J. Microbiol. Biotechnol. (1999), **9**(5), 650–654

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

Characteristics of Metal Biosorption of Oxidized *Undaria pinnatifida*

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Received: August 10, 1999

Abstract *Undaria pinnatifida* oxidized by nitric acid had a high capacity of Cu^{2+} uptake (3.5 mmol Cu^{2+}/g dry mass) at pH 4 and showed high affinity to Cu^{2+} and Pb^{2+}, in a mixed-metal system, compared to Ca^{2+} and Mg^{2+}. The IR spectrum showed increase of carboxylic acid on the surface of *Undaria pinnatifida,* mostly due to the effect of the oxidation reaction.

Key words: Biosorption, oxidation, *Undaria pinnatifida*, IR spectrum, heavy metals

Heavy metals in a natural hydraulic system are known to cause serious problems to both living organisms and human. Biosorption provides an alternative and potentially an economical treatment for heavy metal removal and recovery [2, 14, 16, 17]. Water insoluble starch xanthate (ISX) has been shown to be an effective alternative to heavy metal removal and recovery. ISX is a cereal grain-based product chemically cross-linked to make it insoluble in water and then xanthated to form an anionic polymer. The ISX process was originally developed at the U. S. Department of Agriculture [12]. Although these products are commercially used at the present, the uptake capacities of these materials are similar to or even lower than ion-exchangers. Commercial biosorbents used for the treatment of metals are presently sold and representative ones are as follows: AlgaSORB® is a cross-linked biomass bacteria, and has an uptake capacity of 1.47 mmol Pb^{2+}/g that is in a practical application stage. It should be mentioned that the Sorbex company produces this product [4]. The commercial ion exchange resin has an uptake capacity of 0.83-1.94 mmol (divalent metal ion)/g dry resin for strong acidic ion exchanger and 1.23-3.91 mmol (divalent metal ion)/g dry resin for the weak acidic ion-exchanger [1]. To compete with the commercial ion-exchange resin, biosorbents must have an uptake capacity of 2-3 mmol/g dry mass for a divalent metal ion. The hydroxyl groups on the surface of the cell wall have relatively high pK_a values compared with other functional groups. Therefore, it cannot exchange protons with metal ions at a low pH in an aqueous solution. In a case where the hydroxyl groups of the biomass are changed to other pertinent groups, uptake capacities will be increased and the affinity to heavy metal can be changed as well. Because brown alga *Undaria pinnatifida* has a high uptake capacity and can be obtained easily, it is used as a model material [9]. To increase the uptake capacity, several chemical modifications on the surface of *U. pinnatifida* are employed to change the hydroxyl group to other functional groups and the effects of chemical modifications on the surface functional groups are reported in this paper.

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Materials and Methods

U. pinnatifida was harvested in the west sea of Korea. Cells were collected in a fine powder form by filtering with a 100-mesh sieve. The procedure of chemical modification was as follows. Ten g of *U. pinnatifida* was oxidized in 200 ml 20% nitric acid at 100°C for 3 h. It was rinsed thoroughly 5 times with 200 ml of water. After drying at 50°C, it was stored in a refrigerator for future use. The biosorption experimental procedure was as follows. Predetermined amounts of metal and biosorbent of known weight were mixed together to make a 100-ml solution in a shaking incubator at 30°C. All metals were of nitrate form and were purchased from Sigma-Aldrich (U.S.A.). After pH adjustment with 0.1 M HNO_3 and NH_4OH, the solution was centrifuged at 10,000 rpm for 20 min to remove suspending biosorbent, and then the metal concentration of the supernatant was analyzed using AA (Atomic Absorption spectroscopy: Perkin-Elmer, U.S.A.). In order to investigate the effect of ionic strength on a metal uptake capacity, NaCl was used to control the ionic strength and NaCl inhibition on the AA analysis was corrected. For the experiment of the inhibitory effect by an organic material, NTA (nitrilo triacetic acid) was added to copper solutions ranging from 10 mg/l to 200 mg/l. After the pH adjustment had been completed, the solution was centrifuged at

10,000 rpm for 20 min to remove the suspending solid, and then 10 ml of the supernatant was collected. The pre-treatment method of sample to exclude inhibition of NTA on the analysis was adopted from the 'Standard method' [6].

RESULTS AND DISCUSSION

Several modification methods were tried for the purpose of increasing the uptake capacity of *U. pinnatifida*. Oxidation by nitric acid, phosphorylation, oxidation by potassium permanganate, and sulfonation were used to modify miscellaneous functional groups. Addition of a chelating group (imidodiacetic acid) was attempted to introduce a specific functional group. Attachment of the polyacrylic acid by an amide bond and Grignard reaction was used to make a biosorbent with a space arm that is a part of the carboxylic functional group. Among various chemical modification methods, the oxidation reaction by nitric acid resulted in the highest uptake capacity, as shown in Table 1. The uptake capacity of *U. pinnatifida* increased about 90% compared to the control. Oxidation of nitric acid changed the hydroxyl group to a carboxylic group and produced a phenolic hydroxyl group with an ion-exchange ability [10]. But an oxidation reaction may break the structure of algae, which resulted in the weight loss of algae (data not shown). To reduce the weight loss during the reaction, nitric acid concentration was optimized. Other chemical reactions to modify the hydroxyl group failed, because there was a possibility of overall conversion efficiency to decrease due to the multi-step reaction, and many other groups on the surface by side reactions might have kept the main reaction from proceeding.

Sakaguchi *et al.* (1981) made a modification of phosphorylation on chitosan and chitin, and found that the uptake capacity of UO_2^{2+} was ca. 1 mmol/g at pH 5. The uptake capacity of sulfonated starch, ISX, was 0.55-0.625 mmol/g resin for the divalent metal ion [12]. However, our modification of *U. pinnatifida* showed the uptake capacity

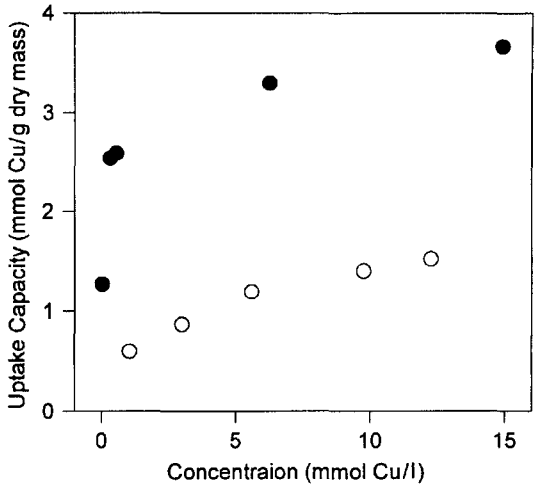

Fig. 1. Copper isotherm of *Undaria pinnatifida* oxidized by nitric acid.
Final pH: 4; ●, *Undaria pinnatifida* oxidized by nitric acid; ○, *Undaria pinnatifida*.

of 2.3 mmol Pb^{2+}/g dry mass, as shown in Table 1 and 3.6 mmol Cu^{2+}/g dry mass, as shown in Fig. 1. Moreover, the uptake capacity was high for each Cu^{2+}, Ni^{2+}, Cr^{2+}, Zn^{2+}, and Pb^{2+} when the initial concentration was the same for all metals at 1,000 mg/l (data not shown). The easiness of the reaction itself and a possibility of reusing the reaction solution of nitric acid and further applicability to other biomass, such as bacteria, fungi, and other algae, were some advantages known for this type of modification.

When Zn^{2+}, Cr^{3+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Ca^{2+}, and Mg^{2+} coexisted, the uptake capacity of each metal was changed by concentrations of metals and pH. When the initial concentration of each metal was set at 1 mmol/l and a final pH was adjusted to 4, Pb^{2+} and Cu^{2+} showed the high selectivity over the other metals, as shown in Fig. 2. Pb^{2+} and Cu^{2+} pre-occupied the uptake sites of *U. pinnatifida*, therefore other metals could not adsorb as well and they showed low uptake capacities. The isotherm of Cr^{3+} diverged, because Cr^{3+} precipitated at pH 4. The effect of pH on a mixed-metal uptake capacity is shown in Fig. 3.

Table 1. Adsorption capacities of chemically modified *Undaria Pinnatifida*.

Chemically modification method	Uptake capacity of lead (mmol/g dry-mass)	Reference
Control	1.20	
Oxidation by nitric acid	2.30±0.05	
Phosphorylation	1.44±0.05	[14]
Oxidation by potassium permanganate	1.44±0.04	
Sulfonation	1.41±0.06	
Attachment of chelating group (imido diacetic acid)	1.57±0.03	[7, 13]
Attachment of acrylic acid by amide bond	1.35±0.07	[8]
Attachment of acrylic acid by substitution reaction of allyl magnesium bromide	1.28±0.07	[5]
Attachment of acrylic acid by amide bond by chlorination of acrylic acid	1.44±0.06	[3]
Attachment of acrylic acid by amide bond by formation reaction of allyl magnesium bromide	1.66±0.08	[16]

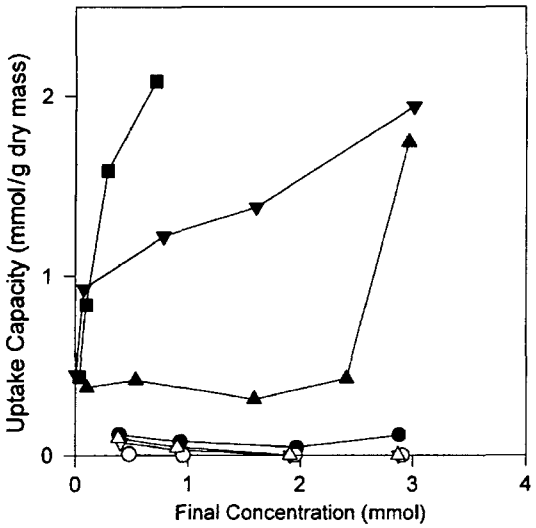

Fig. 2. Effect of metal concentration on the mixed-metal uptake capacities of *Undaria pinnatifida* oxidized by nitric acid.
Final pH, 4; ○, Mg^{2+}; ●, Ca^{2+}; ■, Cr^{3+}; ▽, Ni^{2+}; ▲, Cu^{2+}; △, Zn^{2+}; ▼, Pb^{2+}.

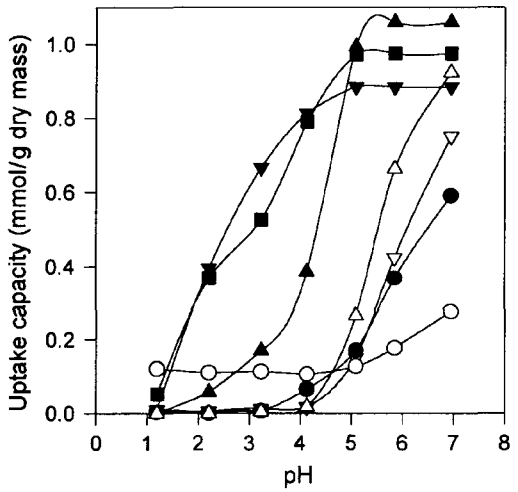

Fig. 3. Effect of pH on the mixed-metal uptake capacities of *Undaria pinnatifida* oxidized by nitric acid.
○, Mg^{2+}; ●, Ca^{2+}; ■, Cr^{3+}; ▽, Ni^{2+}; ▲, Cu^{2+}; △, Zn^{2+}; ▼, Pb^{2+}.

Cr^{3+}, Pb^{2+}, and Cu^{2+} started to adsorb on *U. pinnatifida* at a low pH and all metals except Ca^{2+} and Mg^{2+} adsorbed at a high pH level. However, these uptake capacities included both adsorption and precipitation, because Cu^{2+}, Zn^{2+}, and Cr^{3+} precipitated at above pH 5. For the control test, the soluble portion of metal was measured at above pH 4.

As shown in Fig. 4, all Cr^{3+} and Cu^{2+} precipitated at pH 5, and Zn^{2+}, Pb^{2+}, and Ni^{2+} began to precipitate at above pH 5, while Ca^{2+} and Mg^{2+} did not precipitate until pH 7. This was certified by using the MINEQL software that has a capability of calculating the equilibrium state of metals at various pH levels. Although the uptake capacities showed a precipitation portion at a high pH level, these chemical properties showed that a metal could be recovered or

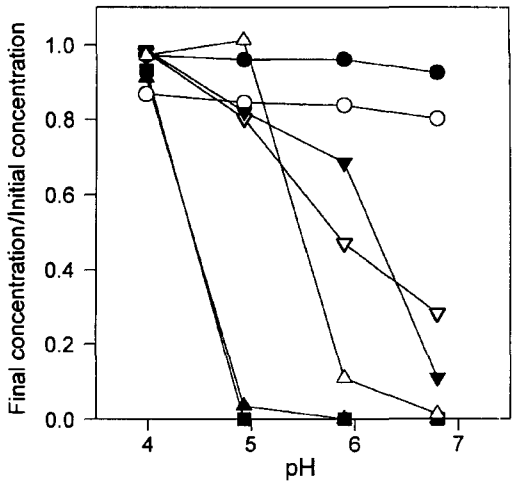

Fig. 4. Soluble portion of mixed-metal at different pHs.
○, Mg^{2+}; ●, Ca^{2+}; ■, Cr^{3+}; ▽, Ni^{2+}; ▲, Cu^{2+}; △, Zn^{2+}; ▼, Pb^{2+}.

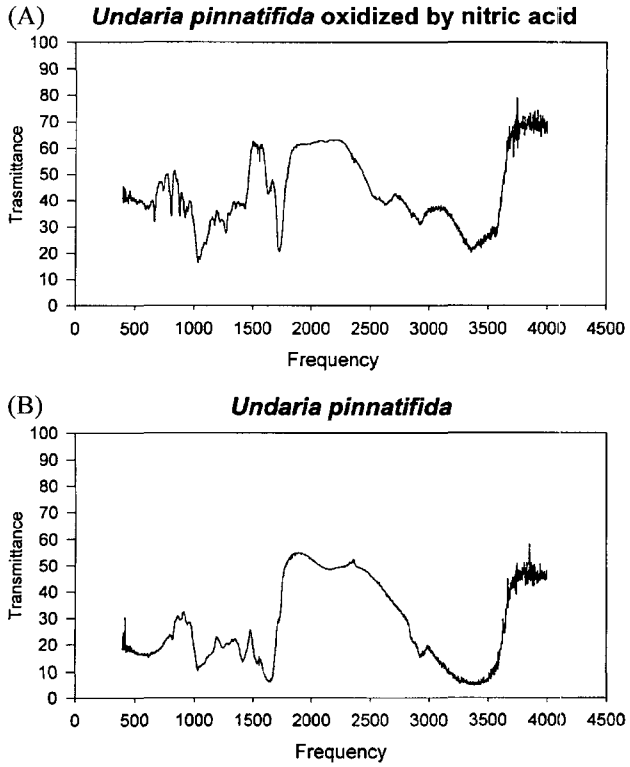

Fig. 5. IR spectrum of *Undaria pinnatifida* and modified *Undaria pinnatifida* oxidized by nitric acid.
(A) IR spectrum of natural *U. pinnatifida* oxidized by nitric acid and (B) IR spectrum of natural *U. pinnatifida*.

easily concentrated to different portions by just changing the pH.

IR spectra of oxidized and native *U. pinnatifida* are shown in Fig. 5. The IR peak of natural *U. pinnatifida* shows a broad peak of unreacted hydroxyl group in a hydrogen bond at a frequency level of 2,500-3,500 cm^{-1} and a characteristic peak of C-O bond at 1,050 cm^{-1}. This

Table 2. Effect of ionic strength and NTA concentration on the copper uptake capacities of *Undaria pinnatifida* oxidized by nitric acid (initial concentration: 300 mg/l, final pH: 4.0).

Ionic strength or NTA conceatration	Uptake capacity (mmol/g)
Ionic strength	
0	2.44
0.01	2.34
0.05	2.3
0.1	2.17
0.5	4.78
1	1.69
2	1.56
NTA conceatration	
0	2.44
10	2.49
50	2.5
100	2.33
200	2.3

explains the existence of alcohol or hydroxyl groups. The peak located in 1,725 cm^{-1} became conspicuous in an oxidized *U. pinnatifida*, which means the carbonyl groups of carboxylic acid increased after the reaction. Moreover, the peak at 2,400-3,400 cm^{-1} shows the OH peak of carboxylic acid in an oxidized *U. pinnatifida*.

Table 2 shows the effect of ionic strength on the uptake capacity. In a real process, different kinds of salts are mixed in wastewater and its ionic strength is high. At high ionic strength, adsorption sites are surrounded by counter ions in which they partially lose their charge, and this weakens the binding force by an electrostatic interaction. In this experiment, ionic strength had only a slight effect on the decrease of uptake capacity at low concentration levels and had a significant effect on the decrease of uptake capacity at high ionic strength. In any event, the ionic strength appears to be ca. 0.1 M in real wastewater and oxidized *U. pinnatifida* was not greatly affected by the ionic strength of 0.1 M.

Another important factor of biosorption in real wastewater is the content of organic materials. Organic materials in water can interact with heavy metals by chelation and complexation reaction so that it can cause the uptake capacity to decrease. NTA (nitrilo tri acetic acid) was chosen as a model compound and at concentrations ranging from 0 to 200 mg/l, according to the analysis result of real plating wastewater. Table 2 shows that *U. pinnatifida* can remove Cu^{2+} up to 200 mg/l with little interference of NTA. A possible reason for low interference might have been due to the fact that its concentration was so low (0.52 mmol/l) that it could not chelate enough metals. From these experiments, it was found that oxidized *U. pinnatifida* could be used in real wastewater treatment.

In conclusion, various chemical methods were attempted to increase the uptake capacity of biomass *U. pinnatifida*. In addition, oxidation by nitric acid was shown to be effective among several methods tested. Oxidation of the surface functional group of *U. pinnatifida* by nitric acid increased the uptake capacity for metal by 2.1 mmol Cu^{2+}/g dry mass and 1.1 mmol Pb^{2+}/g dry mass. This method was advantageous in regards to the uptake capacity of metal along with the fact that it was known to be an economical method in terms of procedure and chemicals used. The modified biomass had a higher affinity to Cu^{2+} and Pb^{2+} compared to Ca^{2+} and Mg^{2+} in a mixed-metal system. This modification method increased the carboxylic groups, which was confirmed by the IR spectrum. Interference by ionic strength and NTA was minimal in the range of the industrial application.

Acknowledgments

This study was supported by a research fund provided by the Ministry of Education, Republic of Korea.

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