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Cadmium Uptake by Non-viable Biomass from a Marine Brown Alga *Ecklonia radiata* Turn.

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Abstract Biomass of non-viable and dried brown marine algae *Ecklonia radiata* Turn. was used to examine its cadmium uptake capability. Twelve different pretreatments on the algal biomass were prepared. Among these pretreatments, the algal biomass, which treated with 0.1 M NaOH and kept in water bath (100°C, 18 h) followed by washing with distilled water and squeezing, showed the highest amount of cadmium uptake as 1634 ± 195 mg/g dry biomass at pH 4.0 and 50°C. Adsorption temperatures and pH levels played some important role in cadmium uptake. However, cadmium uptake decreased dramatically at a lower pH than 4.0. Freundlich adsorption isotherm showed potent cadmium uptake capacity of the non-viable biomass. Pretreatments on the non-viable algal biomass shown in this study may enhance the cadmium removal in the industrial wastewater.

Keywords: cadmium uptake, biomass, *Ecklonia radiata*, pretreatments

INTRODUCTION

Brown seaweeds have been considered as the potent clean-up biomaterials to protect heavy metal contamination in aqueous solution due to their strong ability to metal ion uptake [1]. The brown alga, *Ecklonia radiata* Turn., has been shown to become a strong biosorbent for metal ion uptake [2]. Algal uptake of metal ions is strongly dependent on the content of alginate which is a biopolymer segment consisting of D-mannuronic acid unit (M), L-glucuronic acid unit (G), and alternating D-mannuronic acid and L-glucuronic acid residues. Mizuno *et al.* [3] demonstrate that ion exchange ability for divalent metal ions has been closely related to the M/G ratio. Smidsrod and Grasdalen [4] also suggest that the selective binding of divalent metal ions is mainly associated with the blocks of G in the alginate molecule. However, Kuyucak and Volescky [5] report that cobalt uptake by alginate in *Ascophyllum nodosum* was proportional to the quantity of M residues. In addition, Fourest and Volesky [6] demonstrate that sulfonate group as well as alginate plays an important role in heavy metal biosorption. On the other hand, many studies reveal that proteins and polysaccharides of the algal cell walls contain potential metal ion binding sites, and the number and type of binding sites depend on the chemical composition of the cell walls [5,7].

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Non-living biomass has several advantages used as biosorbent materials because it shows metabolism-independent metal transport into cells, whereas an energy dependent system is sensitive to pH and temperature [8,9]. Kuyucak and Volescky [10] show non-living biomass of *Saccharomyces cerevisiae* and *Phizophus arrhizus* exhibits higher metal uptake capacity than the living biomass for the uptake of copper, zinc, cadmium and uranium. Non-viable algal biomass can be easily produced through physical and chemical pretreatment using heating, autoclaving, freeze-drying, boiling, and acid or alkali treatment. These pretreatment may enhance or reduce in metal adsorption, depending on the algal species and treatment procedures employed [10].

Cadmium is a by-product of zinc or lead mining and it can enter the atmosphere or the marine environment by forming oxides and salts [11,12]. Cadmium contamination can cause several biotoxicity to the renal, pulmonary, skeletal, testicular, and nervous systems. Among them, cadmium exerts long-term and irreversible effects on the developing human brain [13]. With these regards, many studies contribute cadmium removal from aquatic solution by bacteria [14], yeast [15] and algae [16].

The goal of this study is to assess whether several physical and chemical treatments on non-living algal biomass enhance the capacity of divalent metal ion when compared to non-treated algal biomass or do not make a difference between them. In here, we report the rate of cadmium uptake by non-living biomass of *E. radiata* after 12 different pretreatments.

MATERIALS AND METHODS

Organism

Algal biomass, *Ecklonia radiata* Turn., was collected from the Coogee beach, Sydney, Australia. The seaweeds were washed with seawater and distilled water to remove the attached materials such as shells and sand debris. The washed seaweeds were transferred to a room fitted at 30°C for dryness of them with air. After dryness, these seaweeds were subjected to grind to be a fine particle (<1.651 mm) using a hammer mill.

Chemicals

$Cd(NO_3)_2 \cdot 4H_2O$ was purchased from Ajax Chemical Co. (Sydney, Australia). All the chemicals were supplied with high purity. The stock solution of cadmium was 5 g/L.

Pretreatment Methods

Twelve different pretreatments were employed to change physical or chemical composition of non-living biomass of *E. radiata*. The pretreatment methods were described in Table 1.

Table 1. Twelve different pretreatment methods for non-living biomass of *Ecklonia radiata* Turn.

No.	Method	Description for algal biomass treatments
1	Control	Untreated
2	W/S	Washing with distilled water, then squeezing
3	A	Treatment with 0.1 M NaOH and maintaining in water bath (50°C, 18 h), then washing with distilled water and squeezing
4	B	Treatment with 0.1 M NaOH and maintaining in water bath (50°C, 18 h), then adjustment to pH 7.0
5	C	Treatment with 0.1 M NaOH and maintaining in water bath (50°C, 18 h), then air-drying in an oven
6	D	Treatment with 0.1 M NaOH and maintaining in water bath (100°C, 18 h), then washing with distilled water and squeezing
7	E	Treatment with 0.1 M NaOH and maintaining in water bath (100°C, 18 h), then adjustment to pH 7.0
8	F	Treatment with 0.1 M NaOH and maintaining in water bath (100°C, 18 h), then air-drying in an oven
9	G	Treatment with 0.1 M HCl and maintaining in water bath (50°C, 18 h), then washing with distilled water and squeezing
10	H	Treatment with 0.1 M HCl and maintaining in water bath (50°C, 18 h), then adjustment to pH 7.0
11	I	Treatment with 0.1 M HCl and maintaining in water bath (50°C, 18 h), then air-drying in an oven
12	J	Treatment with 0.1 M HCl and maintaining in water bath (100°C, 18 h), then adjustment to pH 7.0
13	K	Treatment with 0.1 M HCl and maintaining in water bath (100°C, 18 h), then air-drying in an oven

Cadmium Uptake Experiments

The desired concentrations of cadmium (5 mg/L to 200 mg/L) were prepared by dissolving $Cd(NO_3)_2$ in deionized distilled water. Short-term metal ion uptake was performed in 50-mL flasks containing 10 mL metal ion solution (5,000 ppm) of five different pHs (1.5, 2.0, 4.0, 6.0, and 6.15) at 50°C, room temperature, and 6°C for 2 h unless otherwise mentioned. These pH values were initial values before the introduction of the metal for binding reaction. In all experiments, the biomass concentration was kept constant to 30 mg dry wt/mL. Metal-free and biomass-free blanks were used as controls. Biomass-free blanks were used to estimate the exact initial concentration of cadmium by dilution. Separation of biomass from metal-bearing solution was achieved through centrifugation at 6,000 rpm for 20 min in Remi RC30 centrifuge. The supernatant was diluted and read for the remaining cadmium content at 228.8 nm wavelength (slit width 3.8 A) using a Varian model-AA-1475 Atomic Absorption Spectrophotometer with an air-acetylene fuel mixture. All experiments were carried out in triplicate and repeated three times.

The metal uptake capacity in mg/g (q) was calculated from the inintial concentration (C_i) and the final concentration (C_f) of the metal according to the following equation [17]:

$$q = V\,(C_i - C_f)\,/\,M$$

where, V is the liquid sample volume and M is the biomass dry weight. The biosorptive metal uptake was evaluated and expressed by use of Freundlich adsorption model [18]. The general form of this model is

$$q = kC^{1/n}$$

where q = uptake of species (metal) and C = equilibrium (final/residual) concentration. The intercept $\ln k$ gives a measure of absorbent capacity and the slope $1/n$ gives the intensity of adsorption.

This can be linearlized by taking natural logarithm in the form of $\ln q = \ln k + 1/n \ln C$.

RESULTS AND DISCUSSION

The experiments on cadmium uptake by the non-viable biomass of *E. radiata* were conducted on five different pH conditions (pH 1.5, 2.0, 4.0, 6.0, 6.15) or three

Table 2. Effect of non-living biomass pretreatments on cadmium uptake at pH 4.0 and 50°C by *Ecklonia radiata* Turn.

Pretreatment	mg cadmium adsorbed/g dry biomass
Control	825 ± 78.5
W/S	1,485 ± 236
A	1,403 ± 153
B	1,452 ± 78.6
C	743 ± 89.2
D	1,502 ± 346
E	1,634 ± 195
F	990 ± 78.5
G	1,360 ± 115
H	1,205 ± 104
I	825 ± 98.6
J	825 ± 72.3
K	495 ± 55.3

different temperatures (6°C, room temperature, and 50°C). Cadmium uptake by the algal biomass was effectively accomplished at 50°C and the three different pHs 4, 6 and 6.15. (data not shown). The alkali pretreatment methods were preferable methods for cadmium adsorption when compared to the acid pretreatment methods (Table 2). Previous report showed our brown alga contained the similar composition of M and G residues (approximately, 1: 1: 1; MM: GM: GG) [19]. It could be proposed that two alginate units serve similarly for divalent metal ion uptake. In our study, alkali pretreatments can contribute to give more chemical changes on G or M units than acidic pretreatments. However, the exact formation of G or M units after changes still needs to be explained. This result is similar to the report reported by Ahuja *et al.* [20]. They showed that cobalt uptake was declined in acidic pretreatments. However, they did not find any improvement after alkali pretreatments on biomass. It may be resulted from the constituent differences of cell surface between cyanobacteria and algae.

A dramatic change on cadmium uptake was found with pH differences (Table 3). At pH 4.0 the algal biomass pretreated with method E showed the highest cadmium adsorption (1634 ± 195 mg cadmium adsorbed/g dry biomass), whereas at pH 1.5 the uptake was worst (660 ± 35.4 mg cadmium adsorbed/g dry biomass). This result showed that cadmium uptake was strongly dependent on the solution's pH value. With cobalt ions, there was no adsorbed by the *Oscillatoria anguistissima* in the pH range 2-4 [20]. In our study, low cadmium uptake was found at a very low pH range such as pH 1.5. However, there was appreciable cadmium uptake at the pH range 2-6 and maximum at pH 4.0 (Table 3). This maximum uptake of cadmium by the algal biomass of *E. radiata* treated with method E may be resulted from the large amounts of generating negatively charged surface on the biomass. Interestingly, method E can give more broad capacity on the algal biomass for cadmium removal with the more broader

Table 3. Effect of pH on cadmium uptake by non-living biomass at 50°C of *Ecklonia radiata* Turn. pretreated by method E

pH	mg cadmium adsorbed/g dry biomass
1.5	660 ± 35.4
2.0	1,564 ± 153
4.0	1,634 ± 195
6.0	1,543 ± 126
6.15	1,513 ± 118

Table 4. Effect of temperature on cadmium uptake by non-living biomass at pH 4.0 of *Ecklonia radiata* Turn. pretreated by method E

Temperature	mg cadmium adsorbed/g dry biomass
6°C	1,581 ± 108
Room temp.	1,573 ± 254
50°C	1,634 ± 195

pH range than control. Usually, metal adsorption has decreased at acidic pH because protons tend to compete with cations to bind the negatively charged surface [21].

In the incubation temperature at 50°C, cadmium uptake was relatively increased by the algal biomass treated with the methods A, B, D, E, and washing with distilled water at the different pH ranges. The algal biomass treated with the methods C, I and K had a poor uptake capacity at the same pH conditions. The method H showed very low uptake at pH 4, whereas at the other pH levels uptake were very high (data not shown).

The alkali pretreatment method E was an outstanding method solely at the three different temperatures and five different pH ranges. The maximum uptake capacity was 1634 mg cadmium/g algal biomass obtained from method E at pH 4 and 50°C, whereas control (non-treated algal biomass) showed 825 mg cadmium uptake at pH 4 and 50°C (Tables 2 and 3). Within a pH range of 2.0 to 6.0, the uptake capacity of method E for cadmium was over 1,500 mg/g dry biomass of *E. radiata*. It is similar to the results reported previously [22]. The non-treated biomass of *E. radiata* exhibited high uptake capacities for lead, cadmium and copper with over 90% of the uptake capacities of the initial concentration. The equilibrium data for the metal ions fitted well to the Freundlich adsorption isotherm and Langmuir isotherm model [22]. In the pretreated biomass of *E. radiata*, the equilibrium data for the cadmium fitted well to the Freundlich adsorption isotherm as shown in Fig. 1. However, the isotherm of non-treated biomass of *E. radiata* for cadmium was about two-fold lower than that of the pretreated biomass of the brown algae treated by method E. Therefore, the alkali pretreatment may increase the uptake capacity of *E. radiata* to cadmium. Moreover, non-treated algal biomass showed 10 times higher uptake

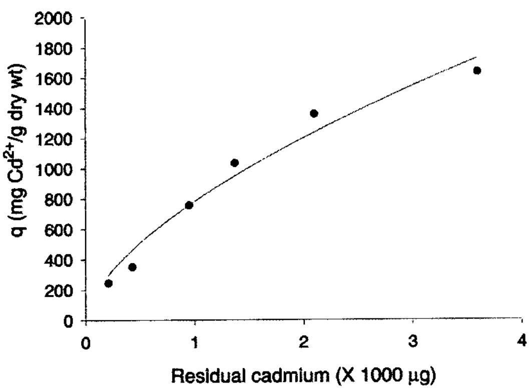

Fig. 1. Freundlich adsorption isotherm for cadmium at 0.3 mg dry weight/mL non-living biomass concentration (pH 4.0). q = 0.7815 (C ^ 1/1.6162).

capacities on lead than those of powdered activated carbon and natural zeolite [22]. These results may be similarly employed on the cadmium removal using the pretreated algal biomass of *E. radiata*. Therefore, we suggest that the non-viable algal biomass of *E. radiata* pretreated with method E can be used in the industrial wastewater cleaning-up containing cadmium as potent biosorbents. Further studies may be on the application of the alkali pretreatments with the other algal species rather than cyanobacteria which has been not affected by alkali pretreatments on cobalt uptake [20].

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