Oriental Aquamarine Biotech India (P) Limited, Coimbatore

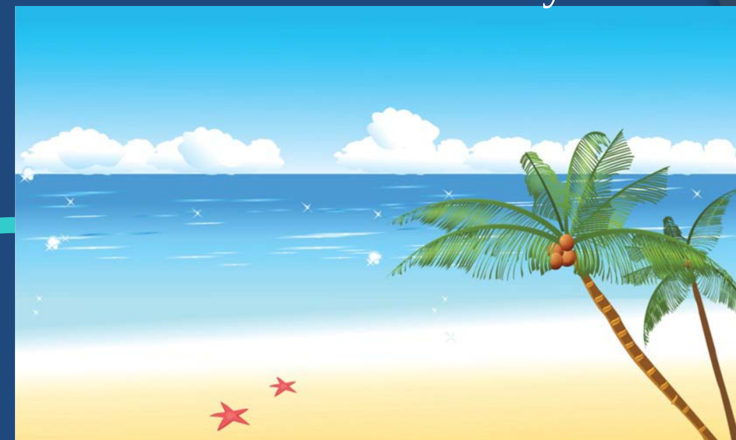
Nitrifying Bioreactor Technology for the Establishment of Biosecure Recirculating Aquaculture Systems

Aquaculture

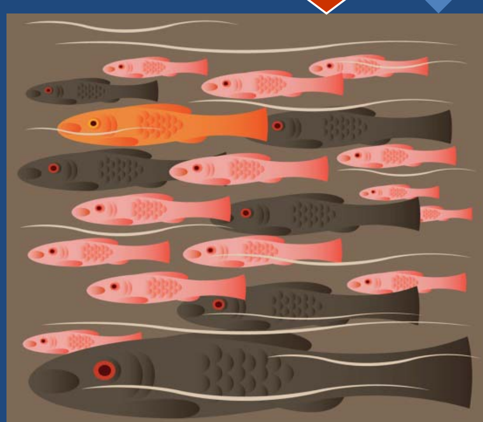
Conventional Systems

Aquaculture Tanks

Ocean / Water body



Ammonia & Nitrites
Excess Feed
Fecal Matter



Conventional systems – problems
Needs large quantities of water

Conventional systems problem

Reduced survival rate

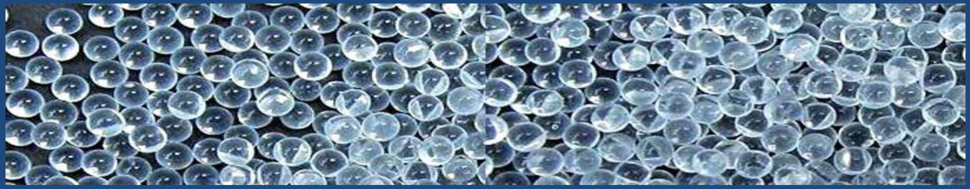
Conventional systems problems

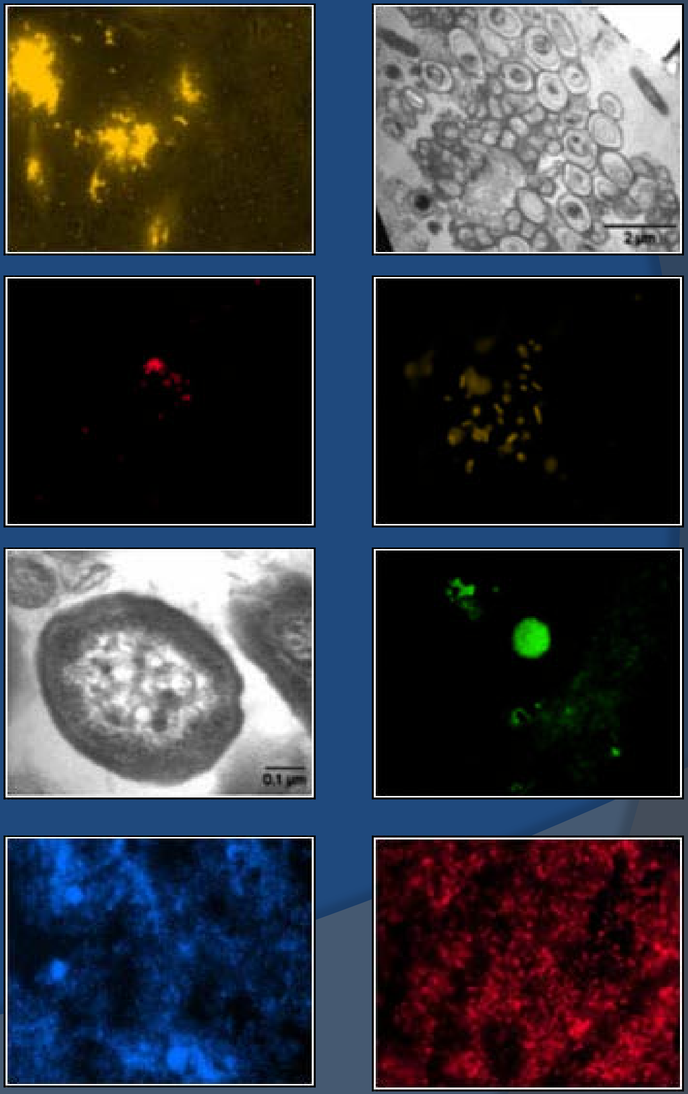
Causes water pollution

Bacterial Consortium

- A consortium of nitrifying bacteria have been identified which break down the nitrites and ammonia

- Consortium grows as biofilm on beads packed and remove Nitrites & Ammonia inside a bioreactor





Nitrifying Bioreactor

- **PBBR**
- **SBSBR**

PBBR

PBBR

SBSBR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India Private Limited

Nitrifying Bioreactor

Stringed Bed Suspended Bioreactor (SBSBR)

-500 liters/day

Packed Bed Bioreactor (PBBR)

-60,000 liters/day

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Water quality parameters in the larval rearing system and treated water stabilized as under :

NH_4^+	< 0.1 ppm
NO_2^-	< 0.5 ppm
NO_3	10 – 100 ppm
Vibrio	$< 2 * 10^2$ / ml
Luminescent Vibrio	Nil
pH	7.5 – 8.5

No water exchange

Reef Quality Water.

PATENT

Patented in India

- Patent no. 241648

PCT

-International Publication no - WO 02/30835 A1

-International patent classification: C02F 3/13.

-International application No. PCT/IN00/00097

Patented in South East Asian Countries.

- Thailand
- Japan
- Philippines
- Indonesia and
- South Korea.

ORIENTAL AQUAMARINE BIOTECH

Publications

25

Indian Journal of Marine Sciences
Vol. 35(3), September 2006, pp. 240-248

Development of nitrifying bacterial consortia for immobilizing in nitrifying bioreactors designed for penaeid and non-penaeid larval rearing systems in the tropics

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Two ammonia oxidizing (AMOPCU-1 and AMONPCU-1) and two nitrite oxidizing (NIOPCU-1 and NIONPCU-1) consortia for activating nitrifying bioreactors and thereby establishing nitrification in penaeid and non-penaeid hatchery systems were developed by enrichment. For further amplification of the consortia a simple medium having seawater (either salinity 30 ‰ or 15 ‰) as base, supplemented with NH_4^+-N/NO_2^--N and PO_4^- and *p*H adjusted to 8 was identified. During the amplification in a fermentor the consortia exhibited excessive wall growth and diminished their yield coefficient posing difficulty in harvesting the cells completely. The consortia consisted of both Gram negative and Gram-positive bacterial cells embedded in a mucilaginous matrix of glycocalyx - like material presumably composed of polysaccharides. The consortia besides being useful in activating nitrifying bioreactors developed for shrimp/prawn hatchery systems can also be used as bioaugmentors in the bioremediation of ammonia and nitrite toxicity in aquaculture systems.

[Key words: Nitrification, bacterial consortia, nitrifying bioreactors, hatchery systems, penaeids, non-penaeids, shrimp, prawns]

[IPC Code: Int.Cl.^{8} (2006)C12Q 1/00]

Introduction

One of the major limiting factors for successful operation of prawn/shrimp hatcheries is the accumulation of NH_4^+-N to toxic levels. NH_4^+-N enters hatchery water through excretion of prawn larvae, bacterial degradation of faecal matter and unused feed and by way of diffusion from atmosphere and subsurface air bubbles during aeration. Nitrite on the other hand gets accumulated due to ammonia oxidation by chemolithotrophic ammonia oxidizers. In hatchery, ammonia accumulation in the rearing water intensifies as a result of high larval population maintained for commercial viability.

An elevated level of $NH4^+$-N has been reported affecting growth and moulting[1], oxygen consumption, NH_4^+-N excretion[2] and Na^+, K^+-ATPase activities of penaeids[3]. The short term (48-96 h) LC_{50} values for unionized ammonia (NH_3) range from about 0.2 to 0.3 mg l^{-1} suggesting that its safe levels in aquaculture systems[4] should be lower than 0.02-0.03 mg l^{-1}. Exposure to toxic levels of ammonia causes gill hyperplasia, a precursor to bacterial infection and in extreme cases death. Unionized ammonia is more toxic as it passes through gill membrane and causes pH alterations in the cytoplasm[5]. Meanwhile the 96-h LC_{50} values of nitrite for crustacean larvae in the studies[6] were 6-12 mg l^{-1}. Under these circumstances, management of the toxic metabolites is indeed a matter of concern in hatcheries as the daily specific excretion of ammonia by post larvae is about five times higher than that in adults on weight basis[3]. Therefore, an appropriate technology for continuous removal of ammonia from rearing waters is an absolute requirement for successful hatchery management. The usual practice followed in hatcheries other than frequent water exchange is introduction of bacteriological filters, where sand grain or gravel-based substrata are held in a container and through which water is circulated. A number of other techniques were also developed in recent years for the control of total ammonia nitrogen (TAN) in aquaculture systems. Rotating biological contactors[7],

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AQUAMARINE BIOTECH

Aquacult Int
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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

crobiol Biotechnol
DOI 10.1007/s11274-008-9905-1

ORIGINAL PAPER

Mass production of nitrifying bacterial consortia for the rapid establishment of nitrification in saline recirculating aquaculture systems

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

Received: 17 July 2008 / Accepted: 29 October 2008

Abstract Two distinct nitrifying bacterial consortia, namely an ammonia oxidizing non-penaeid culture (AMO NPCU-1) and an ammonia oxidizing penaeid culture (AMOPCU-1), have been mass produced in a nitrifying bacterial consortia production unit (NBCPU). The consortia, maintained at 4°C were activated and cultured in a 2 l fermentor initially. At this stage the net biomass (0.105 and 0.112 g/l), maximum specific growth rate (0.112 and 0.105/h) and yield coefficients (1.315 and 2.08) were calculated respectively, for AMONPCU-1 and AMOPCU-1 on attaining stationary growth phase. Subsequently on mass production in a 200 l NBCPU under optimized culture conditions, the total amounts of NH_4^+–N removed by AMONPCU-1 and AMOPCU-1 were 1.948 and 1.242 g/l within 160 and 270 days, respectively. Total alkalinity reduction of 11.7–14.4 and 7.5–9.1 g/l were observed which led to the consumption of 78 and 62 g Na_2CO_3. The yield coefficient and biomass of AMONPCU-1 were 0.67 and 125.3 g/l and those of AMOPCU-1 were 1.23 and 165 g/l. The higher yield coefficient and growth rate of AMOPCU-1 suggest better energy conversion efficiency and higher CO_2 fixation potential. Both of the consortia were dominated by *Nitrosomonas*-like organisms. The consortia may find application in the establishment of nitrification within marine and brackish water culture systems.

Keywords Ammonia oxidizing consortia · Nitrifying bacteria · Mass production · Maximum specific growth rate · Nitrification · Yield coefficient

Introduction

The most prominent requirement of any recirculating aquaculture system (RAS) is an efficient biofilter to prevent the accumulation of toxic metabolites such as ammonia and nitrite. High levels of ammonia and nitrite undermine commercial production objectives, as their toxic impacts are manifested through impaired growth or chronic diseases (Cheng et al. 2004; Svobodova et al. 2005). This is especially true in shrimp/prawn hatcheries where the daily specific excretion of ammonia by larvae and post-larvae is five fold higher than that of adults. To address this issue, fixed film biofilters are commonly employed for total ammonia nitrogen (TAN) removal (Seo et al. 2001; Shnel et al. 2002). Experience has shown that the biofilters in

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DOI 10.1007/s10295-008-0504-9

ORIGINAL PAPER

Activated packed bed bioreactor for rapid nitrification in brackish water hatchery systems

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

Received: 23 September 2008 / Accepted: 11 November 2008

Abstract A packed bed bioreactor (PBBR) was developed for rapid establishment of nitrification in brackish water hatchery systems in the tropics. The reactors were activated by immobilizing ammonia-oxidizing (AMONPCU-1) and nitrite-oxidizing (NIONPCU-1) bacterial consortia on polystyrene and low-density polyethylene beads, respectively. Fluorescence in situ hybridization demonstrated the presence of autotrophic nitrifiers belong to *Nitrosococcus mobilis*, lineage of β ammonia oxidizers and nitrite oxidizer *Nitrobacter* sp. in the consortia. The activated reactors upon integration to the hatchery system resulted in significant ammonia removal ($P < 0.01$) culminating to its undetectable levels. Consequently, a significantly higher percent survival of larvae was observed in the larval production systems. With spent water the reactors could establish nitrification with high percentage removal of ammonia (78%), nitrite (79%) and BOD (56%) within 7 days of initiation of the process. PBBR is configured in such a way to minimize the energy requirements for continuous operation by limiting the energy inputs to a single stage pumping of water and aeration to the aeration cells. The PBBR shall enable hatchery systems to operate under closed recirculation mode and pave the way for better water management in the aquaculture industry.

Keywords Nitrifying bioreactors · Nitrifying consortia · Immobilization · Nitrification · Closed system shrimp hatchery

Introduction

On assuming the dimensions of an industry, aquaculture systems are bound to operate under strict environmental safety standards. With high land and water costs, the systems are destined to maintain high biological carrying capacity in relatively little space with minimal water exchange. These requirements led to the advent of recirculating aquaculture systems (RASs) which allowed 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 labor costs, and (6) improve product

ORIENTAL AQUAMARINE BIOTECH

Nitrification in brackish water recirculating aquaculture system integrated with activated packed bed bioreactor

V. J. Rejish Kumar, Valsamma Joseph, Rosamma Philip and I. S. Bright Singh

ABSTRACT

Recirculation aquaculture systems (RAS) depend on nitrifying biofilters for the maintenance of water quality, increased biosecurity and environmental sustainability. To satisfy these requirements a packed bed bioreactor (PBBR) activated with indigenous nitrifying bacterial consortia has been developed and commercialized for operation under different salinities for instant nitrification in shrimp and prawn hatchery systems. In the present study the nitrification efficiency of the bioreactor was tested in a laboratory level recirculating aquaculture system for the rearing of *Penaeus monodon* for a period of two months under higher feeding rates and no water exchange. Rapid setting up of nitrification was observed during the operation, as the volumetric total ammonia nitrogen removal rates (VTR) increased with total ammonia nitrogen (TAN) production in the system. The average Volumetric TAN Removal Rates (VTR) at the feeding rate of 160 g/day from 54–60th days of culture was 0.1533 ± 0.0045 kg TAN/m^3/day. The regression between VTR and TAN explained 86% variability in VTR ($P < 0.001$). The laboratory level RAS demonstrated here showed high performance both in terms of shrimp biomass yield and nitrification and environmental quality maintenance. Fluorescent in-situ Hybridization analysis of the reactor biofilm ensured the presence of autotrophic nitrifier groups such as *Nitrosococcus mobilis* lineage, *Nitrobacter* spp and phylum *Nitrospira*, the constituent members present in the original consortia used for activating the reactors. This showed the stability of the consortia on long term operation.

Key words | fluorescence insitu hybridization analysis, nitrification, packed bed bioreactor, recirculating aquaculture systems, volumetric TAN removal

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INTRODUCTION

Over the last two decades, aquaculture has gone through major changes, from small scale homestead-level activities to large scale commercial farming, exceeding landing from capture fisheries in many areas (National Research Council 1992; NACA/FAO 2001). The need to increase aquaculture production drives the industry towards more intensive practices because of limitations in quality and quantity of water, availability and cost of land, limitations on water discharge and environmental impacts. Recirculation-zero water exchange system is the only solution for these issues.

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A recirculating aquaculture facility reduces water demands and discharges by reconditioning water to be used over and again (Goldburg *et al.* 2001). Better food conversions are achievable with a recirculating aquaculture system (RAS) which means less waste is generated from the feed (Losordo *et al.* 1998). In recent years, there has been growing concern over the impacts of aquaculture operations (Harache 2002; Cranford *et al.* 2003; Johnson *et al.* 2004) in the surrounding water bodies. Increasing regulatory pressures focusing on discharges to natural water bodies

ORIENTAL AQUAMARINE BIOTECH

Research Article

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Nitrification in a packed bed bioreactor integrated into a marine recirculating maturation system under different substrate concentrations and flow rates

V. J. Rejish Kumar,[a] Valsamma Joseph,[a] R. Vijai,[a] Rosamma Philip[b] and I. S. Bright Singh[a*]

Abstract

BACKGROUND: A packed bed bioreactor (PBBR) activated with an indigenous nitrifying bacterial consortia was developed and commercialized for rapid establishment of nitrification in brackish water and marine hatchery systems in the tropics. The present study evaluated nitrification in PBBR integrated into a *Penaeus monodon* recirculating maturation system under different substrate concentrations and flow rates.

RESULTS: Instant nitrification was observed after integration of PBBR into the maturation system. TAN and NO_2-N concentrations were always maintained below 0.5 mg L^{-1} during operation. The TAN and NO_2-N removal was significant ($P < 0.001$) in all the six reactor compartments of the PBBR having the substrates at initial concentrations of 2, 5 and 10 mg L^{-1}. The average volumetric TAN removal rates increased with flow rates from 43.51 (250 L h^{-1}) to 130.44 (2500 L h^{-1}) gTAN m^{-3} day^{-1} ($P < 0.05$). FISH analysis of the biofilms after 70 days of operation gave positive results with probes NSO 190 ((β ammonia oxidizers), NsV 443 (*Nitrosospira* spp.) NEU (halophilic *Nitrosomonas*), Ntspa 712 (Phylum Nitrospira) indicating stability of the consortia.

CONCLUSION: The PBBR integrated into the *P. monodon* maturation system exhibited significant nitrification upon operation for 70 days as well as at different substrate concentrations and flow rates. This system can easily be integrated into marine and brackish water aquaculture systems, to establish instantaneous nitrification.

Supporting information may be found in the online version of this article.

Keywords: packed bed bioreactor; nitrification; recirculating aquaculture systems; total ammonia nitrogen; flow rate

INTRODUCTION

In recent years, there has been growing concern over the environmental impacts of aquaculture operations,[1–3] and recirculating aquaculture systems (RAS) have emerged as the major environmentally sustainable solution to combat these impacts. A recirculating aquaculture facility reduces water demands and discharges by reconditioning water to be recycled, and increases food conversions resulting in less waste generation from feed.[4,5] The RAS technologies are highly applicable to the production of marine species[6–8] as reliable supply of fingerlings is a bottleneck for their commercial production.[9,10] Biosecurity is another important matter for consideration in the use of RAS by the hatchery operators[11,12] as the water recirculation dramatically reduces the possibility of pathogen introduction.[5,13]

The most prominent characteristic of any RAS is a nitrifying biofilter to prevent accumulation of metabolites like ammonia and nitrite, which at high levels undermine commercial production objectives as their toxic impacts are manifested through impaired growth or chronic diseases.[14–16] However, nitrate is relatively harmless to the aquatic organisms.[17] Fixed film biofilters are commonly used for total ammonia nitrogen (TAN) removal in RAS,[18–20] where attached growth as biofilm offers several advantages as handling convenience, increased process stability to shock loading and prevention of the bacterial population from being washed off.[21,22] However, at least in a few cases, the immobilized nitrifiers in RAS have exhibited low performance, besides demanding too long a start-up period imposing operational difficulties.[23,24] Considering these drawbacks, we developed a specialized nitrifying packed bed bioreactor (PBBR) (Indian Patent no. 241 648) immobilized with an indigenous nitrifying bacterial

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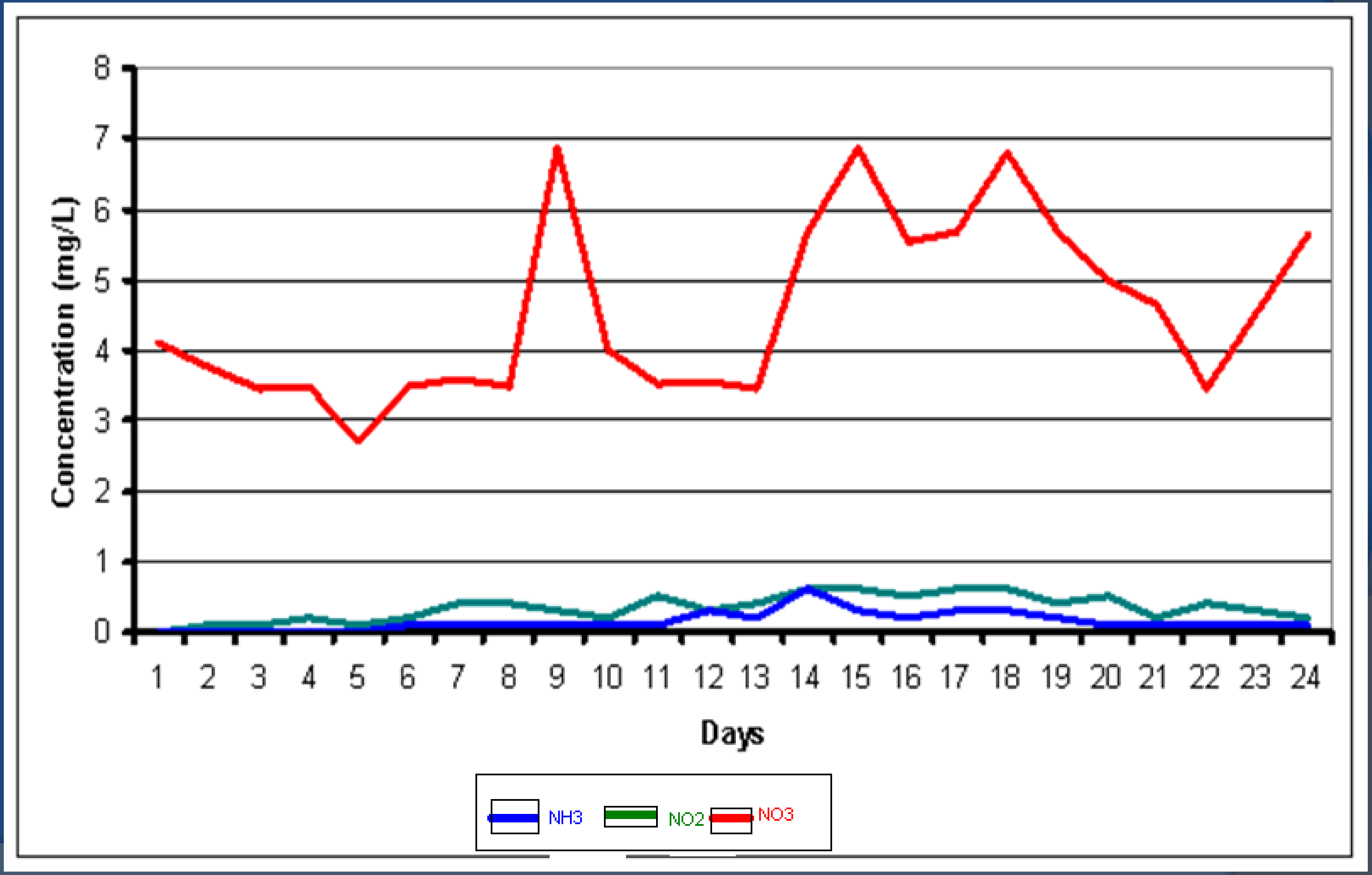
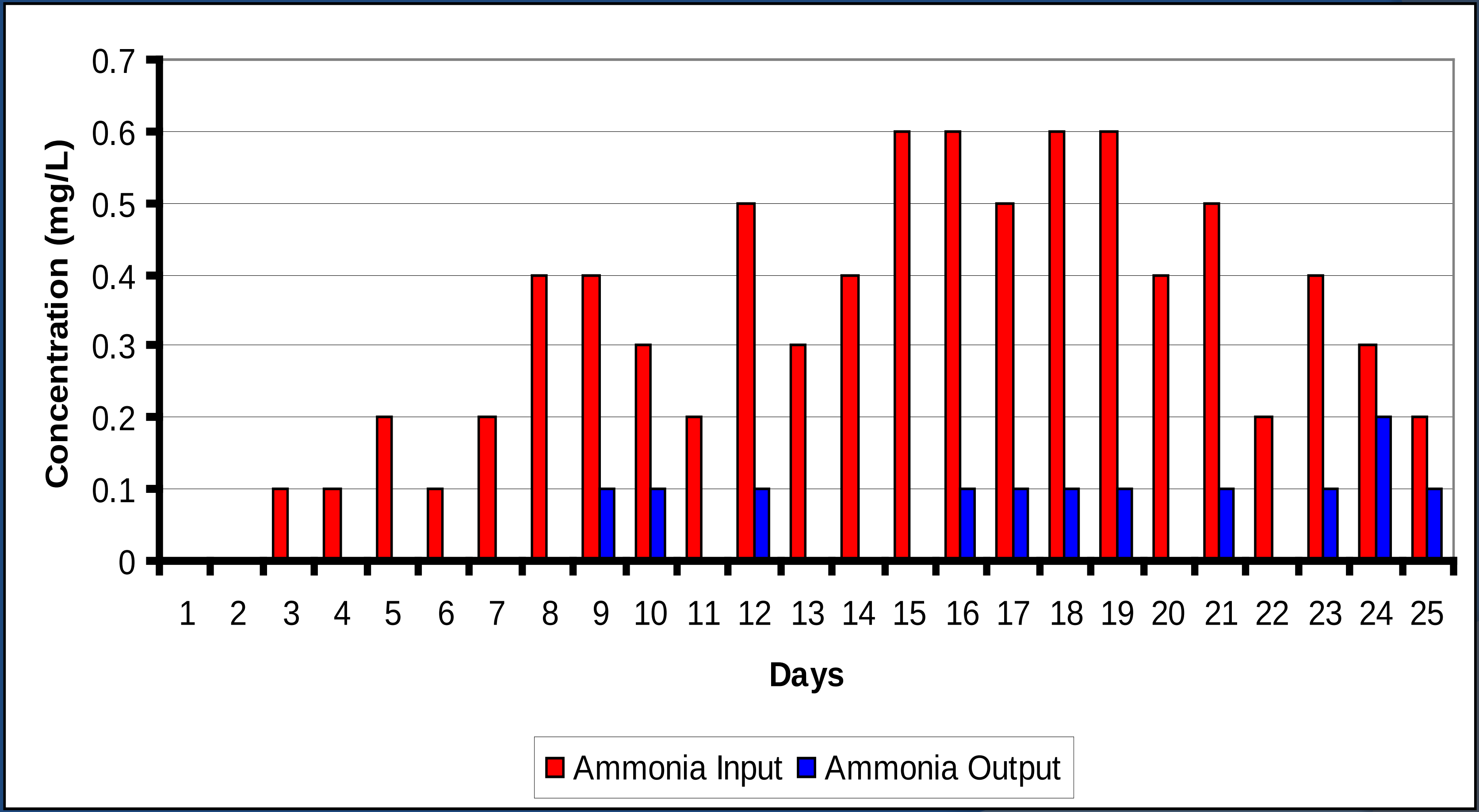
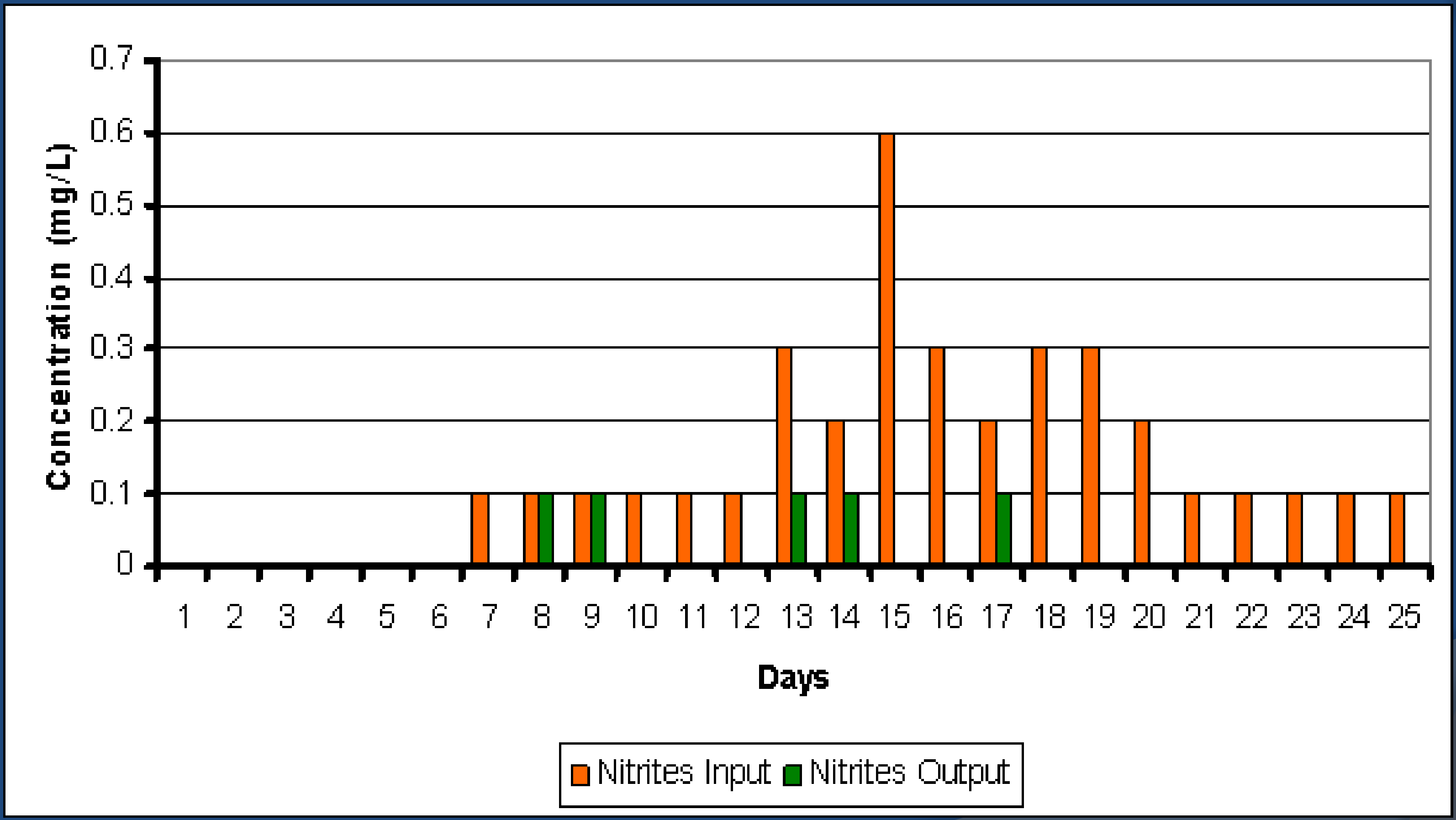
Progress of Nitrification in PBBR integrated *Penaeus monodon* maturation system



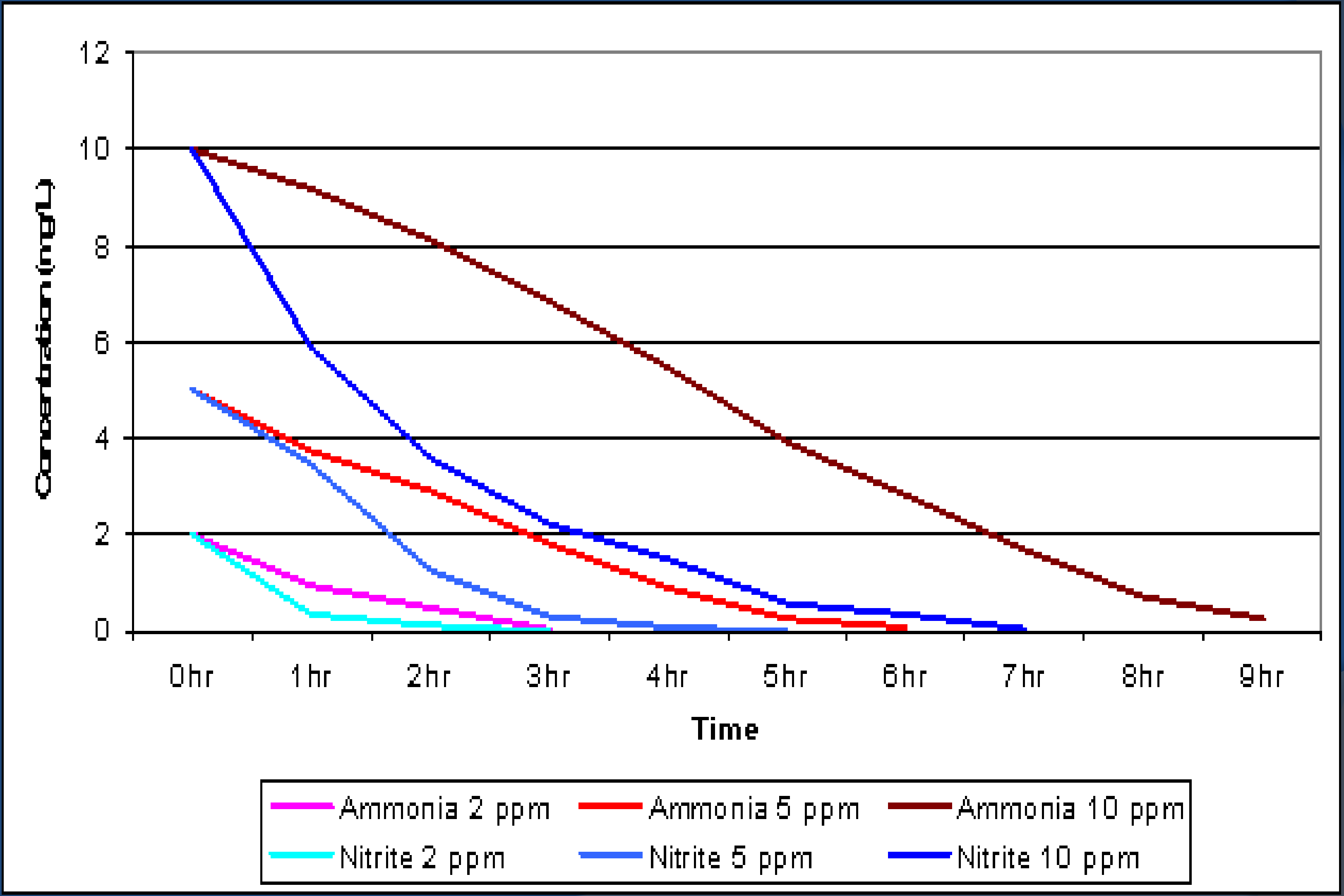
Nitrification Efficiency



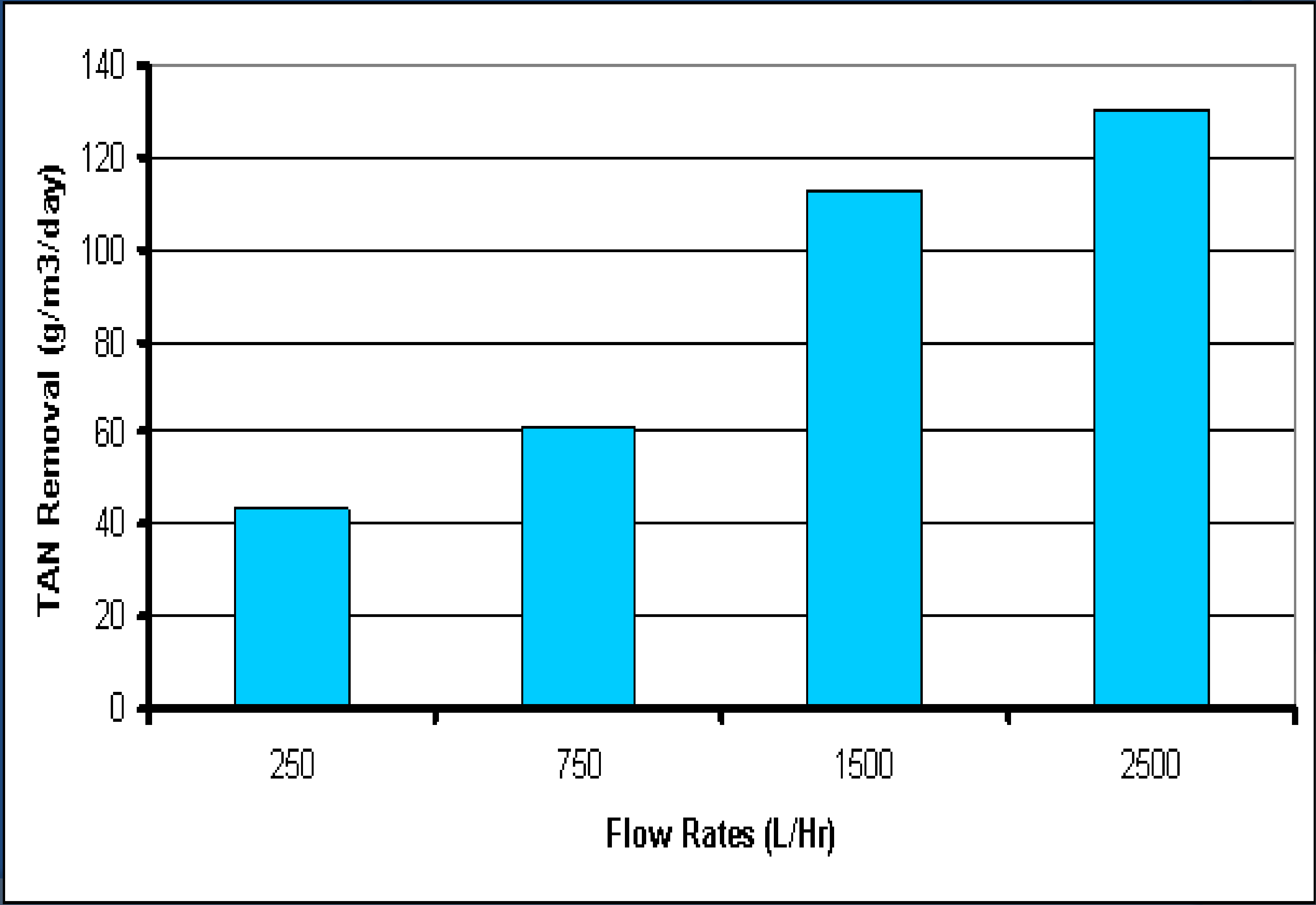
Nitrification efficiency



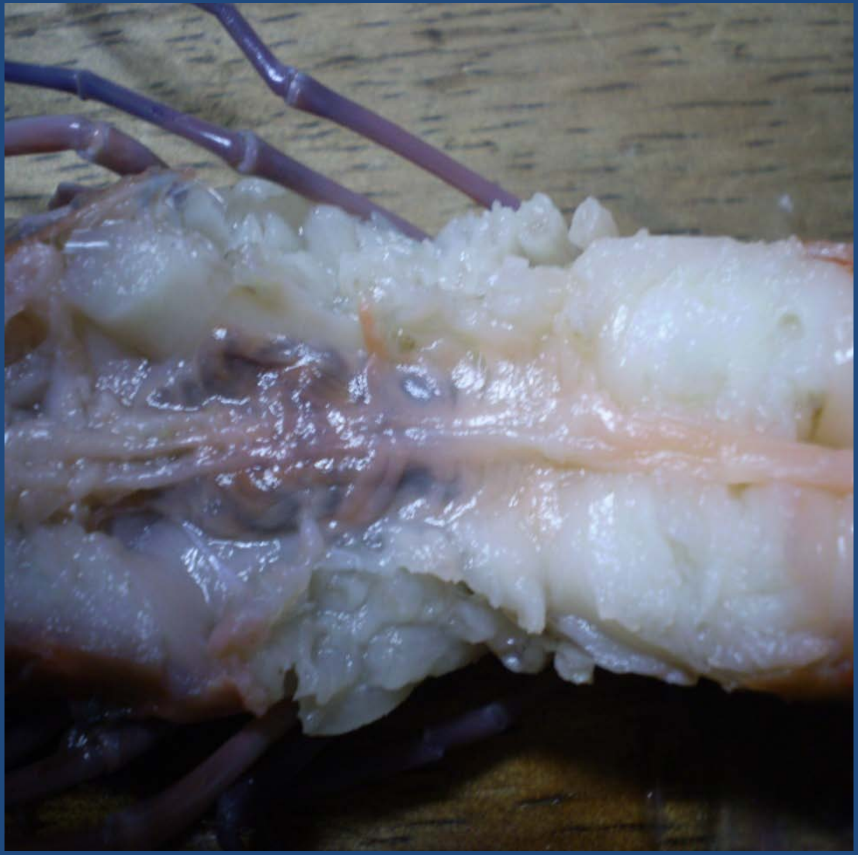
Substrate reduction at varied concentration



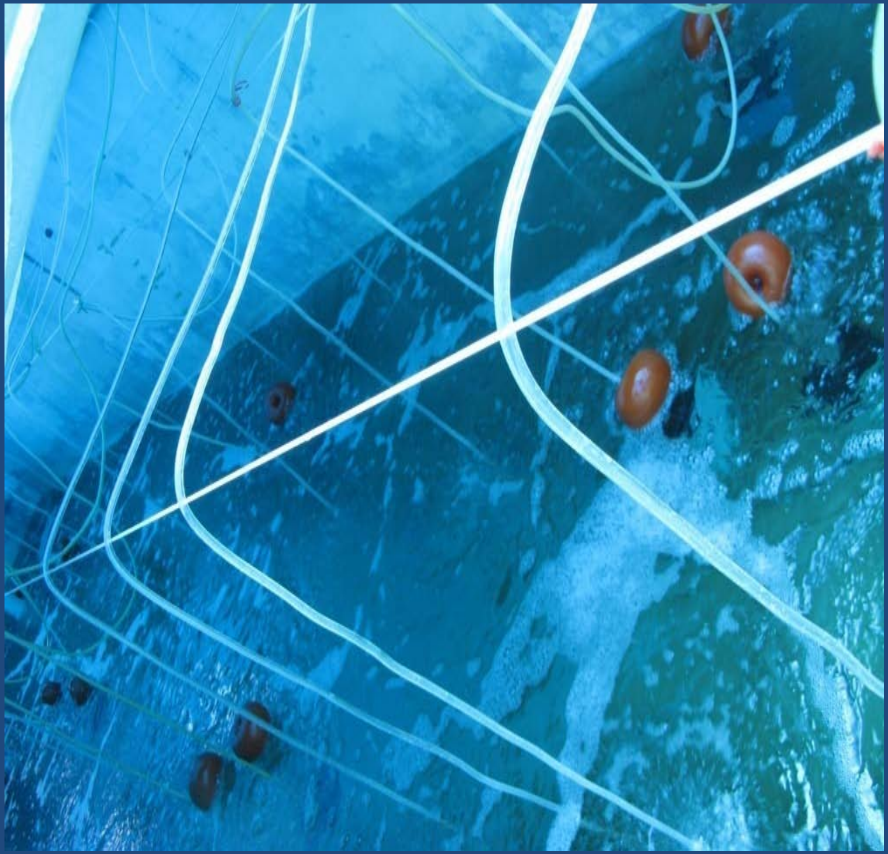
VOLUMETRIC TAN REMOVAL RATES BY THE REACTOR WITH FLOW RATE



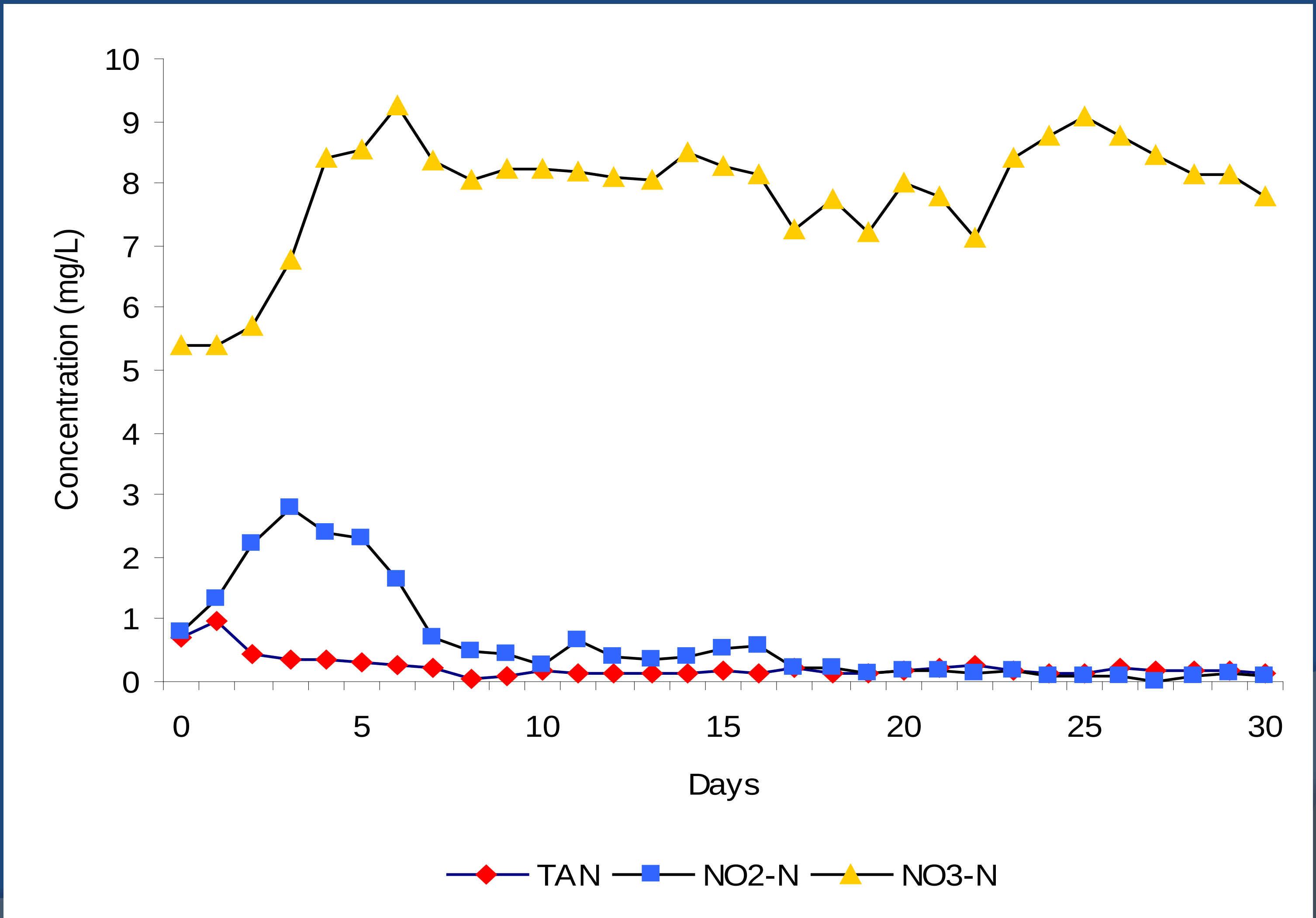
Developing ovary in the animals reared in the recirculating system



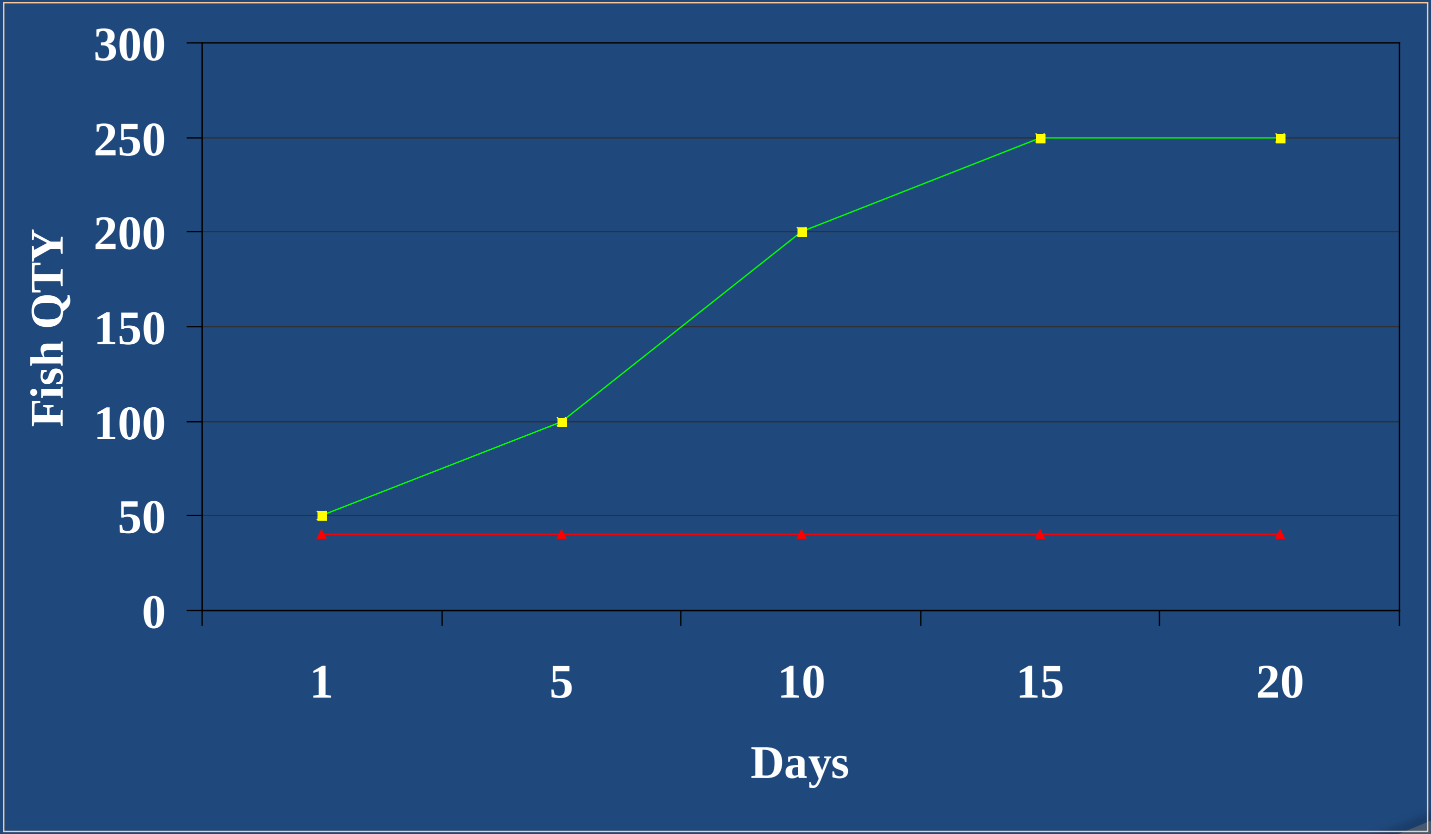
Stringed Bed Suspended Bioreactors deployed in a Larval Rearing System
Queens Hatchery- Kodungalloore



TAN, NO2-N and NO3-N experimental systems integrated with SBSBR rearing *Penaeus monodon* brooders



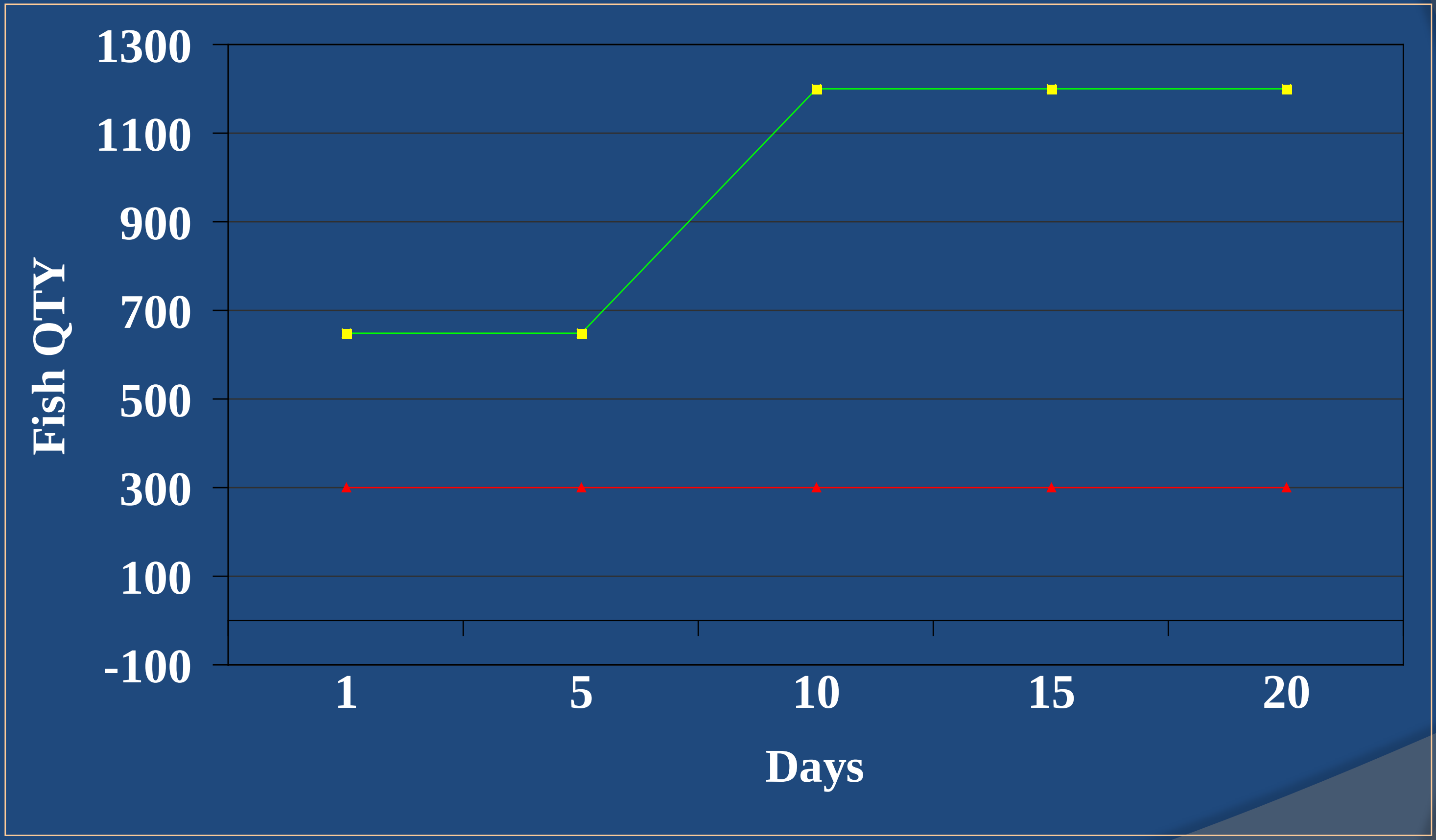
Trial at Aliyar



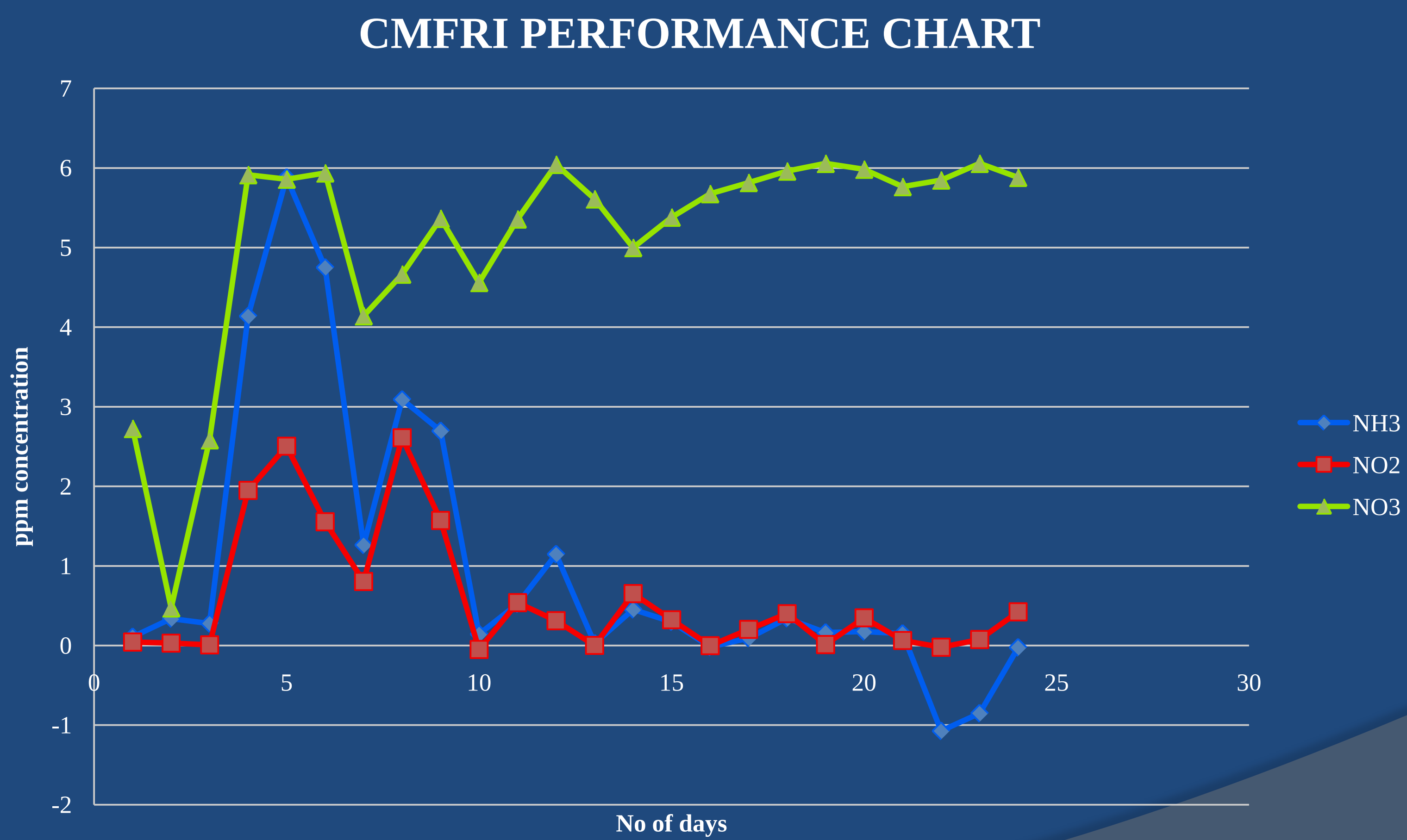
Koi carp (Each 10g)

Golden fish farm ,Calicut

Fish: Angel (Size:1cm to 3cm)



CMFRI-Kochi



PBBR integrated to a Grouper Broodstock Tank.

APPLICATION

- ORNAMENTAL FISH

  FRESH WATER
  MARINE WATER

- EDIBLE FISH

  SEA BASS
  LOBSTER
  SHRIMP
  PRAWN
  CRAB
  COBIA, ETC

NITRIFYING BIOREACTOR – ADVANTAGES

- Improved quality of fish produced (Organic)
- Increase in fish survival rate
- Increase in rate of growth and the size of fish
- Better resistance to diseases
- Reduction in production costs
- Improvement in surrounding environment

NITRIFYING BIOREACTOR - TECHNOLOGY

- Patented technology

- First of its kind in the world

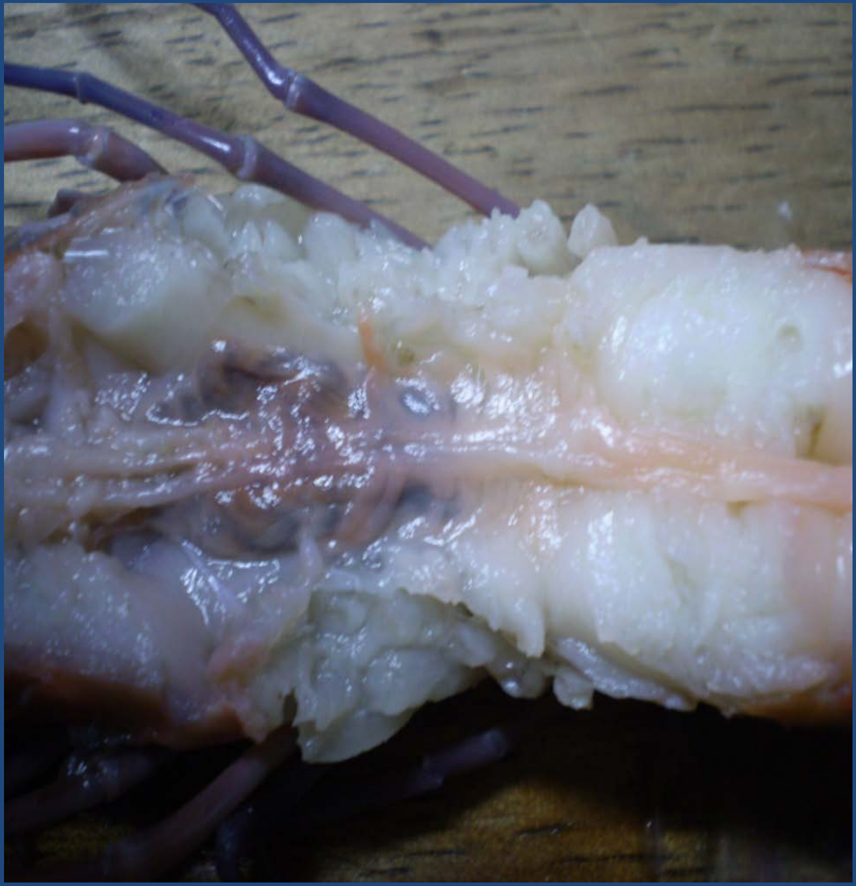
- Maintains reef-like conditions in aquaculture tanks

- Can be used for any level of salinity
  - Fresh Water
  - Salt Water
  - Brackish Water

- Can be used to farm a variety of aquatic animals
  - Shrimp
  - Ornamental Fish
  - Crabs/Lobsters

BENEFITS

Maturation (Developing ovary in the animals reared in the recirculating system)



BROOD STOCK MAINTENANCE & REARING

INCREASE SURVIVAL RATE

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RECIRCULATING AQUACULTURE SYSTEM

- ❑Location flexible – transport costs minimized
- ❑Fewer tanks – less capital outlay
- ❑Temp and other parameters controlled
- ❑Planned production schedules, control supplies of fingerlings to target seasonal market demand by cage farmers Lower mortality, improved FCRs
- ❑RAS allow diversification of the farmed species base for the market substituting seafood imports and opening new markets in live fish sales

THANK YOU

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