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Microbial Conversion of Macroalgae into a Detrital Hatchery Diet

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Abstract

The formation of algal detrital particles in a completely cell-detached form, namely, single cell detritus (SCD) was reported for the first time by the author during the microbial degradation process of macroalgal thalli. SCD display 3 characteristics suitable for feed for aquatic hatchery animals: 1) The size of SCD is in the 2-14 μm range, which is similar to that of dietary phytoplankton; 2) The cell wall components of SCD are partially degraded which facilitates digestion; 3) Bacterial cells are attached to SCD, which modifies the algal detritus to protein-rich particles. The use of SCD as a potential hatchery diet instead of phytoplankton culture, which is labor-intensive, was successfully demonstrated based on feeding experiments with *Artemia*. Further modification of SCD could include the attachement of bacteria to SCD which would exert beneficial effects on the hatchery animals. Use of SCD diets in fish feeding regimes is an attempt to introduce the concept of detrital food web to aquaculture systems, which could contribute to the development of sustainable fish nursery systems.

Discipline: Aquaculture
Additional key words: detritus, food chain, *Pseudoalteromonas, seaweed, Ulva*

Introduction

Aquaculture techniques have markedly progressed over the last decades, and enabled to produce and release millions of seed fishes important to the aquatic environment. On the other hand, however, the prevalence of fish diseases has become a serious problem in many countries, and an increasing number of people consider that current aquaculture systems require significant changes, both technical and conceptual, for sustainable development.

In the natural aquatic environment, larval fish develop by feeding on grazing and detrital food chain systems (Fig. 1). However, the present aquaculture systems utilize only the grazing food chain principle[6]. Since the detrital food web can be considered to be a system whereby biological resources not utilized as feed in the grazing food chain are recycled, a more efficient system could be developed by introducing this recycling system into the present fish-feeding regime. Although our understanding of the detrital food web is still limited, there is an increasing number of studies showing the effectiveness of using detritus[1,2,4]. Since the detrital system has a large input, if it enhances the ecological efficiency, the system could have a large impact on biological production in the natural ecosystems. This paper describes a method of preparing algal detrital materials with a high dietary value to improve the ecological efficiency of the detrital food chain system and introduce it to the present fish culture system.

Initial observations of single cell detritus (SCD)

Our laboratory maintains a collection of over 100 bacterial strains which were isolated from coastal waters of Japan and are able to degrade

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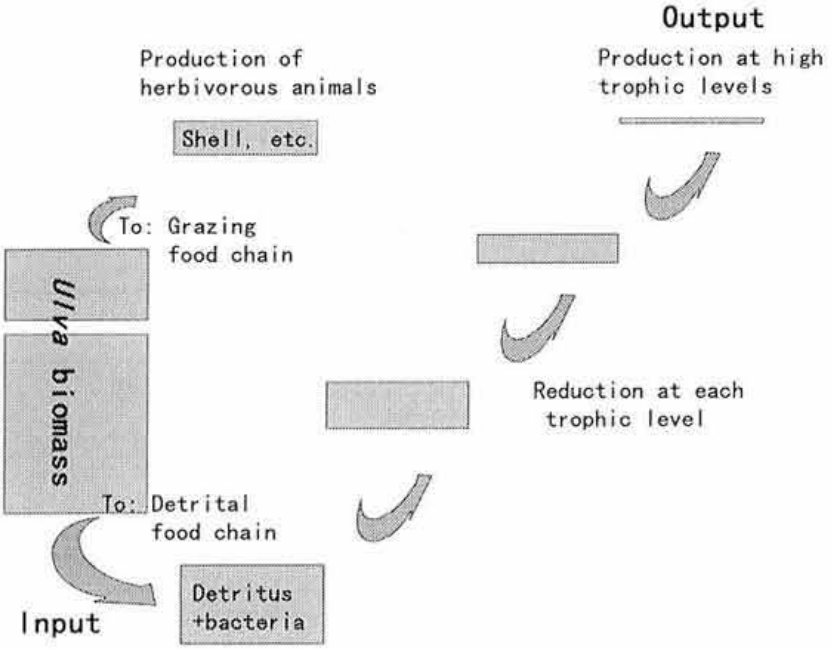

Fig. 1. The detrital food chain as a system to recycle wastes from biological production

The input (the quantity not grazed and decomposed to detrital materials) is large but the output (the quantity linked to the production at higher trophic level) is rather small in the detrital system.

macroalgal thalli. Strain AR06 (MAFF 120006, FERM BP-5024) shows the strongest activity within our collection. AR06 exhibits a decomposing activity toward various kinds of algal polysaccharides such as algin, fucoidan, agar, etc. and is able to degrade macroalgal thalli from brown and green algae, and also presumably from red algae. In our previous studies, we observed the formation of algal detritus in a completely cell-detached form (Fig. 2), namely, single cell detritus (SCD) during the microbial degradation process of *Laminaria japonica*[8] and *Ulva* sp.[12]. AR06 which was assigned to *Alteromonas espejiana* based on the results of biological and physiological tests[8], is presently assigned to *Pseudoalteromonas atlantica* based on the results of phylogenic analysis using 16S rDNA sequence and DNA-DNA hybridization tests with type strains (in preparation). SCD were also found to be formed during the experimental degradation of *Ulva* fronds soaked in natural seawater supplemented with peptone at 5 g/L level. Therefore, SCD can be produced by the activity of indigenous microbial populations without the presence of AR06 which suggests that SCD formation may occur in natural environments.

The size of the SCD formed in these studies is in the 2–14 μm range, which is similar to that of dietary phytoplankton such as *Nannochloropsis* sp., *Isochrysis galbana*, and *Tetraselmis tetrathele*. SCD partially lose their cell wall components by microbial degradation and appear as spheroplasts in the case of *Laminaria* or protoplasts in the case of *Ulva*. Many bacterial cells were attached to the surface of the SCD, especially in the case of SCD produced from *Laminaria*. SCD from *Laminaria* have an algin layer on their surface, which may facilitate the attachment of the bacteria.

It takes about 3–5 days until the production of SCD is observed during the experimental degradation of macroalgal thalli if the degradation is performed by bacterial activity alone. However, the number of particles with the size of SCD increases more rapidly if the degradation is performed with a combination of magnet-stirring during the incubation although the SCD prepared in this manner appear to be more disintegrated compared with intact spheroplasts or protoplasts.

Use of SCD as a hatchery diet

The characteristics of SCD, i.e. a size appropriate

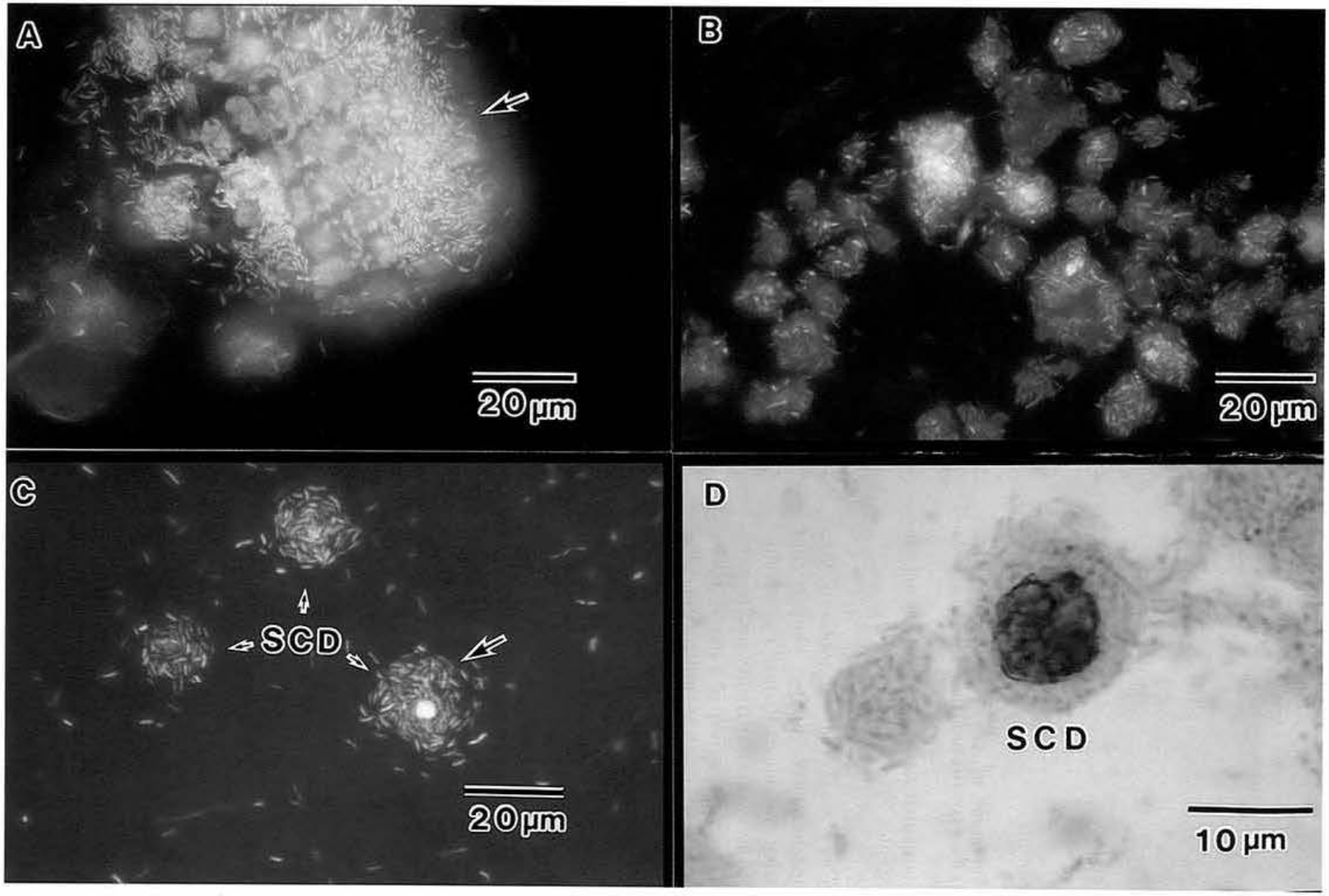

Fig. 2. Microphotographs showing the decomposition process of *Laminaria japonica* (A, B) and the resulting production of single cell detritus (SCD) by the activity of *Pseudoalteromonas atlantica* AR06 (C, D)

The SCD surface is densely covered with bacterial cells (arrow), which were observed after *fluorescent* staining (A, B, C) with 4,6'-diamino-2-phenylindole (DAPI).

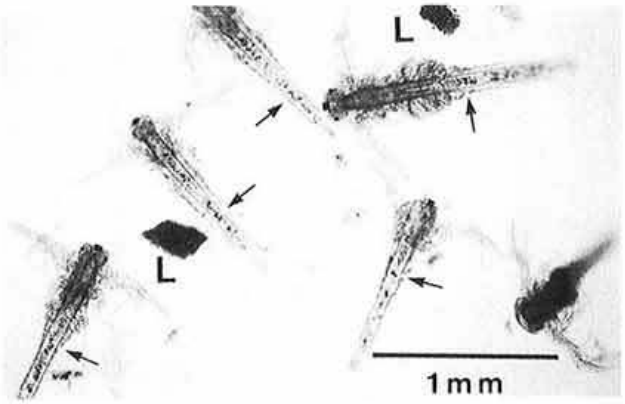

Fig. 3. Microphotograph of *Artemia* larvae ingesting the SCD diets (arrow) prepared from *Laminaria japonica*

Undecomposed *Laminaria* (L) is not suitable for ingestion by *Artemia*.

for ingestion and a structure associated with digestibility for hatchery animals, suggest that SCD can be used instead of dietary phytoplankton. The preparation of SCD is easy and requires only one to a few days, which is a major advantage compared to the use of phytoplankton. The potential use of SCD as a hatchery diet was tested by feeding experiments with *Artemia*[10]. When *Artemia* were given *Laminaria* particles, 105–177 μm in diameter, they could not ingest them because of the size and scarcely grew. In contrast, *Artemia* fed on the same *Laminaria* particles that were degraded to SCD by the AR06 strain exhibited a much better growth and survival rates (Figs. 3, 4a). When the feeding level was compared to that of a commercial phytoplankton diet consisting of *Nannochloropsis* sp. (Marine Alpha, from Nisshin Science), the growth rate was nearly the same, although the survival rate was slightly lower. In another experiment where *Laminaria* particles less than 44 μm in diameter were used as a control diet, it was demonstrated that the macroalgal thalli themselves had a high dietary value if given as a material with a size suitable for ingestion (Fig. 4b). However, the degradation of the *Laminaria* particles by contact with AR06 did not result in an increase of the initial dietary value for *Artemia*. In this experiment, a marked increase in the number of detrital particles suitable for ingestion by *Artemia* was observed in case of axenic control when mechanical degradation by magnet-stirring was used while only a gradual increase of SCD was observed when the diet was prepared by contact with AR06 (Fig. 5). The moderate

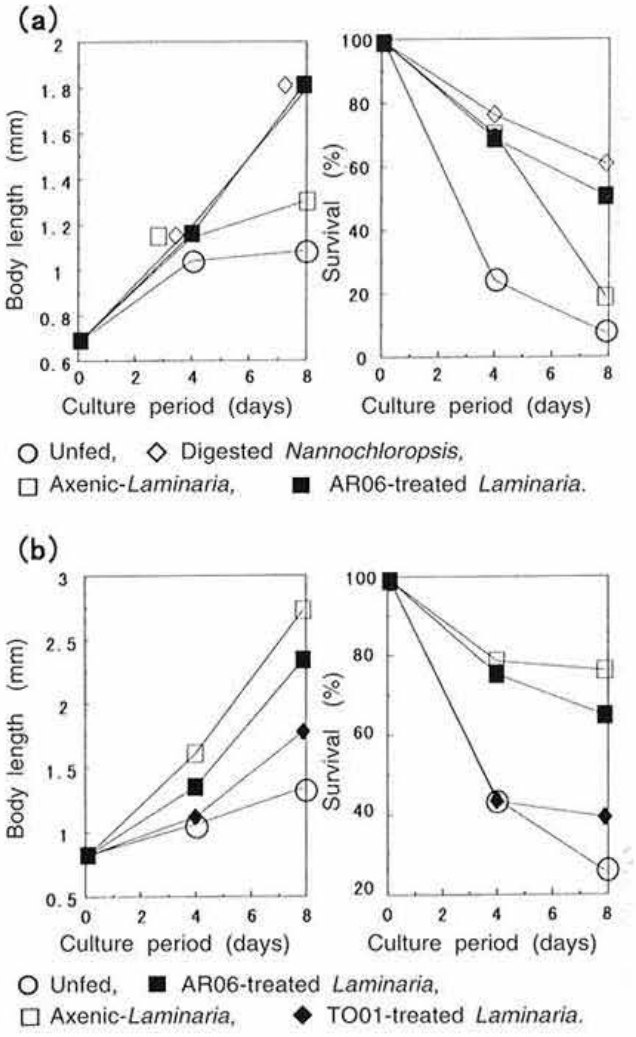

Fig. 4.

(a): Results from the *Artemia* feeding experiment using diets prepared from *Laminaria*, 105–177 μm in diameter

(b): Results from the Artemia feeding experiment using diets prepared from *Laminaria*, <44 μm in diameter

increase in the number of SCD products was ascribed to the characteristics of AR06 and also to the fact that it consumes the SCD. This assumption is supported by the following observations made during a feeding experiment using another detrital diet prepared by contact with *Pseudoalteromonas* sp. strain TO01. The TO01 strain is able to decompose *Laminaria* particles but does not produce SCD. As a result, the number of detrital particles in the size range of that of SCD, 2–14 μm, decreased markedly in TO01-treated *Laminaria*. The dietary value of the detritus was the lowest among the 3 applications of Axenic *Laminaria*, AR06-treated *Laminaria*, and TO01-treated *Laminaria*.

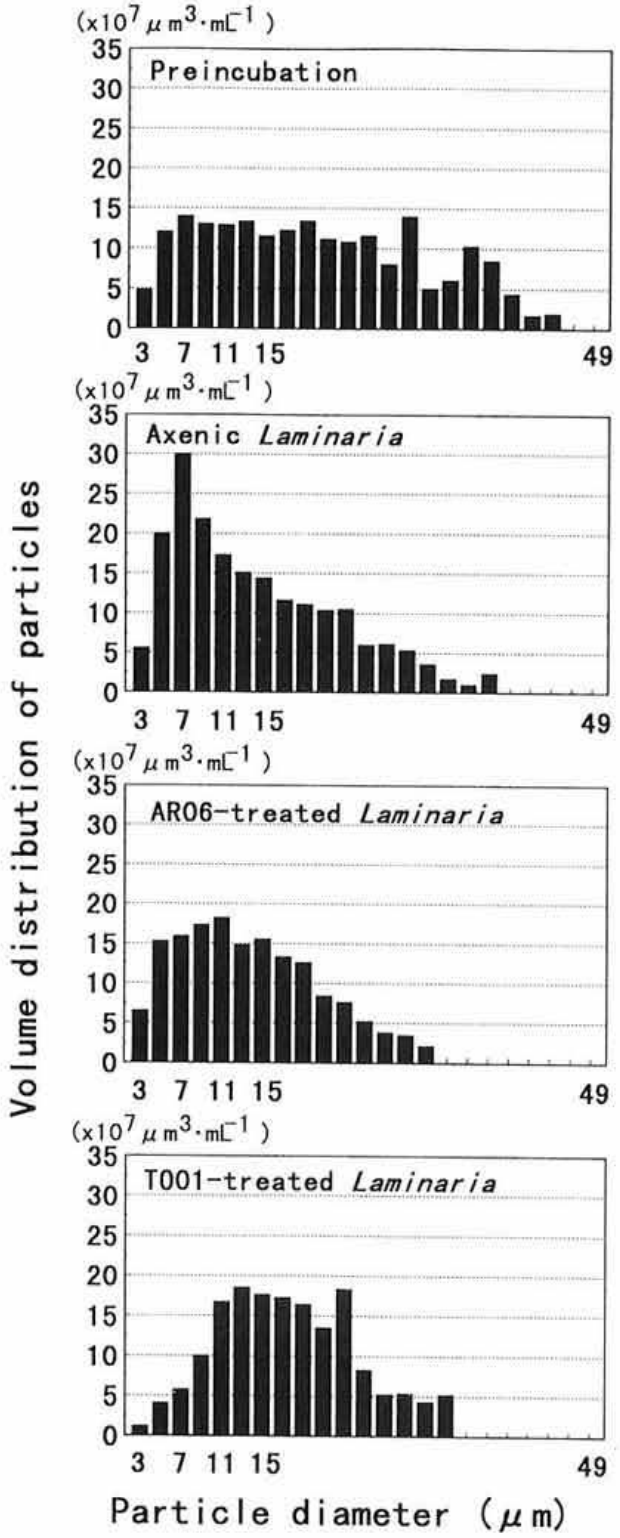

Fig. 5. Volume distribution of detritus particles contained in *Laminaria*-diets after axenic incubation with stirring (preincubation) and following a 24h incubation without (Axenic *Laminaria*) or with the presence of AR06 (AR06-treated *Laminaria*) or TO01 bacteria (TO01 treated *Laminaria*)

The results from this experiment indicated that the dietary value of the macroalgae differs depending on the kind of microorganisms involved in the macroalgal decomposition. The above results show that the dietary value of the macroalgae is maximum if the decomposition of the macroalgae is performed with a view to producing the maximum number of detrital particles with a size suitable for ingestion. We are currently accumulating information for developing algal detrital diets with a high dietary value through feeding experiments with *Artemia*.

Potential use of surface-attached bacteria as a tool for enhancing the nutritive value of SCD

There are 2 main objectives in our attempts to utilize SCD as a hatchery diet (Fig. 6). One is to utilize macroalgal resources as a diet by converting them to microalgae. The other is to utilize the bacteria attached to the detrital materials as a diet. Fish require a high level of protein accounting for 35–50% for their diet[7], while most macroalgae species contain only 10–20% protein. Bacteria have a protein content of about 60–70% on a dry weight basis and therefore have a high potential as a diet[3,5,11]. In the case of the SCD diets, bacteria attached to the algal detritus grow by absorbing nutrients from the environment and, as a result, contribute to modifying the algal detritus to protein-rich materials. In our observations, the protein level of the *Ulva* detritus was nearly double, which accounts for the fact that the SCD diets have such a high dietary value[10,11].

In the case of the present SCD diets, bacteria are utilized while still alive, which is a unique characteristic different from the phytoplankton diets or artificial micro-diets. The bacterial strain AR06, which we used for our serial studies, is specialized in the degradation of macroalgae and is not necessarily used for the enhancement of a diet. Diets with specific activities for feeding of animals can be developed by attaching bacteria with beneficial characteristics for fish, for example, regulation of the growth of pathogenic microorganisms or production of growth-promoting materials such as eicosapentaenoic acid (EPA) and dochosahexaenoic acid (DHA), and growth hormones for fish with or without the application of genetic engineering techniques.

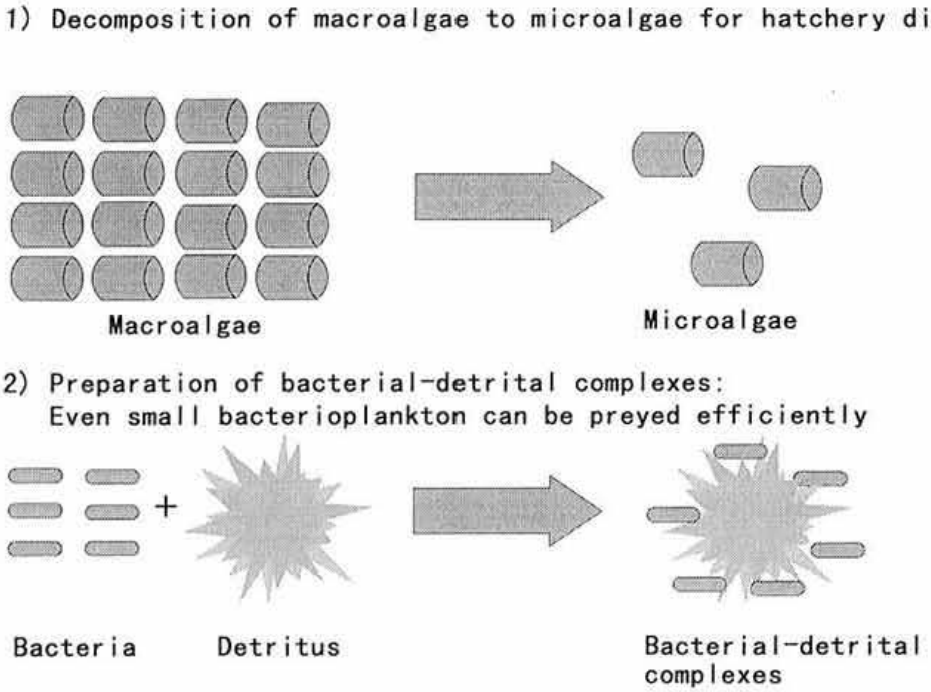

Fig. 6. Two strategies for developing SCD diets

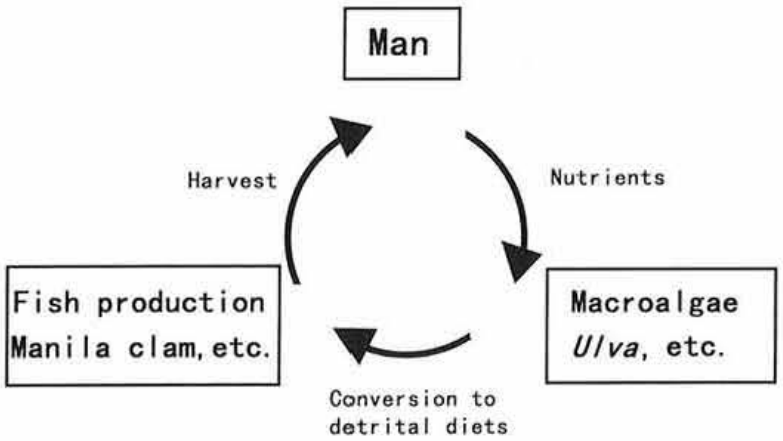

Fig. 7. Concept of trophic enhancement using detrital macroalgal particles

Concept of "trophic enhancement" based on detrital macroalgae

We are presently considering the possibility of converting macroalgal biomass resources to SCD and to utilize them as a hatchery diet in extensive aquaculture systems. This attempt corresponds to the "trophic enhancement" of marine habitats (Fig. 7). Then, what kind of macroalgae could be converted to SCD, and what kind of aquatic animals could be cultured in this system?

Recently, cumbersome blooms of *Ulva* have been reported from coastal waters in Japan and various foreign countries. *Ulva*, after decaying causes oxygen-limited environmental conditions which often kill marine animals, especially bivalves. To address this problem, fishermen and local governments remove these algal accumulations by labor-intensive methods. The collected materials have no commercial value and their disposal is a major problem in several areas in Japan. Against this background, we attempted to utilize the *Ulva* resources. Coastal waters with large *Ulva* blooms are often good fishing grounds for Manila clams, *Ruditapes philippinarum*, a bivalve with a high commercial value. Moreover, the hatchery season of the bivalve larvae, i.e. June to July, corre-

sponds to the period of growth and subsequent decay of *Ulva* fronds. The hatched bivalve larvae have only a limited mobility and are easily transported, depending on the wind and water current, to the same area where *Ulva* fronds are concentrated. Therefore, if we succeed in preparing detritus with a high dietary value from *Ulva* fronds, it may be possible to link this activity to the production of Manila clam larvae. As a preliminary experiment, we prepared hatched larvae from adult Manila clams caught from Uminokouen, Yokohama, and administered a SCD diet prepared from *Ulva* which was also collected from the same area. Some of the larvae ingested the *Ulva* SCD during overnight incubation in a 300 mL-volume beaker. Whether the SCD diet can contribute to the growth of the larvae or not is now being examined.

Blooms of *Ulva* are attributed to eutrophication caused by human activities. Our attempt to culture fishes in "a trophic enhancement system" utilizing bacterially degraded macroalgal resources is different from the conventional aquaculture systems, in that compatibility between commercial fish culture and human activities causing the eutrophication problem could be achieved.

References

1) Azam, F. et al. (1983): The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.*, **10**, 257–263.
2) Bombeo-Tuburan, I., Guanzon, N.G. Jr. & Schroeder, G. L. (1993): Production of *Penaeus monodon* (Fabricius) using natural food types in an extensive system. *Aquaculture*, **112**, 57–65.
3) Gorospe, J. N. et al. (1996): Potential use of *Pseudomonas* as food for brine shrimp *Artemia*. *Jpn. Aqua. Soc.*, **44**, 315–323.
4) Honculada Primavera, J. & Gakutanc, R. Q. (1987): Preliminary results of feeding aquatic macrophytes to *Penaeus monodon* juveniles. *Aquaculture*, **80**, 189–193.
5) Intriago, P. & Jones, D. A. (1993): Bacteria as food for *Artemia*. *Aquaculture*, **113**, 115–127.
6) Sorgeloos, P. (1995): Bioengineering of hatcheries for marine fish and shellfish. *J. Mar. Biotechnol.*, **3**, 42–45.
7) Takeuchi, T., Watanabe, T. & Ogino, C. (1978): Optimum ratio of protein to lipid in diets of rainbow trout. *Bull. Jpn. Soc. Sci. Fish.*, **44**, 683–688.
8) Uchida, M. (1996): Formation of single cell detritus densely covered with bacteria during experimental degradation of *Laminaria japonica* thalli. *Fish. Sci.*, **62**, 731–736.
9) Uchida, M. (1998): Conversion of macroalgae into a detrital hatchery diet—An attempt to introduce detrital food webs into aquaculture. *TechnoInnovation*, **8**(2), 24–29.
10) Uchida, M., Nakata, K. & Maeda, M. (1997): Introduction of detrital food webs into an aquaculture system by supplying single cell detritus produced from *Laminaria japonica* as a hatchery diet for *Artemia* nauplii. *Aquaculture*, **154**, 125–137.
11) Uchida, M., Nakata, K. & Maeda, M. (1997): Conversion of *Ulva* fronds to a hatchery diet for *Artemia* nauplii utilizing the degrading and attaching abilities of *Pseudoalteromonas espejiana*. *J. Appl. Phycol.*, **9**, 541–549.
12) Uchida, M. & Numaguchi, K. (1996): Formation of protoplasmic detritus with characteristics favorable as food for secondary animals during microbial decomposition of *Ulva pertusa* (Chlorophyta). *J. Mar. Biotechnol.*, **4**, 200–206.

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