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Biosorption and bioreduction of Cr(VI) by a microalgal isolate, *Chlorella miniata*

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Abstract

The ability and mechanism of a microalgal isolate, *Chlorella miniata* to remove Cr(VI) were investigated. Kinetic studies indicated that both biosorption and bioreduction were involved in the Cr(VI) removal. The adsorbed Cr(VI) was reduced to Cr(III), and desorption studies indicated that Cr(III) occupied most of the adsorption sites on the biomass. The equilibrium time for Cr(VI) removal was dependent on various factors including initial pH, biomass and Cr(VI) concentrations. Equilibrium study showed that the Cr(VI) removal capacity was negatively related to the initial pH, and the biosorption capacity of total Cr [Cr(III) and Cr(VI)] reached the maximum at initial pH of 3.0. The spectrum of Fourier Transform Infrared Spectrometer analysis (FTIR) further confirmed that amino group on the algal biomass was the main adsorption site for Cr(VI) biosorption in acidic pH while the reduced Cr(III) was mainly sequestered by carboxylate group. The comparison between biosorption–bioreduction and direct bioreduction kinetic models proved that biosorption of Cr(VI) was the first step, followed by Cr(VI) bioreduction and Cr(III) biosorption on the algal biomass.

Keywords: Algae; Biosorption; Hexavalent chromium; Trivalent chromium; Bioreduction

1. Introduction

Chromium pollution in our environment has attracted more and more attention in recent years because of its harmful effects to ecosystems and human beings. Hexavalent and trivalent are two stable states of chromium in nature. These chromium species are commonly found in wastewater produced from leather tanning, dye, wood preservation and electroplating industries and their concentrations could range from tens to hundreds of $mg\,L^{-1}$ [1]. Hexavalent chromium Cr(VI) is more toxic than the trivalent form Cr(III) because of its carcinogenic and mutagenic effects. A variety of diseases such as bronchogenic carcinoma, asthma, pneumonitis and dermatitis have been reported to associate with occupational Cr(VI) exposure [2]. Hence, the discharge of Cr(VI) to surface water is regulated below $0.05\,mg\,L^{-1}$ by the U.S. EPA, and total Cr including Cr(III), Cr(VI) as well as its other forms is regulated below $2\,mg\,L^{-1}$ [3].

Many conventional methods such as chemical precipitation, membrane separation, ion exchange and evaporation have been employed to remove Cr(VI) in industrial wastewater but they are not effective at metal concentrations ranging from 1 to $100\,mg\,L^{-1}$ [4]. The high cost of the chemical reagents and the problems of secondary pollution also make the above physico-chemical methods rather limited in application. In the last two decades, more interests have been focused on using different biosorbents to remove metal ions [5]. Among biosorbents, green algae are attractive as they are ubiquitous in natural environment, have large surface area to volume ratio and high binding affinity to pollutants [6]. *Chlorella miniata*, a green microalgal species, with a spherical to ellipsoidal shape (diameter around 2–3 μm with a surface area to volume ratio of 1.1) was isolated from a municipal sewage treatment plant in Hong Kong SAR by the present research team. Our studies showed that this isolate had a high growth rate in domestic wastewater, and its high biosorption capacity to Ni(II) and Zn(II) ions from contaminated water

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had been reported [6–8]. However, its ability in removing Cr(VI) has never been studied.

The mechanism involved in the removal of Cr(VI) is complex and depends on the properties of biosorbents. Previous studies claimed that the removal of Cr(VI) by biomass was mainly through ion exchange and binding on functional groups [9]. However, the appearance of Cr(III) in solution suggested that Cr(VI) adsorption along with its reduction to Cr(III) may have occurred during the uptake process [3,10–14]. Different mechanisms including ion exchange-redox reaction [12], parallel biosorption and bioreduction [13], direct reduction and a sequential three-step [14] were proposed. The mechanism of Cr(VI) removal by the microalgal species isolated from wastewater may be different from other biosorbents due to the difference in biomass composition.

Various kinetic models for Cr(VI) removal have been proposed, however, they are not correlated well with the corresponding mechanisms. The pseudo-first order kinetic model, assuming only adsorption took place and without any bioreduction, has been widely used in Cr(VI) removal [15]. Park et al. proposed a second order kinetic model based on Cr(VI) reduction but their model showed little correlation with the proposed mechanism [14,16]. Although a parallel reduction and adsorption kinetic model has been proposed for Cr(VI) removal by Cabatingan et al. [13], their results showed that increasing the rate of adsorption would lead to increase of reduction and vice versa, which is the character of consecutive reaction rather than parallel reaction, indicating that something must be wrong in their model. It is necessary to develop a new kinetic model based on the Cr(VI) removal mechanism. The present study therefore aims to: (i) evaluate the mechanism involved in the removal of Cr(VI) by a local microalgal isolate, *C. miniata*; (ii) understand the quantitative relationship between biosorption and bioreduction in Cr(VI) removal through a series of kinetic, equilibrium and desorption studies; (iii) develop kinetic models based on the biosorption–bioreduction mechanism; (iv) identify the possible sorption sites that were involved in the Cr(VI) removal process using the Fourier Transform Infrared Spectrometer analysis (FTIR).

2. Materials and methods

2.1. Mass culture of microalgae and preparation of biosorbent

C. miniata was cultivated in a transparent acrylic column (internal diameter of 140 mm and length of 100 cm) containing approximate 10 L Bristol medium. The composition of the Bristol medium was (g L^{-1} medium): $NaNO_3$, 25; K_2HPO_4, 7.5; KH_2PO_4, 17.5; $MgSO_4{\cdot}7H_2O$, 11.8; NaCl, 2.5; $CaCl_2{\cdot}2H_2O$, 2.5; $FeCl_3{\cdot}6H_2O$, 0.5; $MnCl_2{\cdot}4H_2O$, 0.03; $CoCl_2{\cdot}6H_2O$, 0.002; $CuSO_4{\cdot}5H_2O$, 0.001; $ZnSO_4{\cdot}7H_2O$, 0.004; $NaMoO_4{\cdot}2H_2O$, 0.002 and EDTA, 0.54 (acid form). The culture was illuminated by cool fluorescent light with an average light intensity 4.2 klux in 16-h light:8-h dark cycle at room temperature 25 ± 1 °C. After reaching the stationary phase, the cells were harvested and centrifuged at 5000 rpm for 15 min, the cell pellets were washed with deionized water twice to remove any residues adsorbed on the cell surfaces. The washed cells were then freeze-dried and grounded into fine particles prior to biosorption experiments.

2.2. Preparation of Cr(VI) solution

The stock solution of Cr(VI) (1000 mg L^{-1}) was prepared in deionized water with potassium dichromate ($K_2Cr_2O_7$). All working concentrations were obtained by diluting the stock solution with deionized water, and pH was adjusted to the desired values according to the following experimental design with 1 M HCl and 1 M NaOH solutions.

2.3. Kinetic experiments

The equilibrium time of Cr(VI) removal was determined under different initial pH (from 1.0 to 4.0) and biomass concentrations (from 1.0 to 5.0 g L^{-1}) at an initial Cr(VI) concentration of 100 mg L^{-1}. The effect of initial Cr(VI) concentrations on the kinetic process was investigated by another experiment using 5.0 g L^{-1} biomass, initial pH 2.0, and varied initial Cr(VI) concentrations, 20, 60 and 100 mg L^{-1}. In all experiments, the working volume was 150 mL in a 250 mL conical flask agitated on a shaker at 160 rpm at room temperature (25 ± 1 °C). Liquid solution samples (2 mL from each flask) were collected at regular time intervals and analyzed for residual concentrations of Cr(VI) and total chromium.

2.4. Equilibrium experiments

Equilibrium experiments were carried out to investigate the effect of initial pH on the Cr(VI) removal process. Algal biomass 2.0 g L^{-1} was mixed with water containing 50, 100 and 200 mg L^{-1} Cr(VI) at initial pH varied from around 0 to 4.0. The flasks were agitated on a shaker at 160 rpm for 12 days to ensure that the reaction would reach equilibrium. Samples were then centrifuged at 3500 rpm for 10 min, and the supernatant was used for determination of Cr(VI) and Cr(III).

2.5. Desorption experiments

The Cr-loaded biomass obtained from the above equilibrium experiments was treated with three different desorbents, namely deionized water, 0.5 M HCl and 0.5 M NaOH to elute Cr from the biomass. The working volume was 20 mL and the flasks were agitated on a shaker at 160 rpm for 24 h. After desorption, samples were centrifuged at 3500 rpm for 10 min and the supernatant was analyzed for Cr(VI) and total Cr concentrations.

2.6. FTIR analysis

Infrared spectra of the control (biomass without Cr(VI) treatment) and the biomass mixed with 400 mg L^{-1} Cr(VI) at initial pH 2.0 for 12 days were obtained using a Fourier Transform Infrared Spectrometer (Nocolet, Avatar E.S.P.360). A measured amount of biomass was mixed with KBr (2% potassium

bromide). The mixture was grounded into fine particles and compressed into translucent sample disks by a manual hydraulic press. The disks were then fixed in the FTIR spectrometer for analysis.

2.7. Analysis of chromium

Cr(VI) and total Cr in liquid solution were determined according to the standard method described by Clesceri et al. [17] and Kratochvil et al. [12]. The absorbance of the purple complex formed from reacting Cr(VI) with 1,5-diphenylcarbohydrazide was measured at $\lambda = 540$ nm by a UV spectrophotometer (Shimadzu, UV-1201) and the detection limit was 0.05 mg L^{-1}. Total chromium including Cr(VI) and Cr(III) was determined by atomic absorption spectroscopy (AAS) (Shimadzu, AA-6501) at $\lambda = 357.9$ nm and the detection limit of AAS in the present study was 0.1 mg L^{-1}. The Cr(III) content in liquid solution was obtained by subtracting the content of Cr(VI) from that of total chromium.

3. Results and discussion

3.1. Kinetic studies of Cr(VI) removal

Kinetic results showed that the removal of Cr(VI) by *C. miniata* and the equilibrium time were significantly dependent on both initial pH and biomass concentrations (Fig. 1). A rapid removal of Cr(VI) took place in the first 30 min, and the rate became level off thereafter. Low initial pH as well as high biomass shortened the equilibrium time and enhanced the Cr(VI) removal percentages. At initial pH 1.0, biomass 2.0 g L^{-1}, nearly 100% Cr(VI) was removed within 58 h. At initial pH 4.0, less than 10% Cr(VI) was removed and it was impossible to estimate the equilibrium time (Fig. 1a). The Cr(VI) removal percentages at initial pH 2.0 were 60, 85 and 100% in treatments with 1.0, 2.0 and 5.0 g L^{-1} biomass, respectively, and the respective equilibrium time were 240, 215 and 150 h (Fig. 1b). Previous results also reported that the equilibrium time of Cr(VI) removal by seaweed and fungi varied from tens to hundreds of hours depending on experimental conditions [3,14,16].

Equilibrium time was also dependent on initial Cr(VI) concentrations. The respective equilibrium time under initial Cr(VI) concentrations of 100, 60 and 20 mg L^{-1} was 150, 72 and 30 h, respectively (Fig. 2). Cr(III) appeared gradually with the removal of Cr(VI), indicating that the Cr(VI) adsorbed on the algal biomass was reduced to Cr(III). The amounts of Cr(VI) removed from the contaminated water were more than the amounts of Cr(III) detected, suggesting that not all of the biosorbed Cr(VI) was reduced to Cr(III), some of the reduced Cr(III) was released to the liquid solution while some adsorbed on the biomass. The biosorption and bioreduction processes were likely to be physicochemical transformation as the biomass used in the present

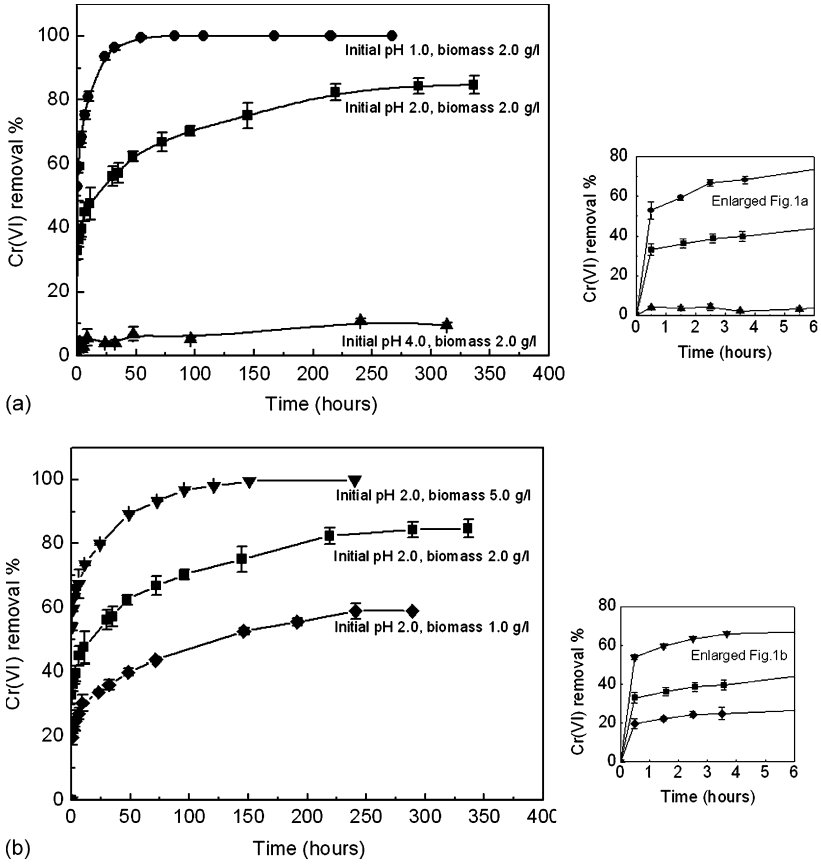

Fig. 1. Kinetic study of Cr(VI) removal under (a) different initial pH and (b) biomass dosage (initial Cr(VI) concentration 100 mg L^{-1}, volume 150 mL; mean and standard deviation values of three replicates are shown).

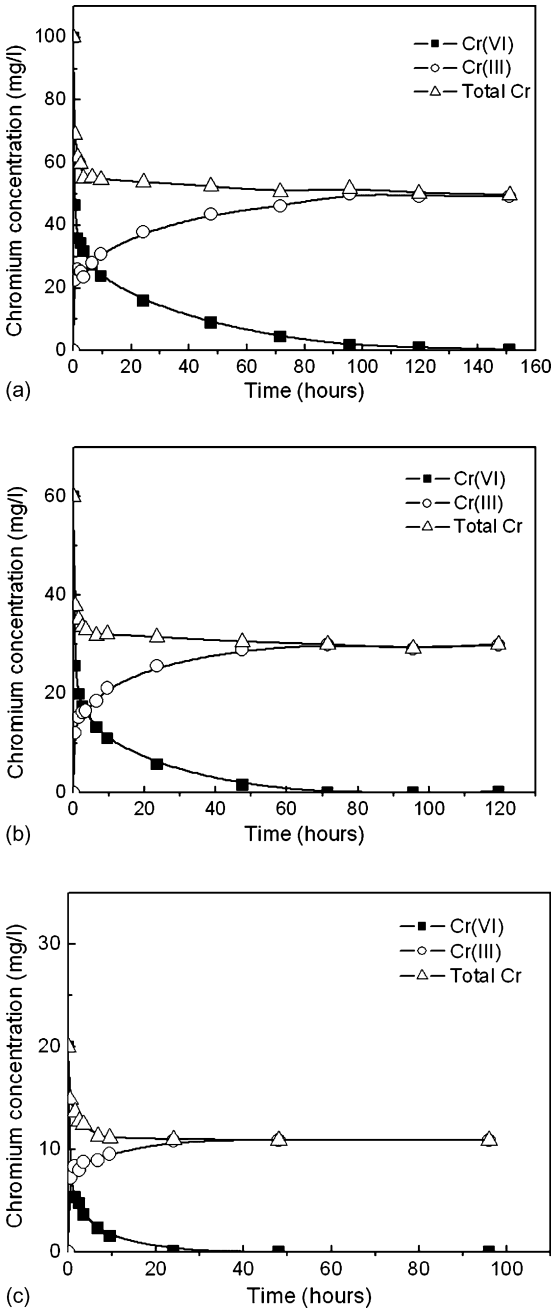

Fig. 2. Cr species and distribution in solution during the removal of Cr(VI) from contaminated water under three initial Cr(VI) concentrations: (a) 100 mg L^{-1}, (b) 60 mg L^{-1} and (c) 20 mg L^{-1} (biomass dosage 5.0 g L^{-1}, initial pH 2.0, volume 150 mL; average of duplicates are shown).

study was freeze-dried, grounded into fine particles, and might have lost its biological activity. The pattern was similar among different initial Cr(VI) concentrations. Zhao and Duncan [11] also reported that Cr(VI) removal was probably a bioreduction along with a biosorption process. Fig. 2 further revealed that the concentration of total Cr [Cr(VI) and Cr(III)] dropped significantly in the first few hours and reached equilibrium at 4–6 h, and the equilibrium time was much shorter than that of Cr(VI) or Cr(III), suggesting that Cr(VI) bioreduction process was the rate limiting step in the removal of Cr(VI) by *C. miniata*.

Cr(VI) forms $HCrO_4^-$, $Cr_2O_7^{2-}$, CrO_4^{2-}, $HCr_2O_7^-$ and H_2CrO_4 in solution, and the relative proportion of each species depends on both pH and Cr(VI) concentration [2]. Cabatingan et al. [13] suggested in the pH range of 1–6.5 and a total Cr(VI) concentration of 7.69×10^{-3} M, $HCrO_4^-$ was the most dominant form with coexistence of H_2CrO_4, $Cr_2O_7^{2-}$ and $HCr_2O_7^-$ in solution, but Cr(VI) was mainly present as the CrO_4^{2-} anion when pH was larger than 6.5. Nieboer and Jusys [18] also found that $HCrO_4^-$ was the predominant form up to the Cr(VI) concentration of 10^{-2} M, and it started to condense yielding the orange-red dichromate ion ($Cr_2O_7^{2-}$) above this concentration. In the present study, the highest Cr(VI) concentration was 200 mg L^{-1} (3.85×10^{-3} M), therefore, in the pH range of 1.0–4.0, $HCrO_4^-$ should be the dominant Cr(VI) species in solution. In neutral or alkali solution, it is difficult for the anion species of Cr(VI) binding to the negatively charged functional groups on the biomass surface. However, in acidic pH, the functional groups on the biomass were protonated and positively charged, thus became available for Cr(VI) anion biosorption. Lowering pH resulted in higher Cr(VI) biosorption due to the increase of electrostatic attraction between sorbate and the protonated groups on the biomass. Similar trend has also been found in Cr(VI) removal by fungal biomass [14], crab shell [19] and chitosan [20], and in biosorption of other anions such as $Au(CN)_2^-$, SeO_4^{2-} and VO_4^{2-} [19].

At low pH, on the other hand, Cr(VI) had a high redox potential and favored Cr(VI) bioreduction [12]. In addition, reductants on the biomass such as carbohydrate and protein could supply electrons for Cr(VI) bioreduction, with partial release of soluble organics or ultimate oxidized product, CO_2 [3]. This explains why increasing biomass dosage and lowering pH of the contaminated water could achieve more Cr(VI) removal within a shorter period of time.

3.2. *Effect of initial pH on Cr(VI) removal at equilibrium*

Equilibrium experiments were conducted at an initial pH varied from around 0 to 4.0 under initial Cr(VI) concentrations of 50, 100 and 200 mg L^{-1} for 12 days. The effects of initial pH on Cr(VI) removal and total Cr bisorption capacity were similar at different initial Cr(VI) concentrations. At equilibrium, the pH changed from initial values of 1.0, 2.0 and 4.0 to 1.03, 2.25 and 5.79, respectively (data not shown). Fig. 3a shows that the Cr(VI) removal capacity of *C. miniata* decreased linearly with increases of initial pH. At initial pH 3.0, 35.42, 38.02 and 40.72% of Cr(VI) at concentrations of 200, 100 and 50 mg L^{-1} were removed, respectively. When initial pH approached 0 and H^+ concentration reached 1.0 M, Cr(VI) was completely removed. This could be attributed to the easier biosorption of Cr(VI) on the protonated biosorbents [20] and the higher reduction–oxidation potential of Cr(VI) at lower pH as reported by Kratochvil et al. [12].

The bisorption capacity of total Cr [Cr(VI) and Cr(III)] of *C. miniata* was also a pH dependent process, and the maximum capacity was obtained at an initial pH 3.0 under all three initial Cr(VI) concentrations (Fig. 3b). Although low pH (0–1) could increase Cr(VI) biosorption on the protonated biomass

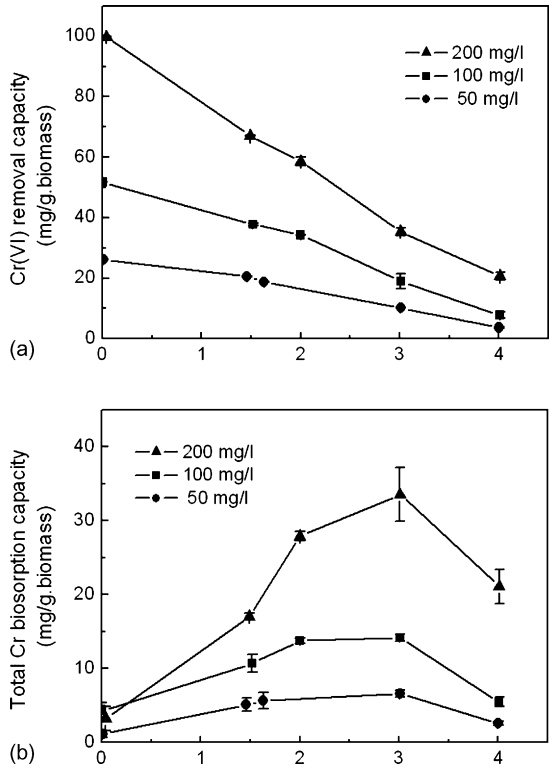

Fig. 3. Initial pH effect on (a) Cr(VI) removal capacity and (b) total Cr biosorption capacity under different initial Cr(VI) concentrations (biomass dosage 2.0 g L^{-1}, volume 20 mL; mean and standard deviation values of three replicates are shown).

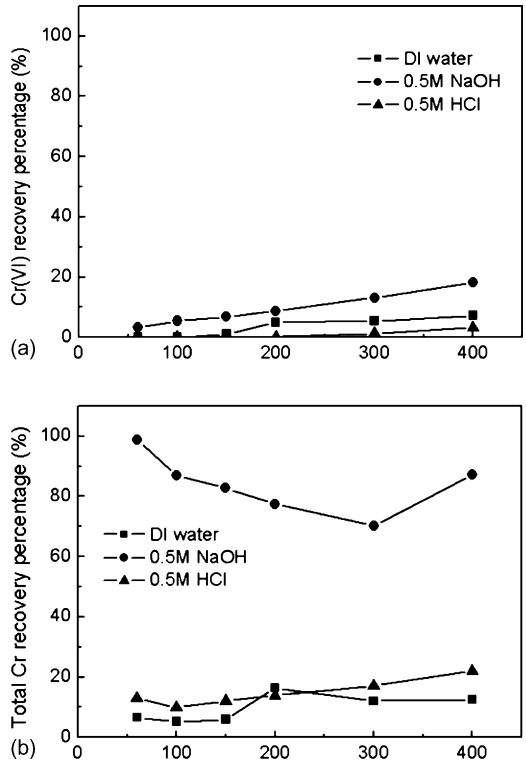

Fig. 4. Effects of different desorbents on (a) Cr(VI) and (b) total Cr recovery under different initial Cr(VI) concentrations (biomass dosage 5.0 g L^{-1}, desorbent volume 20 mL).

and bioreduction of Cr(VI) to Cr(III), the reduced Cr(III) was difficult to be adsorbed on the biomass due to electric repulsion leading to low total Cr biosorption. On the other hand, at high pH such as 3.0 or larger, less Cr(III) was produced due to the sharp decrease of both Cr(VI) biosorption and bioreduction processes. As a consequence, most Cr(VI) would still remain in the contaminated water and the biosorption of total Cr was also low. Previous studies reported that the optimal pH for total Cr biosorption was around 2–3. For instance, the optimal pH for *Sphagnum*-moss peat, leaf, mould and coconut-husk fibre were 1.5, 2.0, 2.05 and 2.0, respectively [11], while 2–2.5 was the optimal pH for *Sargassum* [12,13]. Park et al. [3] further showed that the optimal pH for total Cr biosorption would change according to contact time, the optimal pH was 1.5–2.5 after 6 h contact and changed to pH 4.0 after 480 h (the time for a complete reaction) in *Ecklonia* sp., probably due to the release of organic matter which complexed with Cr(III).

3.3. Desorption studies of Cr species adsorbed on biomass

When the three desorbents were used to elute Cr from the algal biomass, more Cr(VI) were desorbed by 0.5 M NaOH than that by deionized water and 0.5 M HCl (Fig. 4a). Since Cr(VI) adsorbed on the biomass was due to proton bridge, when protons were consumed in alkali wash, the adsorbed Cr(VI) would be released. Boddu et al. [20] also found that NaOH was an effective desorbent to elute Cr(VI) adsorbed on chitosan. The least Cr(VI) recovery percentage by acid wash (0.5 M HCl, pH around 0.3) could be attributed to the further reduction of the adsorbed Cr(VI) to Cr(III) under acidic condition.

Alkali wash by 0.5 M NaOH also had significantly higher percentages of total Cr recovery than the other two biosorbents (Fig. 4b). When NaOH was used as the desorbent, 70–100% of total Cr were recovered while the pecentages of Cr(VI) desorbed were very low (less than 15%), suggesting that most Cr adsorbed on the biomass was in the form of Cr(III) and $Cr(OH)_4^-$ was probably the main composition in NaOH elutant based on the amphotericity of Cr(III). The recovery performance of 0.5 M HCl was not better than deionized water, indicating protons could not replace Cr(III) adsorbed on the biomass.

3.4. FTIR analysis

The infrared spectra of the control biomass of *C. miniata* (not subject to any chromium treatment) and the biomass after mixing with 400 mg L^{-1} Cr(VI) at initial pH 2.0 were shown in Fig. 5. The absorption peaks at 1654 and 1540 cm^{-1} corresponded to the amide I and amide II bands, respectively, as suggested by Yee et al. [21] were found in both control and treated algal biomass in the present study. Glucosamine group had been reported as an important sugar component of the rigid wall in many *Chlorella* species [22]. Under low pH, amino group could be protonated and thus responsible for Cr(VI) adsorption [20,23]. The region between 3200 and 3500 cm^{-1} represented the overlapping peaks of stretching vibration of O–H and N–H [24]. The region between 3000 and 2800 cm^{-1} exhibited the C–H stretching vibrations of $-CH_3$ and $>CH_2$ functional groups,

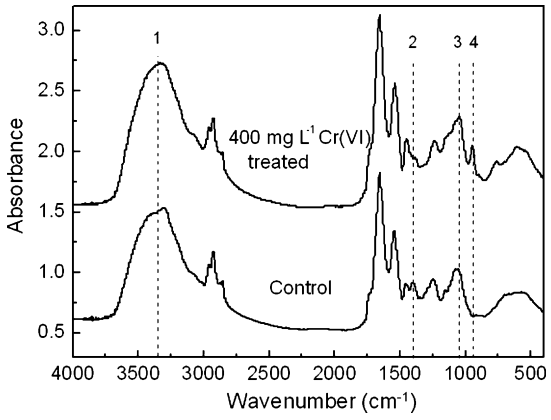

Fig. 5. FTIR analysis of Cr(VI)-treated and control biomass (labels 1, 2, 3 and 4 represent the changes on the algal biomass after Cr(VI) treatment).

and 1300–1470 cm^{-1} was the deformation stretching of C–H, $–CH_3$, and $>CH_2$ functional groups [21]. At 1400 cm^{-1}, it was a characteristic peak of symmetric vibrational COO^- frequencies of terminal amino acid on biomass [25]. The peaks of 1240, 1076 cm^{-1} represented P=O and C–O bands of polysaccharides, respectively [26].

After Cr(VI) treatment, four changes of the functional groups on the biomass were detected from the spectrum. The first change was the enhancement of the intensity at the region 3200–3500 cm^{-1}, indicating an increase of the free hydroxyl group on the biomass (Fig. 5). This could be due to hydrolyzing of some polysaccharides on the cell wall to shorter saccharides such as oligosaccharides, dioses, and monoses under acidic condition [25]. The second change was the weakening of the peak at 1400 cm^{-1}, which was typical of the complexation of the carboxylate functional group by coordination with metal cations [25]. In the present study, this might be due to the complexation of Cr(III) formed from bioreduction of Cr(VI) with carboxylate group. Our previous study confirmed that Cr(III) was mainly sequestered by carboxylate group on *C. miniata* [27]. The third change was the shift of the peak at 1076–1044 cm^{-1}, which could be due to the involvement of the C–O bond of polysaccharides in Cr(III) biosorption. Similar study on using grape stalks to adsorb copper and nickel ions also suggested that lignin C–O might be involved in metal uptake [28]. The last change was the presence of a new peak at around 940 cm^{-1} in the Cr(VI) treated biomass, and it could be attributed to the presence of Cr(VI)–O bond as suggested by Holman et al. [29].

3.5. Mechanism and kinetic modeling of Cr(VI) biosorption and bioreduction

Based on the above results, it is reasonable to conclude that the mechanism involved in the removal of Cr(VI) by *C. miniata* was biosorption–bioreduction. The sequence was: (i) biosorption of Cr(VI) at low pH: protons were adsorbed on the amino group of the algal biomass, the Cr(VI) ions were then adsorbed on the protonated sites; (ii) bioreduction of Cr(VI) to Cr(III): the Cr(VI) adsorbed on the biomass surface was bioreduced to Cr(III) by the reductants on the biomass such as polysaccharides or other reducing organic matters; (iii) release of Cr(III): part of the bioreduced Cr(III) was released from the biomass.

To further confirm the above mechanism, two kinetic models, the biosorption–bioreduction model and the direct bioreduction model were developed and compared in the present study. The chemical equation of the first model could be defined:

$$HCrO_4^- + H^+ + \text{Biomass} \Leftrightarrow HCrO_4^- - H^+ - \text{Biomass} \rightarrow Cr^{3+} + H_2O + \text{Biomass (oxidized)} \quad (1)$$

Since $HCrO_4^-$ biosorption on biomass was a fast step compared to bioreduction, the reaction rate was determined by the bioreduction step. If bioreduction of Cr(VI) on the biomass was thought to be a pseudo-first order reaction, it could be defined:

$$\frac{dq}{dt} = -k_1 q \quad (2)$$

where k_1 is the apparent reaction rate constant for the biosorption–bioreduction model and q is the Cr(VI) adsorbed on biomass.

If Cr(VI) sorption equilibrium was thought to be present during the whole process, q could be expressed by Langmuir isotherm:

$$q = \frac{QbC}{bC + 1} \quad (3)$$

where Q is the maximum sorption capacity, b the sorption constant of Cr(VI) and C is the concentration of Cr(VI) in solution. By combining Eqs. (2) and (3), the following equation was obtained:

$$\frac{dC}{dt} = -k_1 C(bC + 1) \quad (4)$$

with the initial conditions: $t = 0$, $C = C_0$, C_0 is the initial concentration of Cr(VI). After a definite integral, we could get:

$$k_1 t = \ln\left(\frac{C_0}{bC_0 + 1}\right) - \ln\left(\frac{C}{bC + 1}\right) \quad (5)$$

In the direct bioreduction kinetic model, a pseudo-first order reaction was assumed due to its simple form as described by Sparks [30], and the following equation could be defined:

$$\frac{dC}{dt} = -k_2 C \quad (6)$$

where k_2 is the apparent reaction rate constant for the direct bioreduction. Then we could get:

$$k_2 t = \ln(C_0) - \ln(C) \quad (7)$$

The parameters k_1, b in Eq. (5) and k_2 in Eq. (7) were estimated by a nonlinear regression using the Sigmaplot 8.0 software and the results are listed in Table 1. The rate constant k_2 in the direct bioreduction model was larger than k_1 in the biosorption–bioreduction model, which could be attributed to the combined effect of biosorption and bioreduction in direct bioreduction model. The higher R^2 of the biosorption–bioreduction model than the direct bioreduction one indicated the former model was more likely to be involved in the removal

Table 1
Regression parameters of Cr(VI) removal under different Cr(VI) initial concentration by *Chlorella miniata*

Cr(VI) initial concentration ($mg\,L^{-1}$)	Parameters in biosorption–bioreduction model			Parameters in direct bioreduction model	
	$k_1 \times 10^2$ (h^{-1})	$b \times 10^2$ ($L\,mg^{-1}$)	R^2	$k_2 \times 10^2$ (h^{-1})	R^2
100	2.15	6.99	0.973	4.78	0.836
60	5.58	9.15	0.889	7.54	0.847
20	15.24	20.25	0.982	26.27	0.872

of Cr(VI) by *C. miniata*. It further confirmed that Cr(VI) biosorption on the biomass was the first step and it was then bioreduced to Cr(III). The increase of k_1 and b values with decreases of initial Cr(VI) concentrations (Table 1) could be explained by the fact that less proton was consumed in water with lower Cr(VI) contamination and the pH was maintained at lower level throughout the study, thus was easier for Cr(VI) biosorption and bioreduction.

4. Conclusions

The present study shows that the green microalgal isolate, *C. miniata* was capable of removing Cr(VI) from the contaminated water. At an initial pH of 2.0 and biomass of $5.0\,g\,L^{-1}$, 65% Cr(VI) was removed from the contaminated water containing $100\,mg\,Cr(VI)\,L^{-1}$ in the first 2 h, while a complete Cr(VI) removal was obtained at 150 h. The main adsorption site for Cr(VI) in acidic pH was amino group, and the reduced Cr(III) was mainly sequestered by carboxylate group on the algal biomass. The kinetic model developed based on the biosorption–bioreduction mechanism had a significantly higher R^2 than that of the direct bioreduction model, further confirmed that the removal of Cr(VI) was based on Cr(VI) biosorption, bioreduction of Cr(VI) to Cr(III) and Cr(III) biosorption on the biomass.

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