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# Utilization of Anaerobically Digested Cattle Dung Slurry for the Culture of Zooplankton, *Daphnia similis* Claus (Crustacea: Cladocera)

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# Abstract

The nutrient utilization pattern and algal productivity at different concentrations of anaerobically digested cattle dung slurry (0.1, 0.3, 0.6 and 1.0%) were evaluated in outdoor miniponds at a retention time of 6 days. In algal stabilization ponds, 40-56% of total Kjeldahl nitrogen, 85-98% of  $NH_3$ -N and 60-90% of phosphorus were utilized. Types and number of algal forms and Net Primary Productivity (NPP) increased with increasing concentrations (0.1-1.0%) of digested biogas plant slurry. Treatment was for 50 days. Treated water was partially harvested everyday and used for Daphnia similis culture.

Maximum growth increment (0.6 mm) of *D. similis* occurred between first and fourth instar (within 5 days) in all the concentrations tested. Based on the mean number of eggs produced

animal  $^{1}$  day  $^{1}$ , it was observed that concentrations of 0.1-0.3% biogas slurry are suitable for longer maintenance of algae and *D. similis* culture.

# Introduction

As a waste treatment and fuel production system, biogas technology is becoming increasingly popular both in developing and developed nations. Biomethanization provides only a partial answer to the problem of waste treatment and the result of this process is an effluent still rich in inorganic nutrients ( $NH_4^+$ ,  $PO_4^{3-}$ ) with a high Chemical Oxygen Demand (COD). Therefore, it is necessary to further treat the effluent (de la Noue and Basseres 1990). Asian countries have a shortage of fertilizer which is a constraint in feed production, yet they do not fully recycle organic wastes.

In India, a large amount of cattle waste remains unutilized. According to estimates by the National Council of Applied Economic Research, about 1,335

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million tonnes of wet dung are available annually in India (Garg et al. 1971). There are about 1,616,000 biogas plants (Qasim 1993). Interest, however has been directed towards utilizing the solid portion of effluent additive to livestock feed (Hashimoto et al. 1980) and as a crop fertilizer (Dahiya and Vasudevan 1986). Little attention is paid to the proper utilization of anaerobically digested cattle dung slurry, especially for aquaculture.

In this connection, culture of *Daphnia* utilizing organic waste attracted the attention of many aquaculturists over several decades (Banta 1921; Murphy 1970; Venkataraman et al. 1986) due to its importance as a live fish feed. Various types of feed like cow dung, poultry manure, green algae and rice bran were used as feed in *Daphnia* culture.

With this background, the present study investigated the influence of different concentrations of anaerobically digested cattle dung slurry on growth and reproduction of a commonly occurring dominant species of zooplankton of south India, namely *Daphnia similis* Claus (Crustacea: Cladocera) under laboratory conditions after treating the slurry in outdoor miniponds.

# **Materials and Methods**

# **Biogas Plant Effluent**

Biogas plant effluent collected from a family size biogas plant of 4 m<sup>3</sup> capacity, Khadi and Village Industries Commission (KVIC) model, situated inside the premises of the School of Energy, Environment and Natural Resources (Lat:  $9^{\circ}53^{\circ}N$ ; Long:  $78^{\circ}E$ ) was used. Cattle dung was mixed with tap water in the ratio of 1:1 (W/V) and fed semi-continuously to the biogas plant running at a retention time of 30-35 days.

# Algal - Zooplankton Culture

# LABORATORY MINI STABILIZATION PONDS

Laboratory mini stabilization ponds of  $41 \times 30 \times 12$  cm plastic troughs were used as high rate algal stabilization ponds. These were kept outside the laboratory, exposed to sunlight from 0830 to 1730 hours. A soil bed of 1 cm thickness was made in each pond. Different dilutions of digested biogas slurry were made (0.1, 0.3, 0.6 and 1.0%) with tap water by W/V, each in duplicate. Twelve liters of culture volume were maintained in each pond. Everyday, 2 l of culture medium was removed from the pond. From the tenth day onwards this water was utilized for *D. similis* production. At 2-day intervals, 4 l fresh medium of the respective concentrations were added to the stabilization ponds. Retention time was 6 days. At night, the ponds were kept indoors to avoid the influence of external sources.

### Daphnia similis Production

Growth rate, life span and production of young of *D. similis* were studied as described by Venkataraman (1983). Harvested water from slurry-treated algal pond water (0.1, 0.3, 0.6 and 1.0%) was utilized for *Daphnia* culture.

Gravid females of *D. similis* were collected from a seasonal pond near the University campus and maintained in laboratory conditions. Neonates were separated from mother *Daphnia* daily and used in the present study. Culturing was carried out in 100-ml containers, each with 14 replicates. The culture medium was changed daily using the biogas slurry treatment pond water after filtering through a nylon net. Length of the *Daphnia* was measured daily with an ocular micrometer.

Analyses of Total Kjeldahl Nitrogen (TKN), ammonia nitrogen ( $NH_3$ -N), available phosphorus ( $PO_4$ -P), dissolved oxygen (mg·1<sup>-1</sup>) (membrane electrode method), primary productivity (g C·m<sup>-3</sup>·hour<sup>-1</sup>) (light and dark bottle method) and phytoplankton (cells ml<sup>-1</sup>) collection and identification were done according to standard methods (APHA 1985). Dissolved oxygen values were converted to g·C·m<sup>-3</sup>·hour<sup>-1</sup> using the formula mg 0<sub>2</sub>·1<sup>-1</sup>·hour<sup>-1</sup> x 0.375. Alkalinity (mg CaC0<sub>3</sub>·1<sup>-1</sup>) was estimated by acid titration method (Ripely et al. 1986). Sunlight intensity (W·m<sup>-2</sup>) was measured by using a "Suryamapi Sm20" meter (Central Electronics Ltd., India). Statistical analyses of the data using linear regression were carried out utilizing the program COSTAT, version 2.00 (CoHort Software, Borland Inc., Italy).

### Results

## Solar Insolation, Temperature and pH

Solar insolation was 400-580 W·m<sup>-2</sup> in the morning, 600-920 W·m<sup>-2</sup> at midday and 500-800 W·m<sup>-2</sup> in the afternoon during the 50-day culture period. Atmospheric temperature was 28-36°C during the day and 25-28°C during the night. During the day, pond water temperature was 4-5°C higher than the atmospheric temperature, and 2-3°C less than the atmospheric temperature during the night. There was no marked variation of temperature and pH (7.5-9.0) among different concentrations of slurry treatment ponds. Twenty-four-hour observation revealed that pH reached the maximum of 9.28, 9.35, 9.57 and 9.45, respectively, in 0.1, 0.3, 0.6 and 1.0%, at 1800 hours.

## Alkalinity

Alkalinity increased with increasing concentrations of slurry and time of the treatment period. At 0.1, 0.3, and 0.6 and 1.0% concentrations, alkalinity was 345-370, 350-410 and 350-430 mg·l<sup>-1</sup>, respectively.

# Nutrient Pattern, Algal Productivity and Dissolved Oxygen Level in Digested Biogas Slurry Stabilization Ponds

The data are given in Table 1. Daily mean values of 0.27, 0.58, 0.75 and 1.01 mg·l<sup>-1</sup> of TKN were supplied through biodigested slurry to the 0.1, 0.3, 0.6 and 1.0% concentration ponds, respectively. Of this, 40-50, 36-40, 40-44 and 50-56% of TKN was utilized in 0.1, 0.3, 0.6 and 1.0% concentrations, respectively. After 30 days, TKN showed accumulation of 14.4 mg·l<sup>-1</sup> in 1.0% concentration and after 40 days in 0.6% concentration, it was only 8.4 mg·l<sup>-1</sup>.

Daily mean NH<sub>3</sub>-N amounts of 0.16, 0.36, 0.46 and 0.65 mg·l<sup>-1</sup> were supplied to the ponds of 0.1, 0.3, 0.6 and 1.0% concentration, respectively, of which 85-98% was utilized in all the treatment ponds.

Daily mean values of 0.08, 0.11, 0.16 and 0.21 mg·l<sup>-1</sup> phosphorus were supplied to 0.1, 0.3, 0.6 and 1.0% biodigested slurry ponds, respectively. Of this, 77-90% phosphorus was utilized in 0.1 and 0.3% concentrations, 76-86% of phosphorus was utilized in 0.6%, and 50-65% was utilized in 1.0% concentration.

Dissolved oxygen (DO) (measured before 1000 hours) was 5-6 mg·t<sup>-1</sup> in the lower concentrations but low in the 1.0% concentration (0.4-4.4 mg·t<sup>-1</sup>). Oxygen levels decreased gradually until the 50th day when the range was 0-3.2 mg·t<sup>-1</sup>, except in the 1.0% concentration in which  $0_2$  was undetectable by the 30th day.

				1		
	0.1%					
Days	0 day	10th	20th	30th	40th	50th
TKN mg l <sup>-1</sup>	1.6	3.0	2.1- 3.5	2.8	2.8-	2.8- 3.3
Ammonia nitrogen mg 1 <sup>-1</sup>	1.0	0.18- 0.07	0.23- 0.32	0.13	0.15- 0.18-	0.06- 0.14
PO <sub>4</sub> -P mg 1 <sup>-1</sup>	0.50- 0.52	0.03 0.04	0.23- 0.36	0.18- 0.24	0.08- 0.09	0.03- 0.07
NPP mg C m <sup>-3</sup> h <sup>-1</sup>	-30- 19	456- 608	228- 342	114- 228	722- 988	381- 456
0 <sub>2</sub> mg l <sup>-1</sup>	6.00 6.10	7.1	4.8- 5.7	4.2- 4.8	2.6- 3.6	3.2
				0.6%		
TKN mg l <sup>-1</sup>	4.5	4.9 7.7	3.5- 4.4	4.2	5.6	7.2- 8.4
Ammonia nitrogen mg l <sup>-1</sup>	2.8	1.0- 1.2	0.1J- 0.16	0.0- 0.05	0.05	0.05
PO <sub>4</sub> -P mg l <sup>-1</sup>	0.97	0.34 0.54	0.74 0.84	0.81- 0.88	0.50- 0.63	0.32 0.35
NPP mg C m <sup>-3</sup> h <sup>-1</sup>	-38- 151	875- 1,026	342- 912	152- 1,292	760- 2,166	304- 1,748
0 <sub>2</sub> mg l <sup>-1</sup>	5.2-	4.6	3.0- 4.5	1.2- 2.6	0.6- 2.5	1.6 2.0
		6.° 1	1155 A			<b>A</b>

Table 1. Influence of dilution on nutrient and primary productivity of biogas slurry treatment miniponds.

				0.3%		
Days	0 day	10th	20th	30th	40th	50th
TKN mg l <sup>-1</sup>	3.5	4.2	3.7	4.2- 4,9	3.7	3.6
Ammonia nitrogen mg l <sup>-1</sup>	2.2	1.2	0.33	0.03	0.18	0.1- 0.2
PO <sub>4</sub> -P mg l <sup>-1</sup>	0.62- 0.68	0.15- 0.36	0.26- 0.50	0.12- 0.26	0.12- 0.23	0.13- 0.23
NPP mg C m <sup>-3</sup> h <sup>-1</sup>	-38- -76	1,140- 2,740	494- 1,368	342 1,255	1,178- 1,369	950- 5,968
0 <sub>2</sub> mg i <sup>-1</sup>	6.0- 6.2	6.2- 6.7	4.1- 4.3	1.8- 2.8	1.8- 2.0	0- 2.23
				1.0%		11100
TKN mg l <sup>-1</sup>	6.1	4.4	4.2- 4.4	4.6- 5.3	7.7-	5.8- 7.0
Ammonia nitrogen mg l <sup>-1</sup>	3.9	0.80- 1.6	0.1- 0.5	0.02- 0.08	0.05	0.06
PO <sub>4</sub> -P mg I <sup>-1</sup>	1.3	0.5- 0.6	1.2	0.82	0.73	0.92
NPP mg C m <sup>-3</sup> h <sup>-1</sup>	-79- -456	1,368- 2,357	1,064- 1,330	2,470- 3,611	2,509- 2,775	1,824- 2,812
) <sub>2</sub> mg l <sup>-1</sup>	0.4- 4.4	4.5- 6.7	2.2-	0	0	2,812
	line -		1921			

Table 1. Continuation

NPP - Net primary productivity

Variation of DO in the stabilization ponds over 24 hours on the 35th day of the biogas slurry treatment showed that minimum levels at 0600 hours were 5.28, 3.25, 0.81 and 0.00 mg·l<sup>-1</sup>, respectively at 0.1, 0.3, 0.6 and 1.0% concentrations. The maximum  $0_2$  of 16.24 mg·l<sup>-1</sup> was observed at 1600 hours in 0.1 and 0.3%. High level DO of 22.73 mg·l<sup>-1</sup> was observed in 0.6 and 1.0% concentrations at 1600 hours. After 1600 hours,  $0_2$  concentration showed a decreasing trend with increasing concentrations.

The Net Primary Productivity (NPP) (Table 1) was  $114-988 \text{ mg C}\cdot\text{m}^{-3}$ -hour<sup>1</sup> in 0.1% slurry, 494-2,740 mg C·m<sup>-3</sup>-hour<sup>1</sup> in 0.3%, 152-2,166 mg C·m<sup>-3</sup>-hour<sup>1</sup> in 0.6%, and 1,064-3,611 mg C·m<sup>-3</sup>-hour<sup>1</sup> in 1.0%. NPP showed a decreasing trend after 30 days in 1.0% and only after 40 days in 0.6 and 0.1% concentrations.

## Algal Forms Observed in Stabilization Ponds

After the 25th day, samples were observed for algal forms. *Chlorella* was the dominant species observed in all the concentrations, followed by *Anacystis*. At 0.1 and 0.3% concentrations, *Asterionella* was the subdominant form. At 0.6 and 1.0% concentrations, *Ankistrodesmus* and *Asterionella* were the sub-dominant forms. *Scenedesmus* was observed as the subdominant species in 0.6%. Different algal forms and total number increased with increasing concentration of biodigested slurry (Table 2).

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0.1%	0.3%	0.6%	1.0%
Chiorella (D) GA (4-8)	Chlorella (D) GA (7-11)	Chiorella (D) GA (11-12)	Chlorella (D) GA (11-13)
Anacystis (SD) BGA (1-2)	Anacystis (SD) BGA (2-3)	Anacystis (SD) BGA (2-3)	Anacystis (SD) BGA (2-3)
Asterionella (SD) D (1-3)	Asterionella (SD) D (1-4)	Ankistrodesmus (SD) GA (1-3)	Ankistrodesmus (SD) GA (1-3)
Synedra (SD) (3-4)	Phytoconis GA (1-2)	Asterionella (SD) D (3-4)	Asterioneila (SD) D (2-5)
(IInd pond after 30 days)		đ	
Nitzschia D (0-1)	Scenedesmus GA (0-1)	Scenedesmus (SD) GA (2-3)	Navicula D (0-2)
Navicula D (0-1)	Ankistrodesmus GA (0-1)	Setenastrum GA (0-1)	Niteschia D (0-2)
Selenastrum GA (0-1)	Nitzschia D (0-1)	Phytoconis GA (1-2)	Scenedesmus GA (0-2)
Ankistrodesmus GA (0-1)	Navicula D (0-1)	Navicula D (0-1)	Scenedesmus GA (0-2)
Pinnularia D (0-1)	Selenastrum GA (0-1)	Nitzschia D Cocconeis D (1-2)	Comphosphaeria BGA (0-1)
	Actinastrum GA (0-1)	Actinostrum GA (0-1)	Phytoconis GA (2-3)
	Pediastrum GA (0-1)	Paimella GA (0-1)	Actinastrum GA (0-1)
		Pinnularia D (0-1)	Pinnularia D (0-1)
		Gomphosphaeria BGA (0-1)	Coccochloris BGA (0-1)
		Sphaerocystts GA (0-1) Diatoma D (0-1)	Sphaerocystis GA (0-1)

Table 2. Algal forms\* observed in different concentrations of the digested biogas slurry treatment ponds

(D) dominant, (SD) sub dominant, GA - green algae, BGA - blue green algae, D - diatoms. \*- in the order of dominance Numbers in parenthesis X  $10^4 = no.$  of cells  $mt^{-1}$ .

## Daphnia similis Culture in Stabilization Pond Water

#### LIFE SPAN OF DAPHNIA SIMILIS

The life span of *D. similis* in 1.0% concentration of effluent was 38 days, and 30 days in 0.1% concentration. Maximum length growth increment (1.71 mm) occurred in the initial 5-day period at all concentrations. Thereafter, growth was slow and linear, with 1.0% concentration showing highest growth (Fig. 1).

## Egg Production by D. similis

Egg production, in terms of the mean number of young animals released from the brood pouch during the whole life span, was 480 in 0.1% on the 30th

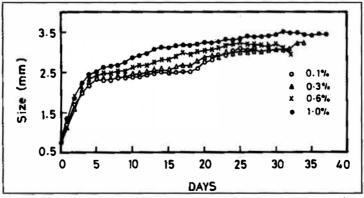


Fig. 1. Growth pattern of *Daphnla similis* in different concentrations (0.1, 0.3, 0.6 and 1.0%) of biogas slurry-treated water (each value represents mean of 14 samples).

day. Cumulative production of neonates was 519, 460 and 690 in 0.3, 0.6 and 1.0%, respectively (Fig. 2). Individual egg production increased (5th day) from 10 neonates animal<sup>-1</sup> day<sup>-1</sup> to 34-36 neonates animal<sup>-1</sup> day<sup>-1</sup> on the 24th day at 0.1% concentration. In 1.0% concentration, neonate production decreased from 24-30 neonates animal<sup>-1</sup> day<sup>-1</sup> during days 6-24, to 15-20 neonates animal<sup>-1</sup> day<sup>-1</sup> day<sup>-1</sup> during days 25-36 (Fig. 3).

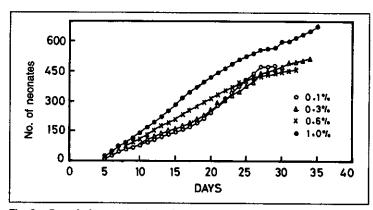


Fig. 2. Cumulative production of neonates by *Daphnia similis* in different concentrations (0.1, 0.3, 0.6 and 1.0%) of biogas slurry-treated water (each value represents mean of 14 samples).

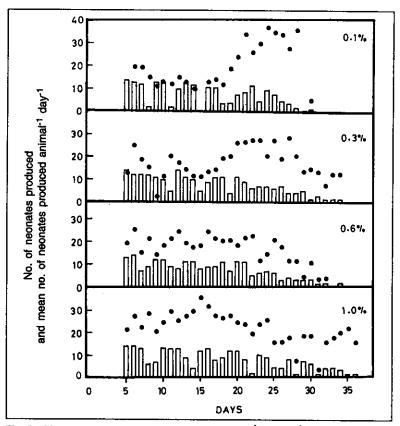


Fig. 3. Mean number of neonates produced day<sup>1</sup> animal<sup>-1</sup> ( $\bullet$ ) and number of neonates produced day<sup>-1</sup> ( $\Box$ ) in different concentrations (0.1, 0.3, 0.6 and 1.0%) of biogas slurry-treated water.

# Mortality Rate of D. similis

Mortality rates exhibited a similar pattern of high initial survival followed by heavy mortality. Longevity was lowest at 0.1% (30 days) and highest at 1.0% (38 days) (Fig. 4).

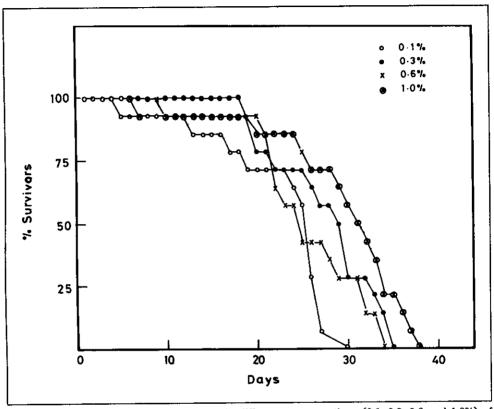


Fig. 4. Survival rate (%) of *Daphnia similis* in different concentrations (0.1, 0.3, 0.6 and 1.0%) of biogas slurry-treated water.

## Discussion

The trends of nutrient (TKN,  $NH_3$ -N, phosphorus) removal observed in the present study have been recorded in similar other systems (Groeneweg et al. 1980; Martin et al. 1985; de la Noue and Basseres 1990).

It has been reported that algal productivity is limited only when concentrations of dissolved phosphorus and dissolved nitrogen are at least four to six times less than the daily requirements (McNabb et al. 1990). In the present study, partial harvesting kept the algal forms and nutrients available throughout the culture period. The same was observed by Martin et al. (1985) with medium renewal every 2 or 3 days in pig manure treatment. The harvested water was further used in *D. similis* culture under laboratory conditions.

The trend here of increased *Daphnia* longevity at higher slurry concentrations was observed in *D. similis* cultured in different concentrations of rice bran and Banta's medium (Venkataraman and Krishnaswamy 1986). However, Arnold (1971) and Venkataraman (1983) found higher survival values at low food levels. Vijverberg (1976) found relatively higher longevity values for poorly fed *D. hyalina* (64 days) than well fed ones (24 days). In the present study, the increased longevity at higher concentrations probably increased egg production.

The mean total number of eggs produced by *D. similis* when cultured in 1.0% slurry was 691 in a lifespan of 38 days, which is almost 2/3 higher than the number of eggs produced in 0.1% slurry (490 in a lifespan of 30 days). The same organism cultured in pond water produced 110 eggs in 57 days (Venkataraman and Krishnaswamy 1986). These results are in agreement with those of earlier workers who showed a positive correlation between changes in individual fecundity and food concentrations (Lambert 1978).

Two key factors influence the number of eggs in the brood chamber: the size of the mother and the food concentration (Richman 1958). Zooplankton may eat the algae (Arnold 1971) or feed directly on waste particles (Schroeder 1980). Other factors which may also influence the number of eggs per female are oxygen concentration, pH and temperature (Vijverberg 1980). In the present investigation, it was felt that oxygen and temperature were generally not limiting. Oxygen concentrations limit egg production only at very low levels (0.2 mg·l<sup>-1</sup>) and this did not usually occur in the present *Daphnia* culture, while temperature was similar (25-35°C) for all the concentrations tested.

As in other members of Cladocera, *D. similis* undergoes a series of molts, and growth occurs shortly after each molt. In the present study, the maximum growth increment (0.6 mm) occurred between the first and fourth instar in all concentrations. Analysis of data on the growth of a few tropical Cladocera reveals that maximum growth occurs in the early preadult instar (Venkataraman 1983). Linear regression analysis of growth of *Daphnia* at different concentrations showed that growth was highly significant at 0.1% (r = 0.88) and 0.3% (r = 0.87) than at 0.6% (r = 0.83) and 1.0% (r = 0.81) (Table 3). Statistical analysis of cumulative egg production by *D. similis* at different concentrations of biogas slurry (0.1, 0.3, 0.6 and 1.0%) were highly significant. At the same time, the statistical analysis of individual egg production was also highly significant and positively correlated with increasing culture period in 0.1% (r = 0.75) and 0.3% (r = 0.63) concentrations of biodigested slurry, whereas negatively correlated and not significant at 0.6% (r = -0.33) and 1.90% (r = -0.64) concentrations.

The results suggest that 0.1-0.3% concentrations of biogas slurry are suitable for longer maintenance of culture of algae and of *Daphnia* in biogas slurrytreated ponds.

Table 3. Linear regression analysis of data on the growth of *Daphnia similis* (Y, mm) at different concentrations of biodigested slurry. X is the mean daily increase of growth (mm) of *D. similis*.

0.1 %	Y = 0.065 X + 1.410	(r = 0.88)
0.3 %	Y = 0.058 X + 1.473	(r = 0.87)
0.6 %	Y = 0.067 X + 1.529	(r = 0.83)
1.0 %	Y = 0.062 X + 1.742	(r = 0.81)
1. 1. (B) 1.		

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