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Culture of Rotifer, *Brachionus plicatilis* (MÜLLER), in an Air Lift Aquarium Using Alcohol Fermentation Slops*1

Osamu FUKUHARA, Hiroshi YUKAWA*2 and Tatsuya NISHIMURA*3

アルコール発酵母液によるシオミズツボワムシのエヤーリフトを用いた培養

福原　修・湯川　宏・西村　辰也

アルコール発酵母液を餌料生物の培養に利用する場合，微生物フロックを形成させて給餌する。その際，餌料培養槽内に発生する懸濁物の除去には従来，特殊な循環ポンプを用いていた。しかしこの方法はポンプの故障の頻度が高いとか，電源の設備を必要とするとか，ワムシが損傷するなど実用化には種々の改良を要する問題を残しているので，信頼性があり，簡易で，ワムシへの損傷が少ない通気装置（エヤーコンプレッサー）のみによる方法を検討した。

沪過水槽を餌料生物培養槽の外部に設置し，３本のエヤーリフト管で循環する装置を試作した。この装置を使用してワムシの培養を1980年夏から1981年春にかけ30～40日間にわたり３回行った。その結果，毎日５～10%収穫しても総個体数を 100～200/ml に維持することができ，従来のポンプによる培養法より優れていることが明らかになった。

Abstract

The pump was employed as a mean of water circulation for removing the suspended particulate matter by filteration when the alcohol fermentation slops were fed to rotifer, *Brachionus plicatilis*. However, the water circulation occasionally stopped due to the malfunction of the pump. Furthermore, the pump has defects such as the extra cost for the electricity and physical damage of the rotifer.

The culture facilities based on the air-lift is designed to overcome the defects of pumping system in the rotifer culture using the alcohol fermentation slops. Three culture experiments, lasting for 30 to 40 days for each, was conducted during the summer through spring in 1980 and 1981.

The air-lift method provided 5 to 10 percent of removal each day while having retained

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*1 Study on fermentation slop from the viewpoint of fisheries resources

*2 Research and Development Center, Duskin Co. Ltd.

*3 Kyowa Hakko Kogyo Co. Ltd.

the density of rotifer at a level between 100 to 200 individuals/m*l*. It is obvious that the air-lift circulation can replace the water circulation by means of pump for practical culture of the rotifers.

The rotifer, *Brachionus plicatilis*, has played an indispensable role as initial food organism for the larval rearing of marine fishes. Therefore a number of studies were conducted to develop the methods to culture the rotifer in the last ten years.

Eventually, it is now possible to use the yeast as well as *Chlorella* as feeding materials of these organism (e.g. WATANABE *et al.*, 1978; FUKUSHO, 1980). Experiments have been undertaken to clarify the artificial ecosystems, and the effect of microorganisms and phytoplankton on rotifers (HIRATA, 1977; HIRATA and YAMASAKI, 1980; TESHIMA *et al.*, 1981; HINO *et al.*, 1981 a, b).

YASUDA and TAGA (1980) studied the utilization of marine bacteria for rotifer culture. The utilization of marine bacteria as a food organism has been of interest for a long time (SEKI, 1966). Little is known about the utilization of bacteria as a practical feed for zooplankton culture.

The CLEAN JAPAN CENTER (CJC, 1978) and ABE *et al.* (1980) studied the production of rotifers fed on marine bacteria which in turn had been cultured on alcohol fermentation slops. Rotifers can thus indirectly cultured with the alcohol fermentation residues as a biological means of waste disposal. CJC and ABE *et al.* adopted pumps to maintain the water quality However the high mortalities of rotifers were occasionally observed due to the malfunction of pumping system, and an alternative circulation method of water was desired.

The present work was designed to improve the circulation system for the rotifer cultures so that the pumping is not required. Elimination of the pump will reduce running cost and will assure reliability of the whole culture system of rotifers.

Materials and Methods

Experiments were carried out in the summer, winter and spring of 1980 and 1981 at the Nansei Regional Fisheries Research Laboratory. Seawater was pumped up on the nearly coast and filtered with the sand before the use. Rotifers were obtained from a wild strain kept in an outdoor tank fed on *Chlorella* sp. The animals ranged from 188 to 286 μm in

length and from 126 to 169 μm in width.

General observations of environmental conditions were usually made at 9:00-10:00 a.m. for water temperature, salinity, pH and dissolved oxygen.

Design and Maintenance of Tanks

The rotifer tank is shown semidiagrammatically in Figure 1. A transparent polycarbonate tank with a capacity of 1 m^3 (1000 *l*) was employed. Surface of the tank was covered with a plastic sheet to help maintaining the temperature of the culture media as stable as possible. The water column was heavily aerated at a rate of 100 to 120 *l*/min. It is cleaned through a filter container of 70 *l* located beside the culture tank. A 30-*l* tank, filled with monofilament fish net, which acted as a filter was placed inside the 70-*l* tank. This filter apparatus removes sediment from the rearing tank. Water was circulated by means of three air-lift devices (PVC pipe, 20 mm in diameter). Approximately 20 *l* of water per minute flowed from the rearing tank into the filter container.

Water temperature was maintained within a range of 25° to 30°C by a 500 W heater. No artificial illumination was provided during the course of the experiments.

The filter materials were washed every day and the air-lift and rearing tank surface, every three or four days.

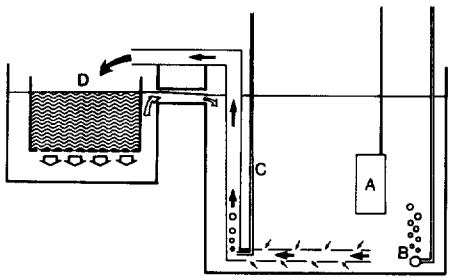

Fig. 1 *Brachionus Plicatilis*. Diagrammatic drawing of the experimental tank used for culture. Closed arrows indicate unfiltered water. Open arrows show filtered water. A, 500 W heater; B, aeration; C, air lift: D, filter container.

Alcohol Fermentation Slops (AFS)

The fermentation slops were transported from an alcohol fermentation factory to the laboratory, where they were stored at ambient temperature. It is known that there are several nutrient substances in alcohol fermentation slops, which are derived from the molasses (CJC, 1978; TERAMOTO and KAWAMORI, 1980).

Culture of Microbial Flock

Two plastic tanks, 300 and 500 *l* in capacity, were used for culturing microbial flock. Technical procedures for growing the microbial flock have been described by CJC (1978). Briefly, alcohol fermentation slops (AFS) and monobasic potassium phosphate were added to warm seawater of about 30°C at a rate of 20 g/*l* and 0.5 g/*l*, respectively. After the bacteria bloomed, 10 to 20% of the microbial flock was harvested for feeding the rotifers. Continuous flock culture was achieved by the further addition of 30 g AFS/*l* and 0.7 g monobasic

potassium phosphate per liter of filtered seawater. An airstone was placed to aerate the water at a rate of 3 *l*/min. in 300-*l* tank, and approximately 6 *l*/min. in the 500-*l* tank.

Feeding Microbial Flock to Rotifers

The optimal rate of microbial flock giving to rotifers may depends upon the volume of water, but no information on optimal bacteria density for rotifer culture on this scale (1 m^3 or 1000 *l*) had been obtained before the present experiment. After several trials we obtained a value of 25 *l*/m^3 as the optimal concentration of the microbial flock.

Counting the Number of Rotifers

Before feeding the microbial flock, 1 m*l* culture medium was sampled with pipette to determine the rotifer density. The animals were anesthetized with MS-222 or fixed in 5% formalin seawater solution. Density of rotifers with emphasis on the brooding dults were determined under a binocular dissecting microscope. For each sample the organisms were counted three times and the averaged.

Results

1. Summer Experiment, June 11 to July 24, 1980

Microbial flock was cultured at a temperature of approximately 30 ± 1°C in a 500-*l* tank. Two plastic tanks (A and B, 1000 *l* each) were employed for rotifer culture. Rotifer density at the start of the experiment was about 50 individuals/m*l*, and the animals were harvested at a rate of 10% beginning the eighth day of the experiment. The rotifer density and the number of gravid adults are given in Figure 2. Figure 3 shows environmental conditions in the rearing tanks. Rotifer density gradually changed from approximately 100 to 300 individuals/m*l*. While 10% were harvested each day.

Daily harvest of rotifers averaged 23.28 million in tank A and 20.95 million for tank B in number, respectively. Gravid adults made up about 15% of the total population in each tank. In the tank A the water temperature ranged from 24.9° to 27.3°C, averaging 25.9°C. The temperature in the tank B ranged from 25° to 28.8°C, and averaged 26.4°C. The pH and salinity varied within the range of natural conditions in the sea near the laboratory. Dissolved oxygen varied from 5 to 6.5 ppm in both tanks.

2. Winter Experiment, December 16, 1980 to January 30, 1981

The microbial flock was cultured at a temperature of about 30 ± 1°C in a 300-*l* tank, as in the proceeding summer experiment.

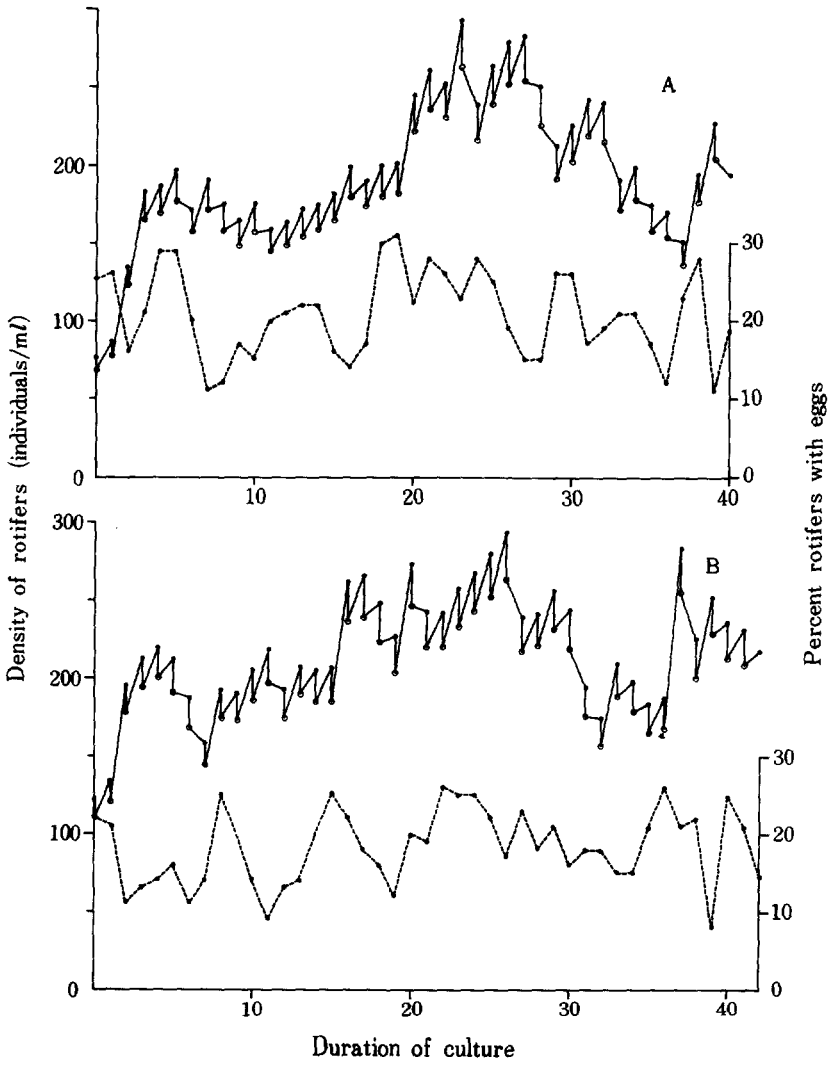

Fig. 2 *Brachionus plicatilis*. Daily changes of rotifer density (solid lines) and percent gravid adults (broken lines) in the culture tank of A and B during summer experiment. Open circles on solid lines show the density of rotifers after harvest.

In our preliminary experiment in the winter 1979, rotifers cultured at a temperature of about 26.0°C sank to the bottom of the tank when fed to larval fishes. Therefore, in this experiment water temperature was controlled in order to avoid this inconvenience.

Figure 4 shows the density of rotifers and the environmental conditions in the culture tank. The rotifers were removed at a rate of 5% every day since seventh day of the experiment. Rotifer density averaged 66 individuals/m*l* at the start of the experiment, rose to between 100 and 150 individuals/m*l* in the initial phase, and thereafter increased gradually

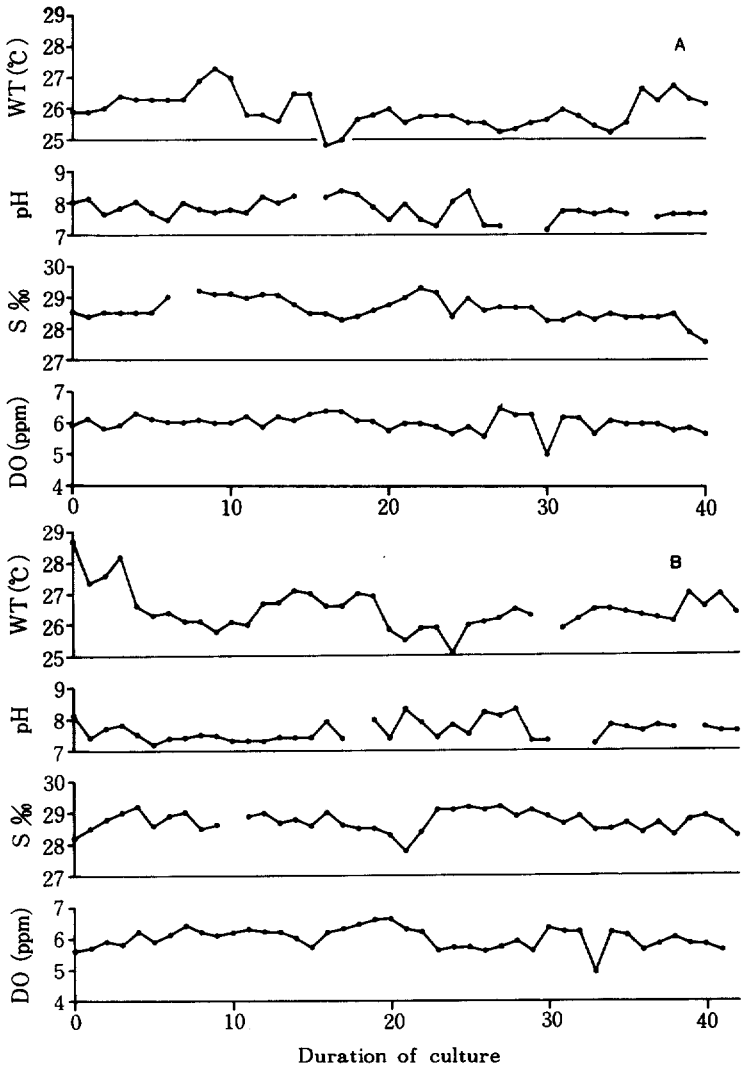

Fig. 3 *Brachionus plicatilis*. Environmental conditions of culture tanks, A and B ((capacity 1 m^3) for rotifers in the summer experiment.

to 150 individuals/m*l* or more. The number of gravid rotifers varied from 5 to 27%. On the average 6.67 million rotifers could be harvested daily.

Water temperature also showed wide variation due to malfunction of the equipment which persisted for one third of the experiment. The temperature was as low as about 17°C during the remainder of the experiment. No continuous measurements of the other physical parameter were made. There was no remarkable change in pH and dissolved oxygen as found in the summer experiment. The salinity was higher in the experiment than in the summer due to intensive evaporation.

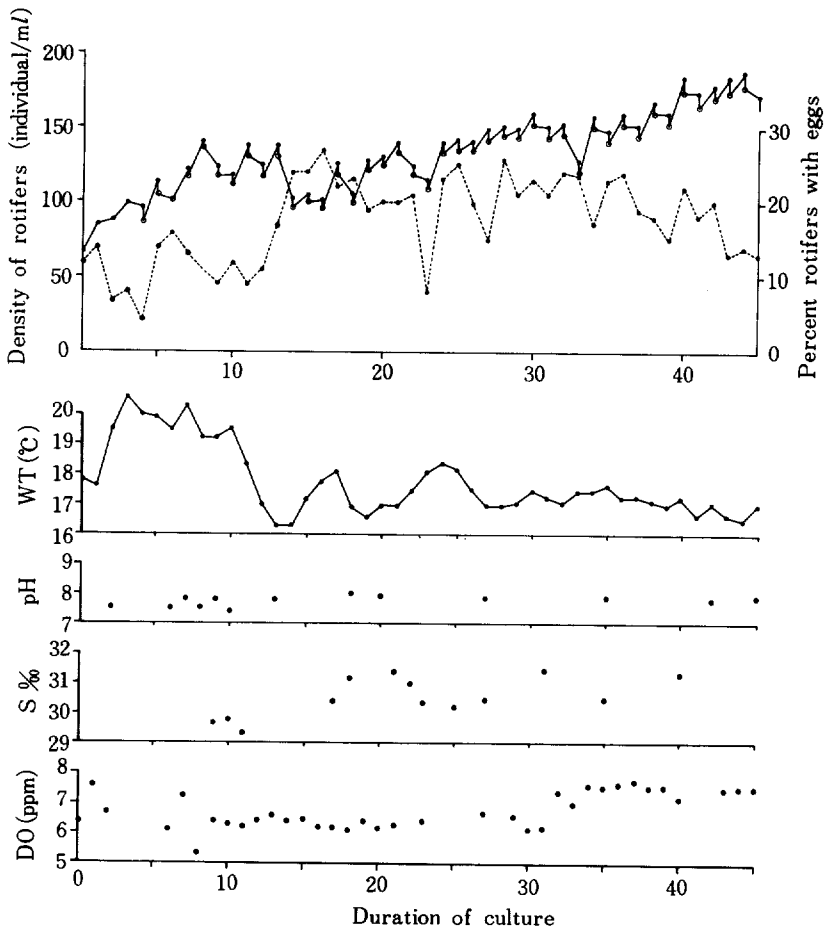

Fig. 4 *Brachionus plicatilis*. Daily changes in rotifer density and percent gravid adults (broken lines) and environmental conditions in the culture tank during the winter experiment.

3. Spring Experiment, February 1 to March 4, 1981

It is benefitable to culture the microbial flock at a lower temperature in order to save the running cost. In this experiment, the temperature was reduced from 30°C as used to be 25°C. Microbial flock culture was done in a 300-*l* tank in which the temperature varied from 24° to 27°C, and averaged 25.5°C. The density of rotifers fed on this microbial flock is shown in Figure 5, together with the environmental conditions in the rotifer culture tank. No feeding was done for the seventh day of this experiment. The average daily harvest of rotifers amounted to 12.20 million. This figure was 10% of the rotifer population. The number of gravid individuals varied from 10 to 40%. On the twelfth day of the experiment, a marked decrease in salinity resulted from the addition of the tap water to the rotifer culture tank. No serious influence on rotifer density was observed.

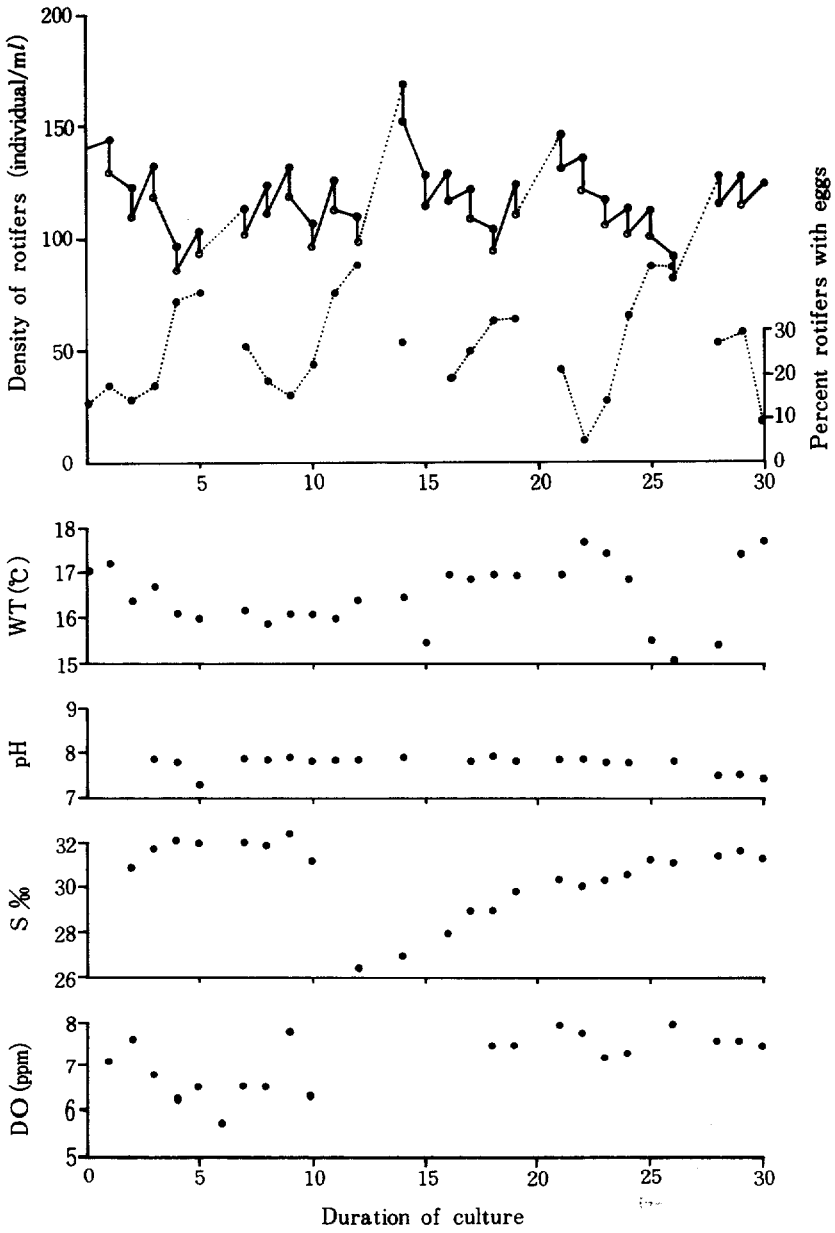

Fig. 5 *Brachionus plicatilis*. Daily changes of rotifer density, percent gravid adults (dotted lines) and environmental conditions in the spring experiment.

Discussion

When microbial flock is fed to zooplankton in a closed system a decrease in dissolved oxygen and the occurrence of sediment is usually observed. In the present experiment, heavy aeration and good water circulation were used to supply oxygen and remove suspended parti-

culate matter. CJC (1978) and ABE *et al.* (1980) described a method for culturing rotifers that used a pump to maintain the water quality in a closed culture system. Unfortunately, the pump in that system malfunctioned occasionally. In the present study, the benefits of an air-lift system (SPOTTE, 1979) were utilized. These experiments also confirmed that air-lift devices are suitable for culturing rotifers fed on microbial flock. It was possible to maintain rotifer densities of more than 150 individuals/m*l* that could be harvested daily to control the population growth.

When comparing the circulation provided by the air-lift versus that provided by pumps several benefits were confirmed:

1. Stoppages in water circulation usually resulted from the malfunction of a pump, because the pump must be used in a condition of high lift from the rearing tank to the filter tank. While, the air-lift proved to be a trouble free means of moving water and reducing running costs. The air compressor used for the air lifts worked well during the experimental periods in 1980 and 1981.

2. Gravid female rotifers were disturbed by the vortex created by the pump. Eggs were often separated from the females after passed through the pump. This phenomenon was not observed in the air-lift.

3. Water exchange between the rearing tank and the filter tank was accomplished better with the air-lift than that of the pump. The air-lift suitably controlled the water level in the rotifer tank and the filter container. In addition, the air-lift effectively supplied oxygen (STICKNEY, 1979) to the rotifer culture.

The successful use of the air lifts is expected to promote the expansion of culture techniques of rotifer by feeding microorganisms for practical purposes.

In the winter experiment, microbial flock cultured at a temperature of about 25°C was, for all practical purposes, useful for rotifer culture. This culture temperature was about 5°C lower than those described by CJC (1978) and ABE *et al.* (1980).

Rotifers reared at a temperature of 26°C sank to the bottom when placed in a larval fish rearing tank which had an ambient temperature of 9° to 12°C (winter experiment). It is assumed that the rapid transition from a high (26°C) to a low (10°C) temperature had a physiological effect upon the rotifers. The result was an inhibition of swimming activity. To prevent such rotifer sinking before fed to larval fishes, a reduction of the temperature in the rotifer culture tanks may be effective. Rotifer culture, fed on microbial flock, can be performed at ambient temeprature in summer and at 17°C in winter and spring.

For plankton culture in closed systems, good results depend largely on the maintenance of water quality during the rapidly growth phase. HIRATA (1977) assumed that micro and macro-algae played an important role in maintaining the homeostasis of artificial ecosystems.

HINO *et al.* (1980 a, b) also described the importance of phytoplankton when activated sewage used as a nutrient source. In the present study it was found likely that the control of oxygen levels is of importance when microbial flock is used as a food source. The constant suspension of the flock assists rotifer production and thus assures constant harvests of the rotifers for feeding.

This work is a part of the studies undertaken by the comprehensive research on reutilization of the alcohol fermentation slops which is sponsered by the grant of the Science and Technology Agency in 1979 and 1980. We wish to thank all member of the project team for their help, especially Dr. Yunosuke SAITO, Director of Aquaculture division organized the plan in the Nansei Regional Fisheries Research Laboratory. Our sincere thanks are due to Mr. Thomas B. MCCORMICK III for helpful comments and criticism.

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