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# Removal of eutrophication factors and heavy metal from a closed cultivation system using the macroalgae, *Gracilaria* sp. (Rhodophyta)

KANG Kyoung Ho (康庆浩) [1], SUI Zhenghong (隋正红) [2,*]

[1] *Department of Aquaculture, Chonnam National University, Yeosu Jeonnam, 550-749, South Korea*

[2] *Key Laboratory of Marine Genetics and Breeding, Ministry of Education, Ocean University of China, Qingdao 266003, China*

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**Abstract** In this study, the ability of macroalgae *Gracilaria* sp. of removing eutrophication factors and toxic heavy metals Al, Cr, and Zn in a closed cultivation system is reported. The results show that the concentration of the three heavy metals decreased significantly during the experimental period in an algal biomass dependent manner. The biofiltration capacity of the alga for Al, Cr, and Zn is 10.1%–72.6%, 52.5%–83.4% and 36.5%–91.7%, respectively. Using more materials resulted in stronger heavy metal removal. Additionally, the concentration of chl-*a*, TN, TP and DIN of water samples from aquariums involving large, medium, and small algal biomass cultivation increased first and then decreased during the experiment. COD value of all three groups decreased with time and displayed algal biomass dependency: more algae resulting in a greater COD value than those of less biomass. Furthermore, changes in COD reflect an obvious organic particles deprivation process of algae. This is the first report on heavy metal removal effect by *Gracilaria* species. The results suggest that macroalgae can be used as a biofilter for the treatment of nutrient-enriched or heavy-metal polluted water, to which an appropriate time range should be carefully determined.

**Keyword**: biofiltration; COD; eutrophication; *Gracilaria* sp.; heavy metal

## 1 INTRODUCTION

Eutrophication of aquatic systems becomes a serious issue during industrialization. In addition, toxic heavy metals in air, soil, and water are becoming a growing threat to humanity. For example, Cr may accumulate in the body after exposure to contaminated environments (Luo et al., 2008; Xu et al., 2008). Cr and As have been classified as priority pollutants by the United States Environmental Protection Agency (USEPA) with a carcinogenicity classification of A (human carcinogen) (USEPA, 1999; Pekey, 2006).

The rapid expansion of aquaculture has led to an excessive increase in eutrophication factors, especially nitrogen and phosphorous, in aquatic ecosystems (Beveridge, 1996). These factors generally originate from pond fertilization, feed and metabolic residues of cultivated animals. Hence, there is a great demand for non-polluting strategies that minimize the negative effects of this activity. The most practical and economical approach to reducing the concentration of eutrophication factors in aquaculture areas is to treat the effluent before it is discharged into the environment. Biological treatment of contaminated effluent using macroalgae for nutrient removal has the potential to successfully reduce the amount of toxic materials in the effluent prior to discharge (Chopin et al., 2001; Neori et al., 2004). A series of studies have demonstrated the potentially beneficial effects of intensive culture of seaweed during wastewater treatment and bioremediation (Schramm, 1999; Chung et al., 2002; McVey et al., 2002). Among the species of seaweed commonly used, *Gracilaria* is one of the most important because of its high yields and commercially valuable extracts (Lapointe et al., 1978; Troell et al., 1999; Wang, 2002). In the present study, we examined the removal of eutrophication factors by

* Corresponding author: suizhengh@ouc.edu.cn

applying macroalgae *Gracilaria* sp. in a closed cultivation system. In addition, we assessed the possible use of this species as a biofilter for the removal of Al, Cr, and Zn. The goal of this study was to monitor the dynamic changes in eutrophication factors and heavy metals in treated water within a limited time and to evaluate the potential of the species for bioremediation of coastal waters.

## 2 MATERIAL AND METHOD

### 2.1 Setting of *Gracilaria* sp. biomass scale

The algae were collected from the Jangseung coast in Chonnam Province, South Korea, in the intertidal zone during low tide periods. After collection, the macroalgae were transferred to the laboratory and immediately cleaned of epiphytes and encrusting organisms. Next, they were placed in a container filled with sea water until the start of the experiment. Three scales of algal biomass were set within different 50 cm×30 cm×30 cm aquariums containing 20 L of sea water and a holding rate based on the algal wet weight of 3:2:1 (Table 1 for details). All experiments were conducted under constant light (250 μmol/m$^2$s), photoperiod (12:12 L: D cycle) and aeration at 20±2°C. Triplicate experiments were conducted under the same cultivation conditions.

**Table 1 Experimental conditions of three levels of algal biomass within 50 cm×30 cm×30 cm aquariums containing 20 L of sea water**

| Item | Large (L) | Medium (M) | Small (S) |
|---|---|---|---|
| Total weight (g) | 170±1 | 107±1 | 54±1 |
| Approximate holding rate on wet weight | 3 | 2 | 1 |

### 2.2 Determination of COD

Water samples from three algal biomass groups were collected at 3, 5, 10, 24, 48, and 72 h. COD analysis was conducted using the sodium thiosulfate method according to the standard methods (APHA, AWWA and WPCF, 1980).

### 2.3 chl-*a* and *N-P* analysis in aquarium

Nutrient removal experiments were conducted on the three algal groups cultivated in seawater collected from a nearby marine animal farm. Water samples were collected after cultivation for 3, 12, 24, and 72 h for the determination of the chl-*a*, TN, TP, and DIN concentrations, which was conducted using an Automatic Analyzer of Water Quality (Micromac Total P and Total N, Italy). The experiment was initiated with the same starting concentration of chl-*a* (0.74 mg/L), TN (1 mg/L), TP (0.03 mg/L) and DIN (0.25 mg/L) as indicated in Figs.2 and 3.

### 2.4 Heavy metal analysis

Water samples of three biomass groups were collected for measurement of the concentration of Al, Cr, and Zn after 3, 12, 24, and 72 h of cultivation. All analyses were conducted using an Inductively Coupled Plasma Mass Spectrometer/ICP-MS (Perkin-Elmer). The experiment was initiated with the same starting concentration of Al (49.923 mg/ml), Cr (21.764 mg/ml) and Zn (13.581 mg/ml) as indicated in Fig.1.

## 3 RESULT

### 3.1 Removal of heavy metal

The concentration of three heavy metals, Al, Cr and Zn, decreased significantly during the experimental period, indicating that macroalgae have a high heavy metal removal capacity (Fig.1). Furthermore, the results revealed that this decrease

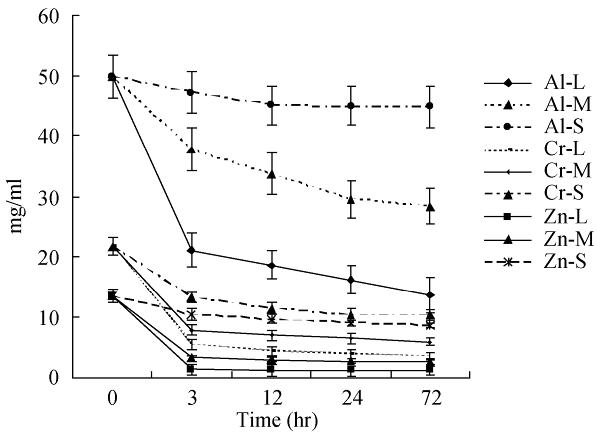


**Fig.1 Changes in the concentration of Al, Cr and Zn of tested groups containing different levels of algal biomass during the study period**

L-Large biomass; M-Medium biomass; S-Small biomass

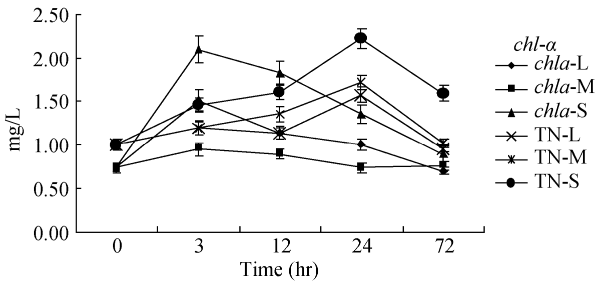


**Fig.2 Changes in the concentration of chl-*a* and TN of tested groups with different algal biomass during the study period**

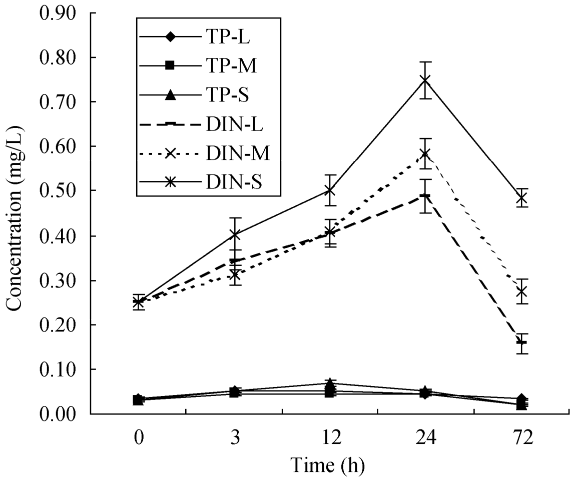


**Fig.3 Changes in the concentration of TP and DIN of tested groups with different algal biomass during the study period**

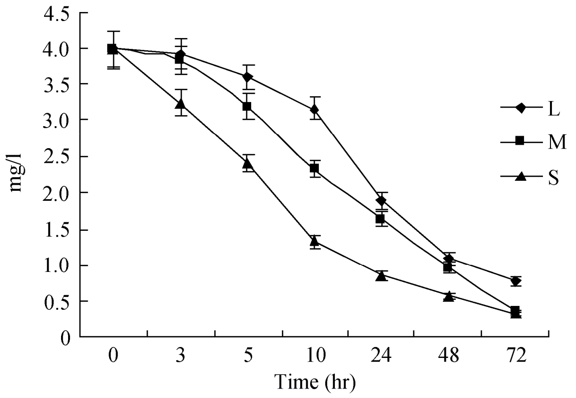


**Fig.4 Changes in the COD of tested groups with different algal biomass during the study period**

occurred in an algal biomass dependent manner. The concentration of Cr decreased from 21.764 to 10.339 and 3.611 mg/ml in the small and large biomass group, respectively, within 72 h, corresponding to a biofiltration capacity of 52.5%–83.4%. There was also a reduction in Zn concentration during the experimental period that corresponded to a biofiltration capacity of 36.5% for the small biomass assay to 91.7% for the large biomass assay. However, a weaker Al removal was observed, with the biofiltration capacity ranging from 10.1%–72.6% in the small and large biomass groups, respectively. In most of the cases, the heavy metal removal effect was obvious within the first 3 h, except for Al in the medium and small biomass groups, which required more time for the heavy metal removal to occur efficiently.

### 3.2 Removal of eutrophication factor

The concentration of chl-*a*, TN, TP, and DIN in the water samples from aquariums containing large, medium and small levels of algal biomass were determined (Figs.2–3). All of the nutrients displayed an initial increase followed by a decline in concentration. The change in concentration was extensive in the small biomass group, while it was insignificant in the medium and large group. The highest concentration of the different tested factors was reached at different time, but was basically coordinated among biomass groups. Specifically, the highest concentration was highly coordinated as for TN and DIN, being attained within 24 h in all of the biomass groups. However, the reduction of most of the eutrophication factors at 72 h was not obvious when compared with their start concentrations. Most of the eutrophication factors fell to their started concentrations after the up-and-down process, except for TN and DIN in the small biomass group, which was increased to a value higher than their initial values. A slight reduction of TP in the small and medium biomass groups and DIN in the large groups was also observed at 72 h when compared with their initial concentrations.

### 3.3 COD monitoring

The COD of each of the groups decreased following the monitoring time and displayed an algal biomass dependent manner, with more algal material being associated with a higher COD value compared with those of less biomass. The COD at 72 h was 0.32, 0.36 and 0.76 mg/L in the small, medium and large biomass group, respectively, corresponding to 8%, 9% and 14% of the original COD.

## 4 DISCUSSION

This is the first report of the heavy metal removal effect of *Gracilaria* species. A strong heavy metal biofiltration capacity was displayed for the different heavy metals involved, with the effect occurring in the order of: Al<Zn<Cr. The high Cr removal efficiency of *Gracilaria* provided a light foreground for the elimination or reduction of this severe pollution. However, an algal biomass dependent effect was also observed in this study. The results also indicated that the heavy metal removal process was nearly completed within the first 3 h of the experiment. Accordingly, an appropriate biomass and operating time must be determined before the process is applied for field treatment.

Contrary to other reports indicating that macroalgae had the potential to remove nutrients from the eutrophication water column during algal cultivation (Xu et al., 2007; Yang et al., 2003), the concentrations of chl-*a*, TN, TP and DIN increased during the early period of the present experiment, although they later decreased. This contradiction may have been due to differences in the experimental time range and different nutrition factors monitored. While in the former, a longer monitoring time longer than 40 days and IN plus IP was checked in those studies (Xu et al., 2007). In addition, this may have been caused by the different biomasses used; however, this cannot be determined for certain because it was not specifically tested in this study and there is insufficient data for such a comparison. Nevertheless, similar changes in the nutrient concentrations in water samples have been observed by others (Marinho-Soriano et al., 2009; Li et al., 2008), but these changes were recorded within a relatively short time range and were not biomass dependent. The results of this study suggest that when macroalgae will be used for the treatment of enrichment water, an appropriate time range should be carefully determined.

Although there have been reports that macroalgae had no degradative effects on organic particles (Jones et al., 2001; Li et al., 2008), our study indicated obvious organic particle deprivation by the algae as reflected by changes in the COD. These findings may be explained by the adsorbing effect of the algae on chemical organic particles. However, the organic particle removal effect was slightly stronger in the small biomass group than in the other two biomass groups. More studies should be conducted to elucidate the function and mechanism of macroalgae in COD removal.

## 5 CONCLUSION

Our study demonstrated a strong heavy metal biofiltration capacity of *Gracilaria* sp. that occurred in an algal biomass dependent manner. The concentrations of chl-*a*, TN, TP, and DIN in water columns treated with different biomasses of algae increased during the initial stages of the experiment, after which they decreased. This alga also displayed a strong COD deprivation effect.

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