Indian J. Fish., ***53****(4) : 469-473, Oct.-Dec., 2006*

Note

Efficacy of commercial shrimp farm bioremediators in removing ammonia in microcosm experiments

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The efficacy of two commercial aquaculture bioremediators to remove ammonia was tested under laboratory conditions. The bioremediators were capable of removal of total ammoniacal nitrogen (TAN) to the tune of 86 - 90% (max) at 1-3 ppm initial level of ammonia (NH_3) and the removal rate decreased thereafter. Significant differences in nitrite (NO_2) levels in treated and control tanks were observed probably due to the differences in the levels of resident nitrifying bacteria that utilize NH_3 and oxidize it to NO_2. The nitrate levels increased in all the treatment tanks, but showed a general decreasing trend in control tanks. Among the tanks with varied NH_3 concentrations, the levels of NO_3 between the control and treatment tanks differed insignificantly ($P>0.05$). The results of the present study revealed that the commercial bioremediators failed to remove majority of the total ammoniacal nitrogen when the NH_3 level is high initially.

Microorganisms play a major role in cleaning up the environment through rapid mineralization of organic matter present in culture ponds. The practice of bioremediation is applied in shrimp culture, but success varies greatly, depending on the nature of the products used and the technical information available to the end user. The bacteria that are added must be selected for specific functions that are amenable to bioremediation, and added at high enough population density, and under the right environmental conditions. Bioremediation is a significant management tool, but its efficacy depends on understanding the nature of competition between species or strains of bacteria. A variety of commercial bioremediators have been used in shrimp aquaculture to increase shrimp productivity but with varying degrees of success. There are a number of reports on the positive and negative effects of the use of bioremediators to remove ammonia (NH_3) in shrimp culture ponds (Boyd *et al.*, 1984; Funge-Smith and Hawthorn, 1996; Moriarty, 1997; Prabhu *et al.*, 1999; Shan and Obbard, 2001). The present communication reports the efficacy of commercial aquaculture

bioremediators under laboratory conditions.

Twenty-four glass aquaria of 50 l capacity were first rinsed in water, treated with 10 ppm chlorine and then thoroughly washed in clean water to remove traces of chlorine and dried. Pond sediment collected from traditional shrimp farms (*Bheries*) of 24 Parganas District (South), West Bengal, was spread uniformly to get a 5 cm thick layer in all the aquaria. Saline water (10 ppt) was added into these tanks slowly without disturbing the sediment layer. All the tanks were kept undisturbed for 3 days. The tanks were divided into 4 sets of six tanks each. In each of the tanks in sets 1 to 4, the ammonia concentration was adjusted to 1 ppm, 2 ppm, 3 ppm and 4 ppm, respectively by the addition of liquid ammonia solution (20%). Each set had six tanks - four as treatment tanks and two as control tanks. The treatment tanks were seeded separately with commercial bioremediatorss, *viz*., Biocult - 0.08g / 40 l at the rate of 20 kg / ha and Epicin - 0.4g / 40 l at the rate of 10 ppm, in duplicate. The control tanks, received no bioproducts. All experiments were carried out at temperatures 30±5°C. The levels of ammonia, nitrite and nitrate were monitored daily for 8 days spectrophotometrically following APHA / AWWA / WEF (1998) methods. One-way ANOVA was followed to test the level of significance among treatments and critical difference calculated to examine which of the treatments varied significantly (Snedecor and Cochran, 1962).

As seen in Table 1, both Biocult and Epicin were capable of removal of TAN to the tune of 86 - 90% (max) at 1-3 ppm initial level of NH_3. At 4 ppm level it could remove only 60% TAN. There was a general reduction in TAN removal rate with increase in initial NH_3 levels in the control tanks. The TAN removal rates of Biocult and Epicin treated tanks and control tanks differed significantly ($P<0.05$) at all the four different initial NH_3 levels. The observed significance of difference ($P<0.05$) in the removal of TAN at varying levels of initial NH_3 concentration thus revealed that at initial high NH_3 levels, the commercial products failed to remove majority of the TAN. This was also observed in the control tanks with varying levels of NH_3. At 1 ppm NH_3 level, nearly 50% of TAN was removed; while in others, it was in the range of 28-37%. The higher rate of removal of TAN in Biocult treated tanks revealed the increased activity of ammonia oxidizers at higher temperature (30±5°C). The relatively high activity of ammonia oxidizers in Biocult was further substantiated by the fact that the percentage increase in NO_2 was higher at all the four different NH_3 levels. The significant differences in TAN removal rates in Epicin treated tanks with 1 - 4 ppm NH_3 levels could be attributed to the variations in the C: N ratio. The efficacy of Epicin was reported to be maximum when a C: N ratio of >10 is maintained (Briggs and Turnbull, 1995). The difference in C: N ratio in tanks with 1 - 4 ppm NH_3 levels was, therefore, the main cause for differences in the TAN removal rates of Epicin. The observed differences in the control tanks could be due to the varying degrees of volatilization of NH_3 or utilization by resident microflora.

The nitrite levels increased in all the experimental tanks treated with commercial products and also in control tanks. The increase in percentage of NO_2 in Biocult treated tanks was highest at 4 ppm level of NH_3 and least at 1 ppm level of NH_3. The increase in percentage of NO_2 in Epicin treated tanks was highest at 3

TABLE 1: *The levels of ammonia (µg at NH_4-N/l), nitrite (µg at NO_2-N/l) and nitrate (µg at NO_3-N/l) as affected by commercial bioremediators in microcosm experiments. (Only the observations of 1st, 4th and 8th day are presented in the table)*

Initial ammonia concentration and Products	Parameters								
	Ammonia (µg at NH_4-N/l)			Nitrite (µg at NO_2-N/l)			Nitrate (µg at NO_3-N/l)		
	Day 1	Day 4	Day 8	Day 1	Day 4	Day 8	Day 1	Day 4	Day 8
1 ppm ammonia									
Biocult	1239.0±4.0	1001.5±41.5	141.0±12.0	31.82±19.54	57.44±22.93	70.83±10.89	0.62±0.06	0.03±0.005	1.64±0.17
Epicin	1170.0±31.0	905.5±33.5	157.0±3.0	22.00±9.00	8.91±1.80	42.06±10.60	0.40±0.003	0.52±0.04	1.12±0.10
Control	991.2±20.0	928.5±34.5	501.6±94.0	15.42±0.93	16.67±8.27	63.09±20.50	1.12±0.34	0.12±0.05	0.81±0.05
2 ppm ammonia									
Biocult	2196.0±64.0	1702.5±73.5	222.0±69.0	33.12±11.50	60.70±11.10	90.72±3.34	0.49±0.02	0.15±0.04	1.64±0.001
Epicin	2195.0±15.0	1835.0±14.0	349.5±138.5	41.06±2.44	24.23±2.00	70.83±2.48	0.86±0.06	0.97±0.05	1.60±0.03
Control	2159.0±126.0	1779.5±268.5	1349.0±165.0	15.29±0.35	10.26±1.86	31.08±8.31	1.40±0.20	0.27±0.04	0.54±0.09
3 ppm ammonia									
Biocult	2961.5±102.5	2290.0±8.0	612.0±168.0	26.11±18.50	44.44±26.16	65.44±19.23	0.66±0.20	0.22±0.11	1.72±0.05
Epicin	3040.5±51.5	2585.0±54.0	564.5±76.5	30.58±3.02	22.84±2.49	81.76±2.55	0.57±0.07	0.77±0.07	1.51±0.12
Control	3078.5±19.5	2794.5±128.5	2209.0±119.0	14.40±0.05	10.08±0.25	35.19±2.55	1.46±0.05	0.23±0.05	0.84±0.15
4 ppm ammonia									
Biocult	4402.5±89.5	3518.0±1.0	963.5±1.50	25.26±12.42	44.80±22.19	69.80±18.92	0.89±0.06	0.39±0.06	1.91±0.2
Epicin	4346.5±35.5	4134.5±123.5	1705.0±140.0	40.79±1.66	23.29±1.00	74.03±2.20	0.90±0.01	1.01±0.01	1.68±0.05
Control	3848.0±2.0	3170.0±211.0	2489.5±119.5	14.67±0.34	12.38±0.21	29.51±3.01	1.45±0.05	0.02±0.002	0.74±0.15

ppm level of NH_3 and least at 2 ppm level of NH_3. The increase in percentage of NO_2 in control tanks was highest at 1 ppm level of NH_3 and least at 4 ppm level of NH_3 (Table 1). Significant differences were noticed among treatments and also among various NH_3 levels ($P < 0.05$). The levels of NO_2 in Epicin treated tanks and control tanks with 1 ppm level of NH_3 were significantly different ($P<0.05$) from those of 2, 3 and 4 ppm levels of NH_3. Biocult treated tanks with 1 ppm NH_3 had significantly ($P<0.05$) high NO_2 than Epicin treated and control tanks. Statistically significant differences ($P<0.05$) existed between the levels of NO_2 at 2, 3 and 4 ppm levels of NH_3 between control tanks and tanks treated with Biocult and Epicin. The observed significant differences in NO_2 levels in treated and control tanks could be attributed to the differences in the levels of resident nitrifying bacteria that utilize NH_3 and oxidize it to NO_2 and also the role of environmental factors could not be ruled out. The results are in sharp contrast to Briggs and Turnbull (1995) who reported on the efficacy of Epicin in removing NO_2 concentration. The significant differences ($P<0.05$) in the levels of NO_2 in Biocult and Epicin treated tanks revealed the differences in the microbial activity. Probably, the Biocult

contained high levels of ammonia oxidizers that oxidize NH_3 effectively to produce NO_2 and Epicin contained bacteria that utilize NH_3 as a source of N_2 for growth. The nitrate levels increased in all the treatment tanks, but showed a general decreasing trend in control tanks (Table 1). Among the tanks with varied NH_3 concentrations, the levels of NO_3 between the control and treatment tanks differed insignificantly ($P>0.05$). The results of the present study also revealed significant differences ($P<0.05$) in NO_2 levels and no marked differences in NO_3 levels ($P>0.05$) between the control and the treated tanks.

A number of techniques have been developed for the control of TAN concentration in aquaculture system in recent years. Submerged flow biofilters (Abeysinghe *et al.*, 1996), high rate linear-path trickling nitrification filters (Twarowska *et al.*, 1997), bench-scale fluidized bed bioreactors (Ng *et al.*, 1996), continuous bioreactors using immobilized alginate beads (Kim *et al.*, 2000), pellet immobilization of nitrifying bacteria (Shan and Obbard, 2001) and other TAN removal approaches / products (Boyd *et al.*, 1984; Funge-Smith and Hawthorn, 1996; Moriarty, 1997; Prabhu *et al.*, 1999) have all been evaluated with varying degrees of success. Achieving a high rate of TAN removal under conditions of continuous TAN production can be a major challenge. At an experimental prawn farm, Chin and Ong (1997) achieved only a 25% TAN removal rate by combining a secondary treatment system with effluent biofiltration. Commercial bioremediator products for aquaculture use are available in plenty and questionable as suppliers of such products often overrate their potential (Young, 1976). Stephenson and Stephenson (1992) opined that inadequate substrate concentration and cell density, interspecific competition with indigenous microorganisms leading to growth inhibition, and an insufficient acclimatization period to affect bioremediation may lead to failure of inocula to function in aquaculture as they do in axenic culture. In general, the results of the present study revealed that the commercial bioremediators failed to remove majority of the total ammoniacal nitrogen when the NH_3 level is high initially.

Acknowledgement

The Indian Council of Agricultural Research, New Delhi under the National Agricultural Technology Project financed this study.

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Date of Receipt : 8-2-06
Date of Acceptance : 17-4-06