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A novel culture system for the ultra-high-density production of the rotifer, *Brachionus rotundiformis*—a preliminary report

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Abstract

A novel culture system for continuous filtration of culture water was developed for ultra-high-density production of a marine rotifer. This ultra-high-density culture system is equipped with a membrane filtration unit (pore size: 0.4 μm) set inside a culture vessel. The culture performance of this system was tested by feeding freshwater *Chlorella* paste as feed in a 4-day batch culture.

To prevent the membrane surface from fouling, the filtration unit was cleaned every day by dipping it in a NaOCl solution. The membrane filter unit was effective in exchanging the culture water with a high level of ammonia with fresh seawater, while rotifers and *Chlorella* cells were retained in the vessel. Using this membrane filtration unit with the supply of oxygen gas, a rotifer density of more than 1.6×10^5 individuals ml^{-1} was obtained on the 4th day of culture. The removal of accumulated ammonia from the culture water enabled an increase of the productivity and of the vitality of the rotifers.

Keywords: Rotifer; Culture; High density; Membrane filter; Ammonia

1. Introduction

In recent years, the aquaculture production in the world has increased. To produce fish and crustacean fry for aquaculture, rotifers play an indispensable role as an initial food item. The culture technology of rotifers used to be dependent on the empirical skills of

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technicians and the productivity of rotifers was comparatively low (Kitajima, 1983; Yoshida, 1989). About 10 years ago, we demonstrated that the major factors of inhibiting or limiting the propagation of rotifers were mainly the lack of feed concentration, shortage of dissolved oxygen and toxicity of undissociated ammonia accumulated in the culture medium (Yoshimura, 1995; Yoshimura et al., 1992, 1994, 1995, 1996a). We developed several culture methods and devices to eliminate these inhibitory factors from the rotifer culture medium. The deficiency of feed was resolved by the use of condensed micro-algal paste, such as freshwater *Chlorella* (Yoshimura et al., 1992). The shortage of dissolved oxygen was also resolved by supplying high purity oxygen gas instead of ambient air to the culture seawater (Yoshimura, 1995; Yoshimura et al., 1994, 1995, 1996a). The toxicity of undissociated ammonia was controlled and successfully reduced by maintaining culture pH at 7.0 with automatic addition of HCl solution to promote the dissociation of NH_3 to $NH_4^+ + OH^-$ (Yoshimura et al., 1995), which is less toxic to aquatic animals. These technological advances enabled the production of high-density cultures at a rotifer density of 10^4 individuals ml^{-1}, which is much higher than the conventional "low density" culture at a density of 10^2 individuals ml^{-1} level (Yoshimura, 1995; Yoshimura et al., 1992, 1994, 1995, 1996a,b, 1997a,b,c, 1998). As a result, the scale of the culture tanks was reduced to 1/100–1/1000 of that of a conventional rotifer culture, and labour costs were reduced as well (Yoshimura et al., 1996a).

To achieve further improvement of the high-density culture method, it is necessary to suppress the inhibitory effects of ammonia and bacteria coexisting with rotifers more completely. Some of the bacteria in the culture medium may be harmful to both rotifers and larval fish. Those bacteria could produce toxic compounds and reduce dissolved oxygen in the culture water. Hence, we developed a novel culture system in which a porous membrane filtration unit is utilized, that renews the culture seawater continuously by removing the substances that are inhibitory to rotifers and which may limit blooms of coexisting bacteria. The high filtering performance of the membrane filtration unit in the culture system successfully enabled ultra-high-density culture at rotifer densities of 1.6×10^5 individuals ml^{-1}. In this paper, we preliminarily report about the effect of membrane filtration on the elimination of ammonia from culture seawater and on the increase in rotifer density.

2. Materials and methods

2.1. Membrane filter unit

The design of the membrane filtration unit used in this experiment is shown in Fig. 1. Each membrane filter cartridge (chlorinated polyethylene filter, Type H3-203, Kubota, Tokyo, Japan) had a size of 315 × 225 mm; thickness was 6 mm; dry weight 0.4 kg; the effective area for filtration 0.11 m^2; and mean pore size, 0.4 μm. The membrane filter unit consists of 10 sheets of membrane filter cartridge framed with stainless steel pipes. Each suction pipe on the upper part of the filter cartridge was connected to a metal branching cock. The slot between each membrane filter was 20 mm. According to the specifications of the filter cartridges, a flux of 120 ml min^{-1} is obtained with pure water at a temperature of 20 °C and a pressure of 5 kPa.

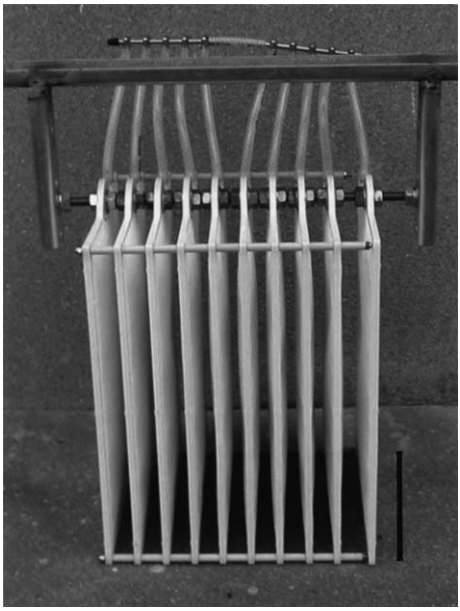

Fig. 1. Photograph of membrane filtration unit. A bar in the picture represents 10 cm.

2.2. Culture system with filtration unit

The culture system with a membrane filtration unit (CMFU) is illustrated schematically in Fig. 2. The culture vessel had a length of 385 mm, a width of 285 mm and a depth of 500 mm. The working volume in the culture vessel, except for the space of the membrane filtration unit, was 40 l. A peristaltic pump, into which rubber tubes from each membrane filter cartridge were introduced, was used to extract supernatant of the culture medium from the vessel. This peristaltic pump for extraction was set to work with an interval of 5-s suction and 10-s pause. Other peristaltic pumps were also used to feed *Chlorella* paste continuously and seawater intermittently. The level of the culture medium in the vessel was maintained using the pumps and a water-level sensor. A dispenser (carbon sparger; Furuhashi, Nagoya, Japan) was installed at the bottom of the vessel to supply oxygen gas into the culture water effectively and to prevent the membrane from fouling. The surface of the membrane filter cartridge was cleaned every day by dipping it into a NaOCl solution (active Cl^-; 1%) for 30 min with vigorous aeration to remove the clogging particles.

2.3. Batch culture with filtration

The species of marine rotifer used in this study was *Brachionus rotundiformis* (S-type rotifer, Fukuoka strain cultured in Fukuoka Mariculture, Japan, with commercial freshwater *Chlorella*) and a paste of freshwater *Chlorella* (*C. regularis*, Packed volume: 600 ml l^{-1}, Yoshimura et al., 1997a) was used as the feed. Two batch culture tests, using CMFU with a supply of oxygen gas or with aeration were carried out, and one batch test without

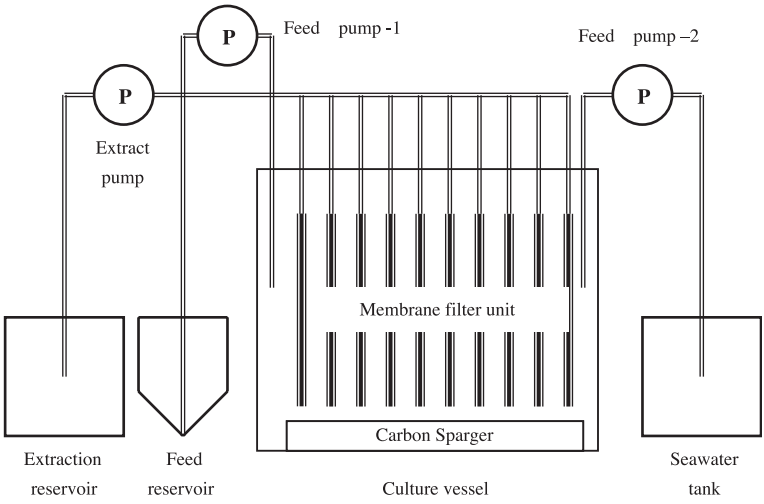

Fig. 2. Schematic diagram of the ultra-high-density culture system with membrane filtration unit (CMFU) (see Materials and methods for the operation of the system).

CMFU were carried out with oxygen supply. Fresh seawater (33–35 ppt) was filtered over an automatic siphon filter (Model 3 type, Shinko Pantec, Kobe, Japan) before use. The culture temperature was always controlled at 32 °C using a water bath. The volumetric rate of supplying oxygen gas or aeration ranged from 0.075 to 0.125 vvm (aeration volume/water volume/min, Yoshimura et al., 1996b). Oxygen gas was supplied with an electric oxygen generator (95% v/v purity; Type SA035NC, Yanmar, Tokyo, Japan). In the cultures with oxygen supply, the initial density of rotifers was set at 20,000 individuals ml^{-1} and the amount of *Chlorella* paste fed to the tank was 4 l (dry weight 560 g) on the 1st day, 6 l on the 2nd day and 8 l on the 3rd day of cultivation. In the culture with aeration, rotifers were inoculated at 880 individuals ml^{-1} and 0.4 l of *Chlorella* per 10^6 rotifers was fed every day.

2.4. Analyses

Concentrations of total ammonia (NH_4^+–N), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3+}), and dissolved oxygen (DO) were monitored every day. DO was measured with an oxygen meter (Model 58, YSI, Tokyo, Japan). Concentrations of the other substances were measured by spectrophotometer colorimetry (Model DR/4000, HACH, CO, USA) after filtering the culture water over a Millipore filter (pore size; 0.2 μm).

3. Results and discussion

Daily changes in rotifer density and concentration of DO and NH_4^+–N are shown in Fig. 3. In the cultures with oxygen supply, DO concentration was always maintained over

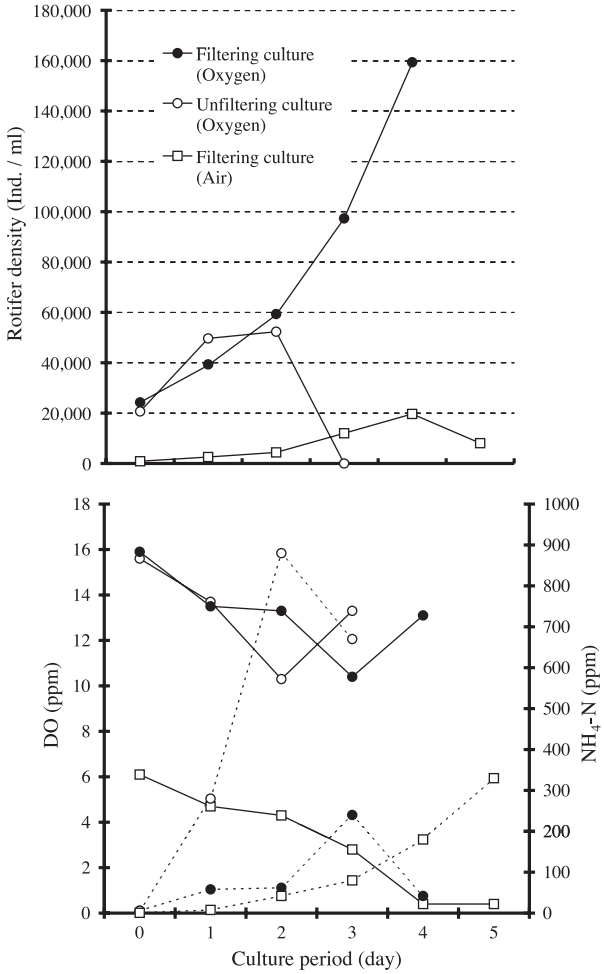

Fig. 3. Daily changes in rotifer densities (upper graph) and environmental parameters DO and ammonia (lower graph) at the different culture conditions. Symbols of culture conditions: solid circle, filtration culture with oxygen supply; open circle, batch culture with oxygen supply; open square, filtration culture with aeration. Symbols of environmental parameters: solid line, dissolved oxygen (DO); dotted line, total ammonia (NH_4^+–N).

10 ppm throughout the culture period. In the culture without CMFU and with oxygen supply, rotifer density increased to 50,000 individuals ml^{-1} on the 3rd day, but the population growth ceased on the 4th day, due to the toxicity of accumulated ammonia (880 ppm on the 3rd day). In the culture using CMFU with oxygen supply, the concentration of NH_4^+–N remained below 240 ppm and a logarithmic growth phase continued until the 5th day of cultivation. The rotifer density finally reached 159,000 individuals ml^{-1} in this trial. In the culture using CMFU with aeration, the concentration of NH_4^+–N was lower than 330 ppm and a logarithmic growth phase continued until the 5th day of cultivation as well. The rotifer density in this culture reached 22,000 individuals ml^{-1} on the 5th day.

However, rotifer growth ceased on the 6th day, mainly due to the shortage of DO (0.4 ppm on the 5th day).

It is known that a shortage of oxygen and toxicity of accumulated ammonia seriously inhibit the population growth of rotifers, and these two inhibitory factors always work simultaneously in high-density cultures (Yoshimura et al., 1994). Therefore, it was difficult to separate the effects of each inhibitory factor on rotifer growth. In this study, we succeeded in controlling the concentrations of NH_4^+–N and DO separately by using CMFU. A rapid increase of the NH_4^+–N concentration obviously caused a collapse of the rotifer culture as shown in Fig. 3. On the other hand, in the culture using CMFU with aeration, though there was a shortage of oxygen for a relatively long period in the second half of the cultivation period, most rotifers still looked healthy under microscopic observation. As reported previously (Imada, 1983; Yamasaki et al., 1987), rotifers were more tolerant to oxygen stress than to high ammonia accumulation.

In the culture using CMFU with oxygen supply, the net production of rotifers was 5.39×10^9 individuals and the yield of rotifers per liter of consumed *Chlorella* paste was estimated to be 3.0×10^8 individuals l^{-1}. The net production and yield of rotifers per volume unit of *Chlorella* paste in the culture without CMFU were almost equal to those in the culture using CMFU until the 3rd day. However, the rotifer density rapidly decreased after it had reached the maximum value on the 4th day. In the culture with aeration, the net production and yield of rotifers also decreased after rotifer density reached its peak on the 5th day. Only the culture using CMFU with oxygen enabled the harvest of healthy rotifers at maximal density with a high efficiency of feed utilization, which should be noted as an important advantage of the use of CMFU in ultra-high-density culture.

Daily changes of the concentrations of phosphate, nitrite and nitrate in the cultures with oxygen supply are shown in Fig. 4 (upper graph). Phosphate concentration changed in the ranges 6.1–99.5 and 10.8–82.0 ppm in the cultures with and without CMFU, respectively. Nitrite concentration ranges were 0.5–2.8 and 0.6–1.7 ppm (with and without CMFU, respectively.); and nitrate ranges 0.024–0.053 and 0.012–0.042 ppm (idem). Though the culture without CMFU crashed on the 3rd day, no major differences had been observed until then in the concentrations of phosphate, nitrite and nitrate between the cultures with or without CMFU. The flux of supernatant ranged from 1.36 to 1.73 ml day^{-1} cm^{-1} in the culture using CMFU, and the total amount of extracted supernatant varied between 150 and 190 l day^{-1} (375–475% of the culture vessel volume) as shown in Fig. 4 (lower graph). Therefore, the total amount of phosphate, nitrite and nitrate generated in the culture using CMFU would be several times greater than in the culture without CMFU. Phosphate accumulated in the culture medium would originate from the *Chlorella* paste, and nitrite and nitrate might be produced by nitrification of NH_4^+–N by nitrifying bacteria in seawater. To reduce the concentrations of these nutrients in the culture medium, it is very important to control the bacterial bloom that might cause the outbreaks of fish diseases (Usuki et al., 1998). As a next step, it is necessary to study in detail the effect of seawater filtering on the bacterial composition and abundance in the rotifer culture medium.

The removal of ammonia from the culture water with CMFU was very effective in enhancing the production of healthy rotifers, which was achieved by continuous renewal of the culture supernatant. Nevertheless, we still had some problems that should be solved in order to establish the ultra-high-density culture using CMFU. At present, the main

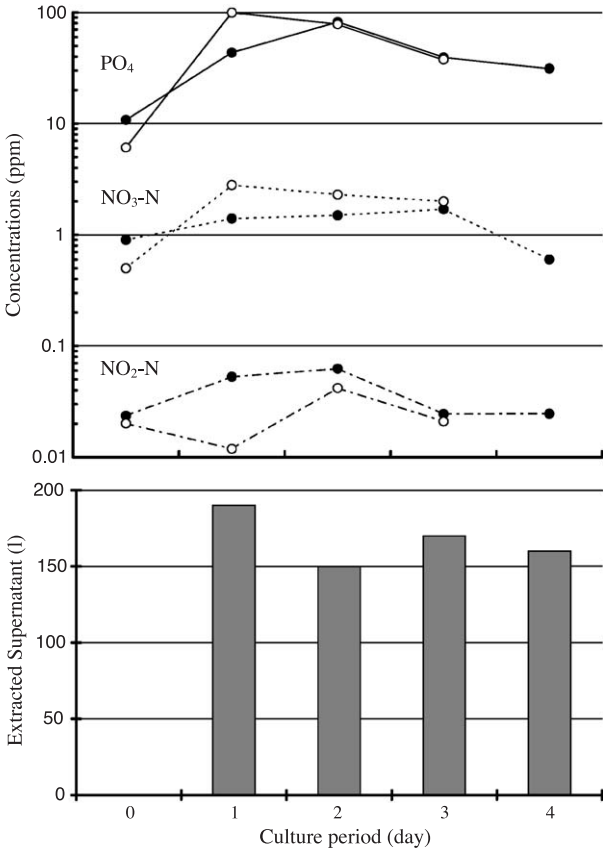

Fig. 4. Daily changes in environmental parameter phosphate, nitrite and nitrate (upper graph) at the different culture conditions and the amount of extracted supernatant (lower graph) in filtration culture with oxygen supply. Symbols of culture conditions: solid circle, filtration culture with oxygen supply; open circle, batch culture with oxygen supply.

problems are the foaming of culture seawater, the increase in culture temperature, fouling of the membrane filter, and the weakness of tubes, solenoid valves and pumps that are used for extraction of culture seawater and feeding of *Chlorella*.

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