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 excreta (0.0, 0.5, 2.0, 3.5 and 5 g l\textsuperscript{-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 l\textsuperscript{-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 ml\textsuperscript{-1} was at 0.5 g l\textsuperscript{-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 goramy. 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 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 x 10⁵ cells 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 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·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 10⁶ cells·ml⁻¹ was observed on day 11 at the concentration of 0.5 g⁻¹ human excreta. The maximum density of Chlorella sp. was lower at concentrations of 2.0, 3.5 and 5.0 g⁻¹ human excreta. The lowest maximum density of Chlorella sp. (5.9 x 10⁶ 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⁻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⁻¹ human excreta, all the rotifers died 2 d after inoculation, and at 0.5 g⁻¹ 4 d after inoculation (Fig. 3). However, B.
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·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·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·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·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. Dahrl (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·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 10⁴ cells·ml⁻¹, the rotifer maintained its filtering rate at the highest and almost con-
Table 1. Water quality parameters in *B. calyciflorus* culture at various concentrations of human excreta without *Chlorella* sp.

<table>
<thead>
<tr>
<th>Treatments (g·l⁻¹)</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>NH₃-N (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>31.0</td>
<td>7.0</td>
<td>0.040</td>
</tr>
<tr>
<td>0.5</td>
<td>30.5</td>
<td>7.0</td>
<td>2.385</td>
</tr>
<tr>
<td>2.0</td>
<td>30.8</td>
<td>7.0</td>
<td>4.033</td>
</tr>
<tr>
<td>3.5</td>
<td>31.0</td>
<td>7.5</td>
<td>4.067</td>
</tr>
<tr>
<td>5.0</td>
<td>30.7</td>
<td>7.5</td>
<td>5.217</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatments (g·l⁻¹)</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>NH₃-N (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>32.0</td>
<td>7.5</td>
<td>0.24</td>
</tr>
<tr>
<td>0.5</td>
<td>32.0</td>
<td>8.0</td>
<td>2.13</td>
</tr>
<tr>
<td>2.0</td>
<td>32.3</td>
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<td>2.57</td>
</tr>
<tr>
<td>3.5</td>
<td>32.5</td>
<td>7.5</td>
<td>3.17</td>
</tr>
<tr>
<td>5.0</td>
<td>32.0</td>
<td>7.5</td>
<td>3.43</td>
</tr>
</tbody>
</table>
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.

Schulte and Groeneweg (1985) reported that un-ionized ammonia affected the growth of Brachionus rubens. In the range of 3-5 mg·l^{-1} of NH₃-N, the reproduction rate decreased, though no mortality was observed. At a concentration over 5.0 mg·l^{-1} NH₃-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


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