaweeds under investigation are: *Fucus vesiculosus* and *Fucus spiralis* (brown), *Ulva lactuca* and *Ulva* spp. (green), and *Palmaria palmata* and *Polysiphonia lanosa* (red). Metal binding studies identify the ability of raw and modified biomasses to remove Cu(II), Cr(III) and total Cr from solution, while Cr speciation studies reveal the ability of the seaweeds to reduce Cr(VI) to Cr(III) on their surface. Potentiometric titrations described carboxyl availability after esterification, while FTIR analysis demonstrated changes in biomass functionality following modification. A novel and comprehensive overview of seven chemical modification techniques on six seaweeds for three heavy metal species is presented in this work. A study with this breadth has not previously been found in the literature, where studies typically involve a number of modifications for a single seaweed species and metal ion [4,12] or one modification technique for a single species and two metal ions [19].

2. Materials and methods

2.1. Biomass preparation

Seaweeds were harvested from Fethard-on-Sea, County Wexford, Ireland (52°11′53.68″N, 6°49′34.36″W), rinsed thoroughly with distilled water, oven-dried at 60 °C for 24 h and subsequently ground and sieved to a particle size of 500–850 μm.

2.2. Treatment with acid, alkali, organic solvents and other chemicals

Solutions of mineral acid (HCl), alkali (NaOH) and other chemicals ($CaCl_2$, CH_3COCH_3 and Na_2EDTA) at a concentration of 0.1 M were selected. Five grams of raw biomass were mixed with 200 mL of these solutions for 6 h at room temperature (RT) (21 ± 1 °C) and 200 rpm. Modified biomasses were vacuum filtered, washed with distilled water and oven-dried for 24 h at 60 °C. Samples were reweighed after treatment to establish any mass losses caused by modification.

2.3. Methylation of amino groups

Five grams of raw biomass was mixed with 100 mL of formaldehyde (HCHO) and 200 mL of formic acid (HCOOH), and the reaction mixture was shaken on a rotary shaker for 6 h at 200 rpm and RT. The biomass was vacuum filtered, washed with distilled water and oven-dried at 60 °C for 24 h.

2.4. Esterification of carboxyl groups

Five grams of the raw biomass was suspended in 500 mL of anhydrous methanol with 5 mL of concentrated hydrochloric acid added to the suspension. The reaction mixture was shaken at 200 rpm and RT for 6 h. The biomass was filtered under vacuum, washed with distilled water and oven-dried for 24 h at 60 °C.

2.5. Metal solutions

Standard metal solutions (analytical grade) each containing 1000 mg L^{-1} (Cu(II) as Cu $(NO_3)_2$, Cr(III) as Cr $(NO_3)_3$ and Cr(VI) as $K_2Cr_2O_7$) were purchased from Sigma–Aldrich (Ireland). Working solutions (250 mg L^{-1}) were prepared by dilution of the stock with distilled water. Adjustment of solution pH to the desired value was achieved using 0.1 M NaOH and HCl. Total solution metal concentrations were determined using Atomic Absorption Spectrophotometry (SpectrAA-600 VARIAN, Software version 4.10, flame mode).

2.6. Batch metal uptake studies

Approximately 100 mg of biomass was dispersed in 50 mL metal solution which had been adjusted to the optimum pH. These values were pH 5 for Cu(II), pH 4.5 for Cr(III) and pH 2 for Cr(VI) as per previous studies [20,21]. In all cases, the solution pH was monitored and controlled at the optimum value using 0.1 M HCl and NaOH solutions, with the change in the working volume due to the addition of acid or base being negligible. Samples were shaken for 4 h to ensure that equilibrium had been reached [20,21] while being maintained at 200 rpm and RT. Samples were vacuum filtered and washed with distilled water. After appropriate dilution, solution metal content was measured using AAS. Three independent replicate samples were analysed in each case.

2.7. Chromium speciation studies

Total chromium, i.e. Cr(III) and Cr(VI), in solution was measured by AAS as outlined in Section 2.5. A colorimetric method, as described by Clesceri et al. [22], was subsequently used to measure the concentrations of the various Cr species in solution. The pink colored complex formed by the reaction of 1,5-diphenycarbazide with Cr(VI) in acidic solution was analysed by UV at 540 nm (Cary 50 UV). The Cr(III) concentration was calculated from the difference between the total Cr and Cr(VI) concentrations. Three independent replicate samples were analysed in each case.

2.8. Titration of esterified seaweeds

Titration of esterified seaweeds was carried out according to a procedure detailed by Murphy et al. [20] with triplicate samples analysed in all cases.

2.9. FTIR analysis

Dried biomass particles were directly analysed by Attenuated Total Reflectance (ATR) using a Digilab Scimitar Series infrared spectrometer (MIRacle^{TM} Single Reflection HATR diamond accessory). Samples were maintained in optical contact with the diamond using a micrometer screw press attachment. A background scan with the diamond in place was run before each analysis. Three independent replicates were analysed for all samples in the range 4000–400 cm^{-1} with spectra generated from a combination of 40 scans at a resolution of 2 cm^{-1}.

3. Results and discussion

3.1. Chemical treatments

In this study, seaweed biomass has been modified to maximise removal of Cu(II), Cr(III) and Cr(VI) from solution. The concept of modification with a view to capacity enhancement is that, once modified, the seaweeds can be immobilised and utilised in fixed columns within an industrial setting. From an industrial cost and environmental perspective, this means that fewer chemicals, e.g. reductants, and lower quantities of raw materials are required for operation, thus making biosorption processes more economically viable. Results of metal binding studies for raw and modified biomasses are summarised in Fig. 1 with mass losses after modification given in Table 1.

Mass loss is a major factor in selecting a suitable treatment methodology, with lower mass losses being desirable for scale-up

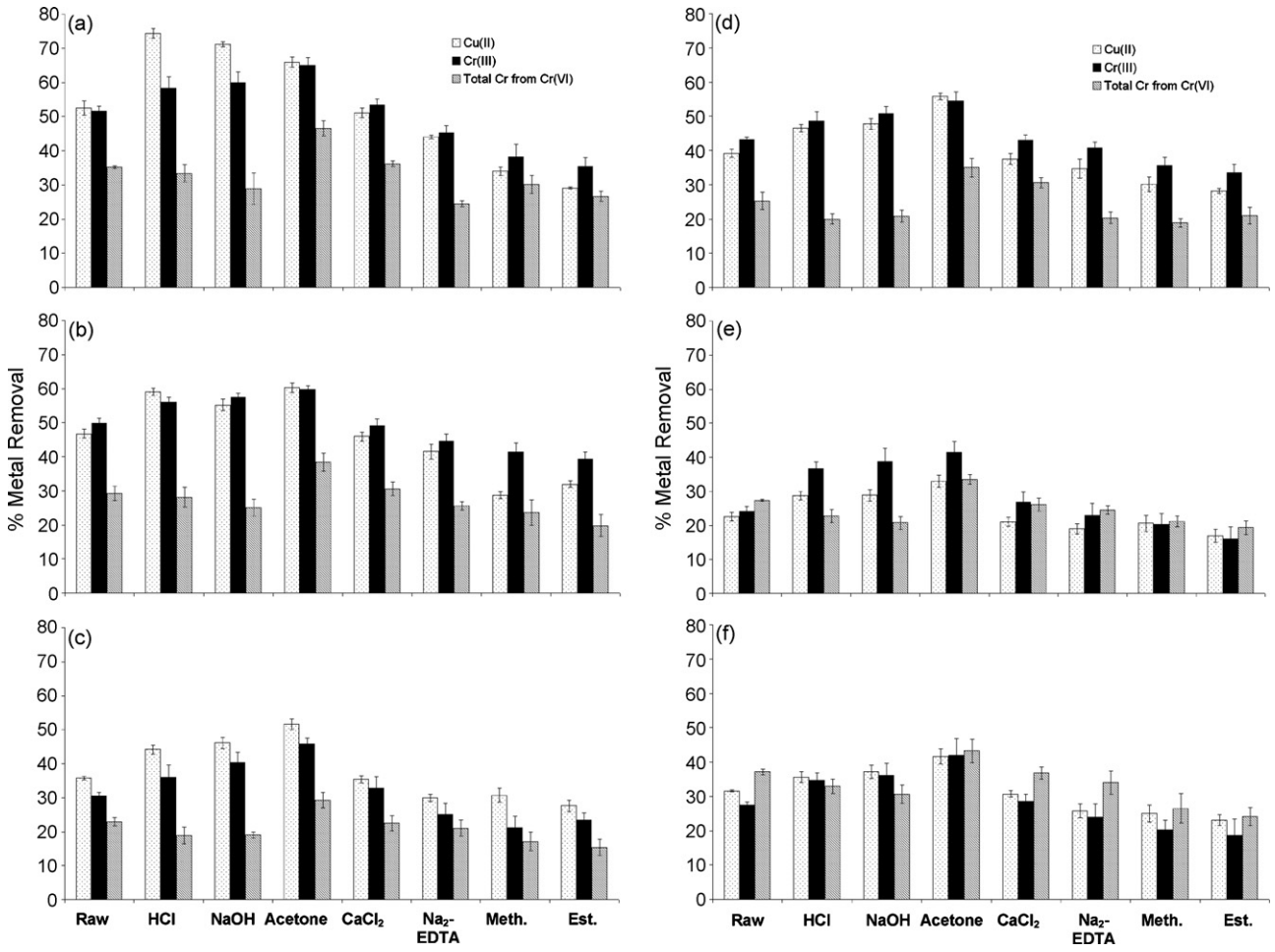

Fig. 1. Effect of chemical treatment on Cu(II), Cr(III) and total Cr removal by (a) *Fucus vesiculosus* (b) *Fucus spiralis* (c) *Ulva lactuca* (d) *Ulva* spp. (e) *Palmaria palmata* (f) *Polysiphonia lanosa*. Meth. = Methylation of amino groups; Est. = Esterification of carboxyl groups. Error bars are calculated based on triplicate analyses with 95% Confidence Intervals.

and industrial operations. From Table 1 it is clear that, in terms of mass loss, acid treatment is most destructive towards (45–49% loss) while $CaCl_2$ is the least (10–21% loss). The low mass losses observed with $CaCl_2$ may also point to its suitability as a desorption agent for regeneration of metal-loaded biomass.

Acid treatment replaces alkali and alkaline earth metals (e.g. Na(I), K(I), Mg(II) and Ca(II)), naturally found in the seaweed cell wall, with protons [7]. This replacement, coupled with the release of soluble biomass material and smaller organic molecules, results in large mass losses after treatment [23]. Therefore it is crucial to balance the benefit associated with enhanced metal removal with the increased demand for raw material to make the process economically viable.

Seaweed functionalities such as sulphonate ($-OSO_3$) and carboxyl (–COOH) display acidic characteristics and therefore, the pH at which maximum metal uptake occurs is related to the pK_a of these groups. The point of zero charge (PZC) of these seaweeds was previously determined by Murphy et al. [20] and on average, a value of approximately 6.18 was observed. Thus, below the PZC the biomass still has a net positive charge despite the presence of dissociated negatively charged functionalities. As a result, pH variation will significantly affect the binding behaviour of metal species. At pH >3.5, dissociation of these functionalities leads to increased negative charge on the biomass, so, whereas anionic Cr(VI) species are repelled at these pH values, cationic Cu(II) and Cr(III) experience increased attraction to the biomass resulting in improved metal

Table 1
Mass loss (%) for six seaweeds after chemical treatment. Error bars are calculated based on triplicate analyses with 95% Confidence Intervals.

Treatment	Mass loss (%)					
	Fucus vesiculosus	*Fucus spiralis*	*Ulva lactuca*	*Ulva* spp.	*Palmaria palmata*	*Polysiphonia lanosa*
HCl	49 ± 2	47 ± 3	45 ± 1	45 ± 2	45 ± 1	49 ± 2
NaOH	41 ± 3	45 ± 4	43 ± 2	41 ± 2	35 ± 3	56 ± 3
Acetone	21 ± 1	15 ± 1	32 ± 4	33 ± 3	24 ± 1	35 ± 2
$CaCl_2$	10 ± 3	19 ± 4	11 ± 3	21 ± 2	12 ± 1	11 ± 2
Na_2-EDTA	29 ± 4	27 ± 2	34 ± 3	20 ± 2	23 ± 2	30 ± 3
Methylation	18 ± 3	14 ± 3	11 ± 1	10 ± 2	20 ± 3	30 ± 4
Esterification	19 ± 2	23 ± 4	39 ± 3	30 ± 5	21 ± 2	36 ± 2

Table 2
Langmuir parameters for Cu(II) biosorption by six seaweeds. Errors bars are calculated based on triplicate analyses with 95% Confidence Intervals.

	q_{max} (mmol g^{-1})	b (mM^{-1})	r^2
Fucus vesiculosus	1.02 ± 0.10	5.45 ± 0.33	0.98
Fucus spiralis	0.91 ± 0.06	4.58 ± 0.28	0.98
Ulva lactuca	0.69 ± 0.08	2.85 ± 0.19	0.99
Ulva spp.	0.74 ± 0.09	2.52 ± 0.15	0.96
Palmaria palmata	0.43 ± 0.04	11.2 ± 0.53	0.97
Polysiphonia lanosa	0.61 ± 0.05	5.57 ± 0.26	0.99

binding. As solution pH decreases, functionalities such as amino (–NH_2) and carboxyl groups may become protonated, thus making the biomass more positively charged and hence creating an electrostatic attraction with Cr(VI) species.

Although modification may affect the pH dependence of these biosorbents, similar pH values were selected for metal binding studies with both the raw and modified biomasses. As previously described in Section 2.6, solution pH values were periodically monitored and re-adjusted to maintain optimum values. The effect of pH on Cu(II), Cr(III) and Cr(VI) binding to these seaweeds has been extensively studied by Murphy et al. [20,21]. Therefore, the use of similar experimental conditions facilitates a direct comparison between metal removal before and after modification.

The authors previously carried out a comprehensive isotherm study where Langmuir and Freundlich models were applied to the six seaweeds in this study. Closer data fits were obtained with the Langmuir model indicating that monolayer sorption dominates for these species. Full Langmuir isotherm data has previously been published for Cr(III) and Cr(VI) binding to these six seaweeds [21] and this data, coupled with Cu(II) isotherm data (Table 2) has been extracted in support of the findings in the present study. Results indicated that *F. vesiculosus* had the largest q_{max} values for Cu(II) and Cr(III), being 1.02 and 1.21 mmol g^{-1}, respectively. However, *P. lanosa* had the largest q_{max} for Cr(VI) binding (0.88 mmol g^{-1}), thus pointing to the increased capacity of the red seaweeds for binding of hexavalent chromium.

On the other hand, binding affinity values (***b*** values) revealed that, in all cases, *P. palmata* had the highest affinity for metal ions, i.e. this seaweed was most suitable for treatment of dilute metal solutions. The ***b*** values obtained for *P. palmata* were 11.2, 4.9 and 8.6 mM^{-1} for Cu(II), Cr(III) and Cr(VI), respectively. Therefore, trends in metal removal for the raw seaweeds in the present study are in agreement with previous results obtained for the same seaweeds.

Fig. 1 shows that, in all cases, acid treatment brought about an increase in Cu(II) and Cr(III) removal (13–51%) but a decrease in total Cr removal (4–19%). Cation removal trends may be explained by a decrease in competition between native ions and heavy metal ions for biomass binding sites after acid treatment. These native ions are substituted by protons on biomass binding sites, thus making them available for subsequent metal binding [23]. Acid hydrolysis of functional groups may also generate more anionic sites for cation sorption [24].

Park et al. [12] reported that alkali pre-treatment improved cation biosorption by removing extraneous materials that may mask binding sites. According to Fig. 1, alkali treatment of the biomass with 0.1 M NaOH brought about significant increases in cation removal (17–59%). However, a decrease in total Cr removal was observed (13–22%) for all seaweeds. Alkali-treatment also causes breakage of cellulose polymers, thus hindering the operational stability of the biomass [25], resulting in large mass losses similar to those observed in Table 1 (35–56%).

Various authors have observed similar favourable effects for metal binding after alkali treatment. Lodeiro et al. [4] reported a 32% increase in Cd(II) binding while Mehta et al. [26] found that alkali treatment increased Cu(II) sorption by *Chlorella vulgaris*. However, Park et al. [12] reported a 14% decrease in total Cr removal by *Ecklonia* sp. after modification with NaOH. This compares well with the range of values (Fig. 1) obtained in the present study (13–22%).

In all cases, acetone pre-treatment resulted in enhanced Cu(II), Cr(III) and total Cr removal ranging from 15 to 69% (Fig. 1). This is in contrast to acid and alkali treatments which increased cation removal only. Ashkenazy et al. [27] and Lodeiro et al. [28] reported that acetone modification of biomasses resulted in increased Pb(II) and Cd(II) removal, respectively. Such treatment may remove protein and lipid fractions of the biomass surface, thus exposing more metal binding sites and improving metal uptake. Park et al. [12] also reported increased binding of total Cr to *Ecklonia* sp. after acetone treatment. Therefore, results obtained in the present study are in agreement with previously published findings for metal removal by a number of biomass types.

From Table 1 it is seen that relatively low mass losses (15–35%) were associated with acetone treatment. These results are significant in terms of identifying the susceptibility of the biomass to solvent effects in an industrial context. While acetone treatment enhances metal removal, it may over time reduce the operational stability of the biomasses, thus reducing the potential for column regenerations and increasing process costs. Therefore, for each seaweed-modification combination it is necessary to balance any increased metal removal with any detrimental effects on biomass stability and reusability.

No significant changes in metal removal after $CaCl_2$ modification were observed in Fig. 1 indicating that this treatment had little effect on metal binding and was not an ideal candidate for enhancement of metal removal for these seaweeds.

In Fig. 1, modification with Na_2-EDTA resulted in decreased metal removal for all seaweed-metal combinations, ranging from 4% for Cr(III) binding to *Ulva* spp. to 29% for total Cr binding to *F. vesiculosus*. Park et al. [12] found that treatment by 1 M Na_2-EDTA caused serious degradation of *Ecklonia* sp. cell walls, resulting in complete solubilisation of the biomass. Breakage of cell wall polymers results in reduced quantities of biomass binding sites, accounting for the decreased metal removal observed in this study [7].

3.2. Methylation of amino groups

FTIR analysis by Murphy et al. [20,21] previously showed the importance of amino groups in metal binding to these seaweeds. Therefore, blocking their availability should significantly decrease metal removal. In Fig. 1, all seaweeds showed diminished Cu(II) removal with brown seaweeds decreasing by 36% for *F. vesiculosus* and 39% for *F. spiralis*. The decreased removal for the remaining seaweeds was much lower; *Ulva* spp. (21%), *P. lanosa* (22%), *U. lactuca* (13%) and *P. palmata* (8%), indicating that amino groups had a less significant role to play in Cu(II) binding to these seaweeds. For Cr(III) (Fig. 1), metal removal decreased in the order: *U. lactuca* (30%) > *P. lanosa* (27%) and *F. vesiculosus* (26%) > *F. spiralis*, *Ulva* spp. and *P. palmata* (17%). In the present study, a decrease in total Cr removal was observed in all seaweeds after methylation: *P. lanosa* (30%), *U. lactuca*, *Ulva* spp. and *P. palmata* (23%), *F. vesiculosus* and *F. spiralis* (13 and 19%, respectively). Results therefore indicate that amino groups play a significant role in total Cr removal by red seaweeds followed by green and brown species.

At low pH, protonation of –COOH and –NH_2 functionalities gives the biomass an overall positive surface charge resulting in the attraction of negatively charged species such as Cr(VI) [29]. However, if amino groups have been methylated, they will no longer become protonated in the low pH range and as a result will be unavailable for Cr(VI) binding [12]. Total Cr removal and the mech-

anism of Cr(VI) binding by these seaweeds will be further discussed in Section 3.4.

3.3. Esterification of carboxyl groups

Based on potentiometric [20] and FTIR studies [20,21], Murphy et al. reported that overall metal removal by the seaweeds is strongly related to their –COOH content. The same authors also reported that these functionalities were the most abundant on the seaweed surface. Thus, esterification of these functionalities should result in significantly decreased metal binding. Fig. 1 clearly reveals decreased metal removal after carboxyl esterification for all species. Cu(II) removal decreased in the order: *F. vesiculosus* (44%) > *F. spiralis* (32%) > *Ulva* spp., *P. palmata* and *P. lanosa* (25%) > *U. lactuca* (23%). The relationship between Cu(II) uptake and quantity of carboxyl groups is further discussed in Section 3.5. Decreases in Cr(III) removal followed the order: *P. palmata* (33%) > *P. lanosa* and *F. vesiculosus* (31%) > *U. lactuca* (22%), *Ulva* spp. (21%) and *F. spiralis* (19%), indicating that –COOH modification has a greater effect on Cr(III) binding in red seaweeds than in brown or green species.

Park et al. [12] observed that esterification of biomass carboxyl functionalities decreased total Cr removal with similar results observed in the present study (Fig. 1). Metal removal decreased in the order: *P. lanosa* (34%) > *U. lactuca* (31%) and *F. spiralis* (30%) and *P. palmata* (28%) > *F. vesiculosus* (22%) > *Ulva* spp. (15%). As previously discussed, while –COOH groups may be protonated at low pH, blocking the availability of these groups through esterification results in a reduction of positive charge on the biomass at low pH, thus decreasing total Cr and Cr(VI) removal.

3.4. Effect of chemical treatment on Cr(VI) reduction

Park et al. [12] previously reported that Cr(VI) can be removed from the aqueous phase by non-living biomass through two mechanisms: direct and indirect reduction. In direct reduction Cr(VI) is reduced to Cr(III) in the aqueous phase by contact with electron-donor groups of the biomass, i.e. groups having lower reduction potentials than that of Cr(VI) (+1.3 V). The alternative mechanism, indirect reduction, consists of a number of steps: (1) binding of anionic Cr(VI) to positively charged biomass sites (2) reduction of Cr(VI) to Cr(III) by adjacent electron-donor groups, and (3) release of Cr(III) ions back into solution due to electronic repulsion, or complexation of Cr(III) with adjacent groups capable of Cr-binding.

Previous XPS studies by Murphy et al. [30] revealed that both Cr(VI) and Cr(III) were present on the biomass surface indicating that binding predominantly took place via indirect reduction, i.e. binding of anionic Cr(VI) with subsequent reduction to Cr(III) on the seaweed surface. At an initial solution Cr(VI) concentration of 2000 mg L^{-1}, *F. vesiculosus*, *Ulva* spp. and *P. palmata* respectively had 64%, 75% and 69% Cr(III) associated with their surface indicating that significant reduction of Cr(VI) had taken place. The study [30] also reported total Cr removal in the range of 14–18% over a 6-h period with solution conversion of Cr(VI) to Cr(III) in the range 34–51%. Therefore, it appears that the rate limiting step of Cr(VI) reduction is the initial electrostatic binding of Cr(VI) to the seaweed surface, as the anionic species predominantly needs to be bound in order to be reduced to Cr(III).

Fig. 1 revealed that acid, alkali, and Na_2-EDTA treatments, as well as amino methylation and carboxyl esterification, had a negative impact on the total Cr removed from solution. Values for percentage conversion of Cr(VI) to Cr(III) in solution before and after chemical treatment are illustrated in Fig. 2.

In raw seaweeds, solution conversion of Cr(VI) to Cr(III) ranged from 58% for *U. lactuca* to 78% for *F. vesiculosus* thus pointing to significant detoxification of Cr(VI) by these seaweeds over a 4-h period. After HCl and acetone exposure, a general increase in the percentage Cr(III) in solution was observed for all seaweeds. Percentage conversions for these treatments were in the range 64–84% and 63–83%, respectively. Increased Cr(VI) reduction after acid treatment may have been due to the removal of native ions which allows seaweed functionalities to be more easily protonated at low pH and bind anionic Cr(VI) or due to removal of extraneous materials which may block binding sites by acetone treatment [7]. Conversely, NaOH and Na_2-EDTA had a negative effect on the conversion of Cr(VI) to Cr(III) in solution with values ranging from 41 to 51% (NaOH) and 35–55% (Na_2-EDTA). NaOH washing may increase the negative charge on the biomass resulting in increased repulsion with anionic Cr(VI). While Na_2-EDTA washing may have destroyed some Cr(VI) binding sites and resulted in a decreased ability of the seaweeds to bind and reduce Cr(VI), treatment with $CaCl_2$ had little effect on the percentage conversion between species.

As expected, methylation and esterification of biomass functionalities had a major negative influence on the conversion of Cr(VI) to Cr(III) by these seaweeds. Conversion of Cr(VI) to Cr(III) in methylated samples ranged from 31 to 45% while that observed in esterified samples was between 28 and 39%. The large negative effect of these modifications is due to blocking of binding sites and clearly illustrates the importance of amino and carboxyl in Cr(VI) removal and reduction. As shown by Murphy et al. [30], Cr(VI) binding is predominately by an adsorption-coupled reduction for these seaweed species. Therefore, the majority of Cr(VI) in solution must be bound to the biomass surface in order to be reduced to Cr(III). Amino and carboxyl groups which are normally protonated at pH 2 are no longer available for binding to anionic Cr(VI) by electrostatic attraction after modification. Therefore, Cr(VI) cannot bind and be subsequently reduced resulting in decreased conversion of Cr(VI) to Cr(III).

Table 3
Surface acidic groups of esterified seaweed biomass as determined by potentiometric titration. Error bars are calculated based on triplicate analyses with 95% Confidence Intervals.

	Acidic groups (mmol g^{-1})		
	Total	Strong	Weak (–COOH)
Fucus vesiculosus			
Raw	2.44 ± 0.22[a]	0.44 ± 0.16[a]	2.00 ± 0.20[a]
Esterified	1.94 ± 0.21	0.19 ± 0.07	1.73 ± 0.14
Ulva spp.			
Raw	1.94 ± 0.13[a]	0.44 ± 0.11[a]	1.50 ± 0.22[a]
Esterified	1.31 ± 0.13	0.19 ± 0.09	1.12 ± 0.11
Polysiphonia lanosa			
Raw	1.81 ± 0.16[a]	0.19 ± 0.06[a]	1.62 ± 0.11[a]
Esterified	1.44 ± 0.09	0.19 ± 0.08	1.25 ± 0.08

[a] Titration data obtained from Murphy et al. [20].

3.5. Titration of esterified seaweeds

Fourest and Volesky [31] used potentiometric titration to establish the extent of carboxyl esterification after chemical modification. In the present study, the relationship between degree of carboxyl blocking and metal uptake is also examined. Fig. 3 compares first derivative plots of titration curves obtained for raw and esterified *F. vesiculosus*, *Ulva* spp. and *P. lanosa* with the quantity of acidic groups summarised in Table 3.

First derivative plots consist of the midpoint of successive amounts of NaOH added (*x*-axis) versus dpH/dV (*y*-axis). Reading the peak location on the *x*-axis gives the number of biomass acidic groups. Strong functionalities (–OSO_3) are determined from the first peak in Fig. 3, the total number of acidic groups is determined from the final peak and the weak acidities (–COOH) are calculated by difference.

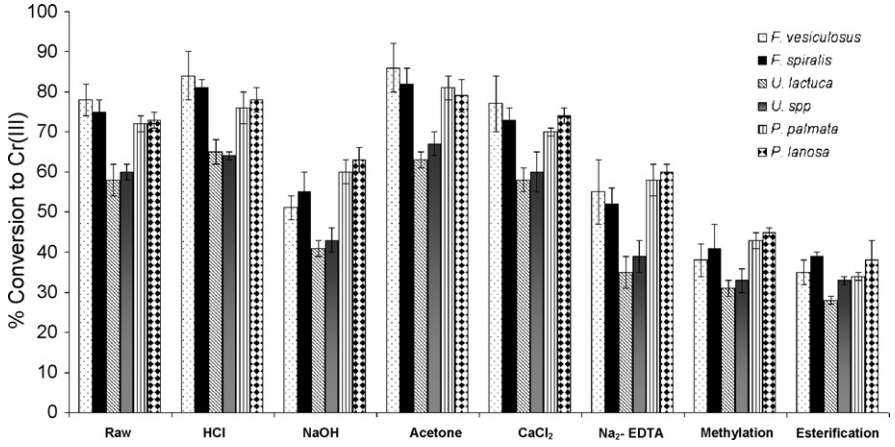

Fig. 2. Percentage conversion of Cr(VI) to Cr(III) before and after chemical modification. Initial Cr(VI) concentration = 250 mg L^{-1}, Biomass concentration = 2 mg mL^{-1}, pH 2. Error bars are calculated based on triplicate analyses with 95% Confidence Intervals.

Loss of complexity in the carboxyl region of the plots in Fig. 3 as well as decreases in the quantity of weak acidic functionalities (Table 3) verifies that blocking of carboxyl groups had taken place after esterification. Murphy et al. [20] reported strong correlation ($r^2 = 0.98$) between Cu(II) uptake and the quantity of acidic (anionic) binding sites for these seaweeds. Therefore, only data for Cu(II) binding shall be discussed here. The decrease in total acidic groups was 0.50, 0.63 and 0.37 mmol g^{-1} for *F. vesiculosus*, *Ulva* spp. and *P. lanosa* respectively, of which 0.27, 0.38 and 0.37 mmol g^{-1} were attributed to carboxyl functionalities (Table 3). Since two carboxyl groups are necessary for chelation of one divalent metal cation [31], it was anticipated that if carboxyl groups decreased by 0.27 mmol g^{-1} (as in *F. vesiculosus*), then the corresponding decrease in Cu(II) binding should be approximately 0.14 mmol g^{-1}. Similarly for *Ulva* spp. and *P. lanosa*, the magnitude of the expected decrease in Cu(II) binding was 0.19 and 0.18 mmol g^{-1}, respectively.

Good agreement between degree of carboxyl blocking and corresponding decrease in Cu(II) binding was found for *Ulva* spp. (0.19 mmol g^{-1}) and *P. lanosa* (0.16 mmol g^{-1}). However, the decrease in Cu(II) binding to *F. vesiculosus* was in fact 0.38 mmol g^{-1} as opposed to 0.14 mmol g^{-1}. Therefore, the decrease in binding must also be related to inhibition of some functionality in addition to carboxyl. Kantor and Schubert [32] described the desulphonation reactions that may accompany esterification and these reactions are represented by a decrease in the quantity of strong acidic groups (Table 3) with a reduction of 0.25 mmol g^{-1} observed for *F. vesiculosus* and *Ulva* spp. Therefore, these reactions may also contribute to decreased metal binding in esterified samples.

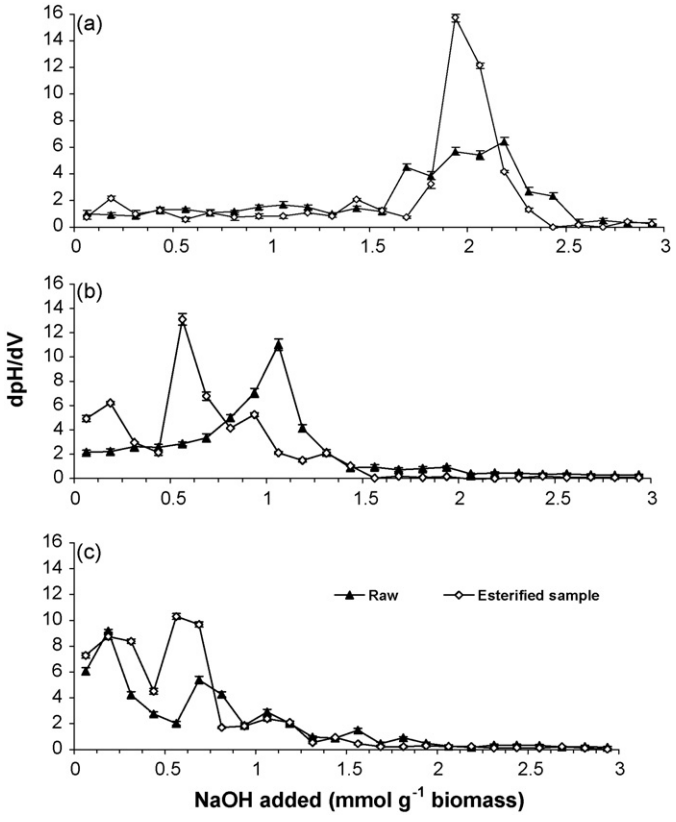

Fig. 3. First derivative potentiometric titration curves for (a) *Fucus vesiculosus* (b) *Ulva* spp. (c) *Polysiphonia lanosa*. Error bars are calculated based on triplicate analyses with 95% Confidence Intervals. Data for raw samples is taken from Murphy et al. [20].

3.6. FTIR analysis

FTIR analysis has been used to identify changes in biomass functionality after metal binding and modification in order to assess their availability and to predict biomass stability. The FTIR spectra of the raw and modified seaweeds (Fig. 4) display a number of absorption peaks, indicating the complex nature of the biosorbent.

Assignment of the FTIR peaks to specific functional groups is based on the work of Clothup et al. [33]. Previous FTIR studies on Cr [21] and Cu [20] binding to these seaweeds indicated that, in all cases, bidentate complexation between metal and biomass ligands was an important binding mechanism. Cu(II) binding was shown to be largely dependent on carboxyl, amino and sulphonate functionalities in all seaweeds, except in the case of *P. palmata,* where, Cu(II) had little effect on biomass amino groups. This is in agreement with results of amino methylation where Cu(II) removal was decreased by 8% only, thus potentially indicating that amino groups are less significant for Cu(II) removal by this seaweed. Only *F. spiralis*, *Ulva* spp. and *P. lanosa* showed any interaction with hydroxyl moieties. Cr(III) and Cr(VI) binding involved participation from amino, carboxyl and sulphonate functionalities. Hydroxyl groups were important for Cr binding to green seaweeds and the red seaweed *P. lanosa*.

Carboxyl functionalities can be attributed to the bands at 1715 cm^{-1} (free C=O), 1624 cm^{-1} (asymmetric C=O), 1451 cm^{-1} (symmetric C=O) and 1207 cm^{-1} (C—O stretch). The amide II band relating to –NH stretching is visible at 1539 cm^{-1} while asymmetric and symmetric stretching of sulphonate groups ($-OSO_3$) occurs at 1364 cm^{-1} and 1146 cm^{-1}, respectively. The band relating to C—O stretching of alcoholic groups occurs at approximately 1027 cm^{-1}.

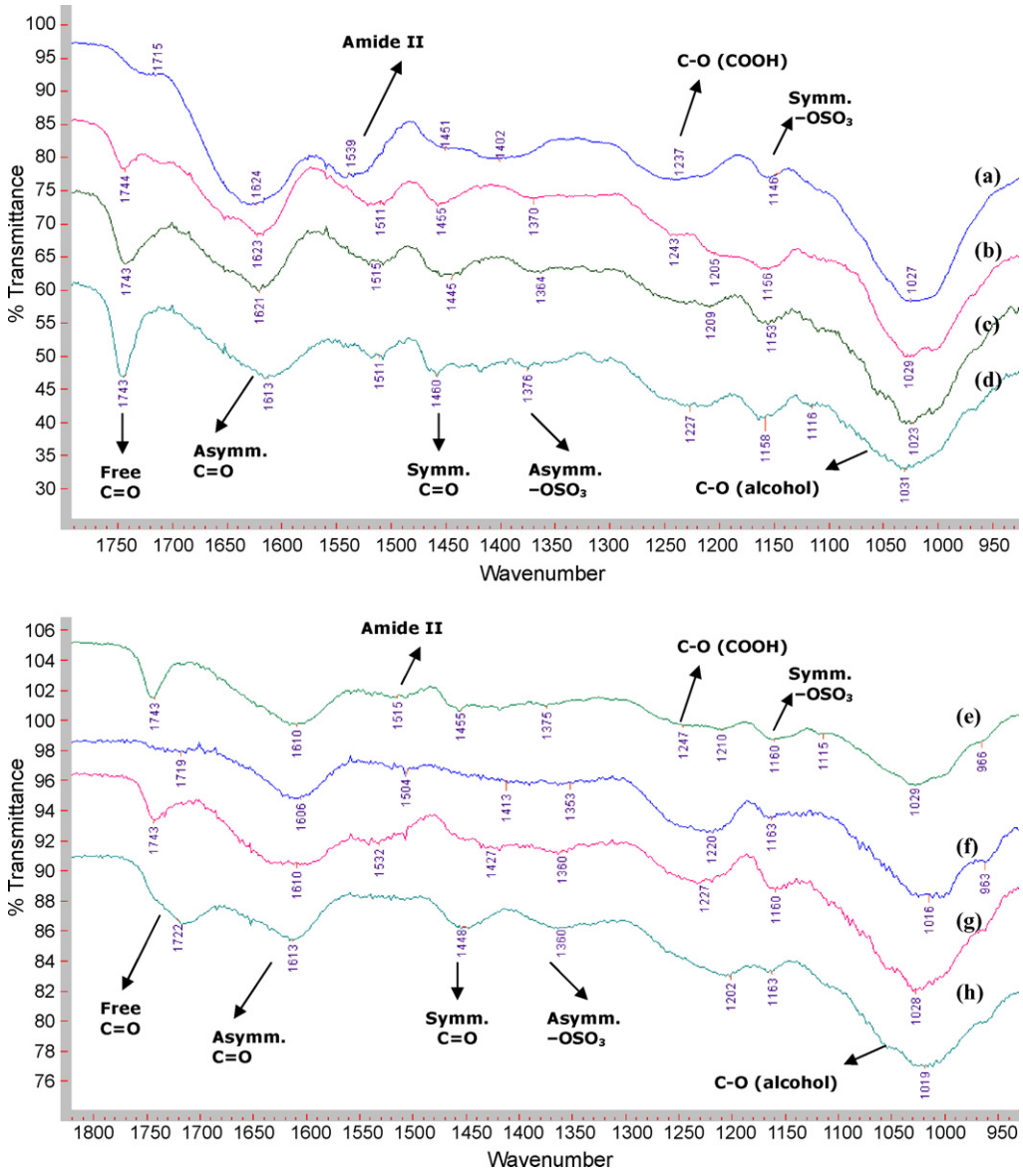

Fig. 4. FTIR spectra of *Fucus vesiculosus* before and after chemical modification (a) raw (b) acid treated (c) alkali treated (d) acetone washed (e) $CaCl_2$ washed (f) Na_2-EDTA washed (g) esterification of carboxyl groups (h) methylation of amino groups. Number of scans: 40, resolution: 2 cm^{-1}. Sample spectra from triplicate analyses are shown.

Significant changes in biomass functionalities are visible after acid treatment (Fig. 4b) which cleans the cell wall, replacing the natural mix of ionic species with protons. Frequency changes of the free C=O stretch (1744 cm^{-1}), asymmetric and symmetric C=O stretches (1623 and 1455 cm^{-1}, respectively) and C—O (COOH) stretch (1402 cm^{-1} decreasing to 1205 cm^{-1}) are due to replacement of alkali and alkaline earth metal ions with H^+ ions during protonation. Replacement of these ions alters the symmetry of biomass functionalities, altering their accessibility for metal binding and causing the observed wavenumber changes.

Treatment with NaOH had a comparable effect on the FTIR spectrum of *F. vesiculosus* to that of HCl (Fig. 4c). Similar frequency changes were observed for the (1) Free C=O stretch (2) asymmetric C=O stretch (3) the carboxyl C—O stretch and (4) the amide II band (1539–1515 cm^{-1}). In the case of NaOH, bound ions may be replaced with Na(I) rather than H^+ as for HCl. Again replacement of divalent metal ions with a monovalent will affect functional group symmetry and availability for metal binding, resulting in altered metal removal as seen in Fig. 1.

As previously discussed, acetone treatment can remove some protein and lipid fractions of the biomass. As these components contain NH_2, –COOH and –OH functionalities amongst others, considerable changes in FTIR band stretching would be expected (Fig. 4d). In general, substantial wavenumber changes were observed for *F. vesiculosus* including shifts of the asymmetric and symmetric C=O stretches (1613 and 1460 cm^{-1}, respectively) as well as the C—O band at 1227 cm^{-1}. A large wavenumber shift of 28 cm^{-1} was observed for the –NH stretch with some shifting of the C—O (alcohol) band also indicating structural changes in these groups.

From Fig. 4e, it is clear that $CaCl_2$ treatment resulted in significant changes in –COOH stretching bands resulting from replacement of existing bound species by Ca(II), thus altering carboxyl symmetry. Changes in sulphonate (1375 and 1160 cm^{-1}) and amino groups were also apparent after biomass washing with the –NH shift from 1539 to 1515 cm^{-1} being most significant. However, despite apparent changes in biomass functionality in the FTIR spectrum, $CaCl_2$ treatment had little effect on metal removal (Fig. 1).

Treatment with Na_2-EDTA resulted in a significant shift in the asymmetric –COOH (1606 cm^{-1}) band of *F. vesiculosus* (Fig. 4f) with a large decrease in intensity of the free C=O band (1719 cm^{-1}) also observed. The symmetric C=O stretch was also no longer visible after Na_2-EDTA exposure indicating major changes in biomass carboxyl groups. It is clear that alteration of these groups impacts significantly on metal removal and detoxification (Figs. 1 and 2). A substantial wavenumber change (1539–1504 cm^{-1}) and decrease in band intensity was observed for the –NH stretch, indicating potential removal of biomass proteins. Considerable wavenumber changes were also observed for biomass sulphonate (1353 and 1163 cm^{-1}) and alcohol groups (1016 cm^{-1}). Changes in biomass structure after Na_2-EDTA treatment, as reflected in changes in intensity and wavenumber in the FTIR spectra, are in agreement with the work of Park et al. [12] who reported that strong chelating agents may damage cell walls and cause disintegration of the biomass.

Fig. 4g reveals significant wavenumber shifts for carboxyl bands after esterification. Kapoor and Viraraghavan [34] proposed that ester formation results in either a decrease or disappearance of the free carboxyl band in the FTIR spectrum. For *F. vesiculosus*, the free C=O band was still visible at 1743 cm^{-1} after esterification, but a significant decrease in band intensity was observed, pointing to the formation of ester groups on the biomass. Wavenumber changes of 14 cm^{-1} and 7 cm^{-1} occurred for the asymmetric and symmetric C=O stretches respectively, while a substantial decrease in the wavenumber of the C—O (–COOH) stretch was observed. This implied that the ester had been formed with the –C—O portion of the carboxyl group thus resulting in a –C—OCH_3 linkage. This obviously alters the symmetry of the carboxyl group and thus affects its observed stretches in FTIR spectra. As previously seen in Figs. 1 and 2, blocking this functionality results in significantly decreased metal removal and conversion in all cases. Wavenumber changes were also observed for the amino (1532 cm^{-1}) and sulphonate functionalities, the latter of which may be related to desulphonation reactions accompanying the esterification. The disappearance of the –NH stretching band at 1539 cm^{-1} (Fig. 4h) after methylation indicated that $-NH_2$ groups initially present have been replaced by $-N(CH_3)_2$ and were not subsequently available for metal binding. Methylation also caused wavenumber decreases for (1) free C=O (2) asymmetric C=O and (3) carboxyl C—O stretches indicating alteration of these groups after modification. Minor changes also took place for sulphonate (1360 and 1163 cm^{-1}) and alcohol functionalities (1019 cm^{-1}).

4. Conclusions

A comprehensive study of chemical modification of seaweeds using numerous treatments and seaweed species has been demonstrated, with results highlighting their significant potential as biosorbents for the remediation of metal contaminated wastewaters. To strengthen this paper's significance, data from a number of other papers by the same authors have been included in support of conclusions [20,21,30]. Treatment with 0.1 M acetone was most effective in increasing the metal removal of all seaweeds and was less harmful to biomass stability than other treatments, thus reducing the quantity of raw material required for operation. Acetone treatment also significantly augmented the Cr(VI) detoxification potential of these seaweeds with Cr(III) conversions of up to 84% achieved for *F. vesiculosus*. Modification of carboxyl and amino functionalities significantly decreased both metal removal and percentage reduction of Cr(VI) for these seaweeds. Thus, the importance of these functionalities in metal removal has been highlighted. Correlation between the degree of carboxyl esterification and reduction in Cu(II) binding was found for *U.* spp and *P. lanosa,* while FTIR analysis pointed to changes in biomass composition and functional group symmetry after chemical treatment which could impact on biomass stability and reusability in industrial waste streams. The results obtained in this study therefore warrant further investigation into the practical applicability of seaweed biomass as a biosorbent for metal-loaded waste streams, and in particular as agents for the detoxification of hexavalent chromium.

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