J. Environ. Syst. and Eng., JSCE, No. 748/VII-29, 25-32, 2003 November

EFFECT OF POTENTIAL ALLELOCHEMICALS EXTRACTED FROM *SARGASSUM HORNERI* ON THE GROWTH OF RED TIDE MICROALGAE

Fumihiko TAKEDA[1], Takashi SAKAMAKI[2], Kaiqin XU[3], Nobuo CHIBA[4], Osamu NISHIMURA[5] and Ryuichi SUDO[6]

[1]Graduate student, Dept. of Civil Eng., Tohoku University
(Aoba06, Aramaki, Sendai 980-8579, Japan)
E-mail:takeda@eco.civil.tohoku.ac.jp

[2]Member of JSCE, Dr. Eng., Postdoctral Fellow, Japan Society for the Promotion of Science
(Aoba06, Aramaki, Sendai 980-8579, Japan)

[3]Member of JSCE, Dr. Eng., Senior Researcher, NIES
(16-2 Onogawa, Tsukuba-Shi, Ibaraki, 305-8506, Japan)

[4]Member of JSCE, Technical Official, Dept. of Civil Eng., Tohoku University
(Aoba06, Aramaki, Sendai 980-8579, Japan)

[5]Member of JSCE, Dr. Eng., Professor, Graduate School of Eng., Tohoku University
(Aoba06, Aramaki, Sendai 980-8579, Japan)

[6]Member of JSCE, Dr. Sci., Guest Professor, Tohoku Institute of Technology
(35-1, Yagiyama-kasumicho Taihaku-ku Sendai 982, Japan)

The allelopathic effects of the extracts of a brown alga *Sargassum horneri* on red tide microalgae were examined using *Skeletonema costatum*, *Chattonella antiqua* and *Heterosigma akashiwo*. The growth of *S. costatum* and *C. antiqua* were inhibited remarkably by the extracts of *S. horneri* while *H. akashiwo* needed higher amount of the extracts than *S. costatum* and *C. antiqua* to attain same level of growth inhibition, confirming the difference in the allelopathic effects with microalga species. The microscopic observation indicated that the growth inhibition effects of the potential allelochemicals produced by *S. horneri* were associated with the change of the body form of the red tide microalgae tested.

Key Words *: Sargassum horneri, red tide microalgae, growth inhibition, allelochemicals*

1. INTRODUCTION

Seaweed bed plays an important role in coastal ecosystems as a place for spawning, feeding and nursery for larvae, juvenile and young fishes. Furthermore, since seaweed absorbs and removes nitrogen and phosphorus from seawater, the preservation and restoration of seaweed bed are important in terms of a countermeasure against eutrophication causing red tide occurrence.

Considering both red tide microalgae and seaweeds are the primary producers in coastal sea areas, their relation would be an antagonism. They compete to get sun light and nutrients like nitrogen and phosphorus in water column. Moreover, there are some reports[1)-6)] that a metabolite released from macrophyte has growth inhibition effect on water bloom or red tide microalgae. It suggests that seaweed bed is useful not only to remove nitrogen and phosphorus but also to prevent red tide occurrence.

Kaziura[5)] and Tanaka and Asakawa[6)] found that Phaeophyceae Sargassaceae *Sargassum horneri*, a representative constitution species of seaweed bed in coastal area in Japan, released allelochemicals inhibiting the growth of microalgae. However, the basic knowledge of the allelochemicals, *i.e.*, chemical identification of the substances, production

characteristics and quantitative evaluation of the inhibition effects, has not been cleared yet. On the other hand, it was reported that the effect of allelochemicals differed with microalga species[1)-6)]. Therefore, evaluation of the inhibition effects of allelochmicals should be conducted using some different microalga species.

In this paper, the growth inhibition effect of the chemicals of *S. horneri* was examined using three kinds of red tide microalgae. The chemicals of *S. horneri* were extracted and fractionated to use for allelopathic effect tests. The results of comparison of allelopathic effect on the different kinds of red tide microalgae were discussed.

2. MATERIALS AND METHODS

(1)Materials

S. horneri was collected from Matsushima Bay, Miyagi Prefecture, on June 2002. Bacillariophyceae *Skeletonema costatum*(NIES-16), Raphidophyceae *Chattonella antiqua*(NIES-1) and *Heterosigma akashiwo*(NIES-293) were used as test microalgae to evaluate the growth inhibition effect of the chemicals of *S. horneri*. These microalgae were obtained from the Microbial Culture Collection of the National Institute for Environmental Studies. Each microalga was cultured at unialgal level with f/2 medium[7)] under a constant illumination condition (5,000lx) with 12/12 of Light/Dark cycle in the incubator(CL-301, TOMY, Tokyo, Japan). The components of f/2 medium were 75mg of $NaNO_3$, 6mg of $NaH_2PO_4 \cdot 2H_2O$, 10mg of $Na_2SiO_3 \cdot 9H_2O$, 0.5 μg of Biotin, 100 μg of Thiamine, 0.5 μg of Cyanocobalamin and 1ml of f/2 metal mixed liquid per 999ml of seawater. f/2 metal mixed liquid were consisted of 4.4g of $Na_2EDTA \cdot 2H_2O$, 3.16g of $FeCl_3 \cdot 6H_2O$, 180mg of $MnCl_2 \cdot 4H_2O$, 21mg of $ZnSO_4 \cdot 7H_2O$, 12mg of $CoSO_4 \cdot 7H_2O$, 7mg of $CuSO_4 \cdot 5H_2O$ and 7mg of $Na_2MoO4 \cdot 2H_2O$ per 1 liter of distilled water. Temperature was maintained at 20℃ for *S. costatum* and *H. akashiwo* and 25℃ for *C. antiqua*.

(2)Methods

a)Extraction and fractionation of the chemicals of *S. horneri*

Extraction and fractionation of the chemicals of *S. horneri* were conducted as shown in **Fig. 1**, referring to Taniguchi *et al.*[8)-10)]. Two kg-wet of *S. horneri* was soaked in acetone for four weeks in the shade at room temperature. The acetone extract was concentrated by evaporation at 30℃. Then it was fractionated into ether-soluble part and water-soluble part using diethyl ether. The ether- soluble part was fractionated into two parts by addition of 5% NaOH solution. The ether-soluble part under alkaline condition was named neutral fraction (N). While the ether-soluble part under acidic condition with 35% HCl solution was obtained as acidic fraction (A). The water-soluble part was supplied to the activated charcoal column so that objective chemicals were adsorbed on the charcoal. The column was washed

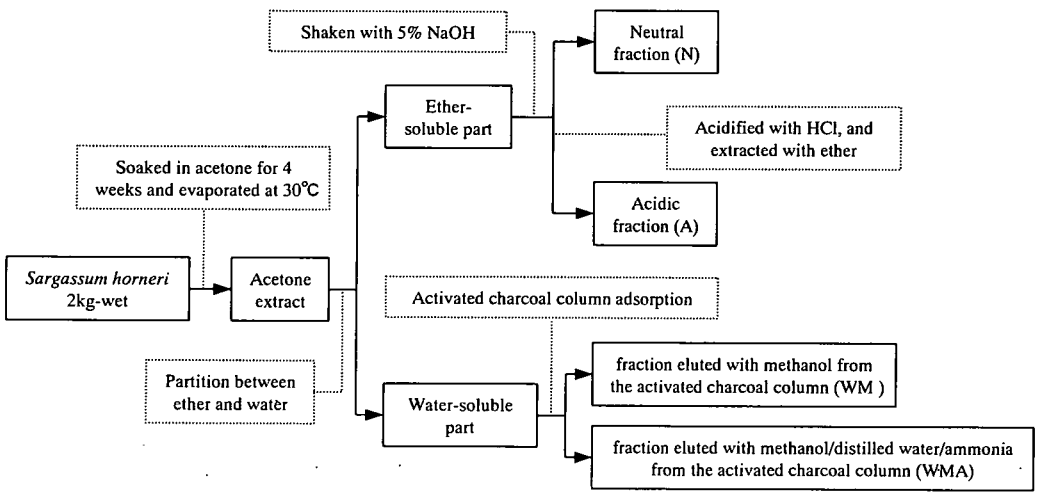


Fig.1 Extraction and fractionation process of chemicals of *Sargassum horneri*

by distilled water to remove non-adsorbed substances, *e.g.* salt. Then the column was eluted by methanol and the fraction eluted with methanol from the activated charcoal column (WM) was obtained. Finally a mixture of methanol/distilled water /ammonia (8:1:1) was used for column elution and the fraction eluted with methanol/distilled water/ammonia from the activated charcoal column (WMA) was obtained. All fractions were concentrated by complete evaporation at 30℃ and stored at -30℃.

b)Evaluation of the allelopathic effect on the growth of red tide microalgae

Initial concentration of *H. akashiwo*, *C. antiqua* and *S. costatum* was adjusted to 10^3cells · ml^{-1}, 500cells·ml^{-1} and 10^4cells·ml^{-1}, respectively. Each red tide microalga was cultured under same condition as described before and used to test the allelopathic effect of the extract of *S. horneri*. The WM and WMA fraction were tested at different concentration, *i.e.*, 25, 50, 100 and 200mg/l. The red tide microalgae mixed with the extract of *S. horneri* were cultured under same condition as described before and the cell concentration of each microalga was monitored. The concentration (cells·ml^{-1}) of the microalgae was determined by microscopic observation to count cell number with regular form or/and looking alive, *i.e.* , swimming. Furthermore, specific growth rate of each red tide microalga was calculated by the following formula.

$$\mu = \frac{ln\,(C_e / C_a)}{d_e - d_a}$$

Where,

μ : specific growth rate (day^{-1})

C_e : the cell concentration when logarithmic growth phase ends (cells·ml^{-1})

C_a : the cell concentration when the extract is added (cells·ml^{-1})

d_e : the day when logarithmic growth phase ends (day)

d_a : the day when the extract is added (day)

3. RESULTS

(1) Extraction and fractionation of the chemicals of *S. horneri*

The yields of each fraction from the extract of *S. horneri* are shown in **Table 1**. The yields of the WM and WMA fraction were much higher than that of the A and N fraction. Although Taniguchi *et al*[8-10] used different kinds of brown algae, similar results were obtained for the WM and WMA fractions. Since their yields occupied more than 90% of the total amount, the evaluation of the allelopathic effect was concentrated on the WM and WMA fractions as described below.

Table 1 Yields (mg/kg wet-weight) for each fraction from the extract of *Sargassum horneri*

WM	WMA	A	N
324.5	1049	98.15	49.20

WM : the fraction eluted with methanol from the activated charcoal column, WMA : the fraction eluted with methanol/distilled water/ammonia from the activated charcoal column, A : acidic fraction, N : neutral fraction

(2) Evaluation of the allelopathic effect on the growth of red tide microalgae

a) Allelopathic effect on the growth of *S. costatum*

Figure 2 and **Fig. 3** show the changes in *S. costatum* cell concentration when the WM and WMA fraction was added, respectively. Based on the growth of *S. costatum*, the specific growth rate and maximum cell concentration of *S. costatum* were calculated as shown in **Table 2**. Regardless of the amount of the WM fraction added, the specific growth rates seemed almost constant. However, the attainable maximum cell concentration tended to low by addition of the WM fraction. Furthermore, each decrease rate of maximum cell concentration was almost similar (about 32%) when the WM fraction was added.

When the WMA concentration was 200 mg/l, no *S. costatum* cells were remained after 5 day. For 100mg/l of the WMA concentration, the growth of *S. costatum* was completely inhibited for 11 days. When the WMA concentration was less than 50mg/l, almost no effect seemed to be observed in the growth curve, resulting in almost same growth curve with the control system. Also the microscopic observation confirmed that the allelopathic effect became obvious when the WMA concentration was higher than 100mg/l. The shape of *S. costatum* cells got longer at 1 day after the WMA fraction was added. **(Photo. 1)**. Although the body color of *S. costatum* in control system was almost constant, those in the WMA added systems were not. Moreover, the number of connecting cell became about

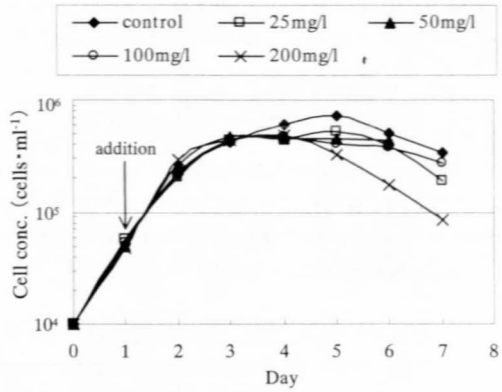


Fig. 2 Effect of the fraction eluted with methanol from the activated charcoal column (WM) on the growth of *Skeletonema costatum*

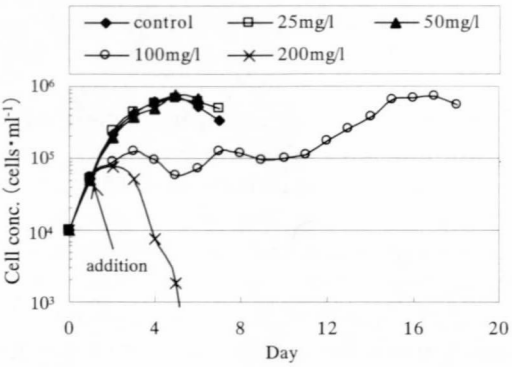


Fig. 3 Effect of the fraction eluted with methanol/distilled water/ammonia from the activated charcoal column (WMA) on the growth of *Skeletonema costatum*

half or one third of that in control system. However, the changes like those were not observed in WMA

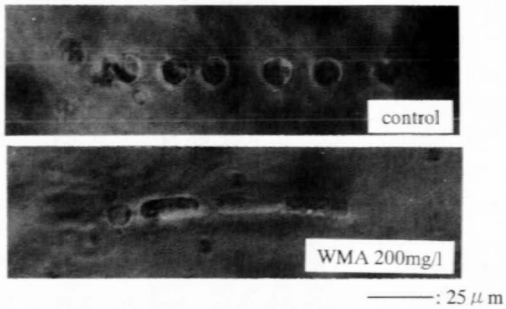


Photo. 1 Appearance of *S. costatum* (after 2 day culture)

25, 50mg/l and all of WM added systems.

When the WMA concentration was higher than 100mg/l, most of *S. costatum* cells became abnormally longer while their growth was stopped. However, the cell returned to regular shape and restarted to grow after 13 day.

b) Allelopathic effect on the growth of *C. antiqua*

The changes in *C. antiqua* cell concentration obtained by addition of the WM and WMA fraction are shown in **Fig. 4** and **Fig. 5**, respectively. **Table 3** shows the specific growth rate and maximum cell concentration of *C. antiqua* on the basis of the time course of the cell concentration. There seemed to be almost no effects on attainable maximum cell concentration. With higher the WM fraction concentration, the specific growth rate became lower.

Maximum cell concentrations attained for the WMA fraction 25 and 50mg/l systems were a little higher (about 20%) than that for control system. However, the specific growth rate became lower

Table 2 Specific growth rate (μ) and maximum cell concentration of *Skeletonema costatum* in each system

	d_a	d_e	C_a ($\times 10^4$)	C_e ($\times 10^5$)	μ	Maximum cell conc. ($\times 10^5$ cells·ml^{-1})
control	0	2	1.00	2.20	1.55	7.19
WM 25mg/l	1	2	5.71	2.00	1.25	5.15
50mg/l	1	2	4.91	2.62	1.67	4.75
100mg/l	1	2	5.36	2.23	1.43	4.72
200mg/l	1	2	4.73	2.87	1.80	4.89
WMA 25mg/l	1	2	5.05	2.38	1.55	6.77
50mg/l	1	2	4.87	2.18	1.37	7.57
100mg/l	1	3	5.47	1.25	0.413	7.03
200mg/l	1	2	5.28	0.775	0.383	0.775

WM : the fraction eluted with methanol from the activated charcoal column, WMA : the fraction eluted with methanol/ distilled water/ammonia from the activated charcoal column, μ : specific growth rate (day^{-1}), C_e : the cell concentration when logarithmic growth phase ends (cells·ml^{-1}), C_a : the cell concentration when the extracted is added (cells·ml^{-1}), d_e : the day when logarithmic growth phase ends (day), d_a : the day when the extract is added (day)

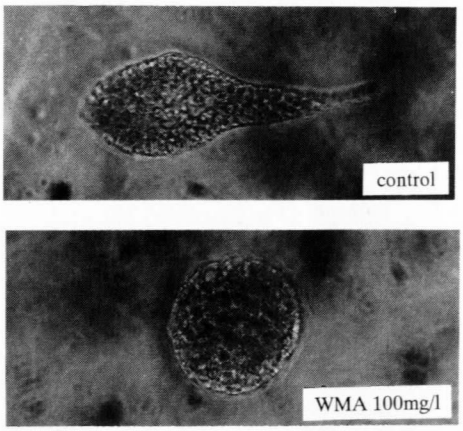
Table 3 Specific growth rate (μ) and maximum cell concentration of *Chattonella antiqua* in each system

	d_a	d_e	C_a ($\times 10^2$)	C_e ($\times 10^4$)	μ	Maximum cell conc. ($\times 10^4$ cells·ml^{-1})
control	0	6	5.00	2.33	0.640	3.42
WM 25mg/l	1	6	10.4	1.83	0.574	3.59
50mg/l	1	6	11.0	1.69	0.546	3.30
100mg/l	1	6	10.1	1.60	0.552	3.36
200mg/l	1	6	10.1	1.34	0.517	4.48
WMA 25mg/l	1	6	9.60	1.50	0.550	3.90
50mg/l	1	7	1.17	0.460	0.228	4.30
100mg/l	1	5	1.06	0.168	0.115	0.168
200mg/l	1	4	1.01	0.162	0.156	0.162

WM : the fraction eluted with methanol from the activated charcoal column, WMA : the fraction eluted with methanol/ distilled water/ammonia from the activated charcoal column, μ : specific growth rate (day^{-1}), C_e : the cell concentration when logarithmic growth phase ends (cells·ml^{-1}), C_a : the cell concentration when the extracted is added (cells·ml^{-1}), d_e : the day when logarithmic growth phase ends (day), d_a : the day when the extract is added (day)

when the WMA concentration was more than 50mg/l. Moreover, the cell growth was almost completely inhibited by 100 and 200mg/l of the WMA fraction.

The additional effect of the WMA fraction appeared also on the body shape of *C. antiqua.* When the concentration of the WMA fraction was higher than 50mg/l, the cell changed to a spherical shape (**Photo. 2**), and it was observed that the cell growth became slower while the cell changed. The cell could not return to their original form when the concentration of the WMA fraction was 100 or 200 mg/l although it could for 50mg/l of WMA fraction. For the WM fraction added system, however, such change of cell shape was not observed.



———: 50 μ m

Photo. 2 Appearance of *C. antiqua* (after 5 day culture)

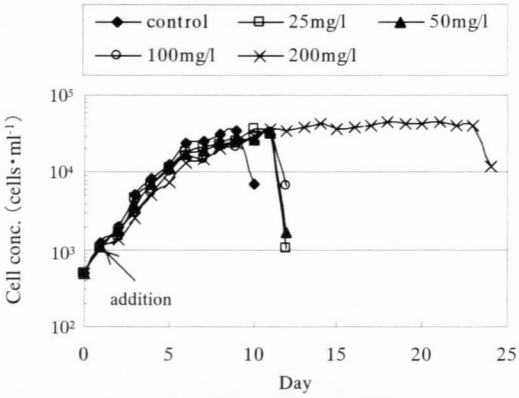


Fig. 4 Effect of the fraction eluted with methanol from the activated charcoal column (WM) on the growth of *Chattonella antiqua*

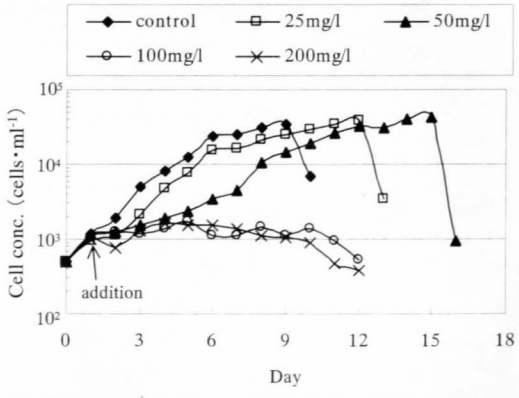


Fig. 5 Effect of the fraction eluted with methanol/distilled water/ammonia from the activated charcoal column (WMA) on the growth of *Chattonella antiqua*

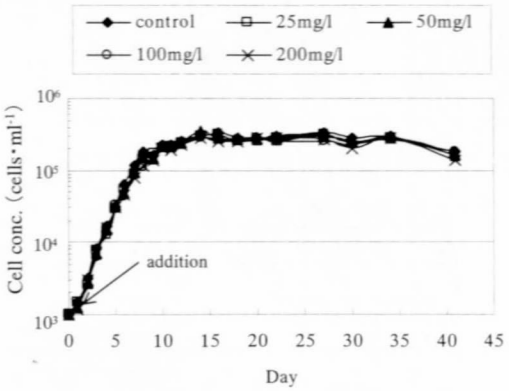


Fig. 6 Effect of the fraction eluted with methanol from the activated charcoal column (WM) on the growth of *Heterosigma akashiwo*

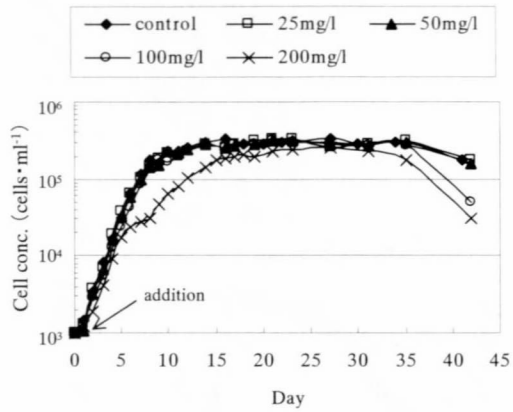
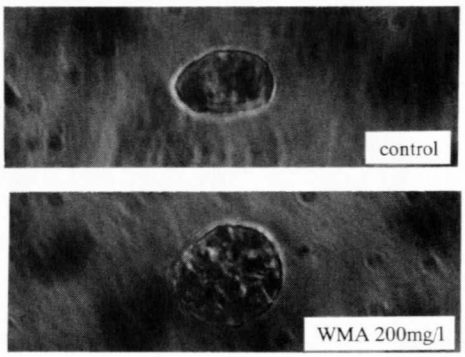


Fig. 7 Effect of the fraction eluted with methanol/distilled water/ammonia from the activated charcoal column (WMA) on the growth of *Heterosigma akashiwo*

c)Allelopathic effect on the growth of *H. akashiwo*

The time course of the cell concentration of *H. akashiwo* obtained for the WM and WMA fraction added systems are shown in **Fig. 6** and **Fig. 7**, respectively. From the growth curve, the specific growth rate and maximum cell concentration of *H. akashiwo* was found as shown in **Table 4**. There seemed almost no effects on the growth of *H. akashiwo* except the WMA concentration of 200mg/l. When the WMA fraction concentration was 200mg/l, the movement of *H. akashiwo* cells became slower after 1 day. The cell size became slightly bigger than that in control system and the shape changed to round although the original cell has oval shape (**Photo. 3**). *H. akashiwo* cell seemed to be expanded. The cell returned to the original shape after 13 day.



——— : 25 μm

Photo. 3 Appearance of *H. akashiwo*(after 2 day culture)

Table 4 Specific growth rate (μ) and maximum cell concentration of *Heterosigma akashiwo* in each system

	d_a	d_e	C_a ($\times 10^3$)	C_e ($\times 10^5$)	μ	Maximum cell conc. ($\times 10^5$ cells·ml^{-1})
control	1	8	1.46	1.76	0.684	3.23
WM 25mg/l	1	8	1.48	1.47	0.656	3.06
50mg/l	1	8	1.22	1.50	0.687	3.44
100mg/l	1	8	1.38	1.12	0.628	2.80
200mg/l	1	8	1.20	1.19	0.657	2.87
WMA 25mg/l	1	8	1.28	1.47	0.677	3.15
50mg/l	1	8	1.08	1.450	0.700	2.85
100mg/l	1	8	1.26	1.35	0.668	2.86
200mg/l	1	4	1.24	0.0914	0.666	2.01

WM : the fraction eluted with methanol from the activated charcoal column, WMA : the fraction eluted with methanol/ distilled water/ammonia from the activated charcoal column, μ : specific growth rate (day^{-1}), C_e : the cell concentration when logarithmic growth phase ends (cells·ml^{-1}), C_a : the cell concentration when the extracted is added (cells·ml^{-1}), d_e : the day when logarithmic growth phase ends (day), d_a : the day when the extract is added (day)

4. DISCUSSION

(1)Allelopathic effects of the extracts of *S. horneri* on the red tide microalgae

The morphology of *S. costatum* observed in control system without the extracts of *S. horneri* depends on the growth phase or the activity. The active cell observed during logarithmic growth phase forms a long filamentous structure by linking each cell. On the other hand, during inactive phase like stationary or decline phase, the number of *S. costatum* cell linking to form a filamentous structure becomes small and the cell tended to make clumps. Such morphological changes depending on the cell activity was caused also by the extracts of *S. horneri.* A significant growth inhibition of *S. costatum* appeared on growth curve was caused only by a high concentration of the WMA fraction, *i.e.*, higher than 100mg/l. Since the morphological change of *S. costatum* cell observed by the microscope was also obvious at 100mg/l of WMA fraction, the growth inhibition effect could be considered to be associated with the change of the body form of the red tide microalga cell.

Similarly as *S. costatum,* the morphology of *C. antiqua* was changed by 50mg/l of the WMA fraction. *C. antiqua* cell became sphere. According to Koga[11], the shape of *C. antiqua* became sphere when cells were exposed to low salinity condition. Therefore, it could be considered that the mechanism of allelopathic effect caused by the extracts of *S. horneri* was to disturb the osmotic pressure control system of *C. antiqua*.

Among three kinds of red tide microalgae tested, *H. akashiwo* revealed the highest tolerance while *C. antiqua* was the most sensitive microalga against the extracts of *S. horneri.* The necessary concentration of the WMA fraction to inhibit the growth of *H. akashiwo* was much higher than that of *S. costatum* and *C. antiqua.* The growth of *C. antiqua* was temporarily inhibited by 25mg/l of the WMA fraction. On the other hand, the growth of *H. akashiwo* was not affected by 100mg/l of the WMA fraction. Such difference in the allelopathic effects on the red tide microalgae by species may be attributed to the permeability of allelopathic substances against the cell wall. To make clear the mechanism of sensitiveness of each microalga against the extracts of *S. horneri,* further investigation concerning the cell wall structure of each microalga is needed.

Although the WMA fraction transformed three kinds of red tide microalgae cell, its effect disappeared after a certain time. *S. costatum* cells transformed by 100mg/l of the WMA fraction returned to regular shape and restarted to grow after 13 day. The microscopic observation confirmed that the red tide microalgae inhibited to grow by the extracts of *S. horneri* restarted to grow when they recovered their original cell shape. The phenomena indicated a possibility that the potential allelochemicals of *S. horneri* would be some consumable substances. As a consequence, the red tide microalgae could restart to grow after a certain time when the concentration of potential allelochemicals decreased to low level enough.

(2)Potential chemicals in the WMA fraction of the extracts of *S. horneri*

In Kaziura[5] 's report, *C. antiqua* was used to test the effect of the allelochemicals released from *S. horneri*, and it was confirmed that the growth of *C. antiqua* was inhibited remarkably. It was similar to the results of the WMA fraction added system in this study.

Tanaka and Asakawa[6] added the mucilage released from living *S. horneri* to the medium of *S. costatum*. The phenomena reported were very similar as this study. The growth of *S. costatum* was inhibited quickly while the maximum cell concentration attained after recovery was equivalent to that in control system. Especially the result obtained for 10% of the mucilage addition was similar to that of 100mg/l of WMA fraction in this study. Also our result that *S. costatum* became extinct by 200mg/l of WMA fraction corresponds to the results obtained for 30% mucilage addition reported by Tanaka and Asakawa[6] .

From these results, it is inferred that the substances in the WMA fraction of *S. horneri* in this study would be one of the potential allelochemicals. Moreover, the extraction and fractionation process used was similar as Taniguchi *et al*[10] except the extraction solution, indicating an enough possibility that the potential allelochemicals belonged to polyphenol compounds. It provides a hint to identify potential allelochemicals in the WMA fraction obtained from *S. horneri.* Based on the results, we are currently working to detect the potential allelochemicals of *S. horneri* by using HPLC analysis.

5. CONCLUSIONS

· The fractionation results of extracts from *Sargassum horneri* showed that the amount of the fraction eluted with methanol and methanol/distilled water/ammonia from the activated charcoal column (WM and WMA, respectively) were about ten times as high as those of acidic fraction (A) and neutral fraction (N).

· The maximum cell concentration of *Skeletonema costatum* tended to low by addition of the WM fraction while its growth was inhibited remarkably by addition of the WMA fraction. When the WMA concentration was higher than 100mg/l, most of *S. costatum* cells became abnormally longer while their growth was stopped.

· The specific growth rate of *Chattonella antiqua* tended to low by addition of the WM fraction. The cell growth was almost completely inhibited by 100 and 200mg/l of the WMA fraction, and became slower while the cell changed to a spherical shape.

· There seemed almost no effects on the growth of *Heterosigma akashiwo* except the WMA concentration of 200mg/l. When the WMA fraction concentration was 200mg/l, movement of *H. akashiwo* cells became slower while the cell size became slightly bigger and the shape changed to round.

· The potential allelochemicals of *S. horneri* would be some consumable substances since its allelopathic effect disappeared after a certain time.

· The growth inhibition effect of the potential allelochemicals by *S. horneri* was found to be associated with the change of body form of red tide microalgae.

ACKNOWLEDGEMENTS: Authors would like to thank Prof. Kazuya Taniguchi and graduate student Akiko Kawashima, Graduate School of Agricultural Science, Tohoku University for helpful advice of extraction and fractionation work.

This study was supported by the Sumitomo Foundation for Grant for Environmental Research Projects.

REFERENCES

1) Nakai, S., Sagehashi, M., Hoshomi, M., Okada, M., and Murakami, A.: Control of Phytoplankton Growth by Macrophyte, *Journal of Japan Society on Water Environment*, Vol. 13, No. 1, pp.33-39, 1994.

2) Nakai, S., Inoue, H., Hosomi, M., Okada, M., and Murakami, A. : Control of Phytoplankton Growth by Bioactive Compounds Extracted from Macrophytes, *Journal of Japan Society on Water Environment*, Vol. 18, No. 12, pp.1012-1019, 1995.

3) Nakai, S., Inoue, Y., Hosomi, M., and Murakami, A. : Growth Inhibition of Blue-green Algae by Allelopathic Effects of Macrophytes, *Japanese Journal of Water Treatment Biology*, Vol. 33, No. 4, pp.215-222, 1997.

4) Nakai, S., Inoue, Y., Hosomi, M., and Murakami, A. : Growth Inhibition of Blue-green Algae (*Microcystis aeruginosa*) by Allelopathic Compounds secreted by *Myriophillum spicatum.*, *Japanese Journal of Water Treatment Biology*, Vol. 34, No. 3, pp.159-170, 1998.

5) Kaziura, T. : Evaluation of water purification function by brown alga *Sargassum horneri*, Master thesis of Graduate School of Engineering, Tohoku University, pp.40-53, 1999.

6) Tanaka, N., Asakawa, A. : Allelopathic Effect of Mucilage Released from a Brown Alga *Sargassum horneri* on Marine Diatoms , *Nippon Suisan Gakkaishi* , Vol. 54, No.10, pp.1711-1714, 1988.

7) Sudo, R.: Experimental Methodology of Microorganisms (Kankyoubiseibutsujikkenhou), pp.268-269, Kodansha Scientific, 1988.

8) Taniguchi, K., Akimoto, Y., Kurata, K., and Suzuki, M. : Chemical Defense Mechanism of the Brown Alga *Eisenia bicyclis* against Marine Herbivores, *Nippon Suisan Gakkaishi*, Vol. 58, No. 3, pp.571-575, 1992.

9) Taniguchi, K., Kurata, K., and Suzuki, M. : Feeding-Deterrent Activity of Some Laminariaceous Brown Algae against the Ezo-abalone, *Nippon Suisan Gakkaishi*, Vol. 58, No. 3, pp.577-581, 1992.

10) Taniguchi, K., Kurata, K., and Suzuki, M. : Feeding-Deterrent Effect of Phlorotannins from the Brown Alga *Ecklonia stolonifera* against the Abalone *Haliotis discus hannai*, *Nippon Suisan Gakkaishi*, Vol. 57, No. 11, pp.577-581, 1991.

11) Koga, F. : Influence of salinity on the morphology and growth rate of *Chattonella antiqua* (Chloromonadophyceae) , *Bull. Nansei Reg. Fish. Res. Lab.*, No. 17, pp.197-205, 1984.

(Received January 6, 2003)