Indian J. Fish., 60(3) : 87-90, 2013

Heterotrophic bacterial populations and dehydrogenase activity in fish ponds under different fertilisation practices

MADHUMITA DAS[1], S. AYYAPPAN[2], BISWAJIT DASH[1] AND H. K.MUDULI[3]
[1]Visakhapatnam Regional Centre of Central Marine Fisheries Research Institute, Ocean View Layout, Panduranga Puram, Andhra University (P. O.), Visakhapatnam - 530 003, Andhra Pradesh, India
[2]Indian Council of Agricultural Research, Krishi Bhavan, New Delhi - 110 012 , India
[3]Central Institute of Freshwater Aquaculture, Kausalyaganga P. O, Bhubaneswar - 751 002, Odisha, India
e-mail: dasmadhumita999@ rediffmail.com.

ABSTRACT

TThe ranges of aerobic heterotrophic bacterial populations in fish culture ponds (0.04 ha) under four manurial treatments, (1) cow manure at 10 t ha^{-1} yr^{-1}; urea at 100 kg N ha^{-1} yr^{-1} and single super phosphate at 50 kg P ha^{-1} yr^{-1}, (2) biogas slurry at 15 t ha^{-1} yr^{-1} and inorganic fertilisers, (3) biogas slurry at 30 t ha^{-1} yr^{-1}, and (4) biogas slurry at 30 t ha^{-1} yr^{-1}, with supplementary feeds assessed over a period of 15 months, were 0.28-4.44,0.30-3.86, 0.48-3.81 and 0.70-4.38 x10^3 ml^{-1} with means of 1.49,1.43,1.94 and 2.08 x10^3 ml^{-1}. The sediment heterotrophic bacterial counts presented a similar pattern of variation with ranges of 1.20-6.71, 1.29-4.13, 2.04-9.92 and 2.66-11.07 x10^5 g^{-1} and mean values of 3.28, 2.62, 4.87 and 5.08 x10^5 g^{-1} in the four treatments. Water temperature and water depth were in the ranges of 20.8-30.2 °C and 0.8 to 1.8 m. The ranges of different water quality parameters were pH, D.O, NH_4-N, NO_2-N, NO_3-N, PO_4-P and dissolved organic matter were 7.4-8.5, 3.5-6.1 mg l^{-1}, 3.25-22.66, 4.63-28.95, 10.04-48.98 mg-at N l^{-1}, 0.03-0.54 and 2.1-9.5 mg l^{-1}. The variations in the dehydrogenase activity of the surface sediment layers, indicative of the total bacterial activity were 0.06-0.46, 0.11-0.36, 0.19-0.87 and 0.02-0.94 mg triphenyl formazone per g sediment per day with the mean levels being 0.48, 0.41, 0.28 and 0.23 mg triphenyl formazone per g sediment per day. It is apparent that the activity was higher in treatments 3 and 4. This corroborates the observations on higher heterotrophic bacterial counts in the sediments of treatments 3 and 4, attributable to availability of processed substrate as compared to the ponds with application of raw cow manure. This also suggests better sediment-water interactions in slurry-applied ponds in comparison to traditional manuring practices.

Keywords: Dehydrogenase activity, Fish ponds, Heterotrophic bacterial populations, Organic fertilisation

Introduction

Microorganisms, forming the linkages between abiotic factors and biotic world are responsible for basic production and decomposition processes in aquatic ecosystems. Microbial activity assumes greater significance in Indian freshwater aquaculture systems, where organic manuring is the most prevalent practice of organic fertilisation. The population of aerobic heterotrophic bacterial communities is the most important component of the microbial coenoses of aquatic ecosystems involved in the mineralisation of organic matter. Estimation of their multiplication potentials is essential for proper quantification of the bacterial productivity (Wolhfarth and Hulata, 1987; Jana *et al.*, 2001).

Spatial and seasonal distribution of heterotrophic bacteria in pond water and sediments under different management practices was studied by different workers (Jana and Patel, 1984; Ampofo and Clerk, 2003). The objective of the present study was to evaluate the variations in the populations of heterotrophic bacteria and their activity that would provide comparative assessment of manurial effects and advantages of processed organic manure like biogas slurry application over raw cow manure.

Materials and methods

Twelve ponds (0.04 ha each) located in the farm of Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Orissa (lat. $20^0$11'06"-$20^0$11'45" N; long. $85^0$50'52"-$85^0$51'35"E), were selected for the study for a period of fifteen months. There were four manurial treatments in triplicates, with bimonthly applications of (1) cow manure at 10 t ha^{-1} yr^{-1}, urea at 100 kg N ha^{-1} yr^{-1} and single super phosphate at 50 kg P ha^{-1} yr^{-1}; (2) biogas slurry at 15 t ha^{-1} yr^{-1} and inorganic fertilisers as in the previous treatment; (3) biogas slurry at 30 t ha^{-1} yr^{-1}; and (4) biogas slurry at 30 t ha^{-1} yr^{-1} with supplementary feed (rice bran-groundnut oil cake in equal proportions at 1% of fish biomass) provided daily in ponds. The ponds were stocked with carp fingerlings at a density of 5000 ha^{-1} in proportions of catla 15% (average initial size 110 mm/15.6 g), rohu 25% (75 mm/6.3 g), mrigal 25% (65 mm/35 g), silver carp 30% (60 mm/4.9 g) and grass carp 5% (40 mm/2.6 g).

Standard plate count method was employed for enumeration of aerobic heterotrophic bacterial populations, once in a month using nutrient glucose agar (Norris and Ribbons, 1970; Collins and Lyne, 1976). While 10^{-3} dilutions with sterile physiological saline (0.85% NaCl) were used for water samples, 10^{-5} dilutions were employed for sediment samples. Samples in sterile petridishes were mixed in agar medium by the pour plate method and the plates were incubated at room temperature (30° ± 2 °C) in a bacteriological incubator (BEC model 1989) for 48 h. The colony forming units (cfu) in the petridishes were counted and from the means in different dilutions, the average counts were derived. The results are expressed as no. x 10^3 ml^{-1} water and no. x10^5 g^{-1} wet weight of the sediment.

The hydrobiological samplings and analysis for different parameters were conducted at monthly intervals throughout the period of study. The temperature and pH of pond water were measured using mercury thermometer and pH meter of water analysis kit (Century, CK-710). Dissolved oxygen content of water was measured with an Oxi-meter, OXI-191(Chemito, India). Water samples from the pond surface waters were collected in polyethylene bottles and brought to the laboratory for analysis. The water quality parameters of NH_4-N, NO_2-N, NO_3-N and PO_4-P were analysed by colorimetric methods using double beam UV-Vis spectrophotometer (APHA, AWWA, WPCF, 1998).

For estimation of dehydrogenase activity, one gram of fresh sediment sample was added with 1 ml of 1.5% triphenyl tetrazolium chloride, 4 ml distilled water and 100 mg of calcium carbonate. The homogenised samples were incubated at 37 °C for 24 h and extracted with 50 ml of methanol. The intensity of pink colour due to reduction of triphenyl tetrazolium chloride to triphenyl formazone due to dehydrogenase activity was determined in a UV-Vis spectrophotometer (Hitachi, 150-20) at 485 nm (Casida *et al.,* 1964). The dehydrogenase activity is expressed as mg triphenyl formazone formed per g sample per 24 h, indicating the total bacterial activity.

Two way analysis of variance (ANOVA) was applied to determine the significance of variations between treatments and periods. A correlation matrix was made between parameters to delineate the relationships and interactions between the parameters.

Results and discussion

The aerobic heterotrophic bacterial populations in waters were in the ranges of 0.28-4.44, 0.30-3.86, 0.48-3.81 and 0.70-4.38 x 10^3 ml^{-1} (Fig. 1) with significant differences between the months ($p< 0.001$). The populations were observed to increase with organic applications, as indicated by mean values in the four treatments (1.49, 1.43, 1.94, 2.08 x 10^3 ml^{-1}). Higher counts were obtained during the summer months of April-June. The populations showed positive relations with dissolved oxygen (r_{12} = 0.18-0.56), specific conductivity (r_{12} = 0.31- 0.44), NO_3-N (r_{12} = 0.26- 0.53), PO_4-P (r_{12} = 0.03- 0.66) and dissolved organic matter (r_{12} = 0.06- 0.26).

The sediment heterotrophic bacterial counts presented a similar pattern of variations with the ranges of 1.20-6.71, 1.29-4.13, 2.04-9.92 and 2.66-11.07 x 10^5 g^{-1} with mean values of 3.28, 2.62, 4.87 and 5.08 x10^5 g^{-1} (Fig. 1) in the four treatments. The variations were significant between the treatments ($p<0.001$). Higher heterotrophic bacterial populations in the water and sediment media of ponds applied with biogas slurry suggested that the increased activity is due to availability of more decomposable organic substrate (Wetzel, 1990).

The water depth in the ponds in all the four treatments ranged from 0.8 to 1.8 m and dissolved oxygen levels were 3.5 to 6.1 mg l^{-1}. Water temperature was in the range of

Table 1. Ranges in the quality of pond water under different treatments

Parameters	Treatments			
	1	2	3	4
Water depth (m)	0.8-1.8	0.9-1.6	0.8-1.8	1.0-1.8
pH	7.4-8.5	8.0-8.4	7.4-8.4	7.4-8.4
Dissolved oxygen (mg l^{-1})	3.6-5.3	3.6-5.0	4.2-5.4	3.5-6.1
Carbonate alkalinity (mg $CaCo_3$ l^{-1})	0-40.0	0-35.4	0-32.0	0-30.0
Bicarbonate alkalinity (mg $CaCo_3$ l^{-1})	60-130	52-128	54-136	50-180
Specific conductivity (mS cm^{-1})	62-250	150-240	165-310	160-305
Ammonium-nitrogen (mg-at N l^{-1})	4.15-11.74	3.25-14.38	5.10-19.88	6.08-22.66
Nitrite-nitrogen (mg-at N l^{-1})	4.87-25.58	4.63-24.32	5.60-26.33	7.94-28.95
Nitrate-nitrogen (mg-at N l^{-1})	10.18-40.11	10.12-33.86	10.04-41.29	10.06-48.98
Phosphate-phosphorus (mg l^{-1})	0.12-0.40	0.08-0.48	0.03-0.40	0.04-0.54
Dissolved organic matter (mg l^{-1})	2.20-5.65	2.10-6.50	4.20-7.40	5.20-9.50

20.8-30.2 °C. The ranges of the different physico-chemical parameters under the four treatments are presented in Table 1.

The variations in the dehydrogenase activity of the surface sediment layers, indicative of the bacterial activity, are presented in Fig.1. The ranges of variations in the four treatments were 0.06-0.46, 0.11-0.36, 0.19-0.87 and 0.02-0.94 mg triphenyl formazone g sediment^{-1} day^{-1}, with significant variations between treatments ($p<0.01$). The activity was higher in sediments of treatment 4, followed by treatments 3, 1 and 2, the mean levels being 0.48, 0.41, 0.28 and 0.23 mg g^{-1}d^{-1}.

et al. (1991). The slurry-applied ponds also showed better sediment-water interactions, as shown by the variations in the two media as also the correlation coefficients ($r_{1,2}$= 0.24-0.42).

The summer peaks in the bacterial counts may be attributed to high temperature and availability of organic matter through the decomposing plankton populations. A similar observation was made by Schroeder *et al.* (1990). Positive correlations of the heterotrophic bacterial populations with water quality parameters indicate the distinct influence of the former on the processing of organic matter and the resultant nutrient status of the water media.

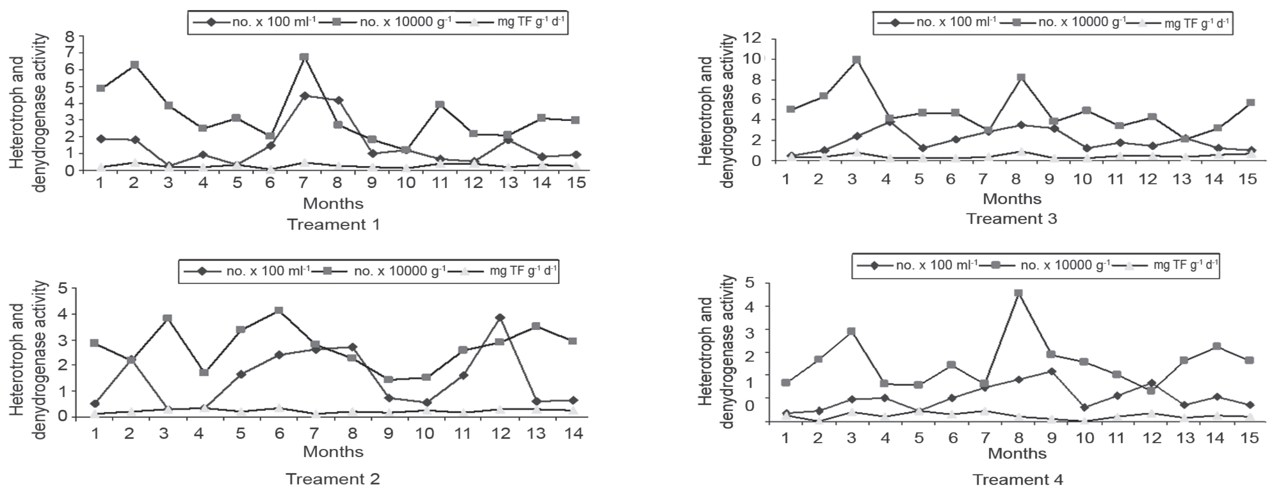

Fig.1. Seasonal variations in aerobic heterotrophs in water (no. x 10^3 ml^{-1}), sediment (no.x10^5 g^{-1} and dehydrogenase activity (mg TF g^{-1} d^{-1}) under different treatments

It is apparent from the above that the activity was higher in treatments 3 and 4. This corroborates the observations on higher heterotrophic bacterial counts in the sediments of treatments 3 and 4, attributable to availability of processed substrate, as compared to the ponds with applications of raw cow manure, as also observed by Ayyappan *et al.* (1991) in ponds under biofertilisation with *Azolla*. The summer peaks in the dehydrogenase activity may be attributed to higher heterotrophic bacterial populations in the sediments (Moriarty, 1997). Similar observations were made by Goyal and Mishra (1992) and Thakre *et al.* (1994).This also suggests better sediment-water interactions in slurry-applied ponds compared to traditional manuring practices**.**

The differences in the heterotrophic bacterial populations in water and sediment media of ponds applied with raw cow manure and biogas slurry pertained to a peak during April-June in the former and a cyclical pattern of fluctuations in the latter. Treatments 3 and 4 also registered higher values. Similar observations have been made in ponds applied with fermented cow manure by Schroeder (1978), Jana and Dey (1990) and Ogbondeminu

The organic processing in the water medium of slurry-applied ponds was conspicuous by the population patterns of heterotrophic bacterial populations. This influenced the nutrient status, particularly in terms of organic matter, where levels of readily decomposable material supported higher bacterial populations in slurry-applied ponds (Bhat, 1984).

With heterotrophic bacterial activity forming the basis of organic mineralisation and nutrient biota-interactions (Freitas and Godinho-Orlandi, 1991; Bhakta *et al.*, 2006), their variations under different treatments influenced the overall hydrobiological conditions. These in turn were largely determined by the type of organic input, in terms of raw cow manure or biogas slurry, the latter being more amenable to bacterial decomposition. A similar observation was made by Goyal and Mishra (1992) and Jana *et al.* (2012) with regard to the use of farm yard manure.

Acknowledgements

The study formed part of the doctoral research (part time) of the first author under Utkal University, Bhubanewsar. The authors thank the Department of

Non Conventional Energy Sources (DNES), New Delhi, for providing financial support for the present work.

References

APHA AWWA, WPCF (American Water Works Association and Water Pollution Control Federation) 1998. *Standard methods for the examination of water and wastewater*, 19th edn. American Public Health Association, Washington, DC, 1038 pp.

Ampofo, A. and Clerk, G. C. 2003. Bacterial flora of fish feeds and organic fertilizers for fish culture ponds in Ghana. *Aquacult. Res.*, 34(8): 677–680.

Ayyappan, S., Purushothaman, C. S., Saha, P. K. and Pani, K. C. 1991. Heterotrophic bacterial populations and primary production in fish ponds under different management practices. *J. Aquacult*., 1: 11-29.

Bhakta, N. J., Bandyopadhyay, K. P. and Jana, B. B. 2006. Effect of different doses of mixed fertilizer on some biogeochemical cycling bacterial population in carp culture pond. *Turkish J. Fish. and Aquat. Sci.*, 6: 165-171.

Bhat, J. V. 1984. Earthworms and microorganisms. *Proceedings of the National Seminar on Organic waste utilization and vermicomposting*, School of Life Sciences, Sambalpur University, 5-8 December, 1984, Orissa.

Casida, C. E., Klein, D. A. and Santaro, T. 1964. *Soil Sci*., 98: 371-376.

Collins, C. H. and Lyne, P. M. 1976. *Microbiological methods*. Butterworth & Co. (Publ.) Ltd., London, 521 pp.

Freitas, E. A. C and Godinho-Orlandi, M. J. L. 1991. Distribution of bacteria in the sediment of an Oxbow tropical lake. *Hydrobiologia,* 211(1): 33-41.

Goyal, S. and Mishra, M. M. 1992. Effect of glucose and farmyard manure addition on the microbial biomass and enzyme activities. *Proceedings of the 32nd Annual Conference Association Microbiologists of India*, Madurai, Jan. 10-12, 1992, Abst. AMO 2-8.

Jana, B. B and Patel, G. N. 1984. Spatial and seasonal distribution of heterotrophic bacteria in pond water and sediments. *Archives fur Hydrobiologie*, 101: 555-568.

Jana, B. B. and De, U. K. 1990. Spatial and seasonal distribution of heterotrophic bacteria in pond water and sediments under different management practices. [J]. *International revder Gesamten Hydrobiologie*, 75: 639-648.

Jana, B. B., Chakrabarti, P., Biswas, J. K. and Ganguly, S. 2001. Biogeochemical cycling bacteria as indices of pond fertilization: importance of CNP ratios of input fertilizers. *J. Appl. Microbiol.*, 90: 733-740.

Jana, B. B., Bag, Sujoy Kumar and Rana Sukanta 2012. Comparative evaluation of the fertilizer value of human urine, cow manure and their mix for the production of carp fingerlings in small holding tanks. *Aquacult. Interntl,* 20: 735-749.

Majumder, S., Biswas, S. and Barat, S. 2002. Abundance of ammonifying and heterotrophic bacterial populations in the water manured with cowdung and distillery sludge in outdoor model tanks. *Asian J. Microbiol. Biotechnol. Environ. Sci.*, 4: 229-233.

Moriarty, D. J. W. 1997. The role of microorganisms in aquaculture ponds. *Aquaculture*, 151: 333-349.

Norris, J. R. and Ribbons, D. W. 1970. *Methods in microbiology*, Volume 3A, Academic Press Inc., London, 505 pp.

Schroeder, G. L. 1978. Autotrophic and heterotrophic production of microorganisms in intensively manured fish ponds and related fish yields. *Aquaculture*, 14(4): 303-325.

Schroeder, G. L.,Wohlfarth,G., Alkon, A., Halevy, H. and Krueger, H. 1990. The dominance of algal-based food webs in fish ponds receiving chemical fertilizers plus organic manures. *Aquaculture*, 86: 219-229.

Thakre, P., Ingle A. O. and Daginawala, H. F. 1994. Dehydrogenase activity as an Index of biological treatment of dairy waste water. *Proceedings of the 35th Annual Conference Association Microbiologists of India*, Aug. 9-12, Mysore.

Ogbondeminu, F. S., Omorinkoba,W. S., Madu, C. T. and Ibikunle, G. F.1991. Bacterial micoflora associated with fish production in outdoor ponds and their feed. *J. Aquacult. Trop*., 6: 55-62.

Qin, J., Culver, D. A. and Yu, N. 1995. Effect of organic fertilizer on heterotrophs and autotrophs, implications for water quality management. *Aquacult. Res.*, 26: 911-920.

Rai, H. and Hill, G. 1980. Classification of Central Amazon lakes on the basis of their microbiological and physico-chemical characteristics. *Hydrobiologia*, 72: 85-99.

Wetzel, R. G. 1990. Detritus, macrophytes and nutrient cycling in lakes. *Mem. Ist. Ital. Idrobiol*., 47: 233-251.

Wolhfarth, G. W. and Hulata, G. 1987. Use of manures in Aquaculture. In: Moriarty, J. W. and Pullin, R. S. V. (Eds.), *Detritus and microbial ecology in aquaculture*. International Centre for Living Aquatic Resource Management, Manila, Philippines, 353 pp.

Date of Receipt : 04.10.2012
Date of Acceptance : 18.04.2013