Chapter 5

VALIDATION OF THE NITRIFYING BIOREACTORS IN RECIRCULATING AQUACULTURE SYSTEMS

Contents

5.1. Introduction
5.2. Materials and Methods

5.2.1. Stringed Bed Suspended Bioreactors in recirculating aquaculture systems
5.2.2. Packed Bed Bioreactors in Recirculating Aquaculture Systems
5.2.3. A Recirculating Aquaculture System for Penaeus monodon: Laboratory Level Demonstration

5.3. Results and Discussions

5.3.1. Validation of Stringed Bed Suspended Bioreactors
5.3.2. Validation of Packed Bed Bioreactors
5.3.3. Laboratory Level Recirculating Aquaculture System for Penaeus monodon
5.1 Introduction

On assuming the dimensions of an industry, the aquaculture systems are bound to operate under strict environmental safety standards. Stringent regulatory guidelines focusing on discharges to natural water bodies will force hatchery operators to adopt methods that are environment friendly (White et al., 2004). With high land and water costs, these systems are designed to maintain high biological carrying capacity in relatively little space with minimal water exchange. Recirculating Aquaculture System (RAS) can reduce the effluent waste stream by a factor of 500-1000 (Chen et al., 1997; Timmons et al., 2001) allowing existing operations to upgrade and expand and comply with future regulations. RAS allow companies to 1) be competitive in both domestic and world commodity markets by locating production closer to markets, 2) improve environmental control, 3) reduce catastrophic losses due to diseases, 4) avoid violation of environmental regulations on effluent discharge 5) reduce management and labour costs, and 6) improve product quality and consistency (Lee, 1995). The use of RAS is gaining wide acceptance in view of the expanding marine production and the demand for biosecure systems to produce larvae, fry and fingerlings for grow out systems (Turk et al., 1997; Watanabe et al., 1998; Malone, 2002; Otoshi et al., 2003; Pruder, 2004). The water quality expectations for these systems can fall well below the 0.3 g-N/m³ TAN standard set for the oligotrophic classification. Most marine hatchery systems require oligotrophic water quality conditions in order to maintain a healthy stock (Table.1). Larval systems typically require TAN concentrations less than 0.1 g-N/m³ and fingerlings typically require a TAN concentration less than 0.5 g-N/m³ (Malone and Beecher, 2000).

The high values associated with fingerlings and marine and freshwater ornamentals are also promoting adoption of recirculating technologies (Howerton, 2001; Palmtag and Holt, 2001; Gutierrez-Wing and Malone, 2006).
Table 1. Ideal water quality ranges for *Penaeus monodon* maturation, hatchery facility and hatchery effluent standards (FAO, 2007)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Maturation/hatchery facility</th>
<th>Hatchery effluent standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp (°C)</td>
<td>28-32</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>7.8-8.2</td>
<td>6-9.5</td>
</tr>
<tr>
<td>Total ammonia-nitrogen (mg/L)</td>
<td>-</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Ammonia (NH₃) (mg/L)</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>Nitrite (NO₂) (mg/L)</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Nitrate (NO₃) (mg/L)</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Oxygen (mg/L)</td>
<td>&gt;4</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Iron (mg/L)</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Hydrogen Sulphide (H₂S) (mg/L)</td>
<td>&lt;0.003</td>
<td>-</td>
</tr>
<tr>
<td>5-day BOD (mg/L)</td>
<td>-</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

However, RAS for producing freshwater commodity food fish are unable to compete directly in terms of cost with pond or flow-through systems and this turns out to be the main obstacle for a wider adoption of RAS technologies in freshwater applications (Timmons and Losordo, 1994; Lorsordo and Westeman, 1994; Malone, 2002). While it may be relatively straightforward to culture large numbers of seed animals in hatcheries, the poor quality of juveniles may limit the effectiveness of any release programme (Vay et al., 2007) as the environmental conditions and husbandry practices within the hatchery as well as broodstock and larval nutrition happen to be the factors which influence the quality of offspring. Under such circumstances maintenance of environmental quality during larval rearing becomes critically important and RAS can satisfy such requirements provided the system is equipped with the necessary devices.
Coming to shrimp culture, its future success as an industry is dependent on the increasing supplies of healthy, high quality seed for stocking ponds. All types of commercial penaeid larval culture systems have been plagued by problems with pathogenic microorganisms (Browdy, 1998). In these systems, high water exchange rates flush out bacterial populations destabilizing microbial community structure and opening up niches for more pathogens. With the greater demand for post larvae, there happened a cascade of events which lead to increased hatchery development, and higher stocking density opening up multitudes of problems in maintaining balance equilibrium of bacterial communities. Sterilization of incoming water in fact destroys all beneficial bacteria and opens niches for whichever relatively pathogenic or benign strain colonizes first. In most cases, antibiotics were used as the treatment of choice with well known negative consequences. These include elimination of both beneficial and pathogenic strains further destabilizing microbial communities and the development and spread of resistant strains which increase problems within hatcheries and pose potential public health concerns. A study by Otoshi et al. (2003) indicated that broodstock shrimp can be cultured in a biosecure RAS while maintaining good growth and high survival. In addition, rearing broodstock in a biosecure RAS does not negatively affect reproductive performance and may facilitate the development of Specific Pathogen Free (SPF) captive breeding programmes. Many recirculating system designs for production of marine shrimp have been published (Mock et al., 1977; Neal and Mock, 1979; Reid and Arnold, 1992; Davis and Arnold, 1998; Tseng et al., 1998); however, are with limitations especially regarding total nitrogen removal. Menasveta et al. (1989, 1991), Chen et al. (1989) and Millamena et al. (1991) reported the development of closed, recirculating seawater systems for black tiger shrimp maturation.

*M. rosenbergii*, though an inhabitant of freshwater, its larval stages require brackishwater for growth and survival. Extensive research carried out in the seed production and culture led to the 'green water' method of seed production and many advances were also made in hatchery technology (Chowdhury et al.,
Recirculating systems indeed have shown to give consistent production of quality post-larvae. In India *M. rosenbergii* is cultured in an area of 34,630 ha with an average production ranging from 880 to 1250 kg/ha. To support the scampi industry in India, there are 71 hatcheries spread across the country supplying 183 billion scampi seed (Bojan, 2007). Two decades ago, most hatcheries were operating on flow-through systems. However, with the establishment of inland hatcheries, the costs of obtaining and transporting seawater or brine, and increasing concerns about the discharge of saline water in inland areas have encouraged many to minimize water consumption through either partial or full recirculating systems (New, 2002). Consequently several attempts have been made to develop and optimize recirculation in aquaculture systems focusing on total ammonia nitrogen (TAN) as the key limiting water quality parameter (Losordo and Westers, 1994; Lyssenko and Wheaton, 2006).

An efficient biofilter is the central component in RAS to maintain the water quality. Proper selection and sizing of biofilters are critical to both the technical and economic success of a recirculating aquaculture system. To satisfy this requirement wide variety of biofilters are available and as such the variability of conditions under which a given biofiltration platform is expected to perform is a matter of concern (Malone and Pfeiffer, 2006). However, there will be an increase in demand for cost effective biofilters with expanded use of RAS (Gutierrez-Wing and Malone, 2006), especially with oligotrophic and ultraoligotrophic technologies for marine nurseries and emphasis needs to be placed on their sizing in support of nursery operations. Biological filters are essential components of recirculation systems for freshwater prawn hatcheries also and many types of biofilters are available for use in freshwater systems (Valenti and Daniels, 2000; New, 2002). Some of the major limiting parameters in the development of biosecure, closed recirculating aquaculture filtration technology are the cost-effective removal of particulates and nitrogen from the recirculating water (Malone and DeLosReyes, 1997). As pointed out earlier, biofilter performance studies are difficult to be conducted due to the large number of parameters that must be controlled and the
Validation of the nitrifying bioreactors in recirculating aquaculture systems

number of measurements that must be completed (Colt et al., 2006). Nitrification capacity of a biofilter is complicated by the sensitivity of the nitrifying population to a variety of water quality factors (Belser, 1979). The loading history and environmental conditions to which the biofilm has been subjected needs to be given careful consideration when evaluating a filter’s nitrification performance (Malone and Pfeiffer, 2006). The simplest rate of nitrification that can be calculated for a biofilter is simply the mass conversion rate of nitrogen from one form to another or the gross nitrification rate. The generation of ammonia within a recirculating system depends on the feeding rate, protein content of the feed, fraction of protein nitrogen that is excreted as TAN and the rate of TAN excretion.

Although great efforts have been made on the investigation of nitrifying biofilters for aquaculture applications, the research have mostly been focusing on performance of an individual component under specific operating conditions using average ammonia removal rate to describe the biofilter nitrification performance. The reported nitrification rates of biofilters varied among systems depending on operating conditions and ammonia loadings. The Volumetric TAN Removal Rates (VTR) is usually used to express the efficiency of the biofilter. The VTR does not require an estimate of the total active surface area of media and can be used directly for system design. Three statistical problems have been identified when reporting VTR values such as the statistical definition of steady-state TAN removal, the randomization of treatments, and the proper replication of experimental units during experimental design (Colt et al., 2006).

Previous studies have shown that an acclimation period is necessary before a biofilter begins to operate properly in marine systems provided they are not inoculated with pure or mixed cultures of nitrifiers. Normal acclimation times can be expected to be of the order of 2–3 weeks for freshwater systems (Masser et al., 1999). However marine systems frequently seem to stop the nitrification in the ammonia oxidizing stage of acclimation. It is not uncommon to see systems with persistent nitrite accumulations for periods as long as 3–4 months (Manthe and
Malone, 1987). It may take 60 days for a new filter to approach steady-state conditions, especially with some category of plastic media in sea water (Colt et al., 2006). However, the Packed Bed and Stringed Bed Suspended reactors described in the previous chapters were found to set up instant nitrification on integrating the activated reactors into tropical hatcheries making theses systems 100% closed recirculation systems (Singh et al., 2007).

The recirculating aquaculture systems are usually operated as closed systems and many essential nutrients progressively getting depleted from water column are replaced/replenished from the digestive/excretory metabolites or feed contaminants and subsequently get accumulated to toxic levels with time (McNeil, 2000). This suggests the requirement of waste management in recirculation systems, a serious concern, as it leads to problems connected with bad water quality and disease outbreak. To tide over the situation the practice of applying viable bacteria and their products has been introduced to regulate water presuming that the added bacteria produce greater quantities of a range of exoenzymes breaking down organic compounds (Moriarty, 1997). Moreover the added organisms stabilize or enhance a microbial community in the gastrointestinal tract and within the culture system favourable to the animal, so as to improve growth, survival, and disease resistance (Douillet, 2000; Karunasagar et al., 2000; Sonnenholzner and Boyd, 2000; Horowitz and Horowitz, 2001). Adequate scientific evidence could be cited concerning the beneficial effects of probiotics in clear water hatchery conditions (Fegan, 2000). Our previous studies have shown that ‘Detrodigest’, an indigenous probiotic preparation containing the bacterium *Bacillus* MCCB101 (GenBank accession no. EF062509), ‘Enterotrophotic’ a gut probiotic preparation composed of *Bacillus* MCCB 101 and *Micrococcus* MCCB 104 (Jayaprakash et al., 2005) could maintain the water quality and animal health and control the *Vibrio* population in penaeid and non-penaeid culture systems. Therefore, the operation of biofilters along with the application of indigenous probiotics can pave the way for a well defined biosecure recirculation larval production system that totally eliminates application of chemical disinfectants,
antibiotics and other chemotherapeutic agents facilitating organic shrimp/prawn seed production (Singh et al., 2004). In the present study both SBSBR and PBBR were validated under various conditions. Validation of SBSBR was accomplished under laboratory conditions in the maintenance of adult and brood stock of *Penaeus monodon* and larval production systems of *P. monodon* and *Macrobrachium rosenbergii*. Meanwhile PBBR was validated in a *P. monodon* commercial maturation system, *M. rosenbergii* larval production system and laboratory level recirculating system for intensive post larval rearing to juveniles to obtain disease free animal for study.

5.2 Materials and methods

5.2.1 Stringed bed suspended bioreactors in recirculating aquaculture systems

5.2.1.1 Laboratory maintenance of *penaeus monodon* adults and brooders

Under this series of study two categories of experiments were conducted. In the initial case adults of *P. monodon* which were brought to the laboratory for various experiments were maintained without water exchange after deploying SBSBRs. The animals (10 numbers) weighing 20 g each were maintained in a volume of 150 L at a salinity of 15 ppt supported with 2 SBSBRs. The animals were fed with pelleted feed together with steamed clam meat. The bioaugmentor Detrodigest and antagonistic probiotic *Micrococcus* MCCB 104 were applied at the rate of 10 mL per week. ‘Detrodigest’ is an indigenous probiotic preparation containing the bacterium *Bacillus* MCCB101 (GenBank accession no. EF062509) having $10^9$-$10^{12}$ cells/mL. Alkalinity destruction due to nitrification was compensated by the addition of Ca$_2$CO$_3$ as and when required. The shrimps were maintained for more than a month till they were taken for experiments.

In the second category of experiments *P. monodon* brooders (4) weighing approximately 150 g were maintained in a 200L tank integrated with 2 SBSBRs at 32 ppt salinity for 30 days. The brooders were fed intermittently with polychaetes (15g), with pelleted feeds (5 g) and steamed clam meat (10 g) in total a day. As described above Detrodigest and *Micrococcus* MCCB 104 were applied at the rate of 10 mL per week. Here also alkalinity destruction due to nitrification
was compensated by the addition of CaCO₃ periodically. The brooders were maintained for a period of one month.

In both the above systems TAN, nitrite and nitrate concentration pH, temperature alkalinity and hardness were monitored every day. The heterotrophic bacterial community of the rearing water was determined once in a week by standard spread plate method employing ZoBell’s Marine agar 2216 E prepared in seawater of salinity 30ppt. *Vibrio* counts were estimated using TCBS Agar plates.

### 5.2.1.2 Larval production of *Macrobrachium rosenbergii* and *Penaeus monodon*

The reactors were validated in two larval production systems such as *P. monodon* hatchery system of Matsyafed, Ponnani, Kerala and *M. rosenbergii* Hatchery of M/s Rosen Fisheries, Trichur, Kerala. As the systems were operating under two saline conditions such as 30 and 15 ppt the reactors were activated with AMOPCU-1 and NIONPCU-1 and; AMONPCU-1 and NIONPCU-1 respectively. 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 and tied securely in a polythene bag. On reaching the site the reactors were suspended from a float through a string 1 foot below the water level. Two tanks of 2000 L capacity each were maintained, one with reactors and the other as control without the reactors. After deploying the reactors to the larval rearing tanks they were connected to the air supply of hatchery and flow regulated using an airflow meter, to 1 L/ min. In this mode, water enters the reactor through the perforations on top of the black lid, passes through the cartridge, and comes out through the airlift pump.

In the *M. rosenbergii* larval production system, the two 2 tonne tanks brought under the study were initially with 250L filtered diluted seawater having a salinity of 15 ppt, stocked with 1,00,000.0 mysis each. The tank without the reactors served as control. The reactors (2 ammonia-oxidizing and 2 nitrite-oxidizing) were deployed and the physical, chemical and biological parameters were
quantified once in three days for 17 days. Every day addition of 200L filtered, chlorinated-dechlorinated seawater having salinity 15 ppt for 10 days brought the water level to the maximum capacity of 2 tonne. During this period the larvae were fed with freshly hatched *Artemia* nauplii. It took 30 days for larvae to metamorphose to post larvae.

In *P. monodon* hatchery the same operation was adopted except in having seawater with 30 ppt salinity instead of 15ppt. Larvae were fed with *Chaetoceros*, *Spirulina* and commercial Zoea/Mysis feed and at post larvae with newly hatched *Artemia* nauplii.

During the period of larval rearing in both the systems no water exchange was provided. The experiment was repeated three times. Physical and chemical parameters measured during the experiment were salinity (using Refractometer, Erma-Japan), pH (using pH probe, Euteck-Singapore), ammonia (Solorzano 1969), nitrite (Bendschneider and Robinson 1952), nitrate (Strickland and Parsons 1972), 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. The reactors after experimentation were brought to the laboratory and tested for their activity by measuring the substrate uptake of the reactor over a period of 24 hr. On completion of the experiment (when the post larvae of *M. rosebergii* attained the stage PL 5 and *P. monodon* at the stage PL 15), overall survivals in both the control and experimental sets of tanks were estimated numerically. Relative percent survival was calculated according to the equation (Gram et al., 1999):

$$RPS = \left(\frac{1 - \% \text{ Mortality in the tank}}{\% \text{ Mortality in the control}} \times 100\right)$$

The validation experiments were performed three times and the data analyzed statistically. One way Analysis of Variance was performed to test the significance of nitrification and survival rates in the control and test tanks.
5.2.2 Packed bed bioreactors in recirculating aquaculture systems

5.2.2.1 Macrobrachium rosenbergii seed production system

The reactors were activated with the consortia AMONPCU-1 and (NIONPCU-1) (Achuthan et al., 2006) and integrated into a Macrobrachium rosenbergii Hatchery of M/s Rosen Fisheries, Trichur, Kerala. The facility used consisted of two larval rearing tanks of 5000 L capacity, one integrated with the activated reactors and the other without any, used as control. The ammonia oxidizing and nitrite oxidizing reactors were connected serially (Fig. 1). The influent from the rearing water tank was pumped in to an overhead tank (282 L) from where water flowed through the two reactors serially by gravitation and got collected in a 140 L collection tank. From the outlet of the collection tank treated water got in to the larval rearing tank. Pumping of the influent from the larval rearing tank was controlled by an automated water level controller (V-guard, Kerala, India) fitted inside the overhead tank. A regulator valve was connected to the overhead tank to maintain a flow rate of 4 L/min to the system attaining a total circulation of 5760 L/day.

Chlorinated-dechlorinated seawater of salinity 15ppt was used during the trial period. The tanks were maintained with 2000 L seawater. Fresh hatched mysis of Macrobrachium rosenbergii before their introduction to the culture system were disinfected by dipping in 0.025 mg/L formalin (SRL, Mumbai, India) for 20 seconds, 0.03 mg/L iodophore (Growel Formulations, Hyderabad, India) for 20 seconds and then washed in running seawater and stocked at 0.2 million/ tank (200/L).

During the experiment, the rearing water was supplemented with 1 mg/L EDTA (Matrix Formulations, Hyderabad, India), 5 mg/L sulphated vitamin C (Matrix Formulations, Hyderabad, India) and 1 mg/L treflan (Growel Formulations, Hyderabad, India). The larvae were fed with fresh hatched Artemia nauplii up to stage 9 (when pleopods with setae appear) and with both Artemia nauplii and egg custard subsequently. The experiment continued for 17 days till the larvae metamorphosed to post larvae, and repeated two more times for concurrent
Validation of the nitrifying bioreactors in recirculating aquaculture systems

results. At the end of the experiment the survival was estimated by counting the larvae manually and the relative percentage survival was estimated as described earlier.

In another experiment, the reactor was tested for its nitrification potential in spent water after the larval culture. Water from the larval rearing tanks, subsequent to harvest of post larvae, was collected and stored in a 5000 L capacity storage tank. This was subsequently circulated through the bioreactor assembly at a rate of 2 L/min. Meanwhile, another system without integration of the reactor was kept as the control. The experiment was repeated two times.

Water samples from the larval rearing tanks were analyzed once in three days for alkalinity, hardness, ammonia (TAN), nitrite and nitrate. The heterotrophic bacterial community of the rearing water was determined once in a week by standard spread plate method employing ZoBell’s Marine agar 2216 E prepared in seawater of salinity 15ppt.

In spent water nitrification experiments, water quality parameters such as phosphate, sulphate, iron, chloride, dissolved oxygen, Biochemical Oxygen Demand (BOD) (APHA,1998) and ammonia, nitrite and nitrate as above were estimated for eight days.

The nitrification efficiency of the control and reactor integrated hatchery systems was analyzed by one way ANOVA. Significance of percentage survival of larvae in the control and reactor integrated tanks was estimated by one way ANOVA, and Least Significant Difference (LSD) at 0.1% level calculated for the delineation of the two treatments. The mean and standard deviation of the water quality parameters of the spent water were estimated.

5.2.2.2 *Penaeus monodon* maturation system

The activated Packed Bed Bioreactors were integrated into a maturation system operated at West Coast Hatchery, Alapuzha, Kerala, India. The maturation tanks were filled with 5 tone 30ppt seawater. The bioreactor was operated at a flow rate
of 400L/min. The tanks were stocked with 40 eyestalk ablated brooders with an average weight of 150 g. The animals were fed with clam, squid and crabs 3 times a day. The bioaugmentor Deterodigest and the gut probiotic Enterotrophotic were applied in to the system at a quantity of 100mL once in 3 days. ‘Enterotrophotic’ is a gut probiotic preparation composed of Bacillus MCCB 101 (GenBank accession no. EF062509) and Micrococcus MCCB 104 (Jayaprakash et al., 2005), blended in equal proportion to attain 10^8-10^9 CFU/mL. The water samples were analyzed daily for pH, alkalinity, total ammonia, nitrite and nitrate following the methods described earlier. Vibrio was enumerated once in three days by plating on TCBS agar.

5.2.3 A Recirculating aquaculture system for *Penaeus monodon*: laboratory level demonstration

A laboratory level recirculating culture system for *Penaeus monodon* was established at this Centre. A series of six activated packed bed bioreactors 3 each in parallel were integrated in to system where in 1000L rearing water was recirculated through the reactors making its 100% recirculating. The reactors were activated with the consortia AMONPCU-1 and NIONPCU-1. The tanks were filled with sea water of salinity of 15 ppt. The reactors were operated at a flow rate of 4L/min. A bag filter was used to filter the incoming water from the rearing tanks to remove detritus. The larvae (PL-20) were tested for WSSV by PCR and MBV by microscopic observation to confirm the absence of pathogen before stocking and the system at a density of 1 larva/L. The larvae were fed with pelleted feed of proper size range and the crude protein content of the feed was calculated by microkjeldhal method (APHA, 1998) as 30%. Detritus management was done using the probiotic Detrodigest and *Vibrio* population was controlled using an anti vibrio probiotic *Micrococcus* MCCB104 added into the system once in three days.

Two trails of recirculation experiments were carried out. In trial 1, the animals were fed in low quantity of feed starting from 1.75g/day and then the quantity slowly increased for a period of 70 days. The average body weight of the animals and the algal counts were determined every 10 days. Total suspend solids and
total dissolved solid were estimated (APHA, 1998) every 10 days to study the
effect of addition of Detrodigest into the culture system. *Vibrio* count was taken
every 10 days to assess the effect of probiotic *Micrococcus* MCCB104.

In the trial 2, the feeding rate was slightly higher starting from 60 g/day. The
animals were reared for 60 days as in trial 1 and the average body weight and
length were calculated every 7 days. In both the trials, TAN, NO₂-N and NO₃-N
were estimated daily.

Since the system was in complete recirculating mode without water exchange, the
source of TAN production in the system was assumed to be only through feed and
excretion by the animals reared. The in situ nitrification was neglected. The total
daily TAN production (Pₜₐₙ) based upon the fish feeding rate was therefore taken
as the TAN into the system and calculated using the following equation (Timmons
et al., 2001):

\[
P_{TAN} = FA \times PC \times 0.092 / \text{day} \tag{5.2}
\]

where,

- PTAN = Rate of ammonia production (g TAN/day)
- FA = Amount of feed per feeding (g)
- PC = Protein Content of the Feed (%)
- 0.092 is the fraction of protein nitrogen that is excreted as TAN.

The volumetric total ammonia nitrogen conversion rate (VTR) is used as the
principal indicator for evaluation of the filter performance (Pfeiffer and Malone,
2006, Colt et al., 2006). The VTR was obtained by using

\[
VTR = \frac{K_c (TAN_i - TAN_f) Q_R}{V_b} \tag{5.2}
\]

where,

- VTR is the g TAN converted per m³ of filter media per day
- QR the flow rate through the filter (L/pm)
- K_c the unit conversion factor of 1.44
TAN_{I} and TAN_{E} the influent and effluent ammonia (mg/L)

V_{b} is the volume of filter media (0.023 m³).

The VTR for each TAN concentration was estimated and regression analysis of TAN versus VTR was carried out

The volumetric biomass capacity of the system was estimated by dividing the biomass (g) by V_{media} (m³) (Colt et. al., 2006).

5.3 Results and discussions

5.3.1 Validation of stringed bed suspended bioreactors

5.3.1.1. Maintenance of Penaeus monodon adults and brooders

The maintenance of adults and brooders of P. monodon in experimental systems without water exchange even at laboratory level was an extremely difficult task as slight alternations in water quality used to lead to mortality. Integration of SBSBRs to the holding system showed that water quality could be maintained to the acceptable level in the rearing systems without water exchange. During the trial average pH was in the range 7.6-7.8, salinity 15ppt and alkalinity 58.5 mg/L. TAN, nitrite and nitrate were within the acceptable levels (Fig. 2). The brooders also could be successfully maintained healthy for longer period with the help of the SBSBRs. The water quality in the brooder rearing system with 10 brooders of 150 g was within the acceptable levels (Table 2, Fig. 3). Based on these results it has been inferred that SBSBRs can be used in live transportation of spawners and adults from the point of collection to the hatchery and for their quarantine till shifted to the maturation or breeding tank. It has to be pointed out that the reactors are sufficiently smaller enough to be incorporated in to the transportation containers.

5.3.1.2 Macrobrachium rosenbergii and Penaeus monodon seed production systems

The water quality parameters in the experimental and control tanks are summarized in Table 3. In both the systems, there were no significant differences in the parameters between the control and test tanks except the moderately higher total bacterial population recorded in the control tanks (Table 3). During
Validation of the nitrifying bioreactors in recirculating aquaculture systems

Validation of the reactors, nitrification was found established in the experimental tanks instantly and progressed rapidly from the 3rd day onwards (Fig. 4 and 5). There was significant ammonia (P<0.001) and nitrite (P<0.05) removal in both prawn and shrimp larval production systems compared to that in the control, where ammonia oxidation was found 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 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 gone up above 2 mg/L and 1.6 mg/L respectively whereas in the experimental tanks both were always below 0.25mg/L.

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 and metamorphosis was found significantly higher (P<0.01) in the experimental tanks with the activated bioreactors (Table 4).

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; Furukwa 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 on the fact 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 important means of overcoming this difficulty.
Validation of the nitrifying bioreactors in recirculating aquaculture systems

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 well below the maximum set for the oligotrophic category (0.3 mg/L N) (Malone and Beecher, 2000). During the validation of the reactors, TAN and TNN could be maintained bellow 0.25 mg/L.

The SBSBRs described here are designed for setting up nitrification in shrimp/prawn larval rearing tanks during the operation. The technology is relatively user friendly in the sense that they can easily be lifted out of water during disinfection and also can 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 stringed 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.

5.3.2 Validation of packed bed bioreactors
5.3.2.1 Macrobrachium rosenbergii seed production system

The minimum and maximum values of pH, temperature, salinity, alkalinity, hardness and total bacterial count in the rearing water of the experimental and control tanks during each treatment are summarized in Table 5. Heterotrophic bacterial community (CFU in ZoBell’s Marine Agar) in the control tank increased substantially and there was no remarkable difference in the other water quality parameters between the tanks. The extent of nitrification during the period is presented in Fig. 6. In the control tanks TAN exhibited progressive increase with its subsequent decline and concomitant increase of NO₂⁻N after 14 days; however, NO₃⁻N was never found built up in the system. Meanwhile, there was significant TAN removal \((P<0.01)\) in the experimental tanks with significant \((P<0.05)\) NO₂⁻N removal. Within 8 days both TAN and NO₂⁻N concentrations were below
detectable levels. NO₃-N exhibited progressive increase to 7.6 mg/L within 17 days of the experiment.

*M. rosenbergii* is an inhabitant of fresh water, however, its larval stages are completed in saline waters with salinity 13-15 ppt and therefore, in the larval rearing systems bioreactors operating in this salinity regime are essential. In saltwater systems RAS plays an important role in the production of healthy and properly sized fingerlings (Fielder and Allan, 1997) and has significant implications as the system demands operation under oligotrophic conditions. In the bioreactor integrated larval rearing systems, ammonia oxidation was established within a day and it took eight days for nitrite oxidation whereas in the control 14 days were required for the initiation of nitrification. The delay in establishing active nitrite oxidation might be due to the lower multiplication rate of nitrite oxidizers compared to that of ammonia oxidizers (Paller 1992). The maximum average TAN and NO₂-N concentrations in the experimental larval rearing tanks were 0.18 and 0.25 mg/L respectively typical of any marine systems. Marine larval rearing systems demands TAN and TNN levels below 0.1 mg/L well below the maximum limit (0.3 mgN/L) under the oligotrophic category (Malone and Beecher 2000). During the progression of the experiment the NO₃-N concentrations increased up to 7.6 mg/L, however, remaining well below the toxic levels for *M. rosenbergii* larval culture (Mallasen et al., 2004). In an experimental study, significantly lower survival rates of *M. rosenbergii* larvae were noticed at total ammonia concentrations ranging from 1 to 8 mg/L at pH 9, whereas at pH 7 and 8 survival rates were high (Mallasen et al., 2004). In the present field evaluation carried out at higher stocking densities (200/L) at a salinity of 15ppt, there was significantly higher percent survival of larvae in the system integrated with the bioreactors when compared to the control. This shall enable hatchery systems to operate as closed recirculating systems.

The overall per cent survival of larvae in the control and test tanks was estimated and presented in Table 6. The tank with the reactor exhibited significantly higher
(P<0.001) percentage survival (LSD at 0.1% =15.19) with an average relative percentage survival (RPS) of 22.86%.

The average water quality parameters of the spent water are given in Table 7. TAN, NO₂⁻ N and NO₃⁻ N were lower in the experimental tanks than in those of the controls (4th day) indicating higher percentage removal of TAN (78%), NO₂⁻ N (79%) and BOD (56%).

Under oligotrophic conditions ammonia diffuses into a relatively thin vertically homogenous biofilm that is dominated by autotrophs, principally due to low BOD (<5 g/m³) of the culture water (Malone and DeLosReyes, 1997). In the present study organic loading to the system was as low as 0.31 mg/L BOD and there has been minimal heterotrophic inhibition of nitrification (Satoh et al., 2000; Zhu and Chen, 2001) evidenced by the progressive increase nitrification rates. On biosecurity perspective (Otoshi et al. 2003; Pruder, 2004) water recirculation dramatically reduces the possibility of pathogen introduction (Goldburg et al., 2001). In this context integration of packed bed bioreactor for nitrification of hatchery spent water with high percentage removal of ammonia (78%), and nitrite (79%) by 4th day strengthens the possibility of reuse of water with limited discharge and reduced intake paving the way for bio-security.

5.3.2.2 Penaeus monodon maturation system

Upon integrating the PBBRs into the maturation system a remarkable reduction in the total ammonia concentration was observed within a week. The total ammonia concentration in the maturation tank was 4.47 ppm during the day of integration of the reactors, within a week this came down to 0.122 ppm, showing the rapid setting up of nitrification (Fig.7). Nitrite also showed a reduction after a slight increase initially. Nitrate concentrations were always bellow 5 mg/L. Bacterial population enumerated on TCBS from the water samples were 30 CFU/mL during the initial days of experiment which gradually declined to zero at the end of the experimental period. Recirculating systems for captive maturation and reproduction of penaeids remain relatively primitive. They depend upon a delicate
balance of environmental parameters, eyestalk ablation and a relatively frequent replacement of broodstock and a diet of expensive fresh frozen feeds to maintain nauplii production levels. In the hatchery, problems with unexplained mortality and the presence of pathogenic bacteria continue to affect the stability of production and seed quality (Browdy, 1998). However, in the present experiments with the integration of PBBR the water quality could be maintained at the desired levels.

5.3.3. Laboratory level recirculating aquaculture system for *Penaeus monodon*

The progress of nitrification in trial 1 with increasing feed rate showed that (Fig.8) TAN, NO\textsubscript{3}-N and NO\textsubscript{2}-N were within acceptable levels. The TDS and TSS (Fig.9) could be maintained in the rearing water during the progression of culture with the addition of Detordigest whereas *Vibrio* population in the system could be maintained by the addition of *Micrococcus* (Fig.10). There was sustained algal production and the animals could gain a weight of 9.4 g in 70 days (Fig.11). The VTR of the reactors increased with increasing TAN concentration (Fig. 12). In trial 1 the average VTR for the largest feed rate of 150 gm was 0.1361±0.0083 kgTAN/m\textsuperscript{3}/day. In trial 2, the average VTR during the highest feeding rate of 160 gm from 54-60\textsuperscript{th} days of culture was 0.1533±0.0045 kgTAN/m\textsuperscript{3}/day. The regression between VTR and TAN explained 98% variability in VTR (P<0.001) (Fig 13). In trial 2 also TAN, NO\textsubscript{3}-N and NO\textsubscript{2}-N were within acceptable levels (Fig.14). The regression between VTR and TAN explained 86% variability in VTR (P<0.001) (Fig 15).

Numerous studies have indicated that increasing the TAN concentration in biofilters results in proportional improvements in a filter's conversion ability (Rogers and Klemetson, 1985; De Los Reyes and Lawson, 1996; Malone et al., 1999; Sandu et al., 2002). In both trials the application of probiotics was successful in breaking down the feed residues and keeping the *Vibrio* population stable.
The volumetric TAN conversion rate (VTR, kg TAN/ m$^3$/d) of biofilters commonly used in RAS are: floating bead filter, 0.07-0.35; fluidized sand filter, 0.1-2.7; trickling filter, 0.02-0.64; moving bed filter, 0.51-2.22; RBC, 0.10-0.13; and submerged filter, 0.01. Backwash frequency has a significant effect on the bead filter nitrification rate and Golz et al. (1999) determined that a high VTR (kg TAN/ m$^3$/d) of 0.37 could be achieved by a bubble-washed bead filter with an 8 hr backwash interval and an optimal VTR (kg TAN/ m$^3$/d) of 0.39 for an aggressively-washed bead filter at a 48 hr backwash interval. Recommended nitrification rates for fluidized bed filters were 0.7 (kg TAN/ m$^3$/d) for applications in cold water systems and 1.0 (kg TAN/ m$^3$/d) for warm water systems based on a series of pilot scale tests (Timmons et al., 2001). However, the nitrification performance of a commercial fluidized sand filter system reported much lower nitrification rates with 0.35-0.49 (kg TAN/ m$^3$/d) in a cold water system (Summerfelt et al., 2004) and 0.1 (kg TAN/ m$^3$/d) in a warm water system (Pfeiffer and Malone, 2006). The values obtained in the present study are comparable to values suggested by Malone and Beecher (2000). Based on over ten years of floating bead filter research, they (Malone et al., 1998; Malone and Beecher, 2000) recommended the use of a VTR (kg TAN/ m$^3$/d) of 0.035-0.105, 0.07-0.18, and 0.14-0.35, for the design of floating bead filters in brood stock, ornamental, and growout systems, respectively, for warm water systems.

The average biomass yield was 9.4 at the end of 70 days culture in trial 1 (Fig 11) and 10.3 g (Fig. 16) in trial 2 at the end of 60 day culture with 60% survival. The mortality was attributed to cannibalism. The maximum volumetric biomass capacity of the system was 68 g/m$^3$ in trial 1 and 75 g/m$^3$ in trial 2. This is only a preliminary study showing the effective usage of bioreactors and probiotics for maintaining a suitable water quality for the culturing of P. monodon. To make this process completely organic we have to supply organically produced feed also. The main problem observed during the culture period was the cannabalism, so the formulation of the diet is a critical component in the recirculating organic culture. The Stringed Bed Suspended Bioreactors an Packed Bed Bioreactors developed can form integral
Validation of the nitrifying bioreactors in recirculating aquaculture systems

parts of Organic Shrimp and Prawn Seed Production system as they are the systems specially designed for tropics using indigenous nitrifying bacterial consortia. Currently organic standards are manly oriented towards temperate species and it is required to develop organic systems for tropical regions (Chen, 2004). This technology is now commercialized through M/S Oriental Aquamarine Biotech India Pvt Ltd, U-140, Kovaipudur, Coimbatore-641 042

Based on the experimental results a novel Recirculation System for Organic Shrimp and Prawn Seed Production could be designed, principally by integrating the reactors at three locations in a larval production system such as, 1. PBBRs in the reservoir system. 2. PBBRs at brood stock maturation system and 3. SBSBRs at the larval rearing tanks. The probiotic Detrodigest can be used for the management of detritus in the systems. This organism, in association with the heterotrophic bacteria in the nitrifying bacterial consortia, rapidly digests the detritus into monomeric substances and helps attain reef quality water. For the prevention of entry of Vibrio sp. through brood stock and nauplii, Micrococcus MCCB104 could be used. The gut probiotic (Enterotrophic- 1) is supplied in all compartments of the larval rearing system such as algal production system, Artemia production unit, larval rearing tank and maturation system so as to prevent colonization of the pathogenic vibrios any were in the production facility (Rejish Kumar et al., 2006).

Quantifiable advantages of implementing organic seed production systems are 1. Increase in output of seed by not less than 20% from the existing level 2. Better performance of seed under field conditions 3. Avoidance of frequent pumping of water from sea and disinfection as the same water is recirculated and reused 4. Decrease in recurring cost and increase in profits as the cumulative effect and 5. Stabilization of maturation system and self-sufficiency in nauplii production.

To sustain the shrimp industry in India, the production process has to match with the global standards and in this requirement the importance of organically produced good quality seed need not require any more emphasis. The
Validation of the nitrifying bioreactors in recirculating aquaculture systems

The technology package presented here is first of its kind globally and is the one developed indigenously. Adoption of the technology in Indian shrimp/prawn production process is the first step towards the beginning of organic shrimp farming in India.

**Table 2.** Water and microbial quality of the rearing tank during the maintenance *Penaeus monodon* brooder in laboratory rearing systems integrated with SBSBR

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8-7.7</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>32</td>
</tr>
<tr>
<td>Temperature (° C)</td>
<td>27-29</td>
</tr>
<tr>
<td>Alkalinity (mg of CaCO₃/L)</td>
<td>72-69</td>
</tr>
<tr>
<td>Hardness (mg of CaCO₃/L)</td>
<td>5820</td>
</tr>
<tr>
<td>TPC (CFU/mL)</td>
<td>$1.43 \times 10^6$ to $1.82 \times 10^8$</td>
</tr>
<tr>
<td>Vibrio count (CFU/mL)</td>
<td>1000-75</td>
</tr>
</tbody>
</table>

**Table 3.** Physico-chemical and microbial quality of rearing water in hatchery systems integrated with SBSBR during the experiment (n=3)

<table>
<thead>
<tr>
<th>Rearing water quality</th>
<th><em>P. monodon</em> system</th>
<th><em>M. rosenbergii</em> system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test tank</td>
<td>Control tank</td>
</tr>
<tr>
<td>pH</td>
<td>7.5-8</td>
<td>7.5-8</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>30-32</td>
<td>30-32</td>
</tr>
<tr>
<td>Temperature (° C)</td>
<td>27-29</td>
<td>27-29</td>
</tr>
<tr>
<td>Alkalinity (mg of CaCO₃/L)</td>
<td>60-69</td>
<td>72-78</td>
</tr>
<tr>
<td>Hardness (mg of CaCO₃/L)</td>
<td>5790-5820</td>
<td>5790-5950</td>
</tr>
<tr>
<td>TPC (CFU/mL)</td>
<td>$1.43 \times 10^6$ to $1.82 \times 10^8$</td>
<td>$1.59 \times 10^6$ to $3.78 \times 10^9$</td>
</tr>
</tbody>
</table>
Table 4. Impact of SBSBR in larval survival and metamorphosis on integration in to hatchery systems (n=3)

<table>
<thead>
<tr>
<th>Hatchery system</th>
<th>Treatment</th>
<th>Survival (%)</th>
<th>P</th>
<th>Relative survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. monodon</em></td>
<td>Control tank</td>
<td>16.33±1.53</td>
<td>&lt;0.01</td>
<td>17.67</td>
</tr>
<tr>
<td></td>
<td>Test tank</td>
<td>31± 1.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. rosenbergii</em></td>
<td>Control tank</td>
<td>19.67±1.53</td>
<td>&lt;0.01</td>
<td>20.67</td>
</tr>
<tr>
<td></td>
<td>Test tank</td>
<td>36.33±3.51</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Physico-chemical and microbial quality of rearing water in control and test tanks with PBBR in *Macrobrachium rosenbergii* hatchery during the experiment (n=3)

<table>
<thead>
<tr>
<th>Water quality parameters</th>
<th>Control tank</th>
<th>Test tank</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.5-8.0</td>
<td>7.5-8.0</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>14-15</td>
<td>14-15</td>
</tr>
<tr>
<td>Temperature(°C)</td>
<td>28-31</td>
<td>28-31</td>
</tr>
<tr>
<td>Alkalinity (mg of CaCO₃/L)</td>
<td>66-70</td>
<td>64-70</td>
</tr>
<tr>
<td>Hardness (mg of CaCO₃/L)</td>
<td>2987-2900</td>
<td>2876-2900</td>
</tr>
<tr>
<td>TPC ( CFU/mL)</td>
<td>1.91 x 10⁵ to 2.51 x 10⁵</td>
<td>1.14 x 10⁹ to 4.21 x 10⁷</td>
</tr>
</tbody>
</table>

Table 6 Larval survival after the integration of PBBR into the *Macrobrachium rosenbergii* hatchery system

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Survival</th>
<th>Average Survival (%)</th>
<th>Relative Survival (%)</th>
<th>ANOVA</th>
<th>Source of variation</th>
<th>df</th>
<th>Mean sum of squares</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control tank</td>
<td>a 18</td>
<td>18.33±1.53</td>
<td>22.86</td>
<td>Between treatments</td>
<td>1</td>
<td>522.67</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b 17</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test tank</td>
<td>a 36</td>
<td>37±2.65</td>
<td></td>
<td>Within treatment</td>
<td>4</td>
<td>4.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b 35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c 40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 7. Mean water quality parameters of the spent water from *Macrobrachium rosenbergii* hatchery during the experiment (N=3).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Tank</th>
<th>Test tank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity (ppt)</td>
<td>15.33±0.47</td>
<td>15±0.41</td>
</tr>
<tr>
<td>pH</td>
<td>8.09±0.07</td>
<td>8.36±0.12</td>
</tr>
<tr>
<td>Eh (mV)</td>
<td>110±0.82</td>
<td>104.33±16.46</td>
</tr>
<tr>
<td>Ammonia (mg/L)</td>
<td>3.71±0.43</td>
<td>0.83±1.46</td>
</tr>
<tr>
<td>Nitrite (mg/L)</td>
<td>2±0.23</td>
<td>0.43±0.55</td>
</tr>
<tr>
<td>Nitrate (mg/L)</td>
<td>0.3±0.09</td>
<td>13.28±6.57</td>
</tr>
<tr>
<td>Phosphate (mg/L)</td>
<td>0.03±0.04</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Sulphate (mg/L)</td>
<td>15.93±1.23</td>
<td>13.35±0.37</td>
</tr>
<tr>
<td>Alkalinity (mg CaCO₃/L)</td>
<td>79.33±0.94</td>
<td>79.83±5.34</td>
</tr>
<tr>
<td>Hardness (mg CaCO₃/L)</td>
<td>2483.33±107.81</td>
<td>2672.83±134.64</td>
</tr>
<tr>
<td>Chloride (mg/L)</td>
<td>10273.513±295.31</td>
<td>10505.83±504.00</td>
</tr>
<tr>
<td>DO (mg/LO₂)</td>
<td>5.73±0.39</td>
<td>5.81±0.64</td>
</tr>
<tr>
<td>BOD (mg/LO₂)</td>
<td>0.71±0.018</td>
<td>0.31±0.29</td>
</tr>
</tbody>
</table>

Fig. 1. Process flow diagram of the reactors integrated into the larval rearing tank (OHT-overhead tank with automatic water level controller, AOB-ammonia oxidizing bioreactor, NOB-nitrite oxidizing bioreactor, CT-collection tank for the treated water, LRT- larval rearing tank)
Validation of the nitrifying bioreactors in recirculating aquaculture systems

Fig. 2. TAN, NO₂-N and NO₃-N in experimental systems integrated with SBSBR rearing *Penaeus monodon* adults

Fig. 3. TAN, NO₂-N and NO₃-N experimental systems integrated with SBSBR rearing *Penaeus monodon* brooders
Fig. 4. TAN, NO$_2$-N and NO$_3$-N concentration in the control and SBSBR integrated *Macrobrachium rosenbergii* hatchery systems.
Validation of the nitrifying bioreactors in recirculating aquaculture systems

Fig. 5. TAN, NO$_3$-N and NO$_2$-N concentrations in then control and SBSBR integrated *Penaeus monodon* hatchery systems.
Fig. 6 Nitrification in the control and PBBR integrated *M. rosenbergii* hatchery systems (N=3)
Validation of the nitrifying bioreactors in recirculating aquaculture systems

Fig. 7 Nitrification in PBBR integrated *Penaeus monodon* maturation system

Fig. 8. Progress of nitrification in recirculatory *Penaeus monodon* culture system (Trial 1)
Validation of the nitrifying bioreactors in recirculating aquaculture systems

Fig. 9 Maintenance of Total Suspended Solids and Total Dissolved Solids in the recirculation system on addition of the probiotic Detrodigest

Fig. 10 Maintenance of Vibrio population in the recirculation system on addition of the probiotic Micrococcus MCCB104
Validation of the nitrifying bioreactors in recirculating aquaculture systems

Fig. 11 Average body weight, algal count in *Penaeus monodon* recirculation system (Trial 1)

Fig. 12. Volumetric TAN Removal rates and TAN in *P. monodon* recirculation system (Trial 1)
Validation of the nitrifying bioreactors in recirculating aquaculture systems

Fig. 13. Regression of Volumetric TAN Removal rates (VTR) versus TAN in *Penaeus monodon* recirculation system- Predicted and measured VTR (Trial 1)

Fig. 14. Progress of nitrification in recirculatory *Penaeus monodon* culture system (Trial 2)
Validation of the nitrifying bioreactors in recirculating aquaculture systems

Fig. 15 Volumetric TAN Removal rates and TAN in Penaeus monodon recirculation system (Trial 2)

Fig. 16. Regression of Volumetric TAN Removal rates (VTR) versus TAN in P. monodon recirculation system- Predicted and measured VTR (Trial 2)
Fig. 17. Average body weight and length in *Penaeus monodon* recirculation system (Trial 2)