ORIGINAL ARTICLE

Urethane foam of sulfated polysaccharide ulvan derived from green-tide-forming chlorophyta: synthesis and application in the removal of heavy metal ions from aqueous solutions

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The major polysaccharide of *Ulva* is ulvan, a sulfated glucurono-rhamno-xyloglycan. Some studies have suggested the potential utility of ulvan and ulvan-based materials. However, procedures for the efficient extraction of ulvan and its structural modification are lacking. Therefore, we attempted to improve the extraction of ulvan from *Ulva*. Specifically, a coagulation–sedimentation process was introduced to shorten the filtration time of residual *Ulva* after the extraction step. Nevertheless, chemical treatment reduced the yield of the acidic polysaccharide due to coprecipitation. Furthermore, ulvan was modified with three diisocyanate derivatives to yield urethane foam; the ability of this foam to remove Cu^{2+} ions from an aqueous solution was investigated. Notably, the foam could remove up to 72.7% of the Cu^{2+} ions. This is the first report of improved extraction, chemical modification and application of ulvan in the removal of heavy metal ions from aqueous solutions.

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INTRODUCTION

As illustrated by Stengel *et al.*,¹ a diverse array of compounds exist within the algal taxonomical group. For instance, phaeophyceae contain alginate, but chlorophyta do not. Even though different species contain so-called ‘alginates’, these compounds exhibit species-specific differences in their gelling properties. Ray *et al.*² revealed three main types of polysaccharide families in *Ulva*. Ulvan, the major polysaccharide, is a sulfated glucurono-rhamno-xyloglycan; other types of these compounds include hemicellulosic polysaccharides, such as glucuronans and glucoxylans. The authors also reported on sulfated polysaccharides containing glucose, xylose and mannose as minor components.² Seasonal changes in the constituents of *Ulva* were also reported by Abdel-Fattah and Edrees,³ who found variations in carbohydrate content depending on the season.

Many ulvan extraction methods have been reported. Yaich *et al.*⁴ revealed that the yield and molecular weight of ulvan varied depending on the pH. It was also reported that stabilization treatments before extraction from *Ulva* affected extraction yields and the properties of ulvan.⁵ Only a few reports describing chemical modifications of ulvan are available; this may be because of the complicated procedures required for extraction and because ulvan is structurally dependent on the season. For example, Qi *et al.*⁶ reported on the acetylation and benzylation of ulvan and the activities of chemically modified ulvan derivatives. Some ulvan-based materials have been developed, such as hydrogels crosslinked with divalent

cations,^{7–9} illustrating the potential utility of ulvan. In order for the potential utility of ulvan to be fully realized, improved extraction procedures must be developed. Such procedures may lead to novel chemical modifications and applications.

There are two main issues encountered during ulvan extraction. First, the coagulation–sedimentation of *Ulva* residue after extraction is necessary because the filtration of residual *Ulva* requires a significant amount of time. Second, the acidic polymers extracted from *Ulva* may act as coagulation–sedimentation reagents on the *Ulva* residue, decreasing the yield of the acidic polymer itself. To avoid the loss of the acidic polysaccharide, we examined the coagulation–sedimentation of *Ulva* residue with inorganic chemicals. Various extraction methods were considered to increase the yield of acidic polysaccharides. After the purification of the polysaccharides, the ulvan was modified with three different diisocyanate derivatives to generate a urethane foam and a nonfoamed urethane/ulvan. The application of the foam in the removal of Cu^{2+} ions from aqueous solutions was also investigated to illustrate the utility of the modified ulvan.

MATERIALS AND METHODS

Materials

Ulva pertusa was collected at the Wajiro mudflats in Fukuoka prefecture in August 2013. The *Ulva* was washed with fresh water, wrung out over nonwoven fabric, dried in an oven at 80 °C for 12 h and further dried by exposure to air.

All of the following reagents were obtained from commercial sources and used without further purification: polypropylene glycol and tolylene 2,4-diisocyanate terminated (TDI terminated) were purchased from Sigma-Aldrich (St Louis, MO, USA); isophorone diisocyanate (IPDI) was purchased from Tokyo Chemical Industry (Tokyo, Japan); 4,4'-methylenebis(phenyl isocyanate) was purchased from Sigma-Aldrich; ulvan was purchased from Wako (Tokyo, Japan); and cellulase was purchased from Wako (0.9 U mg^{-1} , from *Trichoderma Viride*).

Infrared (IR) spectra were measured on an IRPrestige-21 Fourier transform IR spectrophotometer (SHIMADZU, Tokyo, Japan). Absorption spectra were measured on a V-550 UV/VIS spectrophotometer (JASCO, Tokyo, Japan), and atomic absorption spectra were measured on a Z-2300 polarized Zeeman atomic absorption spectrophotometer (HITACHI, Tokyo, Japan).

Rapid coagulation–sedimentation of *Ulva* residue

The rapid coagulation–sedimentation of residual *Ulva* was examined. After the extraction, a mixture of 10 g of *Ulva* in 300 ml 0.05 N sulfuric acid was used for the coagulation–sedimentation test. Filtration of the mixture through non-woven fabric resulted in a suspension. The pH of the filtrate was adjusted to the value listed in Table 1 by the addition of a saturated solution of NaHCO_3 and 0.1 N HCl. Then, 1 ml of 39% ferric chloride or 1 ml of 8% aluminum sulfate was added into the suspended filtrate to produce floc. After the pH of the residue was adjusted, the suspension was stirred at a high r.p.m. for 15 min using a static mixer. The speed of the mixer was then reduced to match the conditions of the flocculation. The suspension was stirred for 45 min and subsequently filtered using filter paper. The acidic polysaccharide mixture was analyzed without further purification.

Extraction of acidic polysaccharides from *Ulva*

Ten grams of dried *Ulva* in 300 ml extracting solvent (listed in Table 2) was ground with an electric mixer. The mixture was stirred at the temperature given in Table 2. The residue was filtered through nonwoven fabric and filter paper under reduced pressure. The filtrate was dialyzed against a solution of $0.1 \text{ mol l}^{-1} \text{ CaCl}_2$ to remove precipitated nonsulfated uronic polysaccharides. The solution was subsequently dialyzed against pure water. The dialyzed solution was subjected to diethylaminoethyl-cellulose column chromatography with a NaCl gradient. The fraction containing ulvan was dialyzed against pure water. This was followed by freeze-drying that yielded ulvan as a beige-colored solid.

Synthesis of a urethane foam of ulvan

Ulvan (with an intrinsic viscosity of 1.12 dl g^{-1}) extracted with sulfuric acid was used for all urethane foam preparations. In 0.2 g of powdered ulvan, 1 g of diisocyanate was added, or the ulvan was swelled in water with 0.1 ml of tetraethylenediamine. The reaction mixture was vigorously stirred for 15 min, and the reaction mixture containing water produced foam. The mixture was subsequently heated at 80°C for 24 h, and the supernatant was removed by centrifugation. The foam body, or powder, was stored for more than 3 h in 20 ml of dimethylformamide, then for 3 h in 20 ml of pure water; this procedure was carried out on three separate occasions to remove

low-molecular-weight salts in the solid. Finally, the urethane was freeze-dried. Alginate urethanes were synthesized in the same manner.

Sulfate group content of the urethane

The sulfate group content of the urethane was determined using the Dodgson–Price method.¹⁰ The results are summarized in Table 3.

Removal of Cu^{2+} ions with the urethane foam

A total of 20 mg of freeze-dried urethane was swelled in 20 ml of a 20 mg l^{-1} solution of CuCl_2 at 30°C . The atomic absorption spectra of the Cu^{2+} ions in the supernatant was measured. The concentration of the heavy metal ions in the supernatants was determined using the calibration curves for the Cu^{2+} ions.

RESULTS AND DISCUSSION

Rapid coagulation–sedimentation of residual *Ulva*

For the rapid coagulation–sedimentation of residual *Ulva*, coagulation–flocculation was applied using two types of reagents, aluminum sulfate and ferric chloride (both are treatment reagents for drinking water). The polysaccharide yield following the coagulation process is shown in Table 1. Ferric chloride, especially at pH 7, induced a good separation of the suspended *Ulva* residue from the extracted solution such that a clear solution was obtained following decantation and filtration. With aluminum sulfate, centrifugation was necessary to separate the suspension before decantation and filtration. However, coagulation with the chemical reagents resulted in a decreased polysaccharide yield compared with yields without chemical treatment (Table 2). The IR spectra of the polysaccharide mixture exhibited a reduced absorption at 1250 cm^{-1} , suggesting that the ulvan coprecipitated with residual *Ulva* during the coagulation–sedimentation process (Figure 1). The adsorption at 1650 cm^{-1} (assigned to uronic acid) suggested that the hemicellulose polysaccharide consisted of uronic acid, as described by Ray and Lahaye.¹¹

Table 2 Ulvan yields

No.	Reagents ^a	Temperature ($^\circ\text{C}$)	Time (h)	Yield (mg) ^b
1	Hot water	100	2	371
2	0.05 M Sulfuric acid	20	24	349
3	0.1 M Sodium oxalate	20	24	306
4	1 mg Of cellulose in pH 6.4 phosphate buffer	45	144	98.0

^aA total of 300 ml of the reaction solvents was used.

^bExtracted from 10 g of *Ulva*.

Table 1 Rapid coagulation–sedimentation of residual *Ulva*

pH	Ferric chloride		Aluminum sulfate	
	Yield of acidic polysaccharide (mg)	Flocculation quality	Yield of acidic polysaccharide (mg)	Flocculation quality
4	61	Fair	94	Null ^a
5.5	58	Fair	66	Null ^a
7	45	Excellent	71	Null ^a
8	55	Fair	53	Null ^a

^aCentrifuged to remove solid from solution.

Table 3 Sulfate group contents of the urethanes

No.	Abbreviation for the product	Sulfate group contents of urethane (mmol g^{-1})	Removal rate of Cu ions at 24 h (%)
1	IPDI-Ulvan formed	0.181	46.1
2	TDI-Ulvan formed	0.298	58.5
3	MDI-Ulvan formed	0.268	67.2
4	IPDI-Ulvan nonformed	0.218	72.7
5	TDI-Ulvan nonformed	0.281	53.1
6	MDI-Ulvan nonformed	0.212	54.5

Abbreviations: IPDI, isophorone diisocyanate; MDI, 4,4'-methylenebis(phenyl isocyanate); TDI, tolylene 2,4-diisocyanate.

Figure 1 Infrared (IR) spectra of the acidic polysaccharide mixture after coagulation-sedimentation.

Figure 2 Infrared (IR) spectra of ulvan extracted using different methods.

Both ferric chloride and aluminum sulfate (Table 1) coprecipitated ulvan and residual *Ulva*.

IR spectra of the acidic polysaccharides obtained by different extraction methods

Sulfated polysaccharides, such as fucoidan, can be extracted under acidic conditions. Uronic polymers, such as alginate, are often extracted under basic conditions. Some chelating reagents, such as sodium oxalate, dissociate the calcium crosslinkage between uronic acids to release ulvan from the extracellular matrix of *Ulva*. As shown in Table 2, 10 g of dried *Ulva* yielded ~300 mg of ulvan. It has been

reported that the yield and structure differ depending on the stabilizing method⁵ and the season.³

The characteristic absorption of sulfates and uronic acids were identified in the Fourier transform-IR spectra (Figure 2). The absorption at ~ 850 , 1250 , 1650 and 3500 cm^{-1} was attributed to the C–O–S bending vibration of the sulfate group in the axial position, the S=O stretching vibration of the sulfate group, the C=O of uronic acids and the OH group, respectively. The IR spectra of the extracted polysaccharides showed the same characteristic absorption as commercial ulvan. However, the spectrum of sample no. 4 showed remarkably different absorption intensities; compared with commercial ulvan, the absorption at 1100 cm^{-1} was greater, and

Table 4 Chemical modifications of ulvan with urethane

No.	Kinds of diisocyanate ^a	Water (ml)	Tetraethylene diamine (ml)	Temperature (°C)	Time (h)	Yield (mg)	Abbreviation for the product
1	Diisocyanate 1	1	0.1	80	24	1.07	IPDI-Ulvan formed
2	Diisocyanate 2	1	0.1	80	24	1.09	TDI-Ulvan formed
3	Diisocyanate 3	1	0.1	80	24	0.910	MDI-Ulvan formed
4	Diisocyanate 1	—	0.1	80	24	0.910	IPDI-Ulvan nonformed
5	Diisocyanate 2	—	0.1	80	24	0.976	TDI-Ulvan nonformed
6	Diisocyanate 3	—	0.1	80	24	1.26	MDI-Ulvan nonformed

Abbreviations: IPDI, isophorone diisocyanate; MDI, 4,4'-methylenebis(phenyl isocyanate); TDI, tolylene 2,4-diisocyanate.

^aDiisocyanate 1: isophorone diisocyanate (mixture of isomers); diisocyanate 2: polypropylene glycol, tolylene 2,4-diisocyanate terminated; diisocyanate 3: 4,4'-methylenebis(phenyl isocyanate).

Figure 3 Infrared (IR) spectra of the six types of urethanes summarized in Table 4.

the absorption at 1700 cm^{-1} was diminished. Sample no. 4 may include hemicellulose with higher neutral monosaccharides.

Urethane foam of ulvan

Ulvan was extracted from 300 g of dried *Ulva* with 0.05 mol l^{-1} sulfuric acid (no. 1 in Table 2). The reaction mixtures for sample nos. 1–3 (Table 4) included water; these samples foamed within a few minutes after the addition of the diisocyanate derivatives. Sample nos. 4–6 did not contain water and did not produce foam. The urethane foams with ulvan were softer than the urethane foams without ulvan. The characteristic absorptions of the ulvan and urethane units were identified in the Fourier transform-IR spectrum (Figure 3). The absorptions representing ulvan at ~ 850 , 1250 , 1650 and 3300 cm^{-1} were attributed to the C–O–S, S=O, C=O and OH groups, respectively. The absorptions representing urethane at 3400 , 3300 , 2900 , 2200 , 1750 , 1700 , 1500 and 1200 cm^{-1} were attributed to the

$\nu\text{N-H}$, $\nu\text{O-H}$, νCH_2 , $\nu\text{N=C=O}$ of the remaining isocyanate, $\nu\text{C=O}$ of uronic acid, $\nu(\text{C=O})$ of amide I, $\nu(\text{C-N}) + \delta(\text{N-H})$ of amide II and $\nu(\text{C-N}) + \delta(\text{N-H})$ of amide III, respectively. The IR spectra suggested that the obtained solids were a mixture of urethane and ulvan. Sample nos. 2 and 5 (crosslinked with 'TDI terminated') showed two strong absorption peaks representing the aromatic groups of the tolylene 2,4-diisocyanate portion of the molecule at 810 and 860 cm^{-1} . An absorption peak characteristic of ether at 1100 cm^{-1} and a methylene group at 2900 and 2800 cm^{-1} (propylene glycol unit) were also observed. Sample nos. 3 and 6 were crosslinked with 4,4'-methylenebis(phenyl isocyanate) and showed a strong absorption peak representing a *para*-substituted aromatic group at 810 cm^{-1} . The presence of absorption peaks identified as urethane units suggested that ulvan-crosslinked urethane was obtained. Table 3 shows that the sulfate group content of the urethanes was between 0.181 and 0.298 mmol g^{-1} . The unmodified ulvan had a sulfate group

Figure 4 Structures of the six types of urethanes summarized in Table 4.

Figure 5 Removal of Cu^{2+} ions with urethane derivatives.

content of 1.71 mmol g^{-1} . This result suggested that the urethanes consisted of 10.1 to 17.4% ulvan, and 'IPDI-Ulvan formed' had the lowest sulfate group content.

The proposed chemical structures of the urethanes are shown in Figure 4. The main difference in the chemical structures between the foamed and nonfoamed urethane is the existence of urea. This is due to the reaction of isocyanate with water to produce urea and carbon dioxide.

Removal of Cu^{2+} ions from aqueous solutions with urethane/ulvan

Ion exchangers are important biomaterials and environmental materials. Christoforou *et al.*¹² reported that ion-exchange beads promote a variety of wound-healing responses in several model systems. We examined the ion-exchange behavior of the urethane derivatives of ulvan. As shown in Figure 5, the amount of Cu^{2+} in an aqueous solution decreased gradually via the ion exchange of the carboxyl and sulfate groups of ulvan in the urethane derivatives. A significant amount of time was required before the maximum removal rate could be achieved. This was because small molecules penetrated the pores of the swelled urethane and diffused between the two water entities. The urethane foam and the nonfoamed urethane with ulvan removed Cu^{2+} ions from an aqueous solution with a

removal rate of up to 72.7%. The 'IPDI-Ulvan formed' showed the lowest removal rate; notably, it had the lowest sulfate group content.

The removal rate of ulvan/urethane was compared with strong chelators. Alginate, a strong chelator of divalent cations, was cross-linked with urethane and the removal rate of Cu^{2+} ions was compared with that of ulvan/urethane. As shown in Figure 5, two types of alginate/urethane, IPDI-Alginate and TDI-Alginate, had a higher removal rate than any ulvan/urethane. There are two possible reasons why alginate/urethane had a higher Cu^{2+} ion removal rate than ulvan/urethane. First, the carboxylic acid content of alginate is higher than that of ulvan. Second, alginate is a strong chelator of divalent cations because of its special structure, termed the 'egg box model'.¹³

CONCLUSIONS

Biodiversity is affected by habitat loss, climate change, overexploitation, invasive alien species and pollution and nutrient loading. For instance, pollution and nutrient loading cause the chlorophyta *Ulva* to form green tide. The utilization of *Ulva* is problematic; however, it should not be discounted as a resource in the reduction of direct pressures on biodiversity and the promotion of sustainability. Some studies have suggested that *Ulva* has potential use in human and animal food. Notably, *Ulva* has been eaten in Japan for some time. Nevertheless, landfills and incineration are popular methods to process *Ulva* waste.

The utilization of *Ulva* as a biomass has been studied by many researchers. Biosorption, especially of heavy metal ions, by dead and live *Ulva* has been previously reported by many groups. Biosorption by *Ulva* has been used to remove organic compounds in aqueous milieu. Notable examples include the removal of bovine serum albumin from industrial wastewater and the removal of agrochemicals from aqueous media. The effective bioremediation of valuable nutrient resources such as nitrogen and phosphorus from wastewater sludge using *Ulva* has also been demonstrated.

The sulfated polysaccharide ulvan is an interesting biomaterial. Many biological activities have been reported for this compound, including antioxidative activity, anti-hypercholesterolemic activity, immunostimulation and the stimulation of turbot phagocytes. In this paper, we focused on the utilization of the green-tide-forming chlorophyta *Ulva* for ulvan. Specifically, we developed an enhanced extraction procedure to obtain ulvan from *Ulva*. Furthermore, we utilized chemically modified ulvan to generate a urethane foam and demonstrated its utility in the removal of Cu^{2+} ions from an aqueous solution. We believe that these results will be useful in developing other novel and useful applications for ulvan.

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