INTRODUCTION

The authors have been developing a new type of artificial algal reef made from sea-bottom sediments (generally called ‘hedoro’ in Japanese) to be employed together with seaweed culturing and the recovery of ‘isoyake’ areas in order to preserve coastal ecosystems.

The two main advantages of this new type of artificial algal reef when maintained continuously are: (i) the low degree of exfoliation from its surface into the sea; and (ii) the beneficial influence of chemical substances eluted from hedoro reefs into the surrounding seawater, as compared to the other conventional type of artificial algal reefs made from cement.

The first advantage was presented in our previous paper.1 The degree of detrition (rate of decrease of block thickness per unit time) from the surface of the hedoro block in still water decreased with the increase in its cement content. In running water at a speed of 2 cm/s, the degree of detrition showed a value 500 times that in still water. In contrast, since the degree of detrition is only $1 \times 10^{-4}$ cm/day or less in our new type of hedoro block, it can be used reliably as an artificial algal reef for cultivation of brown algae.

Until now, artificial algal reefs made from waste materials include those manufactured from fly ash and ferrite, but almost no reports exist describing the use of sea-bottom sediments.2,3 In regard to the growth of seaweed on nutrient salts, Tsukidate4 investigated the optimum conditions for growth (water temperature, chlorinity, illuminance, nitrogen level, and phosphate level) of foliated ‘kurome’, and reported that the contents of nitrogen and phosphate, were 20–200 mmol/L and 0.14–1.4 mmol/L, respectively. Yarish et al.5 showed through an incubator culture that an increased ammonium salt concentration had adverse effects on the growth of gametophytes and sporophytes of Laminaria longicruris. However, there have been very few attempts to study the effects of nutrient salts on the gametophytes and juveniles of brown algae.

In this study, we investigated the concentration of nutrient salts eluted from hedoro blocks, and its effects upon the growth and survival rate of gametophytes and juveniles Eisenia bicyclis.
METHOD

Preparation of blocks

Raw hedoro was taken from the coastal sea bottom off Tomioka-cho, Fukushima Prefecture. The specific gravity, water content, and ignition loss of hedoro were the same as those described in a previous paper (i.e. 2.57, 138%, and 8.4%, respectively). All shells and wood chips were removed from the raw hedoro using a sieve (mesh size: 2 mm), distilled water was added, the mixture was stirred well and then left for 10 days. Only the upper layer of the settled hedoro was skimmed and collected, and was sun dried to yield processed hedoro. This processed hedoro was mixed with ordinary Portland cement, hardener chemicals (NSC Chemical Industry Co. Ltd, Okayama, Japan), and distilled water. Ratios for 500 g of processed hedoro are given in Table 1. Ten minutes after mixing, the mixture was poured into rectangular molds. The hedoro blocks were then removed from the molds after 3 days and cured for a period of 25 days during which time the block surfaces were moistened to prevent cracks from forming. To stabilize the quantity of substances eluted from the hedoro blocks, they were immersed in distilled water for about 15 days.

The cement content of the hedoro block samples was 30%, 50%, 70%, and 100%, and were called C30, C50, C70, and C100, respectively.

In addition to the hedoro blocks mentioned above, a mortar block containing sand was also made as a control block. As shown in Table 1, the mortar block contained 600 g of cement against 2000 g of sand (i.e. it has a 30% cement content). This sample block was called M30.

Nutrient salts eluted from hedoro blocks

The hedoro block samples (C30, C50, C70 and C100) were cut into rectangular specimens (4 × 4 × 2 cm) and sterilized by autoclaving at 120°C and 1.5 at. They were immersed in 1 L of filtrated seawater (0.45 µm; Millipore HA, Bedford, USA), and sterilized by autoclaving at 120°C and 1.5 at, as for the samples. Ten milliliters of the immersion liquids for each sample block were extracted with a pipette on days 1, 3, 7, 14, 21 and 28 from when immersion was initiated. For these immersion liquids and filtrated seawater, concentrations of NO₃⁻–N, NO₂⁻–N, NH₄⁺–N, PO₄³⁻–P were measured using an auto analyzer (TRAACS800; BRAN+LUEBBE Co. Ltd, Hamburg, Germany).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mixing ratios of cement, hardener chemicals and water for the different hedoro blocks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hedoro H (g)</td>
<td>Cement C (g)</td>
</tr>
<tr>
<td>500</td>
<td>150</td>
</tr>
<tr>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>500</td>
<td>350</td>
</tr>
<tr>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>2000¹</td>
<td>600</td>
</tr>
</tbody>
</table>

¹Ratio of hardener chemicals to cement is 0.1.
²r = C/H × 100%.
³Sand weight.

The Petri dish was kept in a dark place. The seawater was collected from the coastal sea bottom off Su-nosaki, Tateyama City, filtrated using a membrane filter (0.45 µm; Millipore HA, Bedford, USA), and sterilized by autoclaving at 120°C and 1.5 at, and after autoclaving at 120°C and 1.5 at, and for the samples. Ten milliliters of the immersion liquids for each sample block were extracted with a pipette on days 1, 3, 7, 14, 21 and 28 from when immersion was initiated. For these immersion liquids and filtrated seawater, concentrations of NO₃⁻–N, NO₂⁻–N, NH₄⁺–N, PO₄³⁻–P were measured using an auto analyzer (TRAACS800; BRAN+LUEBBE Co. Ltd, Hamburg, Germany).

Growth and survival rates of seaweed

Experiments were carried out at the Banda Experimental Station of Tokyo University of Fisheries from November 1997 to January 1998. Mature thalli of Eisenia bicyclis were sampled from the coastal area off Shirahama, Boso Peninsula, and were placed for 1–2 h in the shade and then immersed in filtrated seawater (0.45 µm; Millipore HA) to release zoospores. The released zoospores were immediately put in a dark place for 1 h to separate diatoms, and then a glass slide was placed in the vessel (diameter, 10.5 cm; height, 14.5 cm) filled with the zoospore suspension at a concentration of approximately 4000 individuals/mL. After allowing the suspension to stand for 20 min, zoospores attached to the surface of the glass slide at a density of approximately 46 ind./mm². These glass slides were placed, for the culture experiment, in Petri dishes (diameter, 15 cm; height, 9 cm) filled with the immersion liquids from each block after 30 days of immersion. The cultivation was carried out in an incubator (water temperature, 20°C; light intensity, 7000 lx). During the 40 days of the cultivation period, the immersion liquid used for cultivation was replaced every 10 days. Growth and survival rates were observed every 10 days.

Growth (the maximum length of the gametophytes or juveniles) was measured using vernier calipers (precision, 0.05 mm) after magnifying the glass slide through a universal projector (magnifications, ¥ 100 and ¥ 50). A mean value of 60 individuals (30 male and 30 female gametophytes) was determined.

Survival rate was defined as the number of adhering individuals after a fixed period divided by that of the initial number of adhered individuals and expressed by percentage. Survival rate (Sr) was obtained using the following formula:

\[ Sr = \frac{Dn}{Di} \times 100\% \]

where Di is the individual density of initial adhesion and Dn is the density after the fixed period.

Individual densities on the glass slides (the number of individuals per 1 mm²) were determined from the mean values at 10 locations on the glass slide.
RESULTS AND DISCUSSION

Nutrient salts eluted from blocks

When C30 was left in distilled water for 10 days, it was visually observed that the immersion liquid gradually changed color to give a yellowish tint. This suggested that substances were eluted from the C30 hedoro block. Figure 1 shows the variations of nutrient salts (i.e. NO$_3^-$ -N, NO$_2^-$ -N, NH$_4^+$ -N, and PO$_4^{3-}$ -P) which eluted from each hedoro block.

With each block, the elution of NO$_3^-$ -N increased with immersion time, and reached an approximately constant level after 10 days from the beginning of the experiment (Fig. 1a). It was also observed that the elution levels of the blocks rose with their cement ratios. Furthermore, the trend of elution with NO$_2^-$ -N (Fig. 1b) was similar to that of NO$_3^-$ -N. The elution of NH$_4^+$ -N showed an approximately linear increase with time (Fig. 1c). The elution from the C30 reached a maximum, while the other ratios of C50, C70, and C100 showed almost the same levels of elution.

For the variation of PO$_4^{3-}$ -P, its elution remained almost constant with all sample blocks except C30 (Fig. 1d). However, the C30 showed peak values of approximately 15 times the elution at initiation on day 21 and approximately eight times on day 28. The sharp peak value of 1.91 (µg-at/L) measured on day 28 corresponds to approximately 10 times that of the filtrated seawater. Chen$^6$ investigated the values of total N and PO$_4^{3-}$ -P for a hedoro block using the same materials and methods as those used in the present study. He reported that the values of eluted total N and PO$_4^{3-}$ -P from hedoro blocks are 232.7 and 0.76 (µg-at/L), respectively. These values are approximately 23 times the total N and three times the PO$_4^{3-}$ -P of filtrated seawater. It is, therefore, considered that the highest values may be caused by the properties of C30.

The variation in the elution of NO$_3^-$ -N and NO$_2^-$ -N is characterized by a rapid increase until day 7 of the experiment and a constant level thereafter. The variation of NH$_4^+$ -N, in contrast, shows a linear increase with time. From the elution trends commonly seen in all hedoro blocks, it is considered that there are differences in the elution process between NH$_4^+$ -N and NO$_3^-$ -N, NO$_2^-$ -N. The mechanism of the elution process from the hedoro block is the subject for future studies.

Subsequently, the total amount of nutrient nitrogen salts (NO$_3^-$ -N, NO$_2^-$ -N, NH$_4^+$ -N) from the hedoro blocks on day 28 was assessed. Table 2 indicates that the measured value in the immersion liquid of C30 is slightly higher than those in the other hedoro blocks. When these nutrient values are compared with that of filtrated seawater as shown in Table 2, the measurements of total N with C30, C50, C70, and C100 were 29.7, 19.9, 26.4, and 21.7 times that of filtered seawater, respectively.

In the comparison of the elution of nutrient salts such as NO$_3^-$ -N and NO$_2^-$ -N to cement content, the...
C30 than in the other cases, as mentioned earlier. The pH values during the cultivation period for the C30 immersion liquid and for the filtered seawater were 7.52–8.05 and 7.92–8.45, respectively. Endoh suggested that these pH values do not account for the growth of spores. This, therefore, indicates that growth inhibitors exist in the C30 immersion liquid. The difference of growth between the C30 immersion liquid and filtered seawater may be influenced by the ammonium nitrogen eluted from the hedoro blocks.

Chen conversely found that the growth in the C30 immersion liquid was 1.2 times better than that in filtered seawater. However, in Chen’s report the replacement interval for the culturing liquid was 5 days, which was half that of the interval during this study (i.e., 10 days).

Figure 2 shows the survival rates of gametophytes and juveniles of *E. bicyclis* when cultured in the immersion liquid. The initial adhesion density of zoospores of *E. bicyclis* was 46 ind./mm². The survival rate of *E. bicyclis* in the immersion liquid decreased gradually. For example, the survival rates of zoospores growing on C30 on days 10, 30, and 40 were 63.7%, 29.0%, and 24.8%, respectively. The survival rates differed between the immersion liquids of the hedoro blocks with differing cement contents. The survival rates with M30 decreased sharply to 1.3% on day 20, and reached nearly zero on days 30 and 40. Throughout the experiment, the survival rate of gametophytes and juveniles of this alga was higher on the C50 immersion liquid than in the others. The gametophytes of *E. bicyclis* in the liquid with immersed C50 showed lower growth and higher survival rates than those on the other blocks. The gametophytes in which C100 was immersed also showed lower growth. From these results, it is considered that some substances eluted from the cement in the blocks slowed the growth of gametophytes, but the survival rate was not affected. Further investigation is necessary to confirm this.

A comparison of the growth in the liquid with the immersed C30 hedoro block with that of the mortar block, M30, shows the growth on the mortar block in amount of elution increases with the increase in cement content. The amount of nutrient salt elution NH₄⁺–N was highest with C30, blocks which had the highest hedoro content. As mentioned above, the nature of the elution process may differ between NH₄⁺–N and NO₃⁻–N, NO₂⁻–N. Also, the issue of cement content requires further study.

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>(NO₃⁻ + NO₂⁻ + NH₄⁺)–N (μg/L)</th>
<th>(PO₄³⁻)–P (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C30</td>
<td>160.0</td>
<td>1.92</td>
</tr>
<tr>
<td>C50</td>
<td>107.7</td>
<td>0.24</td>
</tr>
<tr>
<td>C70</td>
<td>142.6</td>
<td>0.16</td>
</tr>
<tr>
<td>C100</td>
<td>117.2</td>
<td>0.29</td>
</tr>
<tr>
<td>Filtered seawater</td>
<td>5.40</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>*(6.64)</td>
<td>*(0.28)</td>
</tr>
</tbody>
</table>

* Values when the experiment began.

Growth and survival rate of gametophytes and juveniles in the immersion liquids

The growth of gametophytes and juveniles of *E. bicyclis* in the immersion liquids is shown in Fig. 2. In the case of C30 in the immersion liquid, the lengths of the gametophytes were 0.09 ± 0.03 mm after 10 days from the beginning of experiment. The lengths of juveniles on days 30 and 40 were 0.24 ± 0.08 mm and 0.41 ± 0.12 mm, respectively. Growth showed a sharp rise on day 20. For the sample blocks C50 and C100, the growth of gametophytes and juveniles was extremely low in sharp contrast to that of C30. In contrast, the growth of gametophytes and juveniles on the mortar block, M30, was lower than that on C30, although similar tendencies were shown.

The highest growth of gametophytes and juveniles was achieved in the liquid with the immersed C30 hedoro block, which had the highest hedoro content, while extremely low values were measured with C50 and C100. According to these observations, it is considered that low cement content and high hedoro content are effective for the growth of gametophytes and juveniles of *E. bicyclis*. In a comparison of the effects on growth between liquids in which the C30 was immersed and filtered seawater, the lengths of gametophytes on day 10 were 0.09 ± 0.03 mm in the C30 liquid and 0.11 ± 0.03 mm in seawater, and the lengths of juveniles on day 40 were 0.41 ± 0.12 mm in the C30 liquid and 0.74 ± 0.25 mm in seawater. Growth in filtered seawater is approximately 1.3 to 1.8 times that in the immersion liquid. The amounts of total N and PO₄³⁻–P in the immersion liquid (30-day immersion) were far greater for C30 than in the other cases, as mentioned earlier. The pH values during the cultivation period for the C30 immersion liquid and for the filtered seawater were 7.52–8.05 and 7.92–8.45, respectively. Endoh suggested that these pH values do not account for the growth of spores. This, therefore, indicates that growth inhibitors exist in the C30 immersion liquid. The difference of growth between the C30 immersion liquid and filtered seawater may be influenced by the ammonium nitrogen eluted from the hedoro blocks.

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Figure 3 shows the survival rates of gametophytes and juveniles of *E. bicyclis* when cultured in the immersion liquid. The initial adhesion density of zoospores of *E. bicyclis* was 46 ind./mm². The survival rate of *E. bicyclis* in the immersion liquid decreased gradually. For example, the survival rates of zoospores growing on C30 on days 10, 30, and 40 were 63.7%, 29.0%, and 24.8%, respectively. The survival rates differed between the immersion liquids of the hedoro blocks with differing cement contents. The survival rates with M30 decreased sharply to 1.3% on day 20, and reached nearly zero on days 30 and 40. Throughout the experiment, the survival rate of gametophytes and juveniles of this alga was higher on the C50 immersion liquid than in the others. The gametophytes of *E. bicyclis* in the liquid with immersed C50 showed lower growth and higher survival rates than those on the other blocks. The gametophytes in which C100 was immersed also showed lower growth. From these results, it is considered that some substances eluted from the cement in the blocks slowed the growth of gametophytes, but the survival rate was not affected. Further investigation is necessary to confirm this.

A comparison of the growth in the liquid with the immersed C30 hedoro block with that of the mortar block, M30, shows the growth on the mortar block in
terms of length corresponds to approximately 30–80% of that on the hedoro blocks throughout the cultivating period. Since the cement content of the sample blocks was the same (30%), it can be assumed that the difference in the growth of gametophyte and juvenile *Eisenia bicyclis* can be ascribed to the effects of nutrient salts contained in hedoro.

**ACKNOWLEDGMENTS**

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**REFERENCES**