Aquacult Int
DOI 10.1007/s10499-008-9218-2

# Stringed bed suspended bioreactors (SBSBR) for in situ nitrification in penaeid and non-penaeid hatchery systems

**V. J. Rejish Kumar · Cini Achuthan · N. J. Manju · Rosamma Philip · I. S. Bright Singh**

Received: 17 January 2008 / Accepted: 1 September 2008


**Abstract** For establishing nitrification in prawn (non-penaeid, salinity 10–15 ppt) and shrimp (penaeid, salinity 30–35 ppt) larval production systems, a stringed bed suspended bioreactor (SBSBR) was designed, fabricated, and validated. It was fabricated with 5 mm polystyrene and low density polyethylene beads as the substrata for ammonia and nitrite oxidizing bacterial consortia, respectively, with an overall surface area of 684 $cm^2$. The reactors were activated in a prototype activator and were transported in polythene bags to the site of testing. Performance of the reactors activated with the nitrifying bacterial consortia AMONPCU-1 (ammonia oxidizers for non-penaeid culture) and NIONPCU-1 (nitrite oxidizers for non-penaeid culture) was evaluated in a *Macrobrachium rosenbergii* larval rearing system and those activated with AMOPCU-1 (ammonia oxidizers for penaeid culture) and NIOPCU-1 (nitrite oxidizers for penaeid culture) in a *Penaeus monodon* seed production system. Rapid setting up of nitrification could be observed in both the static systems which resulted in a higher relative per cent survival of larvae.

**Keywords** Closed system shrimp hatchery · Immobilization · Nitrification · Nitrifying bioreactors · Nitrifying consortia · Shrimp/prawn larval production

**Abbreviations**

| | |
|---|---|
| AMONPCU-1 | Ammonia oxidizers for non-penaeid culture |
| AMOPCU-1 | Ammonia oxidizers for penaeid culture |
| IHHNV | Infectious hypodermal and hematopoietic necrosis virus |

V. J. Rejish Kumar · C. Achuthan · N. J. Manju · I. S. Bright Singh (✉)
National Center for Aquatic Animal Health, Cochin University of Science and Technology, Lake Side Campus, Fine Arts Avenue, Cochin 682016, India
e-mail: bsingh@md3.vsnl.net.in

R. Philip
Department of Marine Biology, Microbiology and Biochemistry, School of Ocean Science and Technology, Cochin University of Science and Technology, Lake Side Campus, Fine Arts Avenue, Cochin 682016, India

| | |
|---|---|
| NBCPU | Nitrifying bacterial consortia production unit |
| NIONPCU-1 | Nitrite oxidizers for non-penaeid culture |
| NIOPCU-1 | Nitrite oxidizers for penaeid culture |
| RAS | Recirculating aquaculture systems |
| RPS | Relative percent survival |
| SBSBR | Stringed bed suspended bioreactor |
| TAN | Total ammonia nitrogen |
| TNN | Total nitrite nitrogen |
| WSSV | White spot syndrome virus |
| YHV | Yellow head virus |

## Introduction

High levels of ammonia and nitrite undermine commercial production objectives in the aquaculture industry as the toxic impacts are manifested through impaired growth or chronic diseases (Manthe et al. 1985; Cheng et al. 2004). The toxic effects of ammonia are more pronounced during the early developmental stages (Wajsbrot et al. 1993; Guillen et al. 1994) as it has been demonstrated that the tolerance of larval *Penaeus monodon* to ammonia increases as the larvae metamorphose to postlarvae. A conservative estimate of the "safe level" of ammonia for rearing larval *P. monodon* on the basis of an estimated 96 h $LC_{50}$ was 0.01 mg $l^{-1}$ $NH_3$–N (Chin and Chen 1987). Nitrite is reported to cause reduction of hemolymph oxyhemocyanin in *P. monodon* with a concomitant increase of $pO_2$ and $P_{50}$, indicating reduced affinity to oxygen (Cheng and Chen 1999). Likewise, ammonia and nitrite toxicity in *Macrobrachium rosenbergii* adults and larvae have been investigated by various researchers (Chen and Lee 1997; Cavalli et al. 2000; Wang et al. 2004; Naqvi et al. 2007) who felt the need for their regulation for successful larval production. Other than water exchange this can be achieved only through biological nitrification, for which it becomes essential to establish a stable nitrifying bacterial system for converting ammonia to nitrate as a mitigation measure of ammonia and nitrite toxicity. Nitrate is relatively harmless to finfish and shellfish (Russo and Thruston 1991).

In aquaculture systems, the serious issue of ammonia and nitrite toxicity can be addressed using rotating biological contactors, trickling biofilters, and fluidized bed bioreactors in RAS mode (Jewell and Cummings 1990; Nijhof and Bonverdeur 1990; Yang et al. 2001). Among them, fixed film biofilters are commonly used for TAN removal (Greiner and Timmons 1998; Singh et al. 1999). Recently, they have been reviewed (Eding et al. 2006; Colt 2006; Gutierrez-Wing and Malone 2006) and rating standards developed (Colt et al. 2006; Malone and Pfeiffer 2006). In such systems, polystyrene micro-bead packed beds are successfully used by several commercial growers (Timmons et al. 2006).

However, for establishing instantaneous nitrification in static larval production systems, a specialized nitrifying bioreactor other than the above was found to be required. This paved the way for the development of Stringed Bed Suspended Bioreactor (SBSBR) (PCT Patent application no. 828/DEL/2000/India), a technology commercialized through M/s Oriental Aquamarine Biotech India (P) Ltd., Coimbatore, India. In this device, specially designed polystyrene and polyethylene beads have been used as the substratum for immobilizing nitrifying bacterial consortia. The reactors were activated under laboratory conditions, transported to the prawn/shrimp hatchery systems, and tested and validated for their potential for rapid setting up of nitrification.

## Materials and methods

### Configuration of stringed bed suspended bioreactor

The reactor has four components: (1) an outer shell of 10 $cm^3$ with conical bottom; (2) an inner cartridge comprising a solid frame work and beads on strings with filter plates both at its top and bottom; (3) an airlift pump at the center of the filter plates; and (4) a black lid with perforations on top. Based on a previous study by Achuthan (2000), polystyrene and low density polyethylene beads of 5 mm diameter and a surface area of 0.785 $cm^2$ with spikes on the surface had been selected as the substrata for immobilizing ammonia and nitrite oxidizing consortia, respectively. On full assembly (Fig. 1), the cartridge with beads was inserted into the outer shell and the black lid was placed on top. The beads stringed in the reactor cartridge provided an overall surface area of >684 $cm^2$ to support the nitrifying biofilm. This is in addition to the inner surface of the shell and cartridge framework.

### The nitrifying bacterial consortia

Two ammonia oxidizing consortia, namely AMOPCU-1 (ammonia oxidizers for penaeid culture) and AMONPCU-1 (ammonia oxidizers for non-penaeid culture), and two nitrite oxidizing consortia, namely NIOPCU-1 (nitrite oxidizers for penaeid culture) and NIONPCU-1 (nitrite oxidizers for non-penaeid culture) (Achuthan et al. 2006) were used

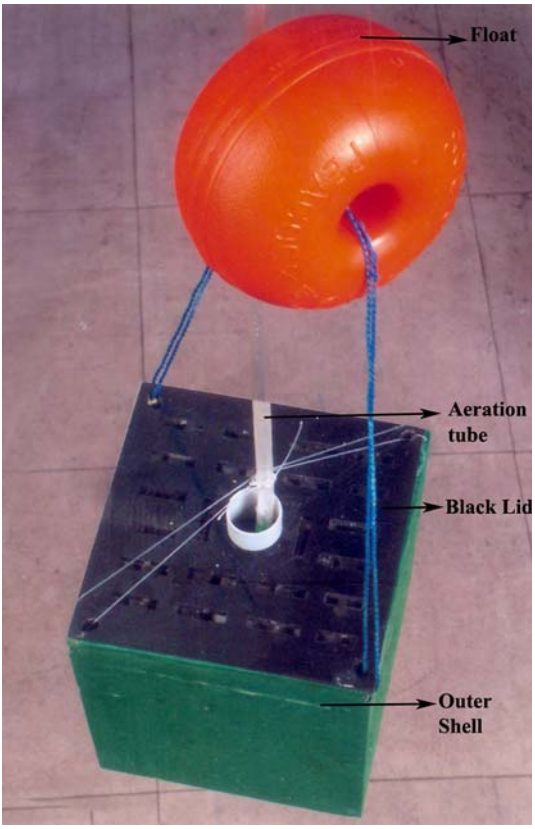


**Fig. 1** Stringed bed suspended bioreactor on full assembly

for activating the reactors. The consortia were acclimated to room temperature (27 ± 0.5°C) from refrigeration (4°C) in 250-ml conical flasks on a rotary shaker for 7 days, and subsequently amplified in a 2-l baby fermentor (New Brunswick, USA) at 28°C for 1 month under optimum pH (8.5 and 7.5) for ammonia and nitrite oxidizers, respectively. This was used as the inoculum for mass production in an indigenous 200-l nitrifying bacterial consortia production unit (NBCPU) under the same conditions (unpublished). A simple seawater base medium (100 ml) supplemented with 10 mg $l^{-1}$ substrate ($(NH_4)_2 \cdot SO_4/NaNO_2$) and 2 mg $l^{-1}$ $KH_2PO_4$ was used. The reactors for penaeid hatchery systems were activated maintaining salinity optima of 25–30 ppt, and non-penaeid systems under salinity of 10–15 ppt.

## Activation of the reactors with nitrifying bacterial consortia

For activation, the lid of the reactor was removed and the void volume filled with an aliquot of 750 ml of the respective nitrifying bacterial consortium, and clamped in place of a thermostatically controlled serological water-bath as the activator. The consortia were circulated through the cartridge by operating the airlift pump at a rate of 1 l $min^{-1}$. For avoiding the escape of aerosols during the operation, the top of the reactor was covered with 'aerosol arrestor', a cup-shaped device made of perspex having a central hole for extending aeration tubing to the airlift pump. The media composition and culture conditions were the same as above. During activation of the reactors, substrate uptake and product formation were monitored depending on the consortia used. When the reactors were under activation with ammonia oxidizers, $NH_4^+$–N and $NO_2^-$–N were determined daily as the substrate and product, respectively (Solorzano 1969; Bendschneider and Robinson 1952). $NO_3$–N was not determined during the period as the consortia used were without the potency to oxidize $NO_2^-$–N. When the reactors were under activation with nitrite oxidizers, $NO_2^-$–N and $NO_3$–N were determined as the substrate and product, respectively, following Bendschneider and Robinson (1952) and Strickland and Parsons (1968). During activation, the substrates were adjusted to the optimum 10 mg $l^{-1}$ (Achuthan et al. 2006). Substrate consumption and product formation, and the disappearance of the nitrifying bacterial biomass from bulk volume (gravimetric determination) were plotted against days of activation. To confirm attachment of the nitrifiers to the substratum and the reactor activation, the fluid in the reactor was drained off and replaced with sea water of the required salinity, supplemented with the substrates, and monitored for nitrification as described above.

## Transportation

Prior to transportation of the activated reactors, the aerosol arrestors were removed, the black lid on top of the reactor replaced, and the medium drained off leaving about 250 ml to maintain moisture inside, then tied securely in a polythene bag. On reaching the site, the reactors were suspended from a float through a string 30 cm below the water level. Two tanks of 2,000-l capacity each were maintained, one with reactors and the other as control without the reactors.

## Demonstration and validation of the reactors

Performance of the reactors activated with AMOPCU-1 and NIOPCU-1 were evaluated in the *P. monodon* hatchery system of Matsyafed, Ponnani, Kerala, India, and the ones with

AMONPCU-1 and NIONPCU-1 in the *M. rosenbergii* hatchery of M/s Rosen Fisheries, Trichur, Kerala, India.

The reactors were deployed in the larval rearing tanks connected to the air supply of the hatcheries with air flow regulated using an airflow meter to 1 l $min^{-1}$. In this operational mode, water entered the reactor through the perforations on top of the black lid, passed through the cartridge, and came out through the airlift pump.

The control and test tanks (2000 l) of *M. rosenbergii* larval production system with 250 l filtered diluted seawater having a salinity of 15 ppt were stocked with 0.10 million mysis individually. The reactors (2 ammonia-oxidizing and 2 nitrite-oxidizing) were deployed, and physical, chemical, and biological parameters were quantified once every 3 days. Every day for 10 days, addition of 200 l filtered, chlorinated–dechlorinated seawater having salinity 15 ppt brought the water level to the maximum capacity of 2000 l. During this period, the larvae were fed with freshly hatched *Artemia* nauplii. It took 30 days for larvae to metamorphose to post larvae.

In the *P. monodon* hatchery, the same operation was adopted except for the use of seawater having 30 ppt salinity. Larvae were fed with *Chaetoceros*, *Spirulina*, and commercial Zoea/Mysis feed, and post larvae with newly hatched *Artemia* nauplii. During the period of larval rearing, there was no water exchange in either system. The experiment was repeated three times.

Physical and chemical parameters measured during the experiment other than the substrates and products of nitrification were salinity (Refractometer, Erma-Japan), pH (pH probe, Euteck-Singapore), alkalinity, and hardness (APHA 1998). Heterotrophic bacterial population was estimated following standard spread plate method employing ZoBell's agar 2216 E prepared in seawater of corresponding salinity. On completion of the experiment (when the post larvae of *M. rosenbergii* attained the stage PL 5 and *P. monodon* PL 15), overall survivals in both the control and experimental sets of tanks were estimated numerically. Relative per cent survival was calculated according to the equation (Gram et al. 1999):

$$\text{R.P.S} = \{1 - (\%\ \text{mortality in the tank with the reactor} / \%\ \text{mortality in the control tank without the reactor})\} \times 100.$$

The validation experiments were repeated three times and the significance of variation in nitrification and larval survival rates between the control and test systems were analyzed using one-way analysis of variance.

## Results

### Activation of the reactors

Progress of nitrification during activation of the reactors is presented in Fig. 2. Nitrification could be established in all reactors within 24 h of initiation of the process, and there was progressive reduction in the suspended biomass and increase in $NO_2^-$–N and $NO_3$–N in the reactors activated with ammonia and nitrite oxidizers, respectively. By the 7th day, more than 90% of the bacterial biomass had disappeared from the activation medium with a removal of 80.7 and 78.1% ammonia and 76.2 and 75.3% nitrite in the reactors meant for *M. rosenbergii* and *P. monodon* larval production systems, respectively. The average

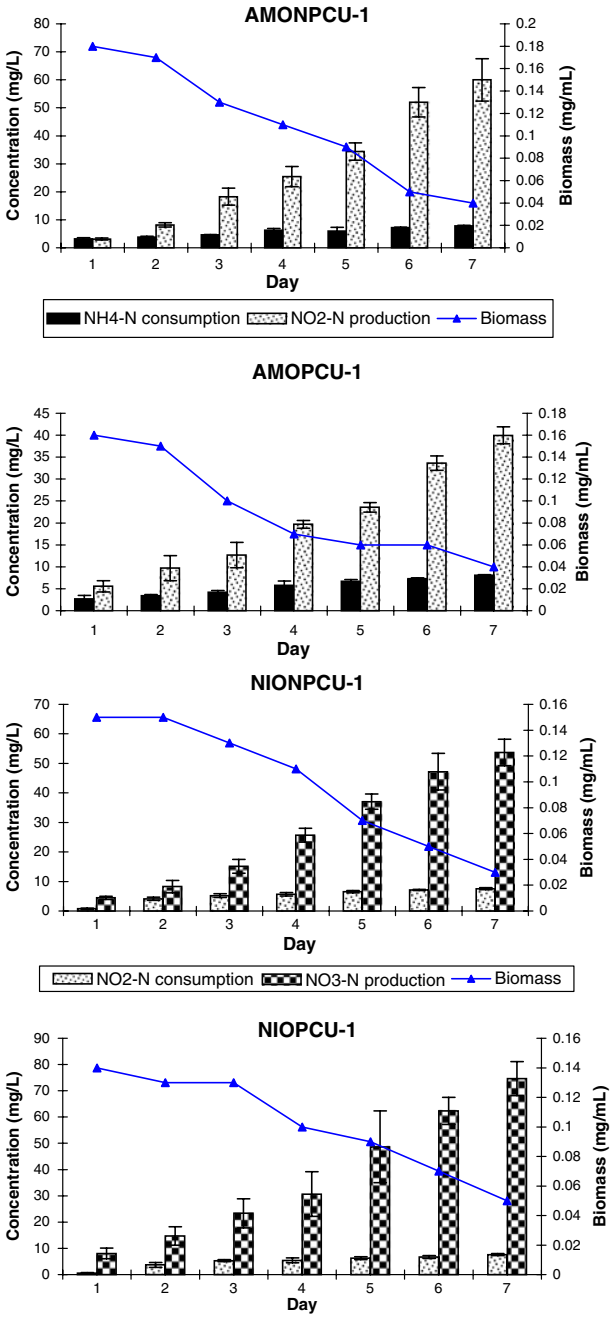


**Fig. 2** Activation kinetics of SBSBR immobilized with nitrifying consortia developed for *Macrobrachium rosenbergii* and *Penaeus monodon* hatchery systems

ammonia and nitrite removal rates at the end of the activation period were 0.88 and 0.86 g TAN $m^{-2}$ $day^{-1}$ and 0.84 and 0.83 g TNN $m^{-2}$ $day^{-1}$, respectively.

The activated reactors subsequent to deployment in the experimental larval rearing tanks their performance in establishing nitrification instantaneously was evaluated. The

water quality parameters measured during the process in the experimental and control tanks are summarized in Table 1. During validation of the reactors, nitrification was found established in the experimental tanks instantly and progressed rapidly from the 3rd day onwards (Figs. 3, 4). There was significant ammonia ($P < 0.001$) and nitrite ($P < 0.05$) removal in both the larval production systems compared to that in the control, where ammonia oxidation was found to have set in only after 8 days of commencement of the experiment with no nitrite oxidation. Meanwhile, in the larval rearing tanks with the reactors, $NH_4^+$–N and $NO_2^-$–N were not found to have built up; instead, there was a steady increase of $NO_3$–N. This proved establishment of the two-step nitrification process in larval rearing tanks deployed with the reactors. During the experiment, ammonia and nitrite concentrations in the control tanks were found to have gone up above 2 and 1.6 mg $l^{-1}$, respectively, whereas in the test tanks both were always below 0.25 mg $l^{-1}$. In both the systems, there were no significant differences in the other parameters between the control and test tanks except a slight decrease in alkalinity in the experimental tanks and the moderately higher total bacterial population in the control tanks (Table 1).

On terminating the experiment at PL 5 in the case of *M. rosenbergii* and at PL 15 in the case of *P. monodon*, the relative per cent survival was found to be significantly higher ($P < 0.01$) in the experimental tanks with the activated bioreactors (Table 2).

## Discussion

In biological ammonia removal systems, nitrifying activity of suspended bacteria has been reported to be extremely low, due to slow growth rate and inhibition of nitrification by free ammonia and nitrite under the alkaline conditions of seawater (Bower and Turner 1981; Furukawa et al. 1993). Without the addition of nitrifiers as start-up cultures, 2–3 months are needed to establish nitrification in marine systems (Manthe and Malone 1987) and 2–3 weeks in freshwater (Masser et al. 1999). There is an agreement among researchers and between laboratory research and commercial applications that saltwater systems need a much longer start-up period. Under such situations, immobilization techniques have been found useful to overcome the delay in the initiation of nitrification (Sung-Koo et al. 2000). Integration of activated SBSBRs to prawn and shrimp hatcheries was found to be an

**Table 1** Physico-chemical and microbial quality of rearing water in the hatchery during the experiment ($n = 3$)

| Rearing water quality | *Penaeus monodon* system | | *Macrobrachium rosenbergii* system | |
|---|---|---|---|---|
| | Test tank | Control tank | Test tank | Control tank |
| pH | 7.5–8 | 7.5–8 | 7.5–8 | 7.5–8 |
| Salinity (ppt) | 30–32 | 30–32 | 14–16 | 14–16 |
| Temperature (°C) | 27–29 | 27–29 | 28–31 | 28–31 |
| Alkalinity (mg of $CaCO_3$ $l^{-1}$) | 60–69 | 72–78 | 62–70 | 65–70 |
| Hardness (mg of $CaCO_3$ $l^{-1}$) | 5,790–5,820 | 5,790–5,950 | 2,190–2,260 | 2,100–2,277 |
| TPC (CFU $ml^{-1}$) | $1.43 \times 10^6$–$1.82 \times 10^8$ | $1.59 \times 10^6$–$3.78 \times 10^9$ | $4.51 \times 10^5$–$2.27 \times 10^7$ | $1.73 \times 10^5$–$1.03 \times 10^9$ |

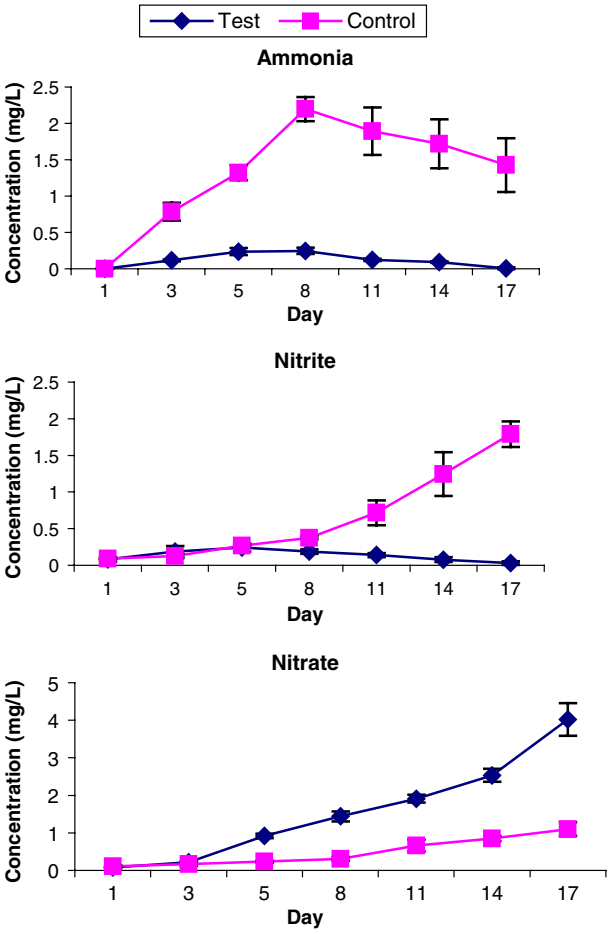


**Fig. 3** Performance of SBSBR in *M. rosenbergii* hatchery systems

important means of overcoming this difficulty in systems which are static where rapid setting up of nitrification is required.

In terms of TAN removal rates per unit of media surface area, Westerman et al. (1993) reported 0.25 g TAN removal $m^{-2}$ $day^{-1}$ for the rotating biological contactors and 0.1–0.15 g TAN $m^{-2}$ $day^{-1}$ for up-flow sand bed filters. For a trickling filter, Van Rijn and Rivera (1990) reported a removal rate of 0.43 g TAN $m^{-2}$ $day^{-1}$. The average TAN removal rates (g TAN $m^{-2}$ $day^{-1)}$ reported for frequently used biofilters in aquaculture systems are rotating biological contactors 0.19–0.79, trickling filters 0.24–0.64, bead filters 0.30–0.60, and fluidized sand filter 0.24 (Crab et al. 2007). The average TAN and TNN removal rates of SBSBRs during the activation period were higher than the reported values.

Maintenance of ammonia and nitrite during larval rearing is crucial as they cause lethal and sublethal toxicity and plays an important role in the production of healthy and properly sized fingerlings (Fielder and Allan 1997). Marine larval systems can demand TAN and TNN levels below 1.0 mg $l^{-1}$ well below the maximum set for the oligotrophic category (0.3 mg $l^{-1}$ N) (Malone and Beecher 2000). During the validation of the reactors, TAN and TNN could be maintained below 0.25 mg $l^{-1}$.

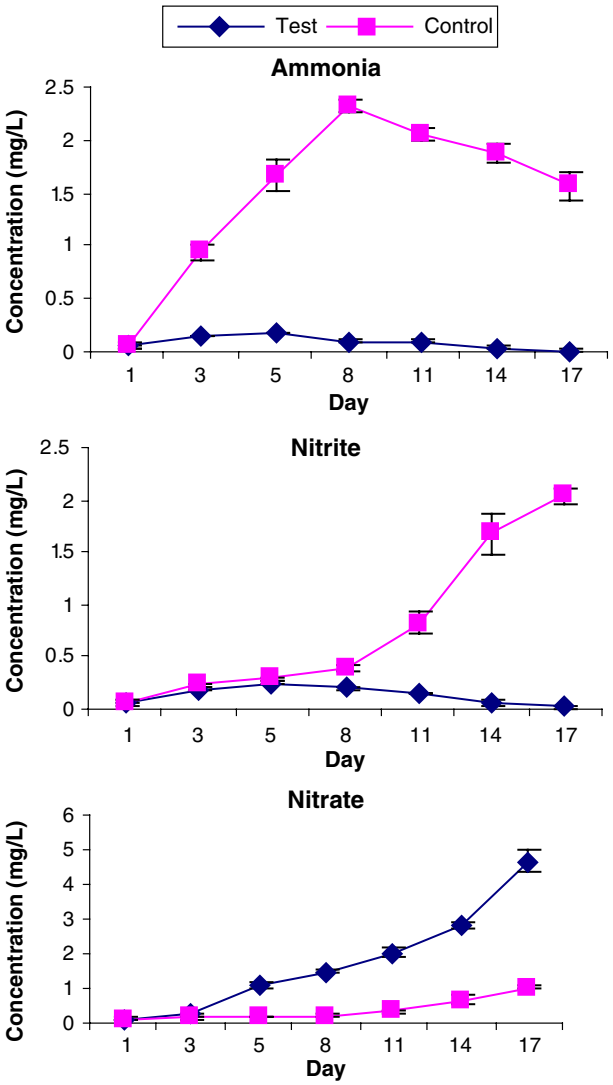


**Fig. 4** Performance of SBSBR in *P. monodon* hatchery systems

**Table 2** Impact of SBSBR in larval survival on integration in to hatchery systems ($n = 3$)

| Hatchery system | Treatment | Survival (%) | *P* | Relative survival (%) |
|---|---|---|---|---|
| *P. monodon* | Control tank | 16.33 ± 1.53 | <0.01 | 17.67 |
| | Test tank | 31 ± 1.73 | | |
| *M. rosenbergii* | Control tank | 19.67 ± 1.53 | <0.01 | 20.67 |
| | Test tank | 36.33 ± 3.51 | | |

The SBSBRs described here are designed for setting up nitrification in shrimp/prawn larval rearing tanks during the operation without either water exchange or under RAS mode. The technology is relatively user friendly in the sense that they can easily be lifted

out of water during disinfection and can also be shifted from one rearing tank to another. Loss of beads encountered in many biofilter systems (Timmons et al. 2006) are not experienced in the case of SBSBRs as the beads are strung together within the reactors. Moreover, the operational costs of the reactors are minimal and no energy costs are added up to the overall production cost as the aeration system already available in the hatchery are used for operating the airlift pumps. Especially, SBSBRs are meant for small-scale larval rearing systems rather than bigger systems where handling of huge quantity of water is warranted. SBSBRs can also find application during the live transportation of spawners as their survival is diminished mainly due to the ammonia they produce (Babu and Marian 1998). The reactors are sufficiently small enough to be incorporated in the transportation containers.

**Acknowledgments** This work was carried out with the financial assistance from Department of Biotechnology, Government of India, under the project no BT/AA/03ds/79/94 and BT/PR 179/AAQ/03/092/99. M/s Rosen Fisheries, Trichur, Kerala and Matsyafed Shrimp Hatchery, Ponnani, Kerala are gratefully acknowledged for extending the larval rearing facility for the demonstration and validation. The technical assistance extended by Mr. Anishmon, Project Assistant, NCAAH, is acknowledged.

## References

Achuthan C (2000) Development of bioreactors for nitrifying water in closed system hatcheries of penaeid and non-penaeid prawns. PhD Thesis, Cochin University of Science and Technology, Kochi, India

Achuthan C, Rejish Kumar VJ, Manju NJ et al (2006) Development of nitrifying bacterial consortia from Indian waters for immobilizing in nitrifying bioreactors designed for penaeid and non-penaeid larval rearing systems in the tropics. Indian J Mar Sci 35:240–248

APHA (1998) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, Washington

Babu MM, Marian MP (1998) Live transport of gravid *Penaeus indicus* using coconut mesocarp dust. Aquacult Eng 18:149–155. doi:10.1016/S0144-8609(98)00022-3

Bendschneider K, Robinson RJ (1952) A spectrophotometric method for the determination of nitrite in seawater. J Mar Res 11:87

Bower CE, Turner DT (1981) Accelerated nitrification in new seawater culture systems: effectiveness of commercial additives and seed media from established system. Aquaculture 24:1–9. doi:10.1016/0044-8486(81)90038-7

Cavalli RO, Berghe EV, Lavens P et al (2000) Ammonia toxicity as a criterion for the evaluation of larval quality in the prawn *Macrobrachium rosenbergii*. Comp Biochem Physiol C 125:333–343

Chen JC, Lee Y (1997) Effects of nitrite on mortality, ion regulation and acid-base balance of *Macrobrachium rosenbergii* at different external chloride concentrations. Aquat Toxicol 39:291–305. doi:10.1016/S0166-445X(97)00029-5

Cheng SY, Chen JC (1999) Hemolymph oxygen affinity and the fractionation of oxyhemocyanin and deoxyhemocyanin for *Penaeus monodon* exposed to elevated nitrite. Aquat Toxicol 45:35–46. doi:10.1016/S0166-445X(98)00090-3

Cheng W, Hsiao IS, Chen JC (2004) Effect of nitrite on immune response of Taiwan abalone *Haliotis diversicolor supertexta* and its susceptibility to *Vibrio parahaemolyticus*. Dis Aquat Organ 60:157–164. doi:10.3354/dao060157

Chin TS, Chen JC (1987) Acute toxicity of ammonia to larvae of the Tiger prawn, *Penaeus monodon*. Aquaculture 66:247–253. doi:10.1016/0044-8486(87)90110-4

Colt J (2006) Water quality requirements for reuse systems. Aquacult Eng 34:143–156. doi:10.1016/j.aquaeng.2005.08.011

Colt J, Lamoureux J, Patterson R et al (2006) Reporting standards for biofilter performance studies. Aquacult Eng 34:377–388. doi:10.1016/j.aquaeng.2005.09.002

Crab R, Avnimelech Y, Defoirdt T et al (2007) Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270:1–14. doi:10.1016/j.aquaculture.2007.05.006

Eding EH, Kamstra A, Verreth JAJ et al (2006) Design and operation of nitrifying trickling filters in recirculating aquaculture: a review. Aquacult Eng 34:234–260. doi:10.1016/j.aquaeng.2005.09.007

Fielder S, Allan GL (1997) Inland production of marine fish. In: Hyde K (ed) The new rural industries: a hand book for farmers and investors. Rural Industries Research and Development Corporation, Australia, pp 108–113

Furukawa K, Ike A, Ryu S et al (1993) Nitrification of $NH_4$–$N^+$ polluted seawater by immobilized acclimated marine nitrifying sludge (AMNS). J Ferment Bioeng 76:515–520. doi:10.1016/0922-338X(93)90251-3

Gram L, Melchiorsen J, Spanggaard B et al (1999) Inhibition of *Vibrio anguillarum* by *Pseudomonas fluorescens* AH2, a possible probiotic treatment of fish. Appl Environ Microbiol 65:969–973

Greiner AD, Timmons MB (1998) Evaluation of the nitrification rates of microbead and trickling filters in an intensive recirculating tilapia production facility. Aquacult Eng 18:189–200. doi:10.1016/S0144-8609(98)00030-2

Guillen JL, Endo M, Turnball JF et al (1994) Skin-responses and mortalities in the larvae of Japanese croaker exposed to ammonia. Fish Sci 60:547–550

Gutierrez-Wing MT, Malone RF (2006) Biological filters in aquaculture: trends and research directions for freshwater and marine applications. Aquacult Eng 34:163–171. doi:10.1016/j.aquaeng.2005.08.003

Jewell WJ, Cummings RJ (1990) Expanded bed treatment of complete recycle aquaculture system. Water Sci Technol 22:443–450

Malone RF, Beecher LE (2000) Use of floating bead filters to recondition recirculating waters in warmwater aquaculture production systems. Aquacult Eng 22:57–74. doi:10.1016/S0144-8609(00)00032-7

Malone RF, Pfeiffer JP (2006) Rating fixed film nitrifying biofilters used in recirculating aquaculture systems. Aquacult Eng 34:389–402. doi:10.1016/j.aquaeng.2005.08.007

Manthe DP, Malone RF (1987) Chemical addition for accelerated biological filter acclimation in closed bluecrab shedding systems. Aquacult Eng 6:227–236. doi:10.1016/0144-8609(87)90006-9

Manthe DP, Malone RF, Perry H (1985) Water quality fluctuations in response to variable loading in a commercial closed blue crab shedding system. J Shellfish Res 3:175–182

Masser MP, Rakocy J, Losordo TM (1999) Recirculating aquaculture tank production systems. Management of recirculating systems. SRAC Publication No. 452 USDA

Naqvi AA, Adhikari S, Pillai BR et al (2007) Effect of ammonia-N on growth and feeding *Macrobrachium rosenbergii* (De-Man). Aquacult Res 38:847–851. doi:10.1111/j.1365-2109.2007.01736.x

Nijhof M, Bonverdeur J (1990) Fixed film nitrification characteristics in sea-water recirculating fish culture system. Aquaculture 87:133–143. doi:10.1016/0044-8486(90)90270-W

Russo RC, Thruston RV (1991) Toxicity of ammonia, nitrite and nitrate to fishes. In: Brune DE, Tomasso JR (eds) Aquaculture and water quality advances in world aquaculture, 3. World Aquaculture Society, Baton Rouge, LA, pp 58–89

Singh S, Ebeling J, Wheaton F (1999) Water quality trials in four recirculating aquacultural system configurations. Aquacult Eng 20:75–84. doi:10.1016/S0144-8609(99)00003-5

Solorzano L (1969) Determination of ammonia in natural waters by the phenol hypochlorite method. Limnol Oceanogr 14:799–801

Strickland JDH, Parsons TR (1968) A practical handbook of seawater analysis, 2nd edn. Fisheries Research Board Canada. Bulletin 167

Sung-Koo K, Kong I, Lee B et al (2000) Removal of ammonium–N from a recirculation aquaculture system using an immobilized nitrifier. Aquacult Eng 21:139–150. doi:10.1016/S0144-8609(99)00026-6

Timmons MB, Holder JL, Ebeling JM (2006) Application of microbead biological filters. Aquacult Eng 34:332–343. doi:10.1016/j.aquaeng.2005.07.003

Van Rijn J, Rivera G (1990) Aerobic and anaerobic biofilteration in an aquaculture unit—nitrite accumulation as a result of nitrification and denitrification. Aquacult Eng 9:217–234. doi:10.1016/0144-8609(90)90017-T

Wajsbrot N, Gasith A, Diamant A et al (1993) Chronic toxicity of ammonia to juvenile gilthead seabream *Sparus aurata* and related histopathological effects. J Fish Biol 42:321–328. doi:10.1111/j.1095-8649.1993.tb00336.x

Wang WN, Wang AL, Zhang YJ et al (2004) Effects of nitrite on lethal and immune response of *Macrobrachium nipponense*. Aquaculture 232:679–686. doi:10.1016/j.aquaculture.2003.08.018

Westerman PW, Losordo TM, Wildhaber ML (1993) Evaluation of various biofilters in an intensive recirculation fish production facility. In: Techniques for modern aquaculture. Proceedings of an aquacultural engineering conference, Wa. ASAE, St. Joseph, MI, 21–23 June 1993, pp 315–325

Yang L, Chou LS, Shiek WK (2001) Biofilter treatment of aquaculture water for reuse applications. Water Res 35:3097–3108. doi:10.1016/S0043-1354(01)00036-7