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Efficiency of Cu(II) Removal From Aqueous Media as a Function of Algal Extract Polysaccharide Content

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Abstract

Proper stewardship of our environment necessitates biodiversity preservation and the wise use of resources. Species that may be pests, such as some algae, may also be used innovatively to mitigate their ecological impact. In this study, we extracted polysaccharide mixtures from three algae-*Undaria*, *Laminaria,* and *Ulva*-under three sets of conditions. The nine extracts contained different polysaccharide compositions and varying carboxylic acid contents. The extracts were converted into their respective algal gels by polyion complex formation with chitosan. The gels were examined for their ability to remove Cu(II) ions from aqueous solutions by ion exchange. The removal efficiency was dependent on the algae extraction conditions, which affected the polysaccharide content of the extracts. Among the gels, those derived from the alkaline extractions of *Undaria* and *Laminaria* exhibited higher Cu(II) removal efficiencies than from the other extracts. Gels prepared from extracts with higher uronic acid contents exhibited better removal Cu(II) efficiencies. We expected that an extract's carboxylic acid content would be proportional to its removal efficiency, because the acidic groups in the polysaccharide bind to the divalent heavy metal ions. However, this proportionality was not observed: extracts that included sulfated polysaccharides were less efficient at ion removal, despite their carboxylic acid content. This can be explained by the structural differences in the adsorption sites between alginate and those of the sulfated polysaccharide. Thus, an environmental deficit was converted into a potential economic benefit in the removal of heavy metals from water.

Keywords: algae, polysaccharide, extraction conditions, removal efficiency, bivalent copper, biodiversity

1. Introduction

1.1 Aichi Biodiversity Targets

The importance of ecosystems and biodiversity is being increasingly appreciated in terms of their economic benefits (Bishop et al., 2010). Intelligent use of biodiverse resources is important for sustainable development because ecosystem services (such as provisioning, regulating, supporting, and cultural) are supported by biodiversity. Scientific approaches to sustainable development have been reported in various fields such as nature conservation (Alvarez & Larkin, 2010), plastics recycling (Chen et al., 2006), research (Wu et al., 2009), and education on the sustainable use of bioresources (Kanno et al., 2011). At the 10th Conference of the Parties of the Convention on Biological Diversity (CBD-COP10) held in Nagoya, Japan, delegates from more than 100 countries agreed on a new strategic plan, known as the "Aichi Biodiversity Targets" (Secretary of the Convention on Biological Diversity, 2010). This plan comprises a shared vision, a mission, strategic goals, and 20 ambitious yet achievable goals. A study on the utilization of species that are both problematic and potential resources is one possible way to achieve strategic goal B of the Aichi Targets, the reduction of direct pressures on biodiversity and promotion of sustainable use.

1.2 Utilization of Algae

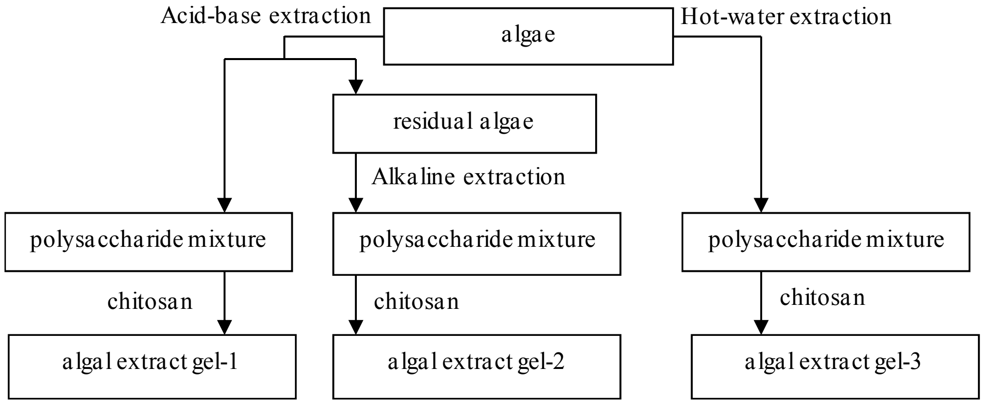
One use for algae, the biosorption of heavy metal ions by both dead and alive *Ulva*, has been reported by many researchers (Areco et al., 2012; Karthikeyan et al., 2007; Kumar et al., 2006; Kumar et al., 2007; Sarı & Tuzen, 2008; Turner et al., 2007). Materials that react with heavy metal ions have the potential not only to be developed into water treatment agents, but also be used in the biomaterials area. For example, Shi et al. reported interesting properties for a low molecular weight polysaccharide extracted from *Ulva* (Shi et al., 2013). They synthesized an iron(III) complex of the extracted polysaccharide and found that it effectively protected mice from radiation-induced damage in bone marrow cells and the immune system. Moreover, they revealed the anti-radiation and anti-oxidative activities of the complex.

1.3 Focus of Study

In this work, we studied water treatment by algal extract gels prepared from three algae-*Undaria*, *Laminaria* and *Ulva*. Although these algae are used as foodstuffs in Japan, they can be environmentally troublesome. For example, *Ulva* can overgrow and result in green tides around the world. It forms algal mats on the Wajiro tideland in Japan, which is an important habitat for the black-faced spoonbill (*Platalea minor*), an endangered species that numbers less than 1,000 worldwide (Figure 1). Algal mats are incinerated or buried in landfills in Hakata Bay. The alga species *Undaria pinnatifida* has been listed in “100 of the World's Worst Invasive Alien Species” by the Global Invasive Species Database (Lowe et al., 2000). Finally, huge amounts of residual *Laminaria* are disposed of every year. For example, in the *Laminaria*-growing district of Hokkaido, Japan, 6,000 tons of *Laminaria* is discarded annually (Sato et al., 2005).

Figure 1. Green tide on Wajiro tideland

Acidic polysaccharides extracted from algae remove heavy metal ions in aqueous solution by ion exchange. We reported the removal of Cu(II) ions from aqueous media via an ulvan-chitosan polyion complex gel (Kanno et al., 2012). However, the extraction and purification of ulvan from the algae was neither easy nor economical. We focused on crude polysaccharide as an ion exchange agent for facile extraction and economical use of residual algae. The efficiency of heavy metal ion removal using algal extracts depends on the algae, because diverse compounds exist within the algal taxonomic group, as illustrated by Stengel (Stengel et al., 2011). For instance, the *Phaeophyceae* produce the anionic polysaccharide alginate, but *Chlorophyta* do not. *Ulva* species produce the sulfated polysaccharide ulvan, whereas the sulfated polysaccharide from the *Phaeophyceae* is fucoidan (Figure 2). The efficiency of heavy metal ion removal with algal extracts also depends on the algae extraction conditions, because this method affects the content of the various polysaccharides in the extracts. As an example, sulfated polysaccharides elute under acidic conditions, in contrast to alginate, a uronic polysaccharide, which needs basic conditions or chelating agents to dissociate the Ca^{2+}-ion crosslinks between the uronic acids.



Scheme 1. Extraction of algal polysaccharide mixtures

As a typical heavy metal, we examined Cu(II) ions for removal; many materials have been reported with respect to Cu(II) removal and the mechanism has been described in detail (Awual et al., 2013, 2014). To determine the extraction conditions preferred for water treatment-i.e., the successful removal of Cu(II) ions by a gelled algal extract-we extracted polysaccharide mixtures obtained from a trio of algae species under three sets of extraction conditions, producing nine different extracts (Scheme 1). We investigated the relationship between the algal polysaccharide extraction conditions and the efficiency of heavy metal ion removal from aqueous media.

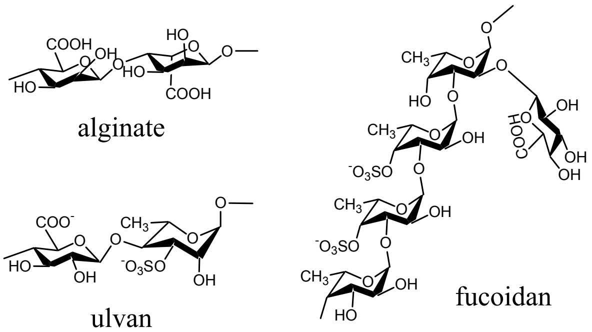


Figure 2. Representative structures of algal acidic polysaccharides

2. Method

2.1 Materials

All reagents were purchased from Wako Pure Chemical Industries, Ltd. *Ulva pertusa* was collected at the Wajiro tideland in Fukuoka prefecture in October, 2012. The samples were rinsed with seawater followed by pure water, and were subsequently dried *in vacuo*. Dried samples of *Undaria pinnatifida* and *Laminaria japonica*, collected in Fukuoka and Hokkaido, respectively, were commercially available. The protein content of the polysaccharide mixtures were assessed using the Biuret reaction. Infrared spectra were measured using an IRPrestige-21 Fourier-transform infrared spectrophotometer (Shimadzu). Absorption spectra were measured using a V-550 UV-vis spectrophotometer (JASCO). Atomic absorption spectra were obtained using a Z-2300 polarized Zeeman atomic absorption spectrophotometer (Hitachi).

2.2 Extraction of Polysaccharide Mixtures

2.2.1 Hot-water Extraction

Ground, dried algae (1 g) in pure water (50 mL) was boiled for 5 h. The residue was filtered through non-woven fabric, and the filtrate was centrifuged at 5,000 rpm for 10 min to remove residual algae. The supernatant was dialyzed against pure water and the resulting solution was freeze-dried to afford the polysaccharide mixture.

2.2.2 Acid-base Extraction

Ground, dried algae (1 g) in 0.05 M H_2SO_4 (50 mL) was stirred at 20 °C for 24 h. The residue was filtered through non-woven fabric, and the filtrate was centrifuged at 5,000 rpm for 10 min to remove residual algae. The supernatant was dialyzed against pure water and the resulting solution was freeze-dried to afford the polysaccharide mixture.

2.2.3 Alkaline Extraction

Ground, dried algae (1 g) in 0.05 M H_2SO_4 (50 mL) was stirred at 20 °C for 24 h. The residue was centrifuged at 5,000 rpm for 10 min to remove residual algae. The precipitate was collected, added into water (50 mL), and the pH was adjusted to 10 by adding sat. Na_2CO_3. The reaction mixture was stirred at 20 °C for 24 h. The residue was filtered through non-woven fabric and then centrifuged at 5,000 rpm for 10 min to remove residual algae. The supernatant was dialyzed against pure water, and the resulting solution was freeze-dried to afford the polysaccharide mixture.

2.3 Carboxylic Acid Content in the Polysaccharide Mixtures

The carboxylic acid content in the polysaccharide mixtures was measured via the carbazole-sulfuric acid method using D-glucuronic acid as a standard (Holzman et al., 1947).

2.4 Preparation of Algal Extract Gel

At 80°C, an aqueous solution of an algal polysaccharide mixture (1.5 wt%, 5 mL) was added into a solution of chitosan (1 mL, 1.5 wt% in 5% acetic acid) to form a hydrogel. The gel was reacted with glutaraldehyde (1 mL, 2.5% in water) at 80 °C for 30 min. The obtained hydrogel was thrice stored for 3 h in water (100 mL) to remove low molecular weight salts in the gel. The algal extract gel was then freeze-dried.

2.5 Removal of Cu(II) by the Algal Extract Gel

The freeze-dried gel (0.05 g) was swelled in 0.01 M $CuCl_2$ (5 mL) 20 °C for 7 d. Atomic absorption spectra of the Cu ions in the supernatant were obtained, and the copper concentration was determined against a $CuCl_2$ calibration curve.

3. Results and Discussion

3.1 Chemical Constituents of Polysaccharide Mixtures

Each extract was examined by the Biuret reaction (Sovago et al., 2012), and did not contain any detectable proteins (data not shown), suggesting that all anionic groups in the extracts are derived from the polysaccharides. Infrared spectra revealed characteristic peaks for the constituents in the mixtures, which typically included sulfated, uronic acid-containing, or neutral polysaccharide structures (Figure 3). The absorptions were attributed as follows: 850 cm^{-1} for the C–O–S bending vibration of a sulfate group in the axial position; 1250 cm^{-1} for the S=O stretching vibration of the sulfate group; 1400 and 1650 cm^{-1} for the C=O group of uronic acid; and 3300 cm^{-1} for the OH groups. The infrared results suggested that *Undaria* afforded no sulfated polysaccharides under any of the three extraction conditions. *Laminaria* afforded a sulfated polysaccharide by the acid-base and hot-water extraction methods, but sulfate groups were not detected from the alkaline extraction samples. The infrared spectra of all the *Undaria* and *Laminaria* extracts revealed absorptions for the carboxylic acids of uronic acid at 1400 and 1650 cm^{-1}. Alginate, fucoidan, and neutral polysaccharides could be extracted from *Undaria* and *Laminaria*. The infrared spectra suggested that all the *Undaria* extracts and the alkaline *Laminaria* extract contained alginate as an ion exchanger. In contrast, *Ulva* afforded sulfated polysaccharides under all extraction conditions. Both the hot-water and acidic extracts of *Laminaria* and all of the *Ulva* extracts contained the uronic acid polymers as well as the sulfated polysaccharide.

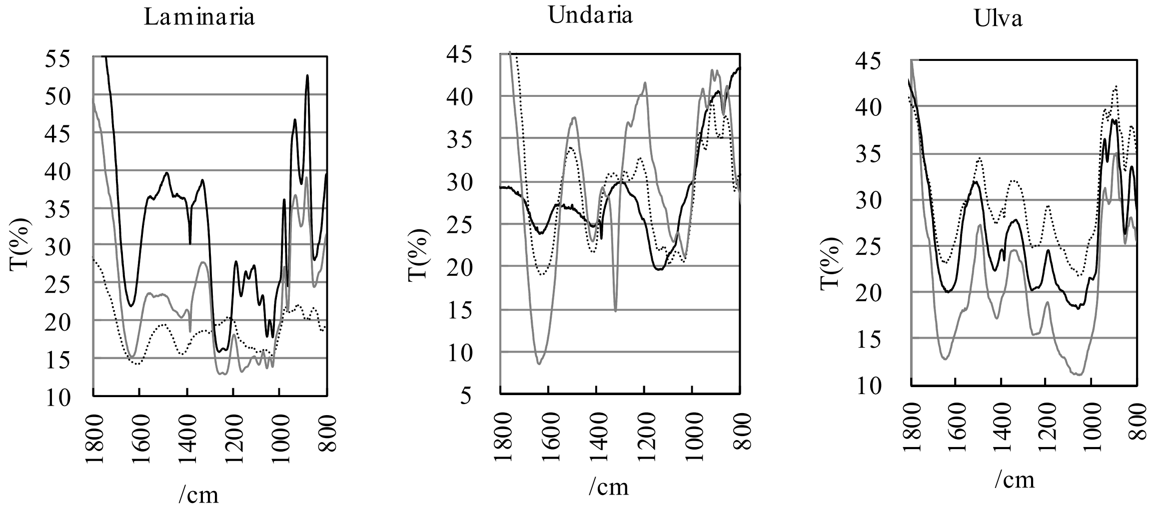
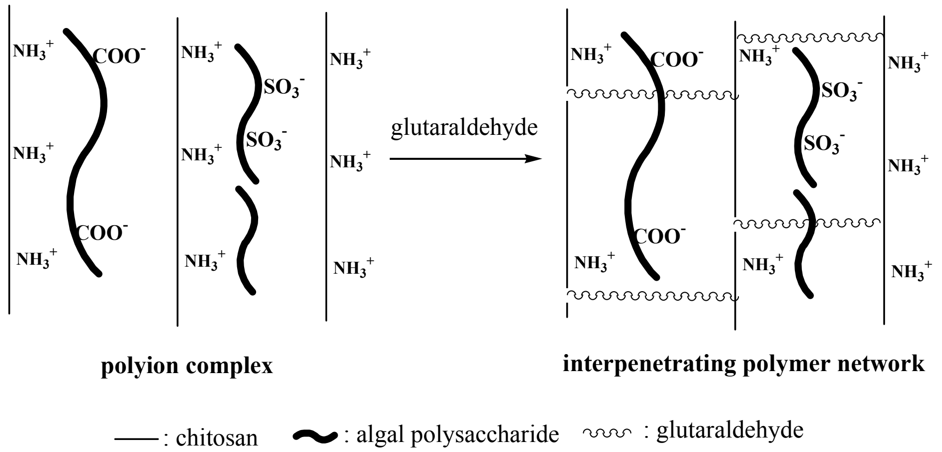


Figure 3. FT-IR spectra of the nine extracts

Hot-water extraction: solid gray line; Acid-base extraction: solid black line; Alkaline extraction: dotted black line.

3.2 Preparation of Algal Extract Gels

The mixed polysaccharide solutions that were extracted from the algae were solidified by mixing with chitosan. This formed a hydrogel that consisted of polyion complexes between the acidic groups of the extracted polysaccharides and the amino groups of chitosan. The chitosan amino groups in the polyion complexes were crosslinked using glutaraldehyde to form interpenetrating polymer networks (IPNs) (Scheme 2). Although dissolution of acidic polysaccharides from the polyion complexes into the supernatant was observed before glutaraldehyde crosslinking, free acidic polysaccharides eluted from the IPNs were not observed by the carbazole-sulfuric acid method. The extracted polysaccharides, i.e., sulfated polysaccharide, uronic polysaccharide, and neutral polysaccharide, seemed to be immobilized in the chitosan network, in the manner shown in Scheme 2, because the glutaraldehyde crosslinking reaction did not crosslink either ulvan or alginate.



Scheme 2. Formation of an interpenetrating polymer network structure of a hydrogel by glutaraldehyde crosslinking

3.3 Removal of Cu(II) by the Algal Extract Gel

Figure 4 shows the relationship between the carboxylic acid content of an extract and its rate of Cu(II) removal by its respective gel. In the figure, diamonds indicate an algal extract gel from an extract having no sulfated polysaccharides. The crosses show algal extract gels from extracts with sulfated polysaccharides. The various extraction conditions resulted in different carboxylic acid contents; however, the carboxylic acid content of an extract was not proportional to the Cu(II) removal rate by its respective gel. Among the various gels, those which were prepared from the alkaline extracts of *Undaria* and *Laminaria* possessed higher Cu(II) removal efficiencies than the other gels.

These results suggested that the gels prepared from extracts having higher uronic acid content tended to exhibit higher heavy metal ion removal efficiencies from aqueous media, whereas the gels derived from extracts with sulfated polysaccharides tended to exhibit lower removal efficiencies. We expected that the carboxylic acid content of the extract would be proportional to the heavy metal removal efficiency in aqueous media, because the acids chelate the divalent heavy metal ions. However, such proportionality was not observed.

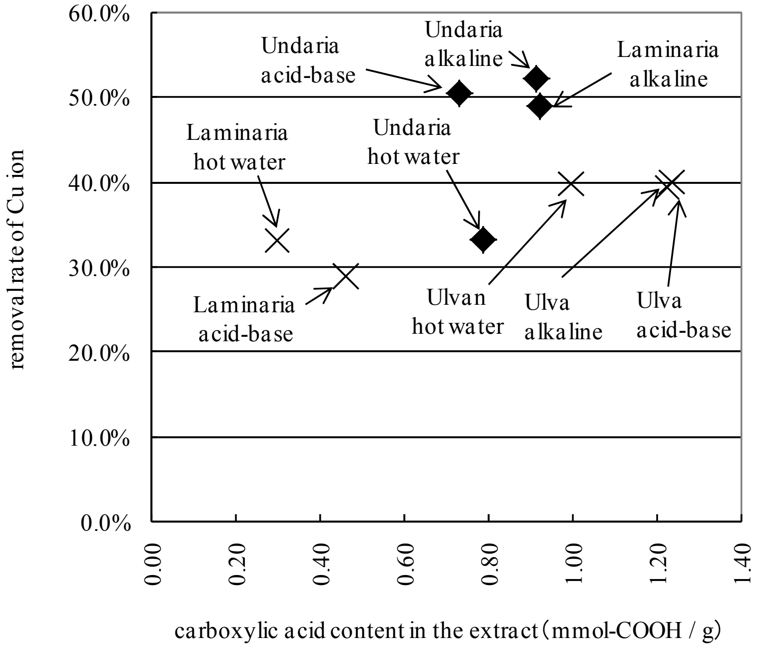


Figure 4. Relationship between the carboxylic acid content of the extract and the removal rate of Cu(II) (diamonds: extracts with sulfated polysaccharides; crosses: extracts with sulfated polysaccharides)

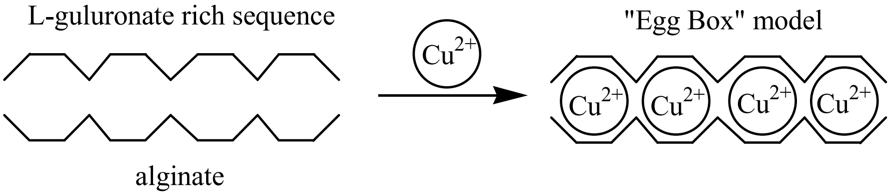


Figure 5. Egg box model for alginate-divalent cation complex

As illustrated by Stengel et al. (2011), many diverse compounds are present within the algal taxonomic group. For example, the *Phaeophyceae* contain alginate but the *Chlorophyta* do not. Although the structures of the polysaccharides are different, both *Phaeophyceae* and *Chlorophyta* have uronic, sulfated, and neutral polysaccharides. As described by Ray and Lahaye (1995), there are three main types of polysaccharide families in *Ulva*. These include hemicellulosic polysaccharides consisting of glucuronans and glucoxylans. Another is the major polysaccharide, ulvan, which consists of sulfated saccharide, uronic acid, and neutral saccharide components. Fucoidan, a sulfated polysaccharide of the *Phaeophyceae*, also comprises sulfated saccharide, uronic acid, and neutral saccharides. In contrast, alginate consists of only uronic acids. The distances between the acidic groups of fucoidan are longer than those of alginate. Divalent cations can bind to alginate by ion exchange. The binding of alginate to divalent cations was described via the "egg box" model as shown in Figure 5 (Grant et al., 1973). Divalent ions are chelated in binding sites located between two l-guluronate-rich sequences. The egg box structure has not been found in fucoidan and ulvan. Therefore, the structures of the adsorption sites in alginate and the sulfated polysaccharide are different. Thus, algal extract gels from extracts with higher uronic acid contents tend to exhibit higher heavy metal ion removal efficiencies from aqueous media compared to the gels from sulfated polysaccharide-laden extracts.

4. Conclusion

Biodiversity loss is effected by habitat encroachment, climate change, overexploitation, invasive alien species, and pollution and nutrient loading. In the latter case, *Chlorophyta ulva* forms green tides which spoil the enjoyment of coastal scenery and thus decreases ecosystem services. *Undaria pinnatifida* has been nominated as one of the world's worst invasive alien species. Huge amounts of residual *Laminaria* are disposed of every year. The utilization of algae that are both resources and environmental nuisances is a way to achieve Strategic Goal B of the Aichi Targets from the Convention on Biological Diversity: to reduce the direct pressures on biodiversity and promote sustainable use.

In this paper, we showed that easily prepared gels of algae extracts were able to achieve good rates of removal of Cu(II) ions from water. We suggested that an extract with a higher uronic acid content and without sulfated polysaccharides produces an algal gel that affords higher Cu(II) ion removal rates. The demonstrated utility of these extracts highlights their promise as potential, sustainable water treatment agents. Further investigations will be required to examine the possible reuses of the Cu(II) ion-adsorbed algal extract gels.

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