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Comparative Study of Cadmium Biosorption by *Eucheuma cottonii* Marine Algae and Carrageenan

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Cadmium adsorption capacity by *Eucheuma cottonii* and carrageenan was investigated. 64.3 % carrageenan was successfully extracted from *E. cottonii* by weakly alkaline solution (pH 8.5) at 95 ºC for 18 h. FTIR spectra of the extracted carrageenan showed the same spectra as the pure carrageenan. Batch experiment was conducted to determine the cadmium biosorption capacities of *E. cottonii* and carrageenan. Cadmium concentration was measured by atomic absorption spectrophotometry. Biosorption capacity of cadmium was 0.95, 3.85 and 3.87 mg/g for *E. cottonii*, extracted carrageenan and pure carrageenan, respectively.

Key Words: Cadmium, Biosorption, *E. cottonii*, Carrageenan, Atomic absorption spectroscopy, FT-IR.

INTRODUCTION

Heavy metal is one of the most important causes of pollution in both aqueous solutions and soils[1]. In order to decrease their concentration in aqueous solution[2], the uptake of metals by agricultural product and by-product appears to be an efficient process[3] due to their numerous functional groups such as carboxyl, amide, thiol, phosphate, hydroxyl and imidazol that can form coordination complexes with metal ions at low capital cost[4,5]. Rice husk[6], algae[7,8] and seaweeds[9,10] have been tested for metal biosorption with very encouraging results.

Cadmium is one of heavy metals that are greatly hazardous to human and environment. Cadmium poses a serious threat to human helath as it accumulates in the environment throughout the food chain. Besides, the industrial uses of cadmium are widespread and increasing in electroplating, paint, plastic, alloy preparation, mining and silver-cadmium batteries[11].

Red algae (*Eucheuma*) are harvested throughout the world as a food sources as well as an export commodity for the production of agar and carrageenan products. *E. cottonii* and *E. spinosum* species were originally harvested from natural stocks growing in Indonesia and the Philippines[12]. In Indonesia, Eucheuma farms can be found in Sulawesi, Bali, Lombok, Sumbawa and West Sumatra[13].

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Carrageenan is a group of natural polysaccharides extracted by hot water or alkaline solution from red marine algae (Rhodophyceae)[14,15]. Its structure is constituted by a linear chain of alternating 1,3-linked α-galactose-4-sulphate and 1,4-linked-3,6-β-anhydrogalactose substance with hydrocolloid properties owing to the presence of sodium, potassium, magnesium and calcium sulfate esters of galactose and anhydro-galactose units[16].

Carrageenans are large, highly flexible molecules with curl forming helical structures, which gives them the ability to form a variety of different gels at room temperature. They are widely used in the food and other industries as thickening and stabilizing agents. Most carrageenan is extracted from *E. cottonii* and *E. spinosum* in water or diluted alkaline solution and may be recovered by alcohol precipitation[17].

The aim of the present studies are to study the cadmium biosorption capacity of *E. cottonii* and extracted carrageenan and its comparison to those of the pure carrageenan. The optimum extraction conditions of carrageenan from *E. cottonii* were also investigated. Cadmium ion concentrations were determined by atomic absorption spectrometry (AAS).

EXPERIMENTAL

Treatment of *E. cottonii*: *E. cottonii* marine algae, which were 40 day-aged, were harvested from countryside of Seurapong district of Pulau Aceh, which is a big Aceh sub-Province of Nangro Aceh Darrusalam, Indonesia. The algae were extensively washed with distilled water to remove the particulate material from their surface and were dried under the sunlight. Dried biomass of *E. cottonii* was cut, ground and then screened to particle size of 150-425 μm.

All reagents used were of analytical grade obtained from Merck (Darmstad, Germany). The apparatus used were screener Octagon 200 (Endcots, London, England), an analytical balance (AA-200 Denver Instrument Company), a shaker (Haake SWB 20), a pH meter (Denver Instrument Company), FTIR (Bio-Rad FTS 60), EDX (Joel 6400 analytical SEM Xray) and atomic absorption spectrometer (AAS Alpha-4, Analys 100, London, England)

Cadmium stock solution (1,000 mg/mL): Cadmium solution was prepared by dissolving 1.00 g of pure cadmium powder with concentrated nitric acid and diluting quantitatively to 1,000 mL using 0.1 mol/L nitric acid.

Carrageenan extraction: Carrageenan was extracted from 10 g dried *E. cottonii* with various particle sizes (150, 180, 250 and 425 μm) into 500 mL of alkaline solution (pH 8.0) with steam boiling to maintain temperature at *ca.* 90 ºC continuous stirring for *ca.* 3 h. The solution was then filtered and precipitated with isopropyl alcohol 1:3 and dried in oven at 60 ºC. After that, it was weighed and milled to fine a powder. These procedures were repeated in order to investigate the extraction efficiency by varying the solution pH, heating time as well as the heating temperature.

Batch biosorption studies: Dried *E. cottonii* were soaked with 0.1 mol/L nitric acid for *ca.* 24 h and then were filtered and washed until neutral by using distilled water before they were dried at room temperature.

All batch experiments were performed by adding 500 mg of each dried red algae and carrageenan to 20 mL of 50 mg/L cadmium solution in 100 mL flasks. The flasks were placed on a rotating shaker (hoakc SWB 20) with constant shaking and at the end of experiment, the flasks were removed from the shaker and solutions were separated from biomass by using filter paper. Cadmium concentrations before and after biosorption were determined by using AAS. The same procedures were done for various pH, particle sizes, shaking time, shaking rate, heating temperature and metal concentration.

FTIR Spectroscopy analysis: For the IR studies, 5 % (wt/wt) of ground and dried extracted carrageenan and pure carrageenan were pressed to form KBr dics. The FTIR spectra were recorded in the 1500-500 cm^{-1} spectral range using a Bio-Rad FTS 60 instrument. A total of 128 scans were averaged for each sample with a resolution of 2 cm^{-1}.

EDX Analysis: Energy dispersive X-ray spectroscopy (EDS), which is also referred to as EDX, is a technique used in conjunction with scanning electron microscopy (SEM). First the area of interest is identified through SEM imaging. X-rays generated by the SEM's focused electron beam are then collected by the EDX detector where the energy of the X-ray is determined. The number of X-rays are counted for particular energy and displayed on a graph of counts *versus* energy. A gun with tungsten filament, probe current 10^{-12}-10^{-5} A and accelerating voltage 0.2-40 kV was used. Biomass before amd after using as adsorbent was analyzed by EDX to indicate the process of adsorption.

RESULTS AND DISCUSSION

Extraction of carrageenan from *E. cottonii*: The extraction of carrageenan from *E. cottonii* using alkaline solution was studied. The yield of carrageenan (%) was influenced by the particle sizes of marine algae biomass, pH of alkaline solution, heating time and heating temperature.

Fig. 1 shows that yield of carrageenan increases with increasing particle size of *E. cottonii* from 150 to 425 μm. At particle size larger than 425 μm, the increase of carrageenan yield was not high and at particle sizes smaller than 425 μm gave lower yield of carrageenan because it was difficult to separate the filtrate and residue by filtering due to the amount of waste produced. Although the yield of carrageenan exracted from *E. cottonii* was optimum at particle size 425 μm or more, satisfactory results were obtained for particles with other sizes. However, considering its yield and easy handling, particle size of 425 μm was selected for further experiment.

The effect of heat treatment on the yield of carrageenan extracted from *E. cottonii* was also examined. The result is shown in Fig. 2. It is indicated that the highest percentage of carrageenan was produced after heating for 12 h at 90 °C. In the

graph, heating for more than 12 h did not show increase of yield. However, heating time less than 12 h gave lower yield of carrageenan because not all carrageenan was extracted-into the alkaline solution and heating time higher than 18 h gave high solution concentration - and it was difficult to separate filtrate and residue.

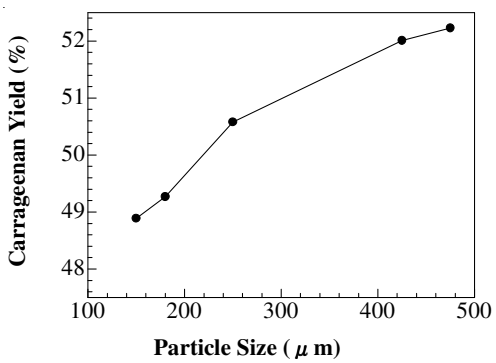

Fig. 1. Yield of extracted carrageenan using various particle size of *E. cottonii* treated with NaOH solution at pH 8.5. Conditions: weight of material =10 g, heating time = 12 h and heating temperature = 90 ºC

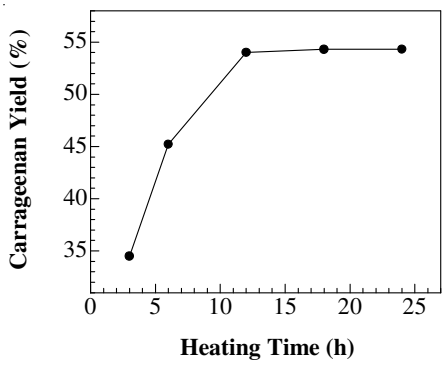

Fig. 2. Yield of extracted carrageenan obtained from various heating time of *E. cottonii* Conditions: weight of material = 10 g, particle size = 425 μm, solution of pH = 8.5 and heating temperature = 90 ºC

The yield of extracted carrageenan from *E. cottonii* also depends on the pH of the extraction solution. As shown in Fig. 3, the yield of carrageenan increased greatly with increasing pH from 8.0 to 8.5 and then stayed close to constant till 9.0 and after that decreased in the range 9-10. The maximum stability of carrageenan[18] is pH 9.0.

The yield of carrageenan was also affected by the heating temperature of *E. cottonii*. The carrageenan was diluted in hot alkaline solution and were heated at 85 to 105 ºC. In this investigation, heating temperature 95 °C gave the highest % of carrageenan yield. At heating temperature less than 95 °C, not all carrageenan was produced. On the other hand, heating temperature more than 95 °C would produce concentrated solution and it was difficult to separate filtrate from the residue.

Under the optimum conditions, 64.3 % carrageenan could be extracted from *E. cottonii*. The galactans extracted with hot water are mainly composed of k-carrageenans 74 % and μ-carrageenans 3 %[19].

FTIR Analysis: The functional groups of extracted carrageenan from *E. cottonii* that produced by alkaline solution extraction and pure carrageenan were determined by FTIR. The spectra in Fig. 5 showed that the functional group of both carrageenans was identical. The study of carrageenans by FTIR spectroscopy shows the presence of strong absorption bands in the 1266 cm^{-1} region (due to the S=O of sulfate esters) and 1068 cm^{-1} region (discribed to the glycosidic linkage) in all carrageenan types. The other chemical groups are characteristics of a given carrageenan type, namely 3,6-anhydro-D-galactose at 929 cm^{-1}, D-galactose-4-sulfate at 846 cm^{-1}, D-galactose-

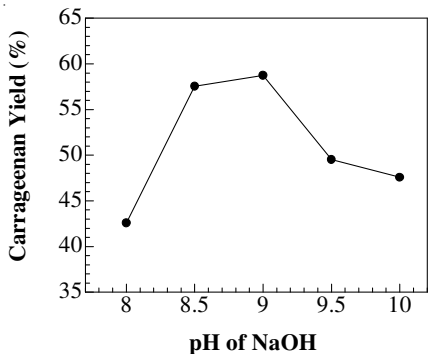

Fig. 3. Yield of extracted carrageenan obtained using various pH of NaOH solution for *E. cottonni*; Conditions: weight of material 10 g, particle size = 425 μm, heating time = 18 h, heating temperature = 90 ºC

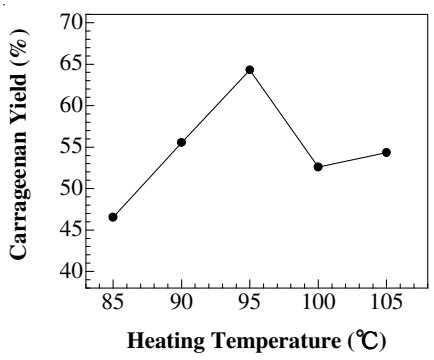

Fig. 4. Yield of extracted carrageenan at various heating temperature of *E. cottonii*; Conditions: weight of material 10 g, particle size = 425 μm, heating time = 18 h

2-sulfate at 830-820 cm^{-1} and 3,6-anhydro-D-galactose-2-sulfate at 805-800 cm^{-1}. The spectra could explain that the carrageenan extracted from *E. cottonii* was almost pure.

Mainly carrageenans of the k-type has strong bands at 933 and 847 cm^{-1} and i-type[20] at 933, 847 and 805 cm^{-1}. The FTIR spectrum of alkali-modified polysaccharide resembled k-carrageenan from red alga *Phacelocarpus peperocarpos* with absorption at 930 cm^{-1} (indicative of AnGal) and 850 cm^{-1} (Gal 4-sulfate) and at 820 cm^{-1} indicating the presence of equatorial sulfate ester substitution at O-6 of Gal residues[21].

EDX Analysis: The percentage of some elements in biomass decreased when biomass was used as metal ion cadmium adsorption. Potasium, calcium, sodium and magnesium were participated in cation exchange process, while chloride participated in cadmium binding. The EDX data of biomass before and after using an adsorbent indicated that the process of adsorption was cation exchange.

The binding mechanism of cadmium and chromium by different biomasses is not fully understood. However, methodologies such as chemical modification, infrared spectroscopy, X-ray absorption spectroscopy, among others, have been used to study the biomass-metal binding mechanisms. Studies have shown that negatively-charged ligands such as carboxyl groups play a major role in the binding of cadmium and chromium to different biomasses, while positively-charged ligands through ligand exchange, ion exchange or reduction mechanisms[21].

Biosorption of cadmium by *E. cottonii* and carrageenan: The influence of several parameters such as pH, particle size, shaking time, shaking rate and metal concentration on biosorption of cadmium by *E. cottonii* and carrageenan were evaluated. The results were expressed as the amount of cadmium uptake on dried *E. cottonii* and carrageenan, Q (mg/g). The effect of pH ions solution on cadmium uptake by *E. cottonii* and carrageenan is shown in Fig. 6.

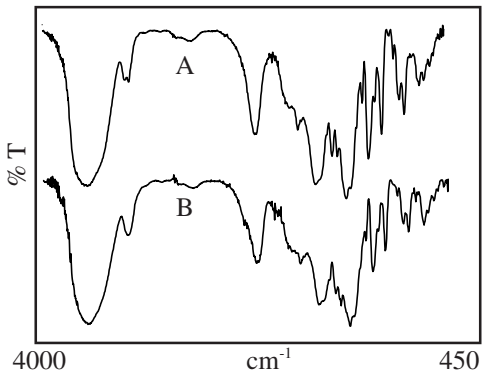

Fig. 5. FT-IR spectra of pure carrageenan (A) and extracted carrageenan (B) from *E. cottonii*

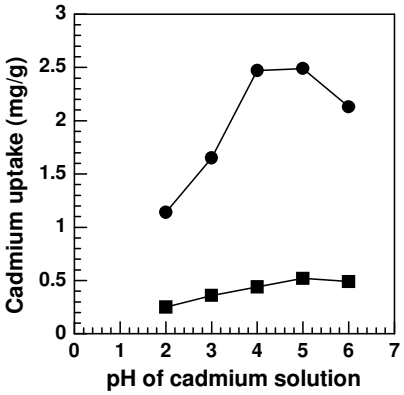

Fig. 6. Effect of pH on cadmium uptake by *E. cottonii* and carrageenan; Conditions: Contact time = 30 min, shaking rate = 180 rpm and cadmium ion concentration = 50 mg/L. ■ = *E. cottonii*, ● = carrageenan

The uptake of cadmium ions depends on the pH, *i.e.*, with increasing of pH from 2.0 to 4.0 cadmium uptake seen that the plot of metal uptake *versus* pH displays and S shape centered at pH 3 and then reaching plateau in the range 5.0 - 6.0. As the pH increases, the ligands such as carboxylate groups in *E. cottonii* and carrageenan would be exposed. Thus increasing the negative charge density on the biomass surface and the attraction of metallic ions with positive charge and allowing the biosorption onto the cell surface[2]. At higher pH, cadmium hydroxide started to precipitate from the solution and at pH below 2, the cadmium uptake is very small, but not negligible, which can be a result of presence of a relatively low amount of strong acid groups such as sulfonic group from fucoidans[22]. At low pH values, the overall surface charge becomes positive, which may inhibit the adsorption of positively-charge metal ions[23,24].

Seven species of brown, green and red seaweeds were examined for their abilities to uptake cadmium ions from aqueous solution. Although all the investigated seaweed types were capable of binding appreciable amounts of cadmium, considerable variability in their biosorption performance was observed. Maximum cadmium uptake capacities at pH 5 ranged from the highest value of 0.74 mmol/g for the brown seaweed (*Sargassum baccularia*) to the lowest value of 0.16 mmol/g for the red seaweed (*Gracilaria salicornia*)[9].

Fig. 7 shows that the optimum adsorption of cadmium by using *E. cottonii* and carrageenan occurred at *ca.* 1 h. Contact time higher than 1 h showed a constant adsorption capacity of cadmium.

Fig. 8 shows the cadmium adsorption capacity of *E. cottonii* and carrageenan *versus* shaking rate. The results indicate that the highest adsorption was obtained

with shaking rate of 150 rpm. Shaking rate of 100 and 200 rpm gave lower ion metal uptake compared to shaking rate of 150-180 rpm, thus the optimum shaking rate, *i.e.* 150 rpm, was used in this study.

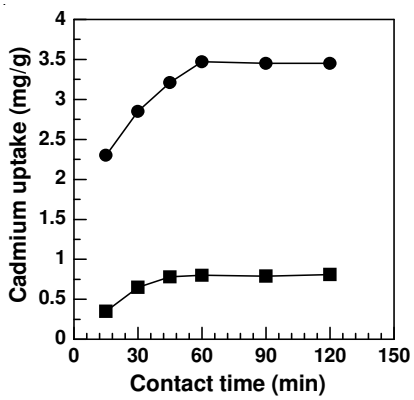

Fig. 7. Effect of contact time on cadmium uptake by *E. cottonii* and carrageenan. Conditions: pH solution = 5.0, shaking rate = 180 rpm and cadmium ion concentration = 50 mg/L. ■ = *E. cottonii*, ● = carrageenan

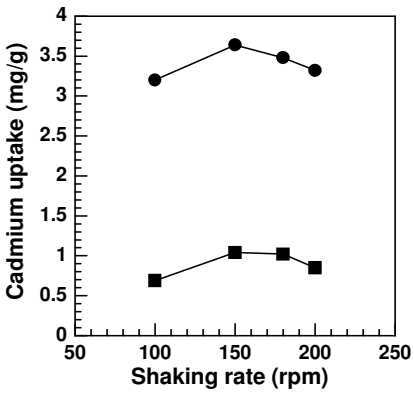

Fig. 8. Effect of shaking rate on cadmium uptake by *E. cottonii* and carrageenan. Conditions: pH solution = 5.0, contact time = 60 min and cadmium ion concentration = 50 mg/L. ■ = *E. cottonii*, ● = carrageenan

Effect of cadmium concentration on cadmium uptake by *E. cottonii* and carrageenan shows that the capacity of cadmium ion uptake increased as the concentration of metals ion increased from 25 to 50 mg/L, but it decreased with increasing metal ion solution from 50 to 100 mg/L. The optimum concentration of cadmium was 50 mg/L. Under the optimum conditions, *i.e.* pH 5.0, particle size 150 μm, contact time 1 h, shaking rate 150 rpm and concentration 50 mg/L, biosorption capacity of cadmium was 3.85 mg/g for extracted carrageenan, 0.95 mg/g for red alga and 3.87 mg/g for pure carrageenan. The adsorption capacity was also affected by the foreign metal ions such as potassium, calcium, sodium, magnesium, *etc.* and sulfate esters which contained in the materials.

Carrageenan contained potassium more than *E. cottonii*. Besides potassium, carrageenan also contain chloride ion that could form a complex with cadmium ion. After using carrageenan as adsorbent of cadmium, chloride ion was not found in carrageenan.

Conclusion

Carrageenan could be extracted from *Eucheuma cottonii* by alkaline solution, followed by precipitation using isopropyl alcohol under the optimum conditions, *i.e.* 425 μm particle size, alkaline solution with pH 8.5, heating time 18 h and heating temperature 95 °C. Based on the FTIR spectra, extracted carrageenan showed the same functional groups as the pure carrageenan. Capacity of cadmium adsorption

under the optimum conditions of pH 5, particle size 150 μm, contact time 1 h, shaking rate 150 rpm and cadmium ion concentration 50 mg/L, carrageenan showed almost the same capacity as the pure carrageenan and its capacity was higher than those of red algae.

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