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Effect of Human Excreta on the Growth of a Freshwater Green Alga (*Chlorella* sp.) and a Rotifer *(Brachionus calyciflorus)*

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Abstract

The effect of human excreta on the growth of *Chlorella* sp. and *Brachionus calyciflorus* was conducted in uni-specific and in two mixed cultures. The excreta were dried in sunlight, then put in 1 l wellwater and boiled for 2 h. The excreta were filtered. Five concentrations of human ex-creta (0.0, 0.5, 2.0, 3.5 and 5 $g \cdot l^{-1}$) were used.

The results of this study indicated that human excreta can promote the growth of *Chlorella* sp. The highest growth was found at 0.5 $g \cdot l^{-1}$ of human excreta. However, the density of *Chlorella* sp. decreased 2 d after *B. calyciflorus* inoculation. Human excreta could not be used directly as feed of *B. calyciflorus*, but could promote the growth of *Chlorella* sp. as food of *B. calyciflorus*. The best growth of *B. calyciflorus* at the density of 109 $ind \cdot ml^{-1}$ was at 0.5 $g \cdot l^{-1}$ of human excreta.

Introduction

One of the main problems in producing fish larvae in breeding stations of Riau Province, Indonesia, has been the limitation of appropriate food material. Often no specific feed is given to the fish larvae. An extremely large larval mortality has been observed.

To increase the survival of fish larvae, it is important to feed a natural food, especially within three weeks after hatching.

Culturing of live food such as rotifers is an important activity for some aquaculture systems. Rotifers are an excellent source of food for culturing fish larvae (Howell 1973; Kafuku and Ikenoe 1983). The mass culture of rotifers is available mostly for cultivation of the brackishwater species, *Brachionus plicatilis* Muller (Furukawa and Hidata 1973; Hirayama et al. 1979; Rothbard 1979), but those for the mass cultivation of freshwater species are limited (Galkovskaya 1963).

B. calyciflorus is a widespread planktonic species in fresh- and slightly saline water (Ruttner-Kolisko 1974). This species has been used as an alternative natural food for fish larvae such as *Cyprinus carpio*, *Leptobarbus hoevenii* and *Osphronemus gouramy*. In our previous field studies, we found that some fishponds in Riau Province, Indonesia, have freshwater *Chlorella* sp. and rotifer *B. calyciflorus*. This rotifer is usually found in ponds with a small latrine. It is expected that human excreta can be used directly as food for the rotifer, or indirectly as fertilizer to support the growth of phytoplankton which in turn serve as food for the rotifer. *Chlorella* sp., a microscopic green alga of about 3 μm in diameter, has the appropriate food size and nutrition for the rotifers. Several studies have reported that marine or freshwater *Chlorella* sp. can support the growth of rotifers (Hirayama and Ogawa 1972; James and Abu-Rezeq 1988). The present study evaluates the effect of human excreta on the growth of freshwater *Chlorella* sp. and *B. calyciflorus*.

Materials and Methods

Experiment I. Chlorella *Growth*

Human excreta were dried in sunlight two weeks before use; 100 g was put into a cooking-pot containing 1 l freshwater and boiled for 2 h. The extract was filtered with a membrane filter (1 μm pore size) to get a 600 ml solution. Water from a fishpond was sampled, filtered and boiled for 1 h and enriched with human excreta extract at the following concentrations of human excreta: 0.0, 0.5, 2.0, 3.5 and 5.0 $g \cdot l^{-1}$.

Freshwater *Chlorella* sp., obtained from the Freshwater Aquaculture Research Center, Depok, West Java, was cultured in 400 ml pond water containing different concentrations of human excreta. A 500-ml glass bottle was used with continuous aeration. All growth studies inoculated with 0.4×10^5 $cells \cdot ml^{-1}$ *Chlorella* sp. were triplicated. *Chlorella* sp. was cultured in a simple incubator illuminated by four daylight fluorescent lamps with a light intensity of 4,000 lux, and with temperatures of 23-28°C, 12 h light:12 h dark photoperiod. The density of *Chlorella* was determined daily for 13 d of the culture periods using a hemacytometer under a binocular microscope.

Experiment II. Rotifer Growth

Culture media in 400 ml containing 0.0, 0.5, 2.0, 3.5 and 5.0 $g \cdot l^{-1}$ of dried human excreta were used. *B. calyciflorus* selected from freshwater ponds in Pekanbaru, Riau Province, in actively grown culture was inoculated directly into each medium with an initial density of 1 $ind \cdot ml^{-1}$. All of the cultures were triplicated and aerated continuously. The population growth of the rotifer was studied by counting the animals daily for 4 d of the culture period under a binocular microscope.

Experiment III. Chlorella *and Rotifer Growth*

Chlorella sp. was cultured by the same method and treatments as in experiment I. After 5 d of culture of *Chlorella, B. calyciflorus* was inoculated into each treatment at a density of 1 ind·ml^{-1}. The concentrations of *Chlorella* sp. before and after rotifer inoculation, and population growth of rotifer were observed daily for 12 d.

Results and Discussion

Chlorella *sp. Growth in Experiments I and III*

Growth curves of *Chlorella* sp. at various concentrations of human excreta are illustrated in Fig. 1. The highest density of about 8 x 10^6 cells·ml^{-1} was observed on day 11 at the concentration of 0.5 g·l^{-1} human excreta. The maximum density of *Chlorella* sp. was lower at concentrations of 2.0, 3.5 and 5.0 g·l^{-1} human excreta. The lowest maximum density of *Chlorella* sp. (5.9 x 10^6 cells·ml^{-1}) was found in the medium without human excreta on day 7.

The growth of *Chlorella* sp. before and after inoculation of *B. calyciflorus* is shown in Fig. 2. Two days after *B. calyciflorus* inoculation, the density of *Chlorella* sp. still increased in the treatment with 0.5 g·l^{-1} human excreta. However, the density of *Chlorella* sp. slowly decreased in other treatments. On the third day after *B. calyciflorus* inoculation, the density of *Chlorella* sp. in all treatments quickly decreased. This may have been caused by filter and ingestion by *B. calyciflorus*.

Rotifer Growth in Experiments II and III

It appears that *B. calyciflorus* was unable to use human excreta directly as feed. At a concentration of 5.0 g·l-1 human excreta, all the rotifers died 2 d after inoculation, and at 0.5 g·l-1 4 d after inoculation (Fig. 3). However, *B.*

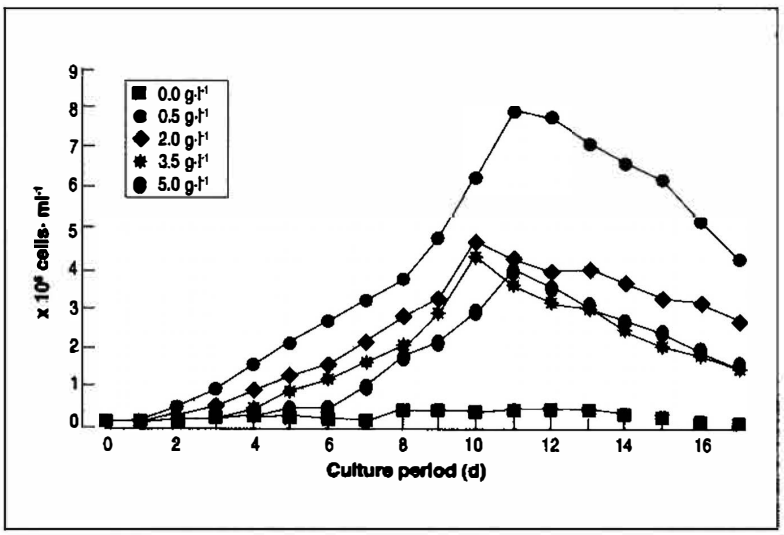

Fig. 1. Growth of *Chlorella* sp. at various concentrations of human excreta.

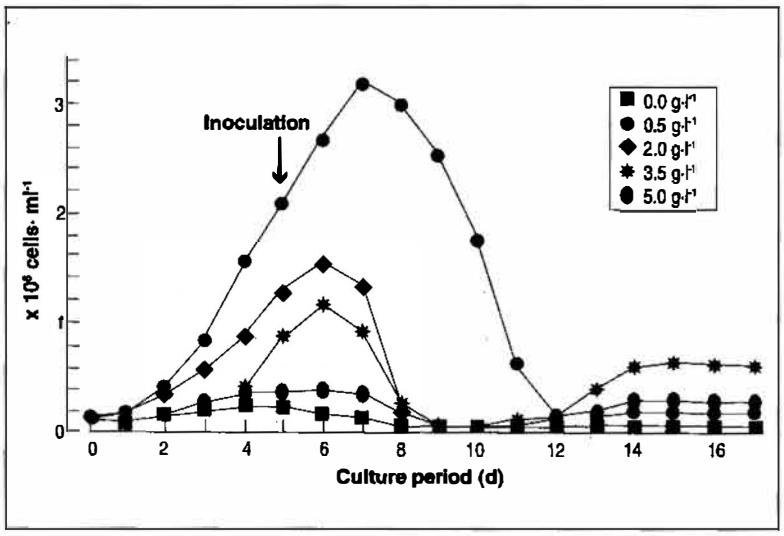

Fig. 2. Growth of *Chlorella* sp. before and after inoculation of *B. calyciflorus* at various concentrations of human excreta.

calyciflorus grew better at various concentrations of human excreta when *Chlorella* was cultured in the same medium before the rotifer was inoculated (Fig. 4). Best growth was observed at the concentration of 0.5 $g \cdot l^{-1}$ with a density of 109·ind·ml^{-1} rotifers 7 d after inoculation. However, growth of *B. calyciflorus* was lower with increased concentrations of human excreta. At a concentration of 5.0 $g \cdot l^{-1}$ human excreta, the maximum density of *B. calyciflorus* was only 26 ind·ml^{-1}. Low growth and production of *B. calyciflorus* at 2.0, 3.5 and 5.0 $g \cdot l^{-1}$ human excreta may have been caused not only by the low density of *Chlorella* sp. as shown in Fig. 4, but also by the high concentration of ammonia in the culture media of the last experiment (Tables 1 and 2).

The results indicate that human excreta can increase the growth of *Chlorella* sp. The optimal concentration of human excreta for growth was 0.5 $g \cdot l^{-1}$. However, with increased concentrations of human excreta, growth of *Chlorella* sp. decreased. Perhaps *Chlorella* sp. was affected by both the discoloration of the culture medium that resulted from propagation, and the overloading of particulate material of human excreta. Dahril (1981) reported that urban wastewater also affected the growth of *Skeletonema costatum* (Grev.) Cleve. However, at high concentrations of urban wastewater, the growth of *S. costatum* decreased, probably due to the high concentration of ammonia in this culture medium.

Human excreta cannot be used as feed for *B. calyciflorus* directly, but it can promote the growth of *Chlorella* sp. which in turn serves as food of *B. calyciflorus*. Best growth of *B. calyciflorus* was observed at 0.5 $g \cdot l^{-1}$. Increased concentrations of human excreta reduced the growth of *B. calyciflorus*. This may have been caused not only by the low density of *Chlorella* sp., but also by the high concentration of ammonia.

This is consistent with the findings of Hirayama and Ogawa (1972) that, when rotifer is fed with *Chlorella* sp. kept at a density of less than 213 x 10^4 cells·ml^{-1}, the rotifer maintained its filtering rate at the highest and almost con-

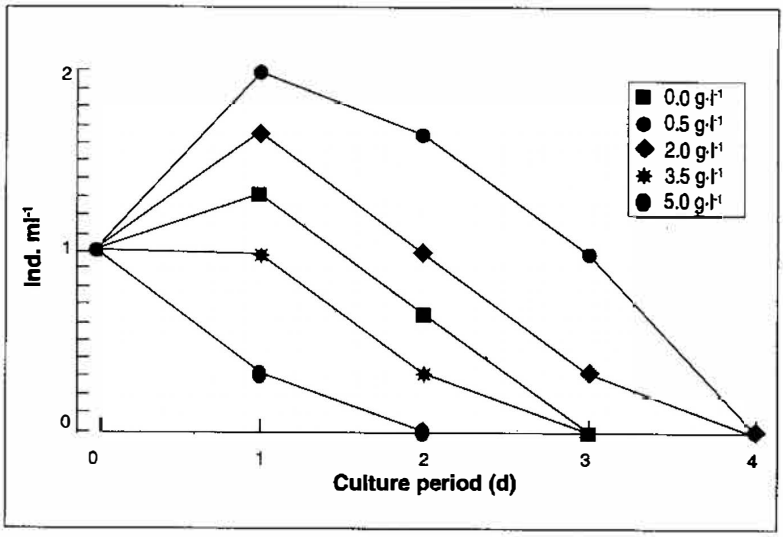

Fig. 3. Growth of *B. calyciflorus* at various concentrations of human excreta without *Chlorella* sp.

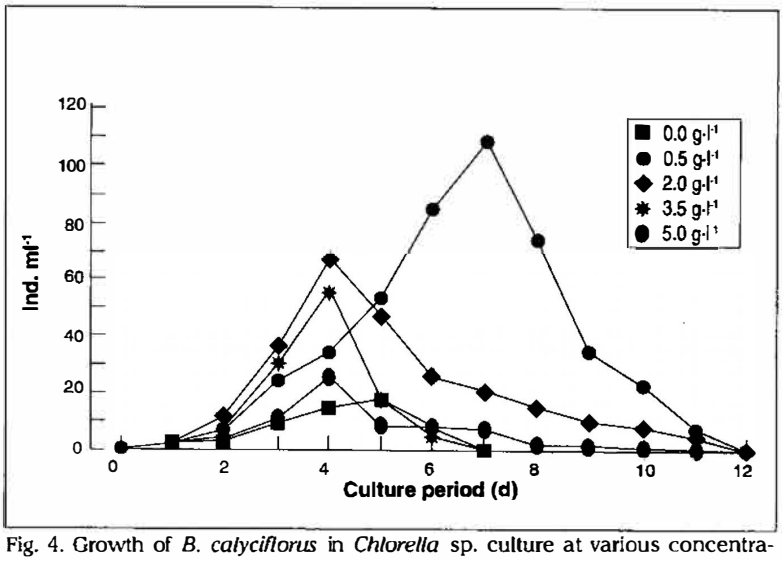

Fig. 4. Growth of *B. calyciflorus* in *Chlorella* sp. culture at various concentrations of human excreta.

Table 1. Water quality parameters in *B. calyciflorus* culture at various concentrations of human excreta without *Chlorella* sp.

Treatments ($g \cdot l^{-1}$)	Temperature (°C)	pH	NH_3-N (ppm)
0.0	31.0	7.0	0.040
0.5	30.5	7.0	2.385
2.0	30.8	7.0	4.033
3.5	31.0	7.5	4.067
5.0	30.7	7.5	5.217

Table 2. Water quality parameters in rotifer culture medium in various concentrations of human excreta with *Chlorella* sp.

Treatments ($g \cdot l^{-1}$)	Temperature (°C)	pH	NH_3-N (ppm)
0.0	32.0	7.5	0.24
0.5	32.0	8.0	2.13
2.0	32.3	7.5	2.57
3.5	32.5	7.5	3.17
5.0	32.0	7.5	3.43

stant level of about 7-10 x 10^5 cells·min^{-1} (empty condition) and 5-7 x 10^5 cells·min^{-1} (satiated condition) while the ingestion rate continued to increase. They also reported that the ingestion rate of rotifer was 200 cells·min^{-1}·ind^{-1} under empty condition.

Schluter and Groeneweg (1985) reported that un-ionized ammonia affected the growth of *Brachionus rubens*. In the range of 3-5 mg·l^{-1} of NH_3-N, the reproduction rate decreased, though no mortality was observed. At a concentration over 5.0 mg·l^{-1} NH_3-N, the rotifers died twice during the culture period. According to Fu and Hirayama (1986) un-ionized ammonia accumulated in the culture medium could be one of the causes for the sudden decrease in rotifer growth.

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References

Dahril, T. 1981. Effect of urban waste water and Urakami river water on the growth of red tide plankton, *Skeletonema costatum*. Faculty of Fisheries, Nagasaki University, Nagasaki, Japan. 36 pp. M.Sc. thesis.

Fu, J.P. and K. Hirayama. 1986. The effect of un-ionized ammonia on the population growth of the rotifer in mass culture. Bulletin. Japanese Society of Scientific Fisheries 52:1509-1513.

Furukawa K. and K. Hidaka. 1973. Technical problems encountered in the mass culture of the rotifers using marine yeast as food organisms. Bulletin of National Research Institute of Aquaculture 2:1-10.

Galkovskaya, F.A. 1963. On the utilization of food for growth and conditions of highest yield in rotatoria, *Brachionus calyciflorus*, Pallas. Zoologicheskij Zhurnal 42:505-512.

Hirayama, K. and S. Ogawa. 1972. Fundamental studies on physiology of rotifer for its mass culture-I. Filter feeding of rotifer. Bulletin. Japanese Society of Scientific Fisheries 38:1207-1214.

Hirayama, K., K. Takagi and H. Kimura. 1979. Nutritional effect of eight species of marine phytoplankton on population growth of the rotifer, *Brachionus plicatilis*. Bulletin. Japanese Society of Scientific Fisheries 45:11-16.

Howell, B.R. 1973. Marine fish culture in Britain. VIII. A marine rotifer, *Brachionus plicatilis* Muller, and the larvae of the mussel, *Mytillus edulis* L. as foods for larval flatfish. Journal du Conseil. Conseil International pour l'Exploration de la Mer 35:1-6.

James, C.M. and T.R. Abu-Rezeq. 1988. Effect of different cell densities of *Chlorella capsulata* and a marine *Chlorella* sp. for feeding the rotifer *Brachionus plicatilis*. Aquaculture 69:43-56.

Kafuku, T. and H. Ikenoue. 1983. Modern methods of aquaculture in Japan. Developments in aquaculture and fisheries of science, Volume 11. Elsevier, Tokyo. 216 pp.

Ruttner-Kolisko, A. 1974. Plankton rotifers. Biology and taxonomi. Binnengewasser. Supplement 26:1-146.

Rothbard, S. 1979. Practical mass-culture of the rotifer *Brachionus plicatilis* Muller. EIFAC Technical Paper No. 35, Supplement 1:194-202.

Schluter, M. and J. Groeneweg. 1985. The inhibition by ammonia of population growth of the rotifer, *Brachionus rubens* in continuous culture. Aquaculture 46:219-220.

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