Vol. 25(3):277-286 | Ocean and Polar Research | September 2003

Article

Organic Matter and Hydraulic Loading Effects on Nitrification Performance in Fixed Film Biofilters with Different Filter Media

Lei Peng[1], Sung-Yong Oh[2], and Jae-Yoon Jo[1]*

[1]*Department of Aquaculture, College of Fisheries Science, Pukyong National University*
599-1 Daeyeon-3-dong, Nam-gu, Busan 608-737, Korea
[2]*Marine Resources laboratory, KORDI*
Ansan P.O. Box 29, Seoul 425-600, Korea

Abstract : Nitrification performance of fixed film biofilters using coarse sand, loess bead, or styrofoam beads in biofilter columns 1 meter high and 30 cm in diameter were studied at different hydraulic and organic matter loading rates. Synthetic wastewater was supplied to the culture tank in order to maintain desired TAN concentrations in inlet water to biofilters. All the biofilters were conditioned 5 months before start of sampling. TAN and NO_2-N conversion rates increased with an increase in the hydraulic loading rate (HLR). However, the improvement in biofilter performance was not linearly correlated to HLR in styrofoam bead filters. This is mainly due to the characteristics of the styrofoam beads used. TAN conversion rates of sand filters increased with the increase of HLR up to 200 m^3/m^2·per day. No increase in the TAN conversion rate was observed at the highest HLR since flooding on the media surface took place. HLR had a significant impact on the TAN conversion rates in loess bead filter up to the highest HLR tested ($P<0.05$). TAN conversion rates were much less at organic matter loading rates of 9 and 18 kg O_2/m^3·per day than those without the addition of organic matter in styrofoam bead filters. The addition of glucose resulted in a reduction of the TAN conversion rate from 540 to 284 g TAN/m^3·per day. No significant difference of TAN conversion rates between the two organic matter loading rates was found ($p<0.05$). This indicates that the impact of organic matter on nitrification becomes less and less sensitive with an increase in the COD/TAN ratio. At an organic matter loading rate of 9 kg O_2/m^3·per day, a great reduction of TAN conversion rates was observed in sand filters and loess bead filters. Clearly, organic matter can be one of the most important impacting factors on nitrification. NO_2-N conversion rates showed a similar trend for TAN. Based on the TAN and nitrite conversion rates, styrofoam beads showed the best performance among the three filter media tested. Also, the low gravity and price of styrofoam beads make the handling easier and more cost-effective for commercial application. The results obtained at the highest organic matter loading rates can be used in the biofilter design in recirculating aquaculture system.

Key words : nitrification, fixed film biofilter, TAN, hydraulic loading rate, organic matter

1. Introduction

It is widely acknowledged that fish supplies from the world fisheries are unlikely to increase substantially and the expansion of the aquaculture sector will probably provide the solution to the problem of projected shortfalls (Chamberlain and Rosanthal 1995). Not only has this led to the quick development of aquaculture systems, but also to a shift towards the intensification of the fish culture system because of the shortage of water resources and high land costs. In an intensive aquaculture system, water quality tends to deteriorate rapidly due to the accumulation of uneaten feed, feces, and inorganic metabolic wastes, especially ammonia excreted by fish or decomposition of organic matter. Recirculating aquaculture systems (RAS) are specially developed to address these problems. The prohibitive release of nutrient water into the environment by environmental regulations, the high expense associated

---

*Corresponding author. E-mail : jyjo@pknu.ac.kr with pumping large amounts of water, and the danger of introducing pathogens also initiated the development of RAS. Besides, recirculating aquaculture systems provides many advantages compared with traditional aquaculture systems, including conserving of water and heat, maintaining better control over environmental factors, and providing a quality controlled product. The advantages have been well documented for many years (Liao and Mayor 1974; Losordo 1991).

Ammonia may lead to suppression of fish growth, sublethal histopathological changes, and even death (Redney and Stickney 1979). Thus, ammonia is considered toxic to fish and has attracted lots of research to study how to remove it from aquaculture systems. By far, biofilters have been most commonly used for ammonia removal in aquaculture systems and have been considered as the main and crucial component of recirculating systems. The key elements for biofilter design are the media to be used and operational parameters (Wheaton *et al.* 1994; Lekang and Kleppe 2000).

Many types of biofilters with different configurations including trickling filters, submerged filters, sand filters, and fluidized bed filters are employed in aquaculture systems. Among them, tricking filters and submerged filters have many advantages including low construction cost, easy management and maintenance, and excellent adaptation to different water and waste loading rates (Lekang and Kleppe 2000). At present, down flow trickling or submerged biofilters are commonly used in aquaculture systems in Korea. However, not much information about the performance of biofilters in seawater systems is available. Hill and Gelman (1977) found that the oxidation of ammonia was almost completely inhibited and that conversion of nitrite to nitrate as a result of the chlorinity of seawater had been brought to a stop. However, complete nitrifications in seawater systems have been reported (Forster 1974; Bower and Turner 1981; Weatherly 1984). Recent studies in shrimp aquaculture systems with different biofilter media showed promising prospects of the seawater recirculating systems (Davis and Arnold 1998; Tseng *et al.* 1998; Menasveta *et al.* 2001).

Various types of filter media are used to provide utilizable surfaces for nitrifying bacteria to grow on in aquaculture and wastewater treatment systems (Nijhof and Bovendeur 1990; Greiner and Timmons 1998; Lekang and Kleppe 2000; Ridha and Cruz 2001). For optimal nitrification and less clogging of the biofilter, biofilter media should have high surface area and low specific gravity (Wheaton *et al.* 1994; Lekang and Kleppe 2000). Coarse sands (2-5 mm) mixed with shell debris, which can provide a high specific surface area, are mainly used in live fish aquarium biofilters in Korea by now. It is believed that this kind of biofilter not only can remove ammonia but also can help maintain the pH of the culture water. Another biofilter media, styrofoam beads, have been tested in freshwater system and showed a reasonable nitrification capacity (data not published). These kinds of beads have a high specific area and low gravity thus making them very suitable as biofilter media. Loess beads, which are a newly developed biofilter media, together with the above-mentioned sand and styrofoam beads, were used in the present experiment.

Many factors influence the performance of biofilters. The ammonia loading rate is commonly considered the crucial factor since the allowable concentration in aquaculture systems is so low that it may become the rate-limiting factor in filter nitrification (Hochheimer 1990). Hydraulic loading rates also affect the performance of biofilters since the substance loading rates usually are determined by the substance concentration and flow rate through the biofilter. Kaiser and Wheaton (1983) observed that for low ammonia concentration, higher flow rates produce higher ammonia mass removal rates. Organic matter is another factor that affects the performance of nitrification filters, including clogging of biofilters and providing substances for heterotrophic bacteria, which compete with nitrifiers for growing space. Pano and Middlebrook (1983) studied RBC's in wastewater treatment and found that ammonia removal was influenced by organic matter loading with ammonia removal rates decreasing as organic matter loading increased.

Nitrification performance of the three media mentioned above were tested in a simulated seawater aquaculture system at various hydraulic, total ammonia nitrogen (TAN), and organic matter loading rates. The obtained data would be useful for selection of the suitable biofilter media for aquaculture systems. Also, the obtained TAN removal rates in present experiments can serve as a database for designing biofilters filled with the tested media.

2. Materials and methods

System description

The configuration of the whole system is shown in Fig. 1. It consists of a culture tank, recirculating pump, three biofilters (sand filter, styrofoam bead filter and loess bead filter), synthetic wastewater feeding tank, metering pump, and thermostatic heating system. Aeration is supplied in

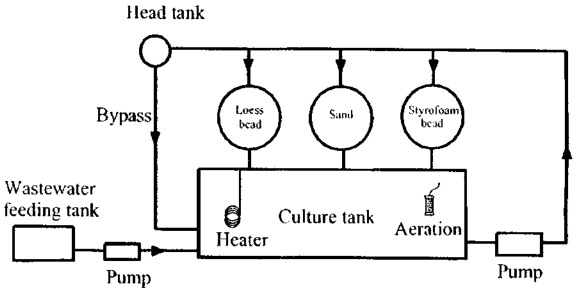

Fig. 1. Schematic diagram of the simulated seawater aquaculture system.

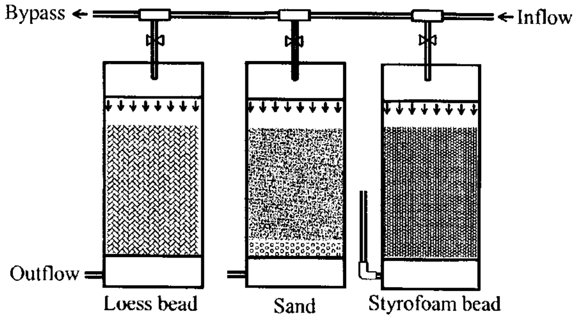

Fig. 2. Configuration and flow patterns of the three biofilters.

the culture tank to increase dissolved oxygen (DO) levels and ensure more efficient mixing of the added synthetic wastewater. The volume of the rectangular culture tank is 880 *l* (1.4 × 0.9 × 0.7 m) and the whole water volume in the system is around 1000 *l*. The three biofilters are placed on a higher level, so the water can flow back to the culture tank by gravity.

Biofilter columns are made of polyvinyl chloride pipes (Fig. 2) and the dimension of each biofilter is 1 m high and 30 cm in diameter. Two gratings are placed inside each biofilter, one near the bottom to prevent the loss of biofilter media and the other at the top to ensure even distribution of inlet water. The high outlet position of the styrofoam bead filter reduced the headloss of this kind of biofilter. Outlets of the other two biofilters are just near the bottom of the filter columns. Approximately 20 *l* of biofilter media was used in each biofilter.

Experiment procedure

The three biofilters were conditioned by feeding synthetic wastewater into them for 5 months. Composition of the synthetic wastewater is shown in Table 1. TAN concentrations in the inlet water were maintained at desired concentrations by changing feeding rate of the synthetic wastewater. Nitrite nitrogen (NO_2-N) and nitrate nitrogen (NO_3-N) concentrations were maintained at low levels by periodic water exchange. Water temperature was maintained at 20°C with a thermostatic heating system.

Table 1. Composition of the synthetic wastewater (modified after Liu and Capdeville, 1994).

Ingredients	Concentration (g/*l*)
NH_4Cl	139
$NaHCO_3$	350
Na_2HPO_4	15.9
KH_2PO_4	15.3
$MnSO_4 \cdot 7H_2O$	3.6

Trial 1 was conducted at different HLRs of 100, 200, and 300 $m^3/m^2 \cdot$ per day for evaluating the effects of HLRs on nitrification rates without addition of organic matter. In trial 2, the biofilters were reconditioned with the addition of organic matter to test the nitrification rates at fixed HLR of 100 $m^3/m^2 \cdot$per day but with different inlet organic matter (COD) concentrations of 0, 25, and 50 mg/*l*, corresponding to COD loading rates of 0, 9, and 18 kg $O_2/m^3 \cdot$ per day, respectively. This trial was only conducted at the lowest HLR tested in the present experiment because at higher HLR, sand filters are easily clogged and make the comparison impractical. The TAN loading rate was set at around 100 g TAN/day$\cdot m^2$ at the column surface area in trial 1 and 2.

In the last trial, the nitrification performance in styrofoam bead filters at various TAN loading rates was tested at fixed hydraulic loading rates of 100 $m^3/m^2 \cdot$per day and fixed organic matter loading rates of 18 kg $O_2/m^3 \cdot$ per day. Since there is a flushing out of styrofoam foam beads and considering the high NO_2-N concentrations in the outlet water at higher hydraulic loading rates, no attempts were made at the higher hydraulic loading rates.

Characteristics of media

Characteristics of the three filter media are shown in Table 2. Styrofoam beads are spherical with an average diameter of 1.4 mm and the calculated specific surface area is 2,820 m^2/m^3. The specific gravity is only one-thirtieth water, so most of the beads in the biofilter tend to float on the water surface. Loess beads are cylindrical (10 mm long and 10 mm in diameter) with some small holes on the surface. These media have the lowest specific surface area of 225 m^2/m^3. Size distribution of sands is within 2 to 4.5 mm and the estimated specific surface area is about 950 m^2/m^3. In sand filters, gravels were placed at

Table 2. Characteristics of the media used in present experiment.

Media	Styrofoam bead	Sand	Losses bead
Specific weight	0.03	2.6	2.2
Size (mm)	1.4	3.5	10.0
Porosity	32%	38%	46%
SSA (m^2/m^3)	2,820	950	225
Total volume (*l*)	20	20	20
PSA (m^2)	0.75	0.75	0.75
TSA (m^2)	57.2	19.8	5.3

PSA, Passive surface area, surface area of filter column and pipes between inlet and outlet of biofilter; SSA, specific surface area; TSA, total surface area.

the bottom layer and then coarse sand was mixed with shell debris in the middle layer. A thin layer of fine sand was added to the surface for even distribution of inlet water.

Sampling and analysis

Water samples were taken randomly after conditioning. All the water samples were taken at the main inlet and the outlet of each biofilter. TAN, NO_2-N, NO_3-N, and COD were measured using the methods described by Strickland and Parsons (1972). Temperature and DO were measured with a DO meter (KDO 5151, KRK Co.). Total alkalinity was measured by the titration method (Grasshoff *et al.* 1999) and pH was measured with a pH meter (Oregon model 720 A).

Performance evaluation

The volumetric TAN conversion rate (VTR), areal TAN conversion rate (ATR), volumetric oxygen consumption rate (OCR), areal nitrite conversion rate (ANR), volumetric nitrite conversion rate (VNR), and volumetric alkalinity consumption rate (VAR) were calculated on a daily basis using the following equations:

$$\text{VTR (g/m}^3\text{·per day)} = 1.44\ (TAN_i\text{-}TAN_e)\ Q/V$$

$$\text{ATR (g/m}^2\text{·per day)} = 1.44\ (TAN_i\text{-}TAN_e)\ Q/A$$

$$\text{VNR (g/m}^3\text{·per day)} = 1.44\ [(TAN_i\text{-}TAN_e) + (NO_{2i}\text{-}NO_{2e})]\ Q/V$$

$$\text{ANR (g/m}^2\text{·per day)} = 1.44\ [(TAN_i\text{-}TAN_e) + (NO_{2i}\text{-}NO_{2e})]\ Q/A$$

$$\text{OCR (g/m}^3\text{·per day)} = 1.44\ (DOi\text{-}DOe)\ Q/V$$

$$\text{VAR (g/m}^3\text{·per day)} = 1.44\ (TA_i\text{-}TA_e)\ Q/V$$

Where, TAN_i and TAN_e, inflow and effluent TAN concentrations (mg/*l*); DO_i and DO_e, inflow and effluent DO concentration (mg/*l*); NO_{2i} and NO_{2e}, inflow and effluent NO_2-N concentrations (mg/*l*); TA_i and TA_e, inflow and effluent total alkalinity concentrations (mg $CaCO_3$/*l*); Q, flow rate through the biofilters (*l*/min); V, filter media volume (m^3); A, total surface area of the biofilter (m^2); 1.44, conversion factor.

Statistical analysis

The sampling data was analyzed by one-way ANOVA (Statistix 3.1, Analytical Software, St. Paul, MN, USA) to test for differences among three filter media. When a significant treatment effect was observed, a Least Significant Different (LSD) test was used to compare means. Treatment effects were considered at a significant level of $P<0.05$.

3. Results

TAN conversion rates

Table 3 shows TAN conversion rates at different hydraulic and organic matter loading rates in the three biofilters in trial 1. TAN conversion rates increased with an increase of HLR in styrofoam bead filters. TAN conversion rates at HLRs of 200 and 300 m^3/m^2·per day were significantly higher than those at HLR of 100 m^3/m^2·per day ($P<0.05$). However, no significant difference was found between HLRs of 200 and 300 m^3/m^2·per day. TAN conversion rates were the same at a high HLR of 200 and 300 m^3/m^2·per day and these values were significantly higher than those at HLR of 100 m^3/m^2·per day in sand filters. TAN conversion rates increased with an increase of HLR in loess bead filters and were significantly higher at HLRs of 200 and 300 m^3/m^2·per day than those at HLR of 100

Table 3. Volumetric TAN conversion rates at different hydraulic loading rates (HLR) and organic matter loading rates (OLR).

Operational parameters		TAN conversion rates (g/m^3·per day)		
HLR (m^3/m^2· per day)	OLR (kg O_2/m^3· per day)	Styrofoam bead	Sand	Loess bead
300	0	737 ± 30^a	264 ± 22^a	911 ± 1^a
200	0	682 ± 35^a	269 ± 8^a	79 ± 5^a
100	0	540 ± 31^b	220 ± 16^b	62 ± 6^b
100	9	301 ± 48^c	129 ± 14^c	40 ± 10^c
100	18	284 ± 23^c	-	-

[1] Values are means ± SD. Values in each column with a different superscript are significantly different ($p < 0.05$).

m^3/m^2·per day. However, no significant differences were found between HLRs of 200 and 300 m^3/m^2·per day.

TAN conversion rates decreased with an increase in organic matter loading rates in all the biofilters tested at HLR of 100 m^3/m^2·per day (Table 3). TAN conversion rates at an organic matter loading rate of zero were significantly higher than those obtained at organic matter loading rates of 9 and 18 kg O_2/m^3·per day in styrofoam bead filters ($P<0.05$). However, no significant differences in TAN conversion rates was found between organic matter loading rates of 9 and 18 kg O_2/m^3·per day. Also, TAN conversion rates in sand and loess bead filters at an organic matter loading rate of zero were significantly higher than those obtained at an organic matter loading rate of 9 kg O_2/m^3·per day. TAN conversion rates at a higher organic matter loading rate of 18 kg O_2/m^3·per day in sand filters and loess bead filters were not measured because sand filters were easily clogged even with frequent backwashing.

NO_2-N conversion rates

NO_2-N conversion rates decreased with an increase of HLR in styrofoam bead filters (Table 4). NO_2-N conversion rates at HLR of 100 m^3/m^2·per day were significantly lower than those at HLRs of 200 and 300 m^3/m^2·per day. However, no significant difference was found between HLR of 200 and 300 m^3/m^2·per day. The increment in NO_2-N conversion rates was not as great as the increment of TAN conversion rates and this caused the increase of NO_2-N concentrations in the outlet water at higher HLRs. In sand filters, NO_2-N conversion rates were the same at high HLRs of 200 and 300 m^3/m^2·per day and these values were significantly higher than those at an HLR of 100 m^3/m^2·per day. NO_2-N conversion rates increased with an increase of HLRs in loess bead filters. No significant differences were found between HLRs of 100 and 200 m^3/m^2·per day or between HLRs of 200 and 300 m^3/m^2·per day.

NO_2-N conversion rates decreased with an increase in the organic matter loading rates in all the biofilters tested at HLR of 100 m^3/m^2·per day (Table 4). NO_2-N conversion rates at an organic matter loading rate of zero were significantly higher than those obtained at organic matter loading rates of 9 and 18 kg O_2/m^3·per day in styrofoam bead filters. However, no significant differences were found between organic matter loading rates of 9 and 18 kg O_2/m^3·per day. Also, NO_2-N conversion rates in sand and loess bead filters at an organic matter loading rate of zero were significantly higher than those corresponding values at an organic matter loading rate of 9 kg O_2/m^3·per day.

Oxygen consumption rates

Oxygen consumption rates increased with an increase of HLRs in all the biofilters tested in zero organic matter load treatments (Table 5). Oxygen consumption rates were higher at higher HLRs of 200 and 300 m^3/m^2·per day and these values were significantly higher than those at HLR of 100 m^3/m^2·per day in styrofoam bead filters ($P<0.05$). No significant differences were found between HLRs of 200 and 300 m^3/m^2·per day. Oxygen consumption rates were significantly higher at HLRs of 200 and 300 m^3/m^2·per day than those at HLR of 100 m^3/m^2·per day in sand filters and no significant differences existed between HLRs of 200 and 300 m^3/m^2·per day. Significant differences existed among all the hydraulic treatments in loess bead filters.

Table 4. Volumetric NO_2-N conversion rates at different hydraulic loading rates (HLR) and organic matter loading rates (OLR).

Operational parameters		NO_2-N conversion rates (g/m^3·per day)		
HLR (m^3/m^2·per day)	OLR (kg O_2/m^3·per day)	Styrofoam bead	Sand	Loess bead
300	0	411 ± 20^a	148 ± 10^a	48 ± 7^a
200	0	384 ± 32^a	154 ± 11^a	41 ± 4^{ab}
100	0	348 ± 18^b	132 ± 9^b	32 ± 6^b
100	9	200 ± 28^c	84 ± 5^c	25 ± 10^c
100	18	178 ± 31^c	-	-

[1]Values are means ± SD. Values in each column with a different superscript are significantly different ($p < 0.05$).

Table 5. Volumetric oxygen consumption rates at different hydraulic loading rates (HLR) and organic matter loading rates (OLR).

Operational parameters		Oxygen consumption rates (g/m^3·per day)		
HLR (m^3/m^2·per day)	OLR (kg O_2/m^3·per day)	Styrofoam bead	Sand	Loess bead
300	0	$3,024 \pm 162^a$	917 ± 24^b	316 ± 24^b
200	0	$2,978 \pm 128^a$	908 ± 32^b	280 ± 7^c
100	0	$2,097 \pm 63^c$	821 ± 69^c	239 ± 27^d
100	9	$2,586 \pm 70^b$	$1,523 \pm 45^a$	505 ± 27^a
100	18	$2,736 \pm 104^b$	-	-

[1]Values are means ± SD. Values in each column with a different superscript are significantly different ($p < 0.05$).

Table 6. Volumetric total alkalinity (TA) consumption rates at different hydraulic loading rates (HLR) and organic matter loading rates (OLR).

Operational parameters		TA consumption rates (g/m^3·per day)		
HLR (m^3/m^2· per day)	OLR (kg O_2/m^3 ·per day)	Styrofoam bead	Sand	Loess bead
300	0	5256 ± 540^a	1898 ± 43^a	648 ± 58^a
200	0	4843 ± 361^a	1922 ± 48^a	574 ± 45^a
100	0	3932 ± 198^b	1620 ± 170^b	446 ± 90^b
100	9	2240 ± 140^c	940 ± 84^c	288 ± 32^c
100	18	2010 ± 104^c	-	-

[1] Values are means ± SD. Values in each column with a different superscript are significantly different ($p < 0.05$).

Oxygen consumption rates increased with an increase in organic matter loading rates in all biofilters (Table 5). Oxygen consumption rates were significantly higher at organic matter loading rates of 9 and 18 kg O_2/m^3 · per day than those at an organic matter loading rate of zero. In styrofoam bead filters, no significant difference in oxygen consumption rates was found between organic matter loading rates of 9 and 18 kg O_2/m^3 · per day. Oxygen consumption rates were significantly higher at an organic matter loading rate of 9 kg O_2/m^3 · per day than those at zero organic matter loading rates in sand and loess bead filters.

Total alkalinity consumption rates

Total alkalinity (TA) consumption rates increased with an increase of HLR in styrofoam bead filter (Table 6). TA consumption rates were significantly higher at HLRs of 200 and 300 m^3/m^2 · per day than those at HLR of 100 m^3/m^2 · per day. TA consumption rates showed the same trends as found for TAN conversion rates at different HLRs in sand and loess bead filters.

TA consumption rates decreased with an increase in the organic matter loading rates in styrofoam bead filters (Table 6). This is a correspondence between the decrease in TAN conversion rates and an increase in organic matter loading rates. TA consumption rates were significantly higher at a zero organic matter loading rate than those at higher organic matter loading rates. TA consumption rates also decreased with increases in the organic matter loading rate in loess beads and sand filters.

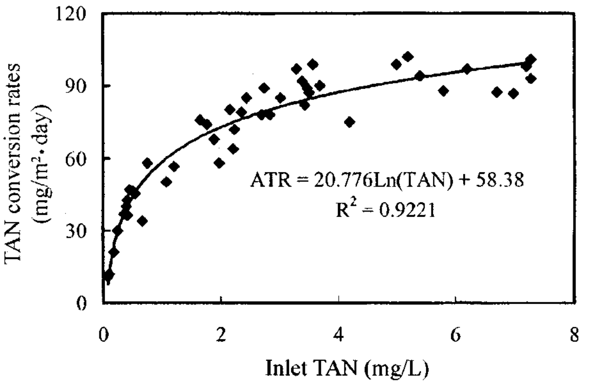

Fig. 3. TAN conversion rates versus various inlet TAN concentrations in styrofoam bead filter (HLR, 100 m^3/m^2·per day; COD loading rate, 18 kg O_2/m^3· per day).

TAN conversion rates at different initial TAN concentrations

TAN conversion rates versus the initial inlet TAN concentrations in styrofoam bead filters are shown in Fig. 3. HLR was set at 100 m^3/m^2 · per day and inlet COD concentration was 50 mg/*l*. At the higher TAN loading rates, relatively stable TAN conversion rates were observed, indicating that conversion rates are independent of TAN loading rates. As inlet TAN concentration declines to a certain value, TAN conversion rates decreased accordingly, indicating dependence on TAN concentration in the inlet water. TAN conversion rates could be described by the 1-order/zero-order kinetic model. Areal TAN conversion rates (ATR) versus inlet TAN concentrations can be described by the equation:

$$\text{ATR } (mg/m^2 \cdot \text{per day}) = 20.776 \text{ Ln (TAN)} + 53.38$$

4. Discussion

TAN conversion

TAN conversion rates increased with an increase of HLRs. Nijhof and Klapwijk (1995) also found an increased performance of trickling filters with an increase of HLR and they concluded that the increase in HLR in combination with the specific filter media used could result in improvement in wettening of media, which might cause the increased performance. This would be true for the styrofoam bead filter used in the present experiment. Higher HLR would induce more even distribution of water through the biofilter media and thus improve the performance. However, the improvement in biofilter performance was not linearly correlated to HLR. At the highest HLR of 300 m^3/m^2 · per day tested, the improvement was not significantly high when compared with those at HLR of 200 m^3/m^2 · per day. This was mainly due to the characteristics of the styrofoam beads. Though the specific gravity of this kind of bead is only one-thirtieth water, part of the beads, around 30% according to the author's observation, would sink in water after a supply of water. This would reduce the impact of HLR on the whole performance. TAN conversion rates of sand filters increased with an increase of the HLR up to 200 $m^3/m^2 \cdot$per day. No increase was observed at the highest HLR due to the factor that at higher water loading rates, flooding on the media surface took place. HLR had a significant impact on the TAN conversion rates of loess bead filters up 200 HRL $m^3/m^2 \cdot$per day. Though loess bead filters operate in real trickling mode, a further increase in the hydraulic loading rate would be of no help when considering the low specific surface area provided by this media. This result was in accordance with the others reported (Forster 1974; Nijhof and Klapwijk 1995).

TAN conversion rates were much less at organic matter loading rates of 9 and 18 kg $O_2/m^3 \cdot$per day than those without organic matter input in styrofoam bead filters. The addition of glucose resulted in reduction of the TAN conversion rate from 540 to 284 g $TAN/m^3 \cdot$per day. At organic matter loading rate of 9 kg $O_2/m^3 \cdot$per day, a great reduction in TAN conversion rates were also found in sand filters and loess bead filters. Clearly, organic matter can be one of the most important impacting factors on nitrification efficiency. This is mainly due to the growth of heterotrophic bacteria, which compete for oxygen, nutrients, and space with the autotrophic nitrifiers. TAN conversion rates were much lower than those reported by Bovendeur *et al.* (1990), Satoh *et al.* (2000), and Zhu and Chen (2001) in freshwater systems.

No significant difference in TAN conversion rates between the two organic matter loading rates was found in styrofoam bead filters. This indicates that the impact of organic matter on nitrification becomes less and less sensitive with an increase in the COD/TAN ratio. This can be explained by the fact that heterotrophic growth followed the Monod kinetics (Bailey and Ollis 1986) with substrate concentration. Heterotrophic bacterial growth rates increase with the increase of organic concentration until it reaches a saturation point. Consequently, the potential for heterotrophic inhibitory impact is significantly reduced with an increase in organic concentrations. Zhu and Chen (2001) also found that the difference of TAN conversion rates between a C/N ratio of 1 and 2 in a freshwater system was not significant and they calculated the corresponding COD/TAN ratios to be 2.7 for a C/N ratio of 1 and 5.4 for a C/N ratio of 2. In the present experiment, the COD/TAN ratios used were 3.2 and 6.4, which were similar to the case reported in their study. Also, they concluded that the biofilters used in recirculating system usually operated under the condition of a C/N ratio equal to 2, so the results obtained at the highest organic matter loading rate, especially the results obtained at various inlet TAN concentrations, can be used in biofilter design in recirculating aquaculture system.

NO_2-N production and conversion

Increment in NO_2-N conversion rates with an increase of HLRs would be due to the fact that the increased conversion of TAN provided more NO_2-N to the nitrafication bacteria. Weatherley (1984) reported that under certain conditions the oxidation of nitrite might be described using first-order kinetics. Sandu *et al.* (2002) found that TAN and NO_2-N removal rates increased with the increase of HLR. Similar results were reported by Malone *et al.* (1999). Though the removal rates increased with an increase of HLRs, NO_2-N concentrations in the outlet water also increased. This would cause the accumulation of nitrites in culture tank water. Sandu *et al.* (2002) also reported an elevated NO_2 concentration in the effluent water with the increase of HLRs. Incomplete removal of NO_2 was found in all the filters tested at various HLRs.

Many factors including pH (Alleman 1985), substrate inhibition, DO (Alleman 1985; Liu and Capdeville 1994), chlorinity (Hill and Gellman 1977), and light (Horrigan *et al.* 1981; Olson 1981) affect ammonia and nitrite oxidizers and thus cause nitrite accumulation. In the present experiment, pH and DO were well within the range for nitrification (Kaiser and Wheaton 1983; Water Pollution Control Federation 1983). Light also should not be a limiting factor since all the media were in dark conditions. Van Rijn and Rivera (1990), in a fresh water system, found that the high inlet ammonia concentrations caused relatively large amounts of nitrite and this nitrite might not be directly available for the nitrite oxidizers. They also found that nitrite removal by the trickling filter took place when ambient ammonia concentrations were lower than 1 mg/*l*, while at higher ambient ammonia concentrations nitrite accumulated. Hao and Chen (1994) observed nitrite accumulation in a submerged-bed system and attributed this to an irreversible inhibitory effect of hydroxylamine on the nitrite oxidizers. These findings may partially explain the accumulation of nitrite in present experiment considering the high TAN loading rate.

On the other hand, the accumulation of nitrite might be due to the depth of the media used and HLR. The filter media were only about 30-cm deep and this depth might not be sufficient for the nitrite oxidizers to fully develop and consume all the nitrite produced. Nijhof and Klapwijk (1995) concluded that the occurrence of high nitrite concentrations in trickling filter effluents could be explained by a diffusional transport mechanism in combination with the characteristics of the biofilm, and biofilms with a relatively low nitrite oxidation capacity induced high nitrite concentrations. Spotte (1992) also found that nitrite sometimes persists at higher than expected concentrations long after it should have disappeared and been replaced by nitrate, and concluded that this might be caused by dissimilatory activities by other bacteria or incomplete nitrification. The accumulation of nitrite in the present experiment also might be induced by an imbalance of ammonia and nitrite oxidation bacteria developed in the biofilters.

Kamstra *et al.* (1998) found that nitrite oxidation capacity in biofilms seems to be variable and sensitive to environmental disturbances after they assessed the performance of trickling filters on 14 eel farms. Nijhof and Bovendeur (1990), after comparing the nitrification of freshwater and seawater biofilms, concluded that the high accumulation of nitrite in seawater system was due to the slow development of nitrite oxidation capacity during the start-up periods. This phenomenon also could have occurred in the present experiment since nitrite accumulation could be noted just after the start of TAN oxidation. This also may indicate a higher growth rate of ammonia oxidation bacteria than nitrite oxidation bacteria as found by Grommen *et al.* (2001).

In the present experiment, the net production of nitrite might be due to the incomplete nitrification caused by the short period of conditioning, high ammonia and hydraulic loading rates, and the shallow depth of media.

Oxygen and total alkalinity

Volumetric oxygen consumption rates at various HLRs and the organic matter loading rate of zero for all the biofilter media tested showed the same trends as with TAN conversion rates since oxygen was mainly consumed by nitrifiers. Oxygen consumption rates increased with an increase in the organic matter loading rates since heterotrophic bacteria also consume oxygen. Bovendeur *et al.* (1990) reported a linear increase of COD removal with an increase of COD loading rates. However, they use low organic matter loading rates of only up to 25 $g/m^2 \cdot$ per day. The high organic matter loading rates may contribute to the less significant difference in oxygen consumption at the two organic matter loading rates. Total alkalinity consumption rates followed the same trends as TAN conversion rates for all the biofilters tested. The ratios of TA consumption to TAN conversion were within the range of 7.10-7.44 with a mean value of 7.19.

Comparison of the nitrification rates

The nitrification rates of the three media tested were between 0.24 and 0.31 $g/m^2 \cdot$ per day. A similar result, 0.28 $g/m^2 \cdot$ per day, was reported in a seawater trickling filter by Nijhof and Bovendeur (1990). In freshwater systems, the reported values were between 0.5-2 $g/m^2 \cdot$ per day (Anderson *et al.* 1994; Boller *et al.* 1994; Parker *et al.* 1997). The nitrification rates in seawater system were considerably lower compared with those in freshwater systems. This probably can be explained by the inhibiting effect of chloride on nitrification, which is reported to occur at chloride concentrations exceeding 10 mg/*l* (Richardson 1985). Salinity is known to affect bacterial metabolic activity, reducing microbial growth and ammonia oxidation rate (Rosa *et al.* 1998). Sakairi *et al.* (1996) observed a six-fold reduction in the nitrification rate of synthetic seawater when it was used in an airlift contactor containing bacteria immobilized in a cellulose carrier. Rosa *et al.* (1998) also have observed that the presence of NaCl seriously affects biofilm development and induced a two-fold reduction of nitrification in aerated biological filters.

5. Conclusion

TAN and NO_2-N conversion rates increased with increase of HLR in styofoam bead filters. TAN conversion rates in sand filters increased with an increase of HLR up to 200 $m^3/m^2 \cdot$ per day. No increase in the TAN conversion rate was observed at the highest HLR since flooding on the media surface took place. HLR had a significant impact on the TAN conversion rates in loess bead filters up to 200 $m^3/m^2 \cdot$ per day. TAN conversion rates were much less at organic matter loading rates of 9 and 18 kg $O_2/m^3 \cdot$ per day than those without organic matter addition in styrofoam bead filters. The addition of glucose resulted in a reduction of the TAN conversion rate from 540 to 284 g $TAN/m^3 \cdot$ per day. No significant difference in the TAN conversion rates between the two organic matter loading rates was found. This indicates that the impact of organic matter on nitrification becomes less and less sensitive with an increase in the COD/TAN ratio. At an organic matter loading rate of 9 kg $O_2/m^3 \cdot$ per day, a great reduction in TAN conversion rates was found in sand filters and loess bead filters. Clearly, organic matter can be one of the most important impacting factors on nitrification. NO_2-N conversion rates showed a similar trend in the case of TAN. Based on the TAN and nitrite conversion rates, styrofoam beads showed the best performance among the three filter media tested. Also, the low gravity and price of styrofoam beads make the handling easier and more cost-effective for commercial application. Usually, the biofilters used in recirculating system operated under the condition of a C/N ratio equal to 2, so the results obtained at the highest organic matter loading rate, especially the results obtained at various inlet TAN concentrations, can be used in biofilter design in a recirculating aquaculture system. In the present experiment, net production of nitrite occurred in all the biofilters tested. This might be due to the incomplete nitrification caused by the short period of conditioning, high ammonia and hydraulic loading rates, and the shallow depth of media. When applying these kinds of biofilters in aquaculture systems, large dimension biofilters would be needed if complete removal of nitrite were desired.

References

Alleman, J.E. 1985. Elevated nitrate occurrence in biological wastewater treatment systems. *Water Sci. Tech.*, 17, 409-419.

Anderson, B., H. Aspegren, D. Parker, and M. Litz. 1994. High rate nitrifying trickling filters. *Water Sci. Tech.*, 9, 47-52.

Bailey, J.E. and D.F. Ollis. 1986. *Biochemical Engineering Fundamentals*. McGraw-Hill, New York. pp. 383-388.

Boller, M., W. Gujer, and M. Tschui. 1994. Parameters affecting nitrifying biofilm reactors. *Water Sci. Tech.*, 29, 1-11.

Bovendeur, J., A.B. Zwaga, B.G.J. Lobee, and J.H. Blom. 1990. Fixed-biofilm reactors in aquacultural water recycle systems: effect of organic matter elimination on nitrification kinetics. *Water Res.*, 24, 207-213.

Bower, C.E. and D.T. Turner. 1981. Accelerated nitrification in new seawater culture systems: effectiveness of commercial additives and seed media from established systems. *Aquaculture*, 24, 1-9.

Chamberlain, G. and H. Rosanthal. 1995. Aquaculture in the next century, opportunities for growth, challenges for sustainability. *World Aquaculture*, 26, 21-25.

Davis, D.A. and C.R. Arnold. 1998. The design, management and production of a recirculating raceway system for the production of marine shrimp. *Aqua. Eng.*, 17, 193-211.

Forster, J.R.M. 1974. Studies on nitrification in marine biological filters. *Aquaculture*, 4, 387-397.

Grasshoff, K., K. Kremling, and M. Ehrhardt. 1999. *Methods of seawater analysis*, 3rd ed. Wiley-VCH, Germany.

Greiner, A.D. and M.B. Timmons. 1998. Evaluation of the nitrification rates of microbead and trickling filters in an intensive recirculating tilapia production facility. *Aqua. Eng.*, 18, 189-200.

Grommen, R., I. Van Hauteghem, M. van Wambeke, and W. Verstraete. 2001. An improved nitrifying enrichment to remove ammonium and nitrite from freshwater aquaria systems. *Aquaculture*, 211, 115-124.

Hao, O.J. and J.M. Chen. 1994. Factors affecting nitrite buildup in submerged filter system. *J. Environ. Eng.*, 120, 1298-1307.

Hill, D.O. and S.R. Gelman. 1977. Effects of chloride on nitrification rates in activated sludge systems. p. 206-213. In: *Proc. Industrial Waste Conference*, eds. by J.M. Bell. Purdue Univ.

Hochheimer, J.N. 1990. Trickling filter model for closed system aquaculture. Ph.D. Thesis. Univ. Maryland, College Park, MD.

Horrigan, S.G., A.F. Carlucci, and P.W. Williams. 1981. Light inhibition of nitrification in sea-surface films. *J. Marine Res.*, 39, 557-565.

Kaiser, G.E. and F.W. Wheaton. 1983. Nitrification filters for aquatic culture systems: state of the art. *J. World. Maricult. Soc.*, 14, 302-324.

Kamstra, K., J.W. van der Heul, and M. Nijhof. 1998. Performance and optimization of trickling filters on eel farms. *Aqua. Eng.*, 17, 175-192.

Lekang, O.I. and H. Kleppe. 2000. Efficiency of nitrification in trickling filters using different filter media. *Aqua. Eng.*, 21, 191-199.

Liao, P.B. and R.D. Mayor. 1974. Intensified fish culture combining water reconditioning with pollution abatement. *Aquaculture*, 3, 61-85.

Liu,Y. and B. Capdeville. 1994. Kinetic behaviors of nitrifying biofilm growth in wastewater nitrification process. *Environ. Tech.*, 15, 1001-1013.

Losordo, T.M. 1991. An introduction to recirculating production system design. p. 32-47. In: *Proceedings, Engineering Aspects of Intensive Aquaculture Symposium*. Cornell Univ., Ithaca, NY.

Malone, R.F., E.B. Lance, and A. Jr. De Los Reyes. 1999. Sizing and management of floating bead bioclarifiers. In: *Aquaculture Engineering and Waste Management, Proceedings from the Aquaculture Exposition and Aquaculture Mid-atlantic Conference*, Washington D.C.

Menasveta, P., T. Panritdam, P. Sihanonth, S. Powtongsook, B. Chuntapa, and P. Lee. 2001. Design and function of a closed, recirculating seawater system with denitrification for the culture of black tiger shrimp brookstock. *Aqua. Eng.*, 25, 35-49.

Nijhof, M. and J. Bovendeur. 1990. Fixed film nitrification characteristic in sea-water recirculation fish culture system. *Aquaculture*, 87, 133-143.

Nijhof, M. and A. Klapwijk. 1995. Diffusional transport mechanism and biofilm nitrification characteristics influencing nitrite levels in nitrifying trickling filter effluents. *Water Res.*, 29, 2287-2292.

Olson, R.J. 1981. Differential photoinhibition of marine nitrifying bacteria. *J. Mar. Res.*, 39, 227-238.

Pano, A. and E.J. Middlebrook. 1983. Kinetics of carbon and ammonia nitrogen removal in RBC's. *J. Water Pollution Control Federation*, 55, 956-965.

Parker, D.S., T. Jacobes, E. Bower, D.W. Stowe, and G. Famer. 1997. Maximising trickling filter nitrification rates through biofilm control: research review and full-scall application. *Water Sci. Tech.*, 36, 255-262.

Redney, B.D. and R.R. Stickney. 1979. Acclimation to ammonia by *tilapia aurea*. *Trans. American Fish. Soc.*, 108, 383-388.

Richardson, M. 1985. *Nitrification Inhibitors in the Treatment of Sewage*. Thames Water, Reading, Great Britain. 103 p.

Ridha, M.T. and E.M. Cruz. 2001. Effect of biofilter media on water quality and biological performance of the Nile tilapia *Oreochromis nilotics* L. reared in a simple recirculating system. *Aqua. Eng.*, 24, 157-166.

Rosa, M.F., A.A.L. Furtado, R.T. Albuquerque, S.G.F. Leite, and R.A. Medronho. 1998. Biofilm development and ammonia removal in the nitrification of a saline wastewater. *Bioresource Tech.*, 65, 135-138.

Sakairi, M.A.C., K. Yasuda, and M. Matsumura. 1996. Nitrogen removal in seawater using nitrifying and denitrifying bacteria immobilized in porous cellulose carrier. *Water Sci. Tech.*, 34, 267-274.

Sandu, S.I., G.D. Boardman, B.J. Watten, and B.L. Brazil. 2002. Factors influencing the nitrification efficiency of fluidized bed filter with a plastic bead medium. *Aqua. Eng.*, 26, 41-59.

Satoh, H., S. Okabe, N. Norimatsu, and Y. Watanabe. 2000. Significane of substrate C/N ratio on structure and activity of nitrifying biofilm determined by in situ hybridization and the use of microelectrodes. *Water Sci. Tech.*, 41, 317-321.

Spotte, S. 1992. *Captive Seawater Fishes: Science and Technology*. Wiley-interscience, New York, 71 p.

Strickland, J.D.H. and T.R. Parsons. 1972. *A Practical Handbook of Seawater Analysis*. Fish. Board. Canada.

Tseng, K.F., H.M. Su, and M.S. Su. 1998. Culture of *Penaeus monodon* in a recirculating system. *Aqua. Eng.*, 17, 138-147.

Van Rijn, J. and G. Rivera. 1990. Aerobic and anaerobic in an aquaculture unit - nitrite accumulation as a results of nitrification and denitrification. *Aqua. Eng.*, 9, 217-234.

Water Pollution Control Federation. 1983. *Nutrient Control, Manual of Practice*. Washington D.C.

Weatherly, L.R. 1984. Rate models for a marine biological filter. *Aqua. Eng.*, 3, 15-29.

Wheaton, F.W., J.N. Hochheimer, G.E. Kaiser, M.J. Kornes, G.S. Libey, and C. Easter. 1994. Nitrification principles. p. 101-126. In: *Aquaculture Water Reuse Systems: Engineering Design and Management*, eds. by M.B. Timmons and T. Losordo. Elsevier, Amesteram.

Zhu, S.M. and S. Chen. 2001. Effects of organic carbon on nitrification rate in fixed film biofilters. *Aqua. Eng.*, 23, 1-11.

Received Jul. 22, 2003
Accepted Sep. 16, 2003