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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]

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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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expanded bed treatment system[8], fixed film nitrification system[9], submerged flow biofilters[10], high-rate linear path trickling nitrification filters[11], bench-scale fluidized bed bioreactors[12] and continuous bioreactors using immobilized alginate beads[13] are the documented pilot scale treatment systems with varying degrees of success. The main drawback of such filters is the long conditioning period required for nitrifying bacteria to get established as biofilm on the substrata. It has been reported that the start up period of a biofilters/bioreactors in aquaculture systems[14] ranges from 28 to 60 days at temperatures between 21° and 26°C. Perfettini & Bianchi[15] demonstrated that, without seeding the system with nitrifying bacteria, even up to 40 days are required for the establishment of a nitrifying community.

Considering the above requirement of speeding up the conditioning of biological filters Spotte[16] applied freeze-dried inoculates of nitrifying bacteria in freshwater aquaria, but with inconsistent performance. Past studies of Timmermans and Gerard [17] showed that such commercial preparations were poorly effective under field conditions for unknown reasons. Meanwhile, Grommen *et al.*, [18] reported the availability of ammonia binding inoculum - liquid (ABIL) useful for freshwater fish aquarium. But such category of commercial nitrifying bacterial cultures developed for fresh water aquaria are not useful for shrimp/prawn hatcheries due to their sensitivity to salinity variations. A nitrifying bacterial consortium developed for fresh water environment may not function as such under saline conditions unless they are acclimated properly. Moreover, nitrifiers enriched from temperate waters may not function in the tropics due to variations in temperature optima. Meanwhile Young[19] reported that efficacy of commercial bioaugmentation products is questionable and suppliers of such products often overstate their potential. Shan & Obbard [20] reported ammonia removal from prawn aquaculture water using nitrifying bacteria immobilized on to porous clay pellets developed from a tropical environment by enrichment technique. The immobilized cultures were able to achieve a high total ammonia-nitrogen (TAN) removal under laboratory conditions and studies are underway to evaluate their usefulness under field conditions. However, these cultures are not available commercially for applying in shrimp/prawn hatcheries.

As the long duration start-up time of any of the above devices in a commercial hatchery configuration is not viable, it has become the requirement to develop nitrifying bacterial consortia to grow as biofilms on substrata that would function under the hydrographical conditions of tropical shrimp/prawn hatcheries. The conditions include salinity of 15 ‰ for non-penaeids and 30 ‰ for penaeids, *p*H ranging from 7.5 to 8.5, and temperature between 25° to 28°C. To satisfy such requirements viable cultures of nitrifying bacteria at high cell density in the active growth phase are required. This requirement prompted us to implement a carefully planned process to develop such indigenous start-up cultures taking in to consideration of the specific requirements of target hatchery systems. This paper deals with the outcome of the process followed in developing and partially characterizing nitrifying bacterial consortia having the requisites for establishing nitrification in prawn/shrimp hatchery systems. Besides, efficacy of the consortia in establishing nitrification within a short duration start up period was also demonstrated by immobilizing them in a Packed Bed Bioreactors (PBBR)[21].

Materials and Methods

Samples

For enriching nitrifying bacterial consortia required for penaeid hatchery systems, samples were collected from sites where salinity 30‰ had been experienced throughout the year. The sampling stations were (1) coastal zone off Kannamali, Kochi, (2) Model Shrimp Farm and Training Centre, Aquaculture Development Agency (ADAK), Maala, Trichur, (3) prawn grow out system, Aayiramthengu, Alleppey, and (4) biological filter of Recirculation Aquaculture System (RAS) used for maintenance of shrimps in this laboratory (NCAAH). Similarly samples for enriching nitrifiers required for non-penaeid hatchery systems, especially for *Macrobrachium rosenbergii*, were collected from stations and systems which were perpetually experiencing salinity of about 15‰. They were (1) prawn grow out system, Maradu, Kochi, (2) biological filter of RAS maintained for prawn larval rearing system, Sherthallai, and (3) biological filter of RAS used for prawns in this laboratory (NCAAH).

Water samples were collected in 20 L polythene carboys from all above locations and filterent grains (100 g) in polythene bags from the biological filters of

RAS. The water samples were transported to laboratory under ambient conditions and the sand grains at 4°C in thermocol boxes.

Pre-enrichment

Pre-enrichment of nitrifiers was achieved in a sand-based biological filter facility set up in the laboratory in which the water samples collected from various locations were enriched (Fig. 1). Basins of the assembly were filled with the collected water samples and airlift pumps operated. $(NH_4)_2SO_4$ was added to achieve 10 mg l^{-1} final concentration NH_4^+-N. Once in every 24 hours, estimations of NH_4^+-N[22], NO_2^--N[23], and NO_3^--N[24] were carried out along with adjustment of *p*H to 8.0 using 10 % aqueous Na_2CO_3. The substrate was added as and when 90 % of which got exhausted. The process was continued till 80-300 mg l^{-1} NH^+-N was consumed over a period of 90 to 160 days and at that stage an aliquot of 1 ml water sample and 1g filterent grains were removed for primary enrichment of nitrifiers in synthetic media. While the pre-enrichment was progressing with the water samples as mentioned above, filterent grains collected from the biological filter of RAS for penaeids in this laboratory, the one at Shertallai and the filter facility attached to the non-penaeid RAS setup in this laboratoy were inoculated with 1g aliquots to the synthetic media. Accordingly seven samples were subjected for the primary enrichment.

Adhesion of nitrifiers to filterent grains

A portion of filterent grains taken out from the pre-enrichment system was washed gently with sterile seawater of the same salinity and fixed in 3 % v/v glutaraldehyde for 24 hours at 4˚C and dehydrated using graded acetone (30 to 70 %), air dried, gold coated in sputter coater and observed under scanning electronmicroscope (H 600, Hitachi Ltd., Japan).

Media for primary and secondary enrichment

Three media were employed for the enrichment of nitrifiers. Medium I was prepared[25] in the water samples themselves (100 ml) by supplementing with autoclaved $(NH_4)_2SO_4$ or $NaNO_2$, 10 mg; K_2HPO_4, 2 mg; $NaHCO_3$, 5 mg and adjusting *p*H to 8. The medium II contained[26] seawater (either salinity 30‰ or 15‰), 100 ml; $(NH_4)_2SO_4$ 4.72 mg or $NaNO_2$, 4.93 mg; K_2HPO_4, 0.88 mg; *p*H adjusted to 8. This was autoclaved at 15 lb for 15 min and inoculated with 1 ml water and 1g filterent grains, independently

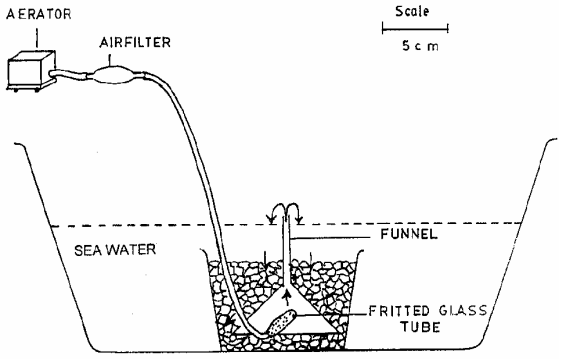

Fig. 1 — Pre-enrichment of nitrifiers- sand based biological filter facility setup

from the pre-enrichment systems. Medium III contained[26] seawater 100 ml (either salinity 30‰ or 15‰), $(NH_4)_2SO_4$ or $NaNO_2$, 132 mg; $MgSO_4.7H_2O$, 20 mg; $CaCl_2$, 2 mg; K_2HPO_4, 11.4 mg; chelated iron, 13 mg; Na_2 $MoO_4.2H_2O$, 0.0001 mg; $MnCl_2.4$ H_2O, 0.0002 mg; $CoCl_2.6$ H_2O, 0.0002 mg; $CuSO_4.$ H_2O, 0.002 mg; $ZnSO_4.7$ H_2O, 0.01 mg; *p*H adjusted to 8 .The medium was autoclaved at 15 lb for 15 min and inoculated with water (1 ml) or sand grains (1g) from the pre- enrichment system as described above.

Primary enrichment was done invariably in 100 ml media in 250 ml conical flasks, by incubating in dark at room temperature (28±1˚C) on a rotary shaker. The samples included water and filterent grains from the four pre-enrichment system and the filterent grains from three biological filters of RAS. All flasks were monitored daily for the oxidation of NH_4^+-N to NO_2^--N in the media meant for ammonia oxidizers and NO_2^--N to NO_3^--N in the case of nitrite oxidizers. Replenishment of the substrate and *p*H adjustment were made wherever necessary.

When the cultures under primary enrichment entered near stationary phase, marked with cessation of substrate consumption, a comparison of the cultures was made with respect to total quantity of the substrate consumed over a period of 231 days. Based on the comparative performance data, the most efficient cultures were segregated from each sampling station/site and subjected for secondary enrichment. This was done by inoculating 1 ml of the primarily enriched cultures to freshly prepared Medium II[26], and incubating over magnetic stirrers at 100 rpm at room temperature (28±1°C). The Medium II[26] was chosen for the secondary enrichment by virtue of its simple composition, and comparatively better performance observed among the three media.

Selection of the best consortia

The secondarily enriched cultures were spiked with 10 mg l^{-1} NH_4^+-N and NO_2^--N respectively and the substrate consumed for 24 hours was assessed. The consortia which consumed the highest quantity of NH_4^+-N and NO_2^--N were segregated for amplification and characterization. They were named as AMOPCU-1 (ammonia oxidizing consortium for penaeid culture), NIOPCU-1 (nitrite oxidizing consortium for penaeid culture), AMONPCU-1 (ammonia oxidizing consortium for non-penaeid culture) and NIONPCU-1 (nitrite oxidizing consortium for non-penaeid culture).

Amplification of the selected consortia in fermentor

Amplification of the consortia was carried out in a 2 litre capacity New Brunswick fermentor (BIOFLO 2000). Uniformly for all consortia, Medium II[26] prepared either in seawater having salinity 15‰ or 30‰ was used. The fermentor vessel along with the media was autoclaved at 10 lb for 10 min, *p*H set at 8, temperature at 28°C and agitation at 200 rpm and aeration at 0.6 l.min^{-1} and the vessel was covered with black cloth. From the final enrichment cultures, the fermentor vessel was inoculated with 20 ml consortium and incubated under conditions described. Whenever *p*H decreased, it was adjusted to 8 by automatic addition of 10 % sterile Na_2CO_3 through the base addition port.

Once in every 24 hours the uptake of substrate and formation of product were monitored, and depending on the requirement, the substrate was added exponentially through the substrate addition port. When the rates of substrate uptake and product formation declined, indicating attainment of stationary phase, the cultures were harvested and concentrated by centrifugation at 7000 g at 4ºC in a refrigerated centrifuge (Remi Instruments, Bombay). The centrifuged culture pellet was washed and resuspended in fresh medium supplemented with 10 mg. l^{-1} substrates (NH_4^+-N or NO_2^--N). Dry weight of the concentrated biomass of the consortia was determined gravimetrically, from which the cell yield was determined for each consortium, following Sharma & Ahlert[27]. Yield coefficient is defined as the weight of cells generated to the weight of the substrate oxidized or as the ratio of the weight of product formed to the weight of substrate utilized. In this study the latter relationship was considered for reasons discussed elsewhere. The concentrated consortia were stored in tightly stoppered sterile glass bottles at 4°C with intermittent addition of the substrates.

Primary characterization

The consortia amplified in the fermentor were characterized primarily by Gram staining for cellular morphology, staining reaction and zoogloeal formation. They were observed under phase contrast microscope and subjected for scanning electron microscopy (SEM). For SEM, 1ml aliquot each of the consortia were centrifuged at 8000 g fixed in 2.5% glutaraldehyde at 4°C for 8 hr, post - fixed in 1% osmium tetroxide, dehydrated in acetone, dried in critical point drying apparatus, gold coated and observed under scanning electron microscope (H 600, Hitachi Ltd., Japan).

Efficacy of the consortia in a reactor configuration

To demonstrate efficacy of the developed consortia for activating reactors designed for shrimp/prawn hatcheries, two consortia AMONPCU-1 and NIONPCU-1 were amplified in a baby fermentor as described elsewhere, harvested and used for immobilizing on cartridges of the Packed Bed Bioreactor (PBBR). Briefly, configuration of the reactor is as follows: the reactor has a fiberglass shell with a base of 30 cm^2 and an overall height of 45 cm, packed with polystyrene beads. Nine airlift pumps are positioned at equidistance. The PBBR package consists an overhead tank, reactors, collecting tank, water and air pumps, air distribution system and flow regulators. The above consortia were mixed together and introduced into the reactors. Rest of the volume of the reactors was filled with seawater having salinity 15‰ supplemented with 10 mg.l^{-1} NH_4-N in the form of NH_4 $(SO_4)_2$ and 2 mg. l^{-1} PO_4-P in the form of KH_2 PO_4. The reactors were operated for 7 days till the consortia were fully immobilized on the substratum. This was assured from the extent of removal of the consortia and NH_4^+-N from the bulk volume. Subsequently the system was spiked with 10 mg.l^{-1} NH_4^+ -N and the performance of the reactors was monitored for 4 hr by determining the residual NH_4^+-N, NO_2^--N, and NO_3-N.

Results

Quantity of NH_4^+- N consumed over a period of 160 days in the pre-enrichment system (Table 1) indicated that nitrifiers had colonized the filterent grains. SEM studies of the filterent grains revealed a thin biofilm on their surface, presumed to be composed of both nitrifiers and heterotrophs (data not presented).

Figure 2 summarizes the cumulative consumption of NH_4^+–N and NO_2^-–N during the primary

Table 1 — Total NH^{+}_{4}-N consumed over a period of 160 days by the biological filter setup with water from Kannamaali, Maala, Aayiramthengu and Maradu

Sample	Salinity (‰)	NH_4^+-N consumed over a period of 160 days (mg l^{-1})
Kannamaali	30	185.31
Maala	30	206.54
Aayiramthengu	30	274.14
Maradu	15	89.06

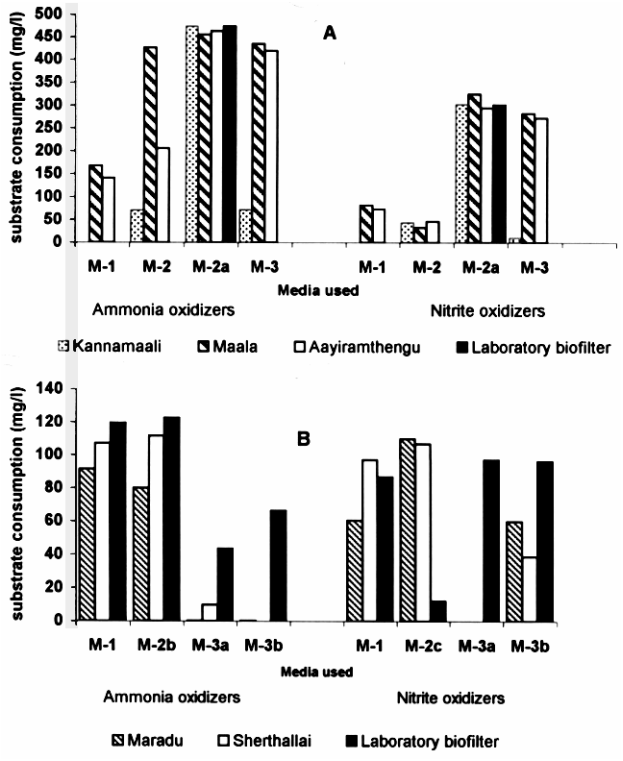

Fig. 2 — Substrate consumption during primary enrichment in three media by the (A) nitrifying bacterial consortia developed from various locations for penaeid hatchery system over a period of 230 days and (B) nitrifying bacterial consortia developed from various locations for non-penaeid hatchery systems over a period of 100 days.

M1 - Medium I prepared in water sample as such. M2 - Medium II inoculated with water samples from biological filters. M2a - Medium II inoculated with filtrant grains from biological filters. M2b - Medium II inoculated with water samples from pre enrichment system. M2c - Medium II inoculated with filtrant grains from the pre-enrichment system. M3 - Medium III inoculated with water samples from biological filters. M3a - Medium III inoculated with water samples from the pre enrichment system. M3b - Medium III inoculated with filtrant grains from the pre enrichment system.

enrichment of nitrifiers required for both penaeid and non-penaeid larval rearing systems. In general the consortia developed in Medium II from the filterent grains of the pre enrichment systems (salinity 30‰) and biological filter of RAS (salinity 15‰) were the ones, which exhibited the highest level of activity.

Substrate uptake by the consortia that exhibited the highest level of activity during the secondary enrichment and the 24-hour short-term consumption assay are presented in Fig 3. Based on the results the ammonia oxidizing and nitrite oxidizing consortia developed from the Model Shrimp Farm and Training Centre, ADAK, Maala and the one developed from the biological filter of RAS at Sherthalai were segregated and named as AMOPCU-1, NIOPCU-1 and AMONPCU-1 and NIONPCU-1 respectively.

Amplification kinetics of the four consortia are summarized in Table 2**.** Among the ammonia oxidizing consortia the yield coefficient of

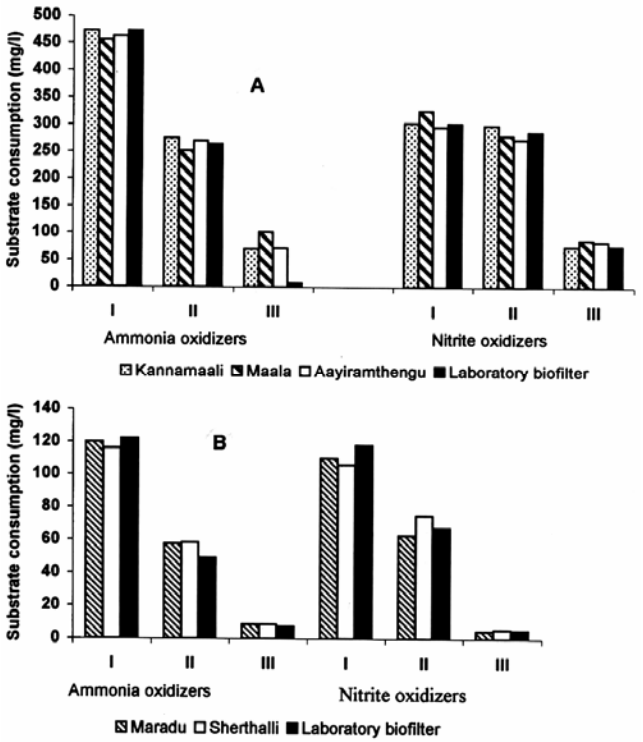

Fig. 3 — Substrate consumption during primary and secondary enrichment and 24 hr substrate consumption assay by the nitrifying bacterial consortia developed from various locations for (A) penaeid hatchery system and (B) non penaeid hatchery system.

I - Substrate consumption of the nitrifying bacterial consortia during primary enrichment (230 days) in medium II from filterant grains of the pre enrichment system. II - Substrate consumption during secondary enrichment in medium II. III - Substrate consumption during the 24 hrs short-term consumption assay.

AMONPCU-1 was higher than AMOPCU-1 and that of NIOPCU-1 and NIONPCU-1 were more or less the same. Growth pattern of all the four consortia documented during the amplification is presented in Fig. 4. Stationary phase in the growth curve is marked by cessation in the product formation.

The amplified consortia contained Gram-negative and Gram-positive bacteria clumped together. Phase contrast microscopy revealed the presence of cells and cysts embedded in a mucilaginous material (Fig. 5) and scanning electron micrographs revealed the biofilm nature of the consortia with the cells embedded in a glycocalyx like material.

Nitrifying potential of the activated Packed Bed Bioreactor using the developed nitrifying consortia AMONPCU-1 and NIONPCU-1 is summarized in Fig. 6. Within 4 hr of operation the reactor could convert 10 mg. l^{-1} NH_4^+-N to NO_3^--N with negligible

Table 2 — Substrate uptake, product formed, biomass generated and total number of days required for attaining stationary phase for the four consortia amplified in fermentor

Code name of consortia	Substrate uptake (mg l^{-1}) (a)	Product formed (mg l^{-1}) (b)	Biomass generated (mg dry weight l^{-1}) (c)	Number of days for attaining stationary phase (d)	Yield coefficient b/a	Yield coefficient c/a
AMOPCU I	1403.6[a]	292.4[c]	65	27	0.208	0.05
NIOPCU I	1976.2[b]	1775.2[d]	59.4	53	0.898	0.03
AMONPCU I	303.7[a]	421[c]	30	37	1.386	0.09
NIONPCU I	441.9[b]	356.2[d]	73.75	48	0.806	0.17

A = NH_4^+-N as substrate, b = NO_2^-–N as substrate, c = NO_2^-–N as product, d = NO_3^-–N as product

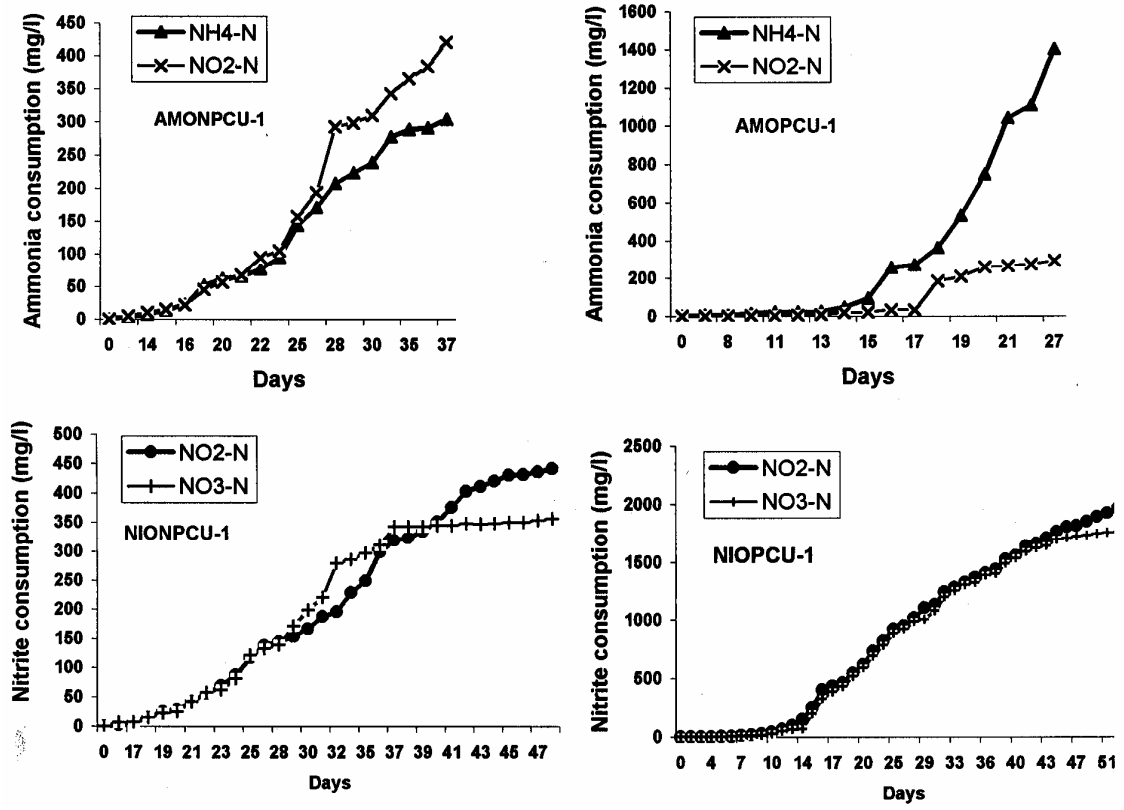

Fig.4 – Growth pattern of the consortia of nitrifiers segregated

AMONPCU-1 – Ammonia oxidizing consortium for non penaeid culture system. AMOPCU-1 – Ammonia oxidizing consortium for penaeid culture system, NIONPCU-1 – Nitrite oxidizing consortium for non penaeid culture system, NIOPCU-1 – Nitrite oxidizing consortium for penaeid culture system.

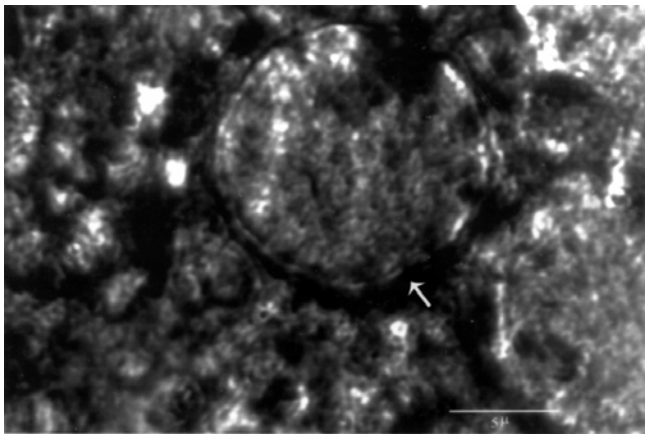

Fig. 5 — Phase contrast photomicrograph of the nitrifying consortium NIOPCU-I (Note the formation of cysts)

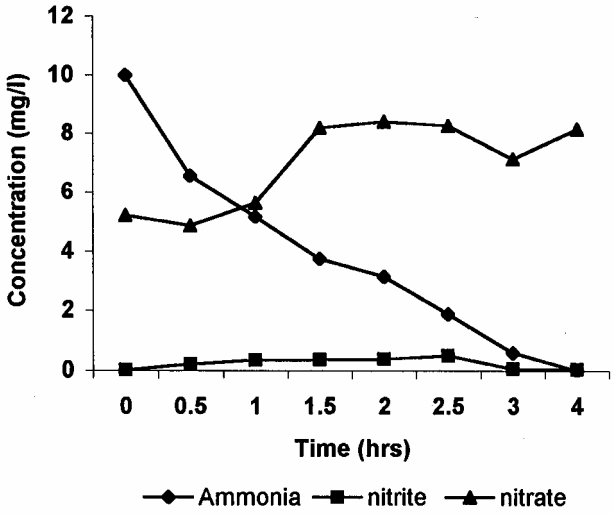

Fig. 6 — Nitrification in a packed bed bioreactor activated by AMONPCU-1

residual nitrite, suggesting that the two-step nitrification process could be set in as per the requirement.

Discussion

Through a series of enrichment systems (pre, primary and secondary enrichments) two ammonia oxidizing and two nitrite oxidizing consortia could be developed for establishing nitrification in penaeid and non-penaeid hatchery systems. In this process the pre-enrichment phase played a significant role in selecting and enhancing the growth of those nitrifiers, which could form biofilm on a support material. Spotte[5] & Kawai *et al.*[28] reported that biological filter beds provided greater surface area for nitrifiers to attach to and colonize making them 100 times more plentiful on sand grains than suspended in water. Scanning electron microscopy revealed a slimy material surrounding the colonies attached to the substratum, which Cox *et al.*[29] had demonstrated as poly-saccharide in nature. This slime material appeared to 'cement' cells to the surface and offered protection from unfavorable environmental conditions. From these observations, it was apparent that the process of pre-enrichment of nitrifiers in a biological filter configuration was a good method of enhancing growth of biofilm forming nitrifiers in a limited space facilitating easy subsequent enrichment and isolation. Also, it is possible to maintain the nitrifiers in the biological filter for a long period just by adding sufficient quantity (10 mg l^{-1}) of NH_4^+–N periodically and by removing and replacing one third of the water with aged and autoclaved seawater to prevent inhibition of nitrification by the accumulated nitrate. Precisely, the filterent grains of the biological filters (pre-enrichment system) were found to be the best source of nitrifiers as during the subsequent primary and secondary enrichments the most potent consortia obtained were found to have emerged from them. As the nitrifiers were derived from the biofilm formed around the filterent grains, this property could be taken advantage of in developing nitrifying bioreactors where the nitrifiers were immobilized on desirable substrata to form biofilm.

Among the three media employed for enrichment, growth of the consortia was comparatively higher in Medium II suggesting suitability of its composition in enriching nitrifiers from varied sources. Incidentally this medium happened to be the one with the simplest composition. Henrikson[30] had pointed out the usefulness of such simple media, composed of seawater enriched with NH_4Cl and $KH_2\ PO_4$ for the cultivation of nitrifying bacteria from marine environment. Undoubtedly, utilization of a medium with simplest composition for enrichment has obvious commercial benefits.

Growth pattern of the consortia in a baby fermentor suggested a decline in the rate of product formation after a period of amplification while the substrate uptake was on its rise till the day of harvest. Obviously this could be considered as the attainment of stationary phase by the consortia in a batch process. If amplification had been continued in this

mode, ratio of the product formation to the substrate consumption might have declined resulting in poor quality consortia with less active nitrifiers. To avoid this situation the consortia was harvested readily.

The overall substrate taken up, product formed, biomass generated and the total number of days essentially required for attaining stationary phase by each consortium indicated tremendous wall growth of nitrifiers that had taken place during the culture. This obviously resulted in a low biomass recovery. In one situation the nitrite produced by AMONPCU-1 (Fig. 4) was found to be more than the ammonia it had consumed. Such situations were generally seen under experimental conditions when continuous aeration was provided. In such situations NH_3-N from atmosphere might have entered the system and served as the substrate over and above provided in the medium.

Gram stained preparations showed cells embedded in a matrix of mucilaginous glycocalyx - like material supposed to be composed of polysaccharides. Along with the majority of Gram-negative rods, Gram-positive bacteria also could be seen, suggesting that they were mixed culture consortia. In enrichment cultures, ammonia-oxidizing bacteria frequently occur as cell aggregates, referred to as zoogloea or cysts, and such aggregates are seldom seen in pure cultures[31]. However, nitrification has been reported much faster in mixed cultures than in pure form [32]. Therefore it was decided to maintain the consortia as such without resolving or purifying, considering them as biological entities having the unique characteristic of nitrification for all practical purposes in shrimp/prawn hatcheries.

As part of demonstration of usefulness of the developed consortia in activating bioreactors the consortia developed for non-penaeid hatchery systems (AMONPCU-1 and NIONPCU-1) were immobilized in a packed Bed Bioreactor and examined their efficacy. Rapid disappearance of NH_4^+–N and concomitant building up of NO_3-N with traces of residual NO_2^-–N suggested the establishment of two-step nitrification, much needed for the hatchery configuration. The data indicated that if such reactors could be integrated to prawn hatchery systems, it would be possible to maintain NH_4^+-N and NO_2^--N near to zero. As the larval rearing systems are supplemented with diatoms as live feed it is unlikely to have substantial NO_3-N build up, as the diatoms consume the latter as the nitrogen source. These consortia are maintained as liquid cultures at 4˚C with intermittent addition of the substrates. The nitrifying consortia form part of the culture collection of National Centre for Aquatic Animal Health (Cochin University of Science and Technology, Cochin, India). For large-scale production of the consortia an indigenous 200 L fermentor has been developed and a mass production protocol standardized. This facilitates availability of the consortia in shrimp/ prawn hatcheries in the tropics[33].

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