Culture of *Brachionus plicatilis* (L-type) using anaerobically treated organic wastes

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Abstract

Considering the high cost of production of Chlorella sp. as a source of food for live feed Branchionus plicatilis (L-type), an alternative cheap and waste organic substances such as cow dung (A), cabbage (B), poultry manure (C) and a mixture of the above three (D) at 1:1:1 ratio (anaerobically treated) have been tested as a food source to grow B. plicatilis at different salinity (10, 20, 30 and 40 ppt) conditions. The population density of B. plicatilis was determined for every two days up to ten days. Among the tested dietary sources, B. plicatilis fed with mixed diet (D) showed a maximum population density (183±12 no/ml) on tenth day at 10 ppt salinity. But the other dietary sources exhibited relatively low density (P< 0.05). B. plicatilis had highest specific growth rate (36%) when fed with mixed diet (D) at 10 ppt salinity than other tested dietary sources and salinities (P< 0.05). The result is compared with the result obtained with other food organisms like algae, yeast and inert food used for the culture of B. plicatilis.

Keywords: *Brachionus plicatilis*; cowdung; cabbage; poultry manure; organic wastes

Introduction

The prime requirement of the aquaculture practice is the production of appropriate nutritionally balanced, non polluting, economically viable and acceptable feed in order to release optimum growth and survival of the cultured stock [1]. Live feed organisms are preferred by most of the cultured larvae compared to artificial feed: because in nature, they select one or the other live food organism as their principal food [2,3,4]. Live food organisms like *Tubifex tubifex* [5,6,7]. Daphnia [8] and *Artemia* [9,10,11,12] have been cultured using organic waste like cow dung, poultry manure, cabbage wastes, coconut mesocarp waste and pig dung in order to reduce the production cost as well as to utilize the waste in useful way for food production. The above mentioned live food organisms are the suitable candidate for raising them on the waste organic materials. But because of their relatively larger size, they are less/not preferred by newly born fish/crustacean larvae. In the mean time, the rotifer *B. plicatilis* and *Artemia* nauplii have been widely used as the food for newly born larvae due to its small size, nutrient condition as well as its tolerance to wide range of salinity. One of the main problems encountered with large scale production of the rotifer is the large scale requirement of unicellular algae like *Chlorella* sp. which requires substantial space, nutrient and time to produce sufficient quantities to feed the rotifer [13]. Hence, an alternative food like yeast has been used as a sole food [14,15] or in combination with several other nutrients such as algae [16,17] as well as inert food [18,19]. Marian *et al*. [11] found that anaerobically treated (12 to 20 days retention time) cow dung, poultry manure and cabbage (200-300g/l) supported growth of life food organisms. Hence I made a preliminary attempt to use these organic wastes as food source for the culture of the rotifer *B. plicatilis.*

Materials and Methods

A factorial design of experiment was undertaken for raising the rotifer *B. plicatilis* (L-type) at four salinities (10, 20, 30 and 40 ppt) and four food sources, *viz* cow dung (A), cabbage (B), poultry manure (C) and mixture of the these three in 1:1:1 ratio (D). For this, 12-20 days anaerobically fermented cow dung, cabbage and poultry manure supplemented with vitamins B12 (10mg/l) (loading rate: 200-300g/l) were used as they supported more bacterial growth, more nutrient and less toxic substances [11].

A stock *B. plicatilis* (L-type) with average lorica length of 220 ± 18 µm was selected for the present study. *B. plicatilis* was cultured in one liter capacity container with 0.5 *l* water. In all trials, triplicates were kept in each treatment and mild aeration was given to keep the oxygen concentration above 4mg/*l*. Initially, five *B.*

plicatilis /ml were inoculated in to the culture medium. Water volume was maintained between 500-700 ml. The different salinity levels were prepared and maintained either by dilution of filtered sea water with filtered fresh water or by addition of solar salt dissolved in sea water. The feeding of respective fermented organic matter and their feeding regime adopted in the experiment is given in table 1. Population of *B. plicatilis* was recorded daily by taking triplicate samples (1 ml each) from the culture media after stirring gently. The whole experiment was conducted for a period of ten days. Specific growth rate (SGR - %) of *B. plicatilis* was calculated using the formula

$$SGR\ (\%) = \frac{In\ Nt - In\ No}{t}$$

Where, No = Initial number of rotifer
Nt = Number of rotifer after t days
t = Number of days (10 days)

Doubling time was calculated by dividing log e^2 by Specific growth rate.

Table 1. Feeding regime adopted in the culture of *B. plicatilis* using the anaerobically fermented organic wastes.

Culture period (Days)	Food Concentration (g/l)	Flow rate (ml/mt)	Duration of feeding (hours of the day)			
1-2	3	5	08-10	12-14	16-18	20-22
3-4	3	10	08-10	12-14	16-18	20-22
5-7	6	15	08-10	14-18	20-24	-
8-10	6	20	08-10	14-18	20-24	-

Salinity was measured daily by using a refractometer and oxygen by Winkler's method. The experiments were conducted at 26±2^{0}C and the photoperiod was 16L: 8D. Salinity and water volume were maintained at the desired level one hour after feeding.

Statistical analysis

All experiments were performed in triplicate. The result obtained in the present study were analysed through Two way ANOVA test following Zar [20].

Results

Among all the fermented dietary sources, *B. plicatilis* cultured using the fermented cabbages (B) as food grew slowly in all the tested salinities and the population density reached to a maximum of 104±8 no/ml at the end of the experiment (10 th day) in 10 ppt salinity. Whereas *B. plicatilis* fed on fermented mixture (D) dietary source exhibited a maximum density of 183±12 no/ml on 10th day at 10 ppt salinity. The other dietary source like fermented cow dung (A) and fermented poultry manure (C) showed the population density of 146±7.0 and 166±14.0 no/ml respectively in the same salinity at the end of the experiment. In the entire experimental dietary source, the growth of *B. plicatilis* was slow up to fourth day, but from sixth day onwards, the multiplication was faster.

In all cases, the maximum density was observed at 10 ppt salinity level and the density become decreased with increasing salinity i.e. it was 96±8 to 26±2; 126±6 to 39±3; 161±11 to 39±3 and 143±10 to 48±4 no/ml. in the test salinities from 20- 40 ppt respectively in the test group of cabbage, cow dung, poultry manure and mixed group respectively (Fig. 1). A two way ANOVA test made on the effect of dietary source and salinity on population density of *B. plicatilis* revealed that the variation between them was statistically more significant ($F(2) = 22.3$ to 240.0; $P<0.0001$).

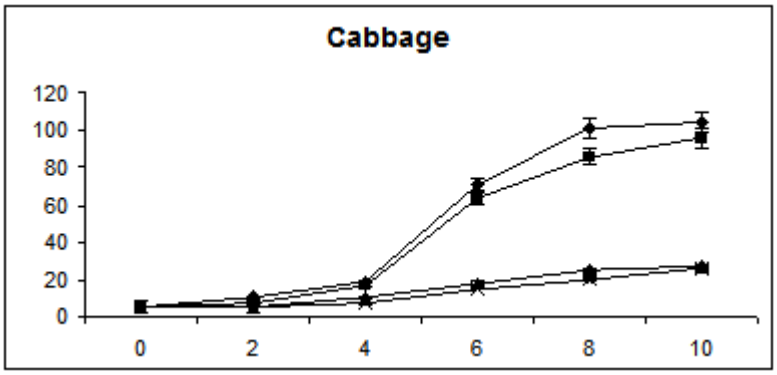
Likewise, among all the tested groups, the specific growth rate (%) of *B. plicatilis* was maximum (36%) in the mixed group (D) in the series containing 10 ppt salinity, whereas it was decreased in other salinities (33.5 to 22.6% in 20 to 40 ppt salinities). Similarly the specific growth rate of cabbage (B) was 30.3% at 10 ppt salinity, whereas it was reduced from 29.5 to 16.4% in the tested salinities of 20 to 40 ppt respectively. In

cowdung (A) and poultry manure (C) also the specific growth rate was more (33.7 and 35.0%) at 10 ppt. Salinity, but it was decreased in the order of 32.2 to 20.5% and 34.7 to 20.5% in the tested salinities of 20 to 40 ppt respectively (Table 2). The Two way

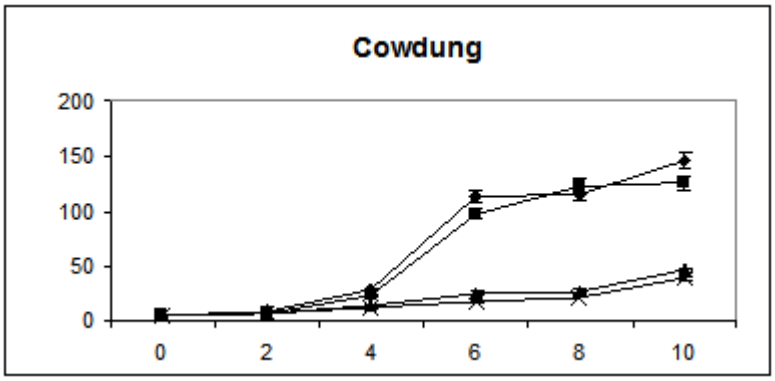
ANOVA test carried out for the specific growth rate of *B. plicatilis* revealed that the variation between the tested salinities and dietary sources were statistically more significant (F (2) 14.1371 to 89.7546; P< 0.001).

Table 2. Specific growth rate (%) of *B. plicatilis* reared at different salinity concentrations using the anaerobically fermented organic wastes.

Fermented Organic wastes	SGR (%) a t different Salinity concentrations			
	10 ppt	**20 ppt**	**30 ppt**	**40 ppt**
Cow dung (A)	33.7	32.2	22.4	20.5
Cabbage (B)	30. 3	29.5	16. 8	16. 4
Poultry manure (C)	35. 0	34. 7	21. 2	20. 5
Mixture (D)	36. 0	33. 5	27. 7	22. 6



Density (no/ml)



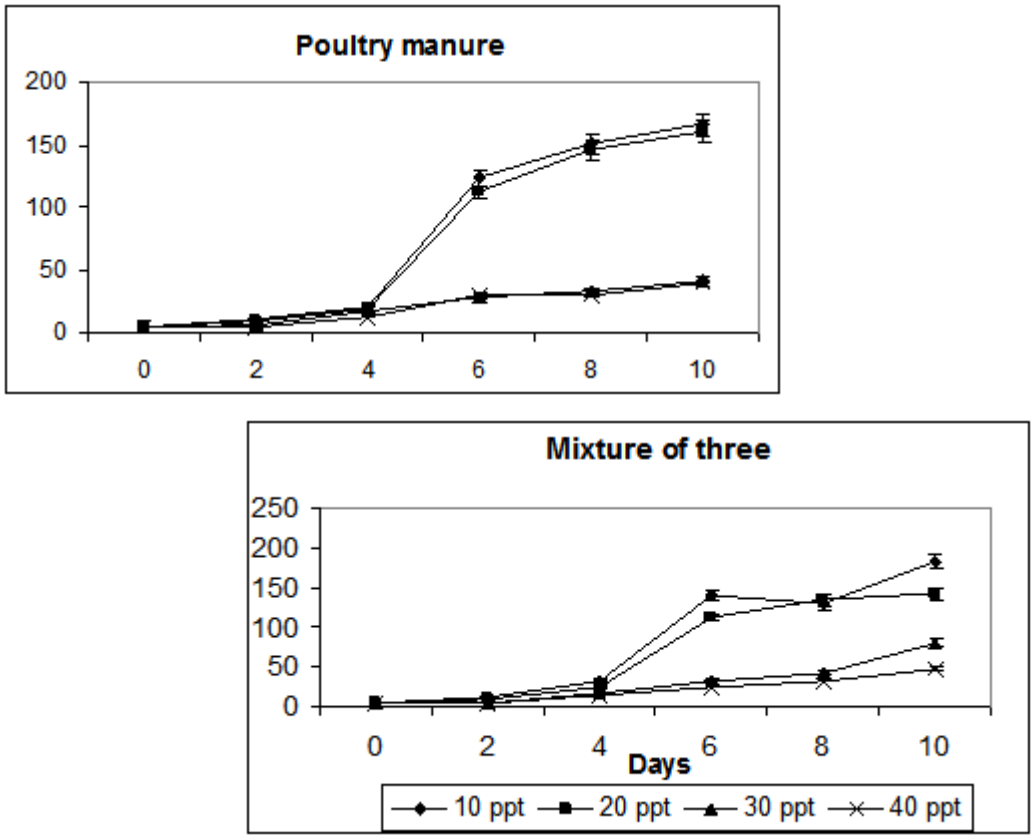


Figure 1. Density (no/ml) of *B. plicatilis* cultured using fermented organic wastes as food at different salinity concentrations (10-40 ppt).

Discussion

The filter feeding habits of *B. plicatilis* led the possibilities of raising the organism on the variety of food types such as algae, yeast, bacteria and inert food. Algae of several species such as *Chlorella* sp., *Synechococcus* sp., *Monochrysis lutheri*, *Dunaliella teriolecta*, *Cyclotella cryptica*, *Nitzschia clostelium*, *Tetraselmis terethe* [16,21] have been used as food for *B. plicatilis*. Others have used the green algae with blue green algae. For instance, Snell *et al*. [22] used the blue green algae, *Schzothrix* with *Chlorella* to increase of production.

Normally, to maintain rotifer culture, one has to subsequently maintain 5-10 times volume of algal cultures [23]. In order to alleviate/ reduce the problem of using expensive algae- culture system, an alternative, inexpensive, commercially available food like yeast and other inert foods have been tried by scientists. Hirata and Mory [23] first used the yeast *Saccharomyces cereviciae* to raise the rotifer, *B. plicatilis*. Later on, several species of marine yeast have been used [15,24]. Though Mastuda *et al*. [24] could raise *B. plicatilis* on isolated specific marine yeast more than 2-3 times higher than those achieved with *Chlorella*, the relatively expensive production cost due to specific techniques led a limited applicability as foods. The bacteria *Pseudomonas* (P-1 and P-7) have been found to improve the growth of *B. plicatilis* by Ushiro *et al*. [25] and Yamasaki and Hirata [26].

The inexpensive supplies of bacteria produced in waste water treatment plants or from alcohol fermentation industries have been used successfully by Hino *et al*. [27] and Fukuhara *et al*. [14] for *B. plicatilis*. Groeneweg and Schluter [28] raised *B. plicatilis* in the effluent of the algal ponds used for the treatment of piggery waste. Another way adopted to reduce the cost was using inert food, eg. Spray dried *Chlorella* or *Platymonas succica* [29]; commercial *Spirulina, Chlorella* and methanol grown yeast [18]. Utilization of microencapsulated diet, some time has the limitation of constant and continuous support of growth to the rotifers [19]; however it opens the chance for permitting the enrichment of rotifers with micronutrients required by the fish and prawn larvae. Comparison of the growth characteristics of *B. plicatilis* reared by giving different foods like algae, yeast, inert food with that of the waste organics used in the present study revealed that the growth rate

and doubling time were considerably equal to the values reported by James *et al*. [13] for yeast food, and for *Chlorella* by Matunog [30] at > 20ppt. The high growth rate obtained in the lowest salinity may be the reason for the comparable growth rate obtained with other works; however, it is important to note that James *et al*. [13] obtained the growth rate at 30 ppt (Table 3). If they have raised the rotifer in low salinity, they might have raised the rotifer ay faster rate. Hence, further research is required before stepping into a large scale production of *B. plicatilis* using the organic wastes by changing the feeding regime, supplementation with other algae /yeast in order to obtain a very good growth rate and continuous production.

Table 3. Comparison of growth characteristics of *B. plicatilis* fed with different kinds of food

Kinds of food	Feeding regime frequency	Food quantity	Temp (^{0} C)	Salinity (ppt)	Culture periods (days)	SGR (%)	Doubling Time (Days)	Source
Algae								
Tetraselmis	Once /	5x10	20-25	35	10	24-	2.9-1.4	Okauchi and
Tetrathele	day	cells/ml	20-25	35	10	49	4.3-1.5	Fukusho (1985)
Chlorella	Once /	15x10	25-31	35	6-7	16-	3.4	Matunog (1977)
sp	day	cells/ml	--	10	--	47	1.2	
,,	--	25x10	--	15	--	20.0	1.3	
,,	--	cells/ml	--	20	--	59.0	2.0	
,,	--	--	--	25	--	52.0	2.3	
,,	--	--	--	30	--	37.0	5.0	
,,	--	--	--	40	--	28.0	5.3	
,,	--	--				16.0		
	--	--				13.0		
		--						
Yeast								
Marine yeast	20 h/day	38±8 mg/l	25-27	30	10	46.0	1.7	James *et al.,* (1986)
Baker's yeast	20 h/day	23±1 mg/l	25-27	30	10	31.0	2.3	
Inert Food								Gatesoupe and
Artificial diet & algae (4:1)	Continuous	-	22-23	18 35	15 27	37.0 --	1.9 --	Luquet (1981)
Organic wastes	3-4	3-6 g/l	26±2	10	10	33.7	2.1	
	times/day			20	10	32.2	2.3	Present study
Cow dung				30	10	22.4	3.3	
(A)				40	10	20.5	3.8	
Cabbage	3-4	3-6 g/l	26±2	10	10	30.3	2.3	
(B)	times/day			20	10	29.5	2.4	Present study
				30	10	16.8	4.0	
				40	10	16.4	4.3	
Poultry	3-4	3-6 g/l	26±2	10	10	35.0	2.0	
manure	times/day			20	10	34.7	2.1	Present study
(C)				30	10	21.2	2.6	
				40	10	20.5	3.3	
Mixture	3-4	3-6 g/l	26±2	10	10	36.0		
diet	times/day			20	10	33.5	2.1	Present study

(D)	30	10	27.7	2.1
	40	10	22.6	3.6
				3.6

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