J. of Aquaculture
Vol. 16(4) : 203-209, 2003

한국양식학회지
Journal of Aquaculture

Nitrification Efficiency in Fixed Film Biofilters using Different Filter Media in Simulated Seawater Aquarium System

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Nitrification efficiency of fixed film biofilters with sand, loess bead, and styrofoam bead in biofilter columns of 1-m height and 30 cm width was studied. Synthetic wastewater was continuously supplied to the culture tank to maintain total ammonia nitrogen (TAN) concentration in the inflow water at around 8 mg/L. The hydraulic loading rate was set at 200 m^3/m^2/day. TAN conversion was stabilized after about 90 day conditioning for all the selected filter media but with net accumulations of nitrite. On the volumetric basis, conversion rates of TAN and nitrite were the highest in styrofoam bead filter. Mean volumetric TAN conversion rates in the final samples were 682, 269, and 79 g TAN/m^3/day in the styrofoam bead, sand and loess bead filters, respectively. Low gravity and cost of styrofoam bead render the handling easier and more cost-effective.

Keywords: Fixed film biofilter, Nitrification, Filter media, Aquaculture

Introduction

Due to accumulation of uneaten feed, feces, and metabolic wastes excreted by fish, especially ammonia, water quality in a closed aquaculture system deteriorates rapidly. Accumulation of ammonia may lead to suppression of growth, histopathological changes, and even death (Redney and Stickney, 1979). Toxicity of ammonia to the fish has attracted many studies on its effective removal from aquaculture systems.

Biofilters have been most commonly used for ammonia removal in aquaculture systems. From the point of configuration, many types of biofilters like trickling filter, submerged filter, sand filter, and fluidized bed filters have been employed in aquaculture systems. Of these, tricking and submerged filters have many advantages including: low construction cost, easy management and maintenance, and their adaptability to different loading rates of water and waste. In Korea, down flow trickling or submerged biofilters are at present commonly used in live fish aquaria. However, no investigation has so far been undertaken to study the nitrification efficiency of these biofilters.

Different types of filter media are used to provide more utilizable surfaces for nitrification bacteria to grow on in aquaculture and wastewater treatment systems (Greiner and Timmons, 1998; Nijhof and Bovendeur, 1990; Lekang and Kleppe, 2000; Ridha and Cruz, 2001). For optimal nitrification and less clogging of the biofilter, the biofilter media should have large surface area and low specific gravity (Wheaton et al., 1994; Lekang and Kleppe, 2000). In Korea, coarse sand (2~5 mm) mixed with shell debris, which can provide large surface area, has mostly been used as a biofilter in live fish aquarium. It is believed that this kind of biofilter not only removes ammonia but also maintains the pH of the culture water. Another biofilter media, styrofoam beads with reasonable nitrification capacity have been tested in freshwater biofilters. The large specific surface area and low gravity of these beads render them very suitable as biofilter media. Loess beads, which are newly developed biofilter media, together with the above-mentioned sand and styrofoam beads was also used in present experiment. The nitrification efficiency was tested in a simulated seawater aquarium system.

Materials and Methods

System description

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

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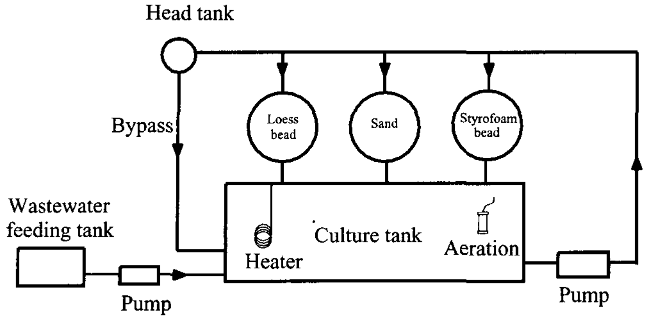

Fig. 1. Aquarium system with sand filter, loess bead filter, and styrofoam bead filter used in present experiment.

Table 2. Characteristics of the media used in present experiment

	Styrofoam bead	Sand	Loess bead
Specific weight	0.033	2.6	2.2
Diameter (mm)	1.4	3.5 (2-4.5)	10 (10 long)
Porosity (%)	32	38	46
Specific surface area (m^2/m^3)	2820	950	225
Total volume (L)	20	20	20
Passive surface area (m^2)	0.75	0.75	0.75
Total surface area (m^2)	57.2	19.8	5.3

Passive surface area: surface area of filter column and pipes between inflow and outflow of biofilter.

culture tank to increase the dissolved oxygen (DO) level and to ensure good mixing of the added synthetic wastewater. The volume of the rectangular culture tank is 882 L (Length: 1400 mm×width: 900 mm×height: 700 mm) and the entire water volume in the system is around 1,000 L. The three biofilters are placed on a higher level, so the water can flow back to the culture tank by gravity.

The biofilter columns are made of PVC pipes. Each biofilter column measures 1 m height and 30 cm diameter. Two gratings are placed inside each biofilter column, one near the bottom to prevent the loss of biofilter medium and the other at the top to ensure the even distribution of inflow water. The high outlet position of styrofoam bead filter reduces the headloss of this kind of biofilter. But the outlets of the other two biofilters are positioned just above the bottom of the filter column. Approximately, 20 L biofilter media is used in each biofilter column.

The biofilters were conditioned by supplying synthetic wastewater. The composition of the synthetic wastewater is shown in Table 1. TAN removal rate was tested at high ammonia loading rates, around 100 g N/m^2 column surface area, to minimize the inhibitive effect of low ammonia levels on nitrification performance. The ammonia concentration in the inlet water was maintained around the set point by changing the supplying rate of the synthetic wastewater to culture tank. Nitrite and nitrate concentrations were maintained at lower level by water exchange. Water flow through the biofilters was set at 10 L/min, equivalent to a hydraulic loading rate of 200 m^3/m^2/day. This is over the recommended minimum hydraulic loading rate for biofilter medium (Roberts, 1985). Water temperature was maintained at 20 °C with thermostatic heating system.

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

Ingredient	*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

Characteristics of media

The characteristics of the three filter media are shown in Table 2. Specific surface area is calculated by multiplying the number of media in 100 ml beaker with the mean surface area of the medium. Styrofoam bead is round-shaped with an average diameter of 1.4 mm and the calculated specific surface area is 2,820 m^2/m^3. As the specific gravity is only one thirtieth of water, most of the beads in the biofilter tend to float on water surface. Loess bead is cylindrical (1 cm long and 1 cm in diameter) with some small holes on the surface. This medium has the lowest specific surface area of 225 m^2/m^3. The size distribution of sand is between 2 and 4.5 mm and the estimated specific surface area is about 950 m^2/m^3. For the sand filter, gravels are put at the bottom layer and then coarse sand mixed with shell debris in the middle layer. A thin layer of fine sands was put on the surface for even distribution of water.

Sampling and analysis

Water samples were taken once a week during the conditioning period and once every three days after a relatively stable nitrification level was observed. All the water samples were taken at the inlet and outlet of each biofilter. TAN, NO_2-N (nitrite nitrogen), and NO_3-N (nitrate nitrogen) were measured using the methods described by Strickland and Parsons (1972). DO and temperature were measured with DO meter (KDO 5151, KRK Co.). Total alkalinity was measured by the titration method (Grasshoff et al., 1999) and pH with 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 NO_2-N conversion rate (ANR), volumetric NO_2-N conversion rate (VNR), and volumetric alkalinity consumption rate (VAR) were calculated on a daily basis using the following equations:

$$\mathrm{VTR}\ (g/m^3/day)=1.44\ (TAN_i-TAN_o)\ Q/V$$
$$\mathrm{ATR}\ (g/m^2/day)=1.44\ (TAN_i-TAN_o)\ Q/A$$
$$\mathrm{VNR}\ (g/m^3/day)=1.44\ [(TAN_i-TAN_o)+(NO_{2i}-NO_{2o})]\ Q/V$$
$$\mathrm{ANR}\ (g/m^2/day)=1.44\ [(TAN_i-TAN_o)+(NO_{2i}-NO_{2o})]\ Q/A$$
$$\mathrm{OCR}\ (g/m^3/day)=1.44\ (DO_i-DO_o)\ Q/V$$
$$\mathrm{VAR}\ (g/m^3/day)=1.44\ (TA_i-TA_o)\ Q/V$$

TAN_i and TAN_o, the TAN concentrations in inlet and outlet water (mg/L); DO_i and DO_o, the DO concentrations in inlet and outlet water (mg/L); NO_{2i} and NO_{2o}, the NO_2-N concentrations in inlet and outlet water (mg/L); TA_i and TA_o, the total alkalinity concentration at inlet and outlet water (mg $CaCO_3$/L); Q, the flow rate through the biofilters (L/min); V, the filter media volume; A, the total surface area of the biofilter; 1.44, the conversion factor.

Statistical analysis

The final three sampling data were analyzed by one-way ANOVA (Statistics 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 Difference (LSD) test was used to compare the means. Treatment effects were considered significant at $P<0.05$.

Results

Changes of water quality

Decrease in TAN occurred in about 4 weeks of conditioning in styrofoam bead filter and sand filter, whereas it occurred after 6 weeks in loess bead filter. After 3-month conditioning, nitrification in all the three biofilters were stabilized with maximum in loess bead filter (Fig. 2). The mean inlet TAN level was 7.8 mg/L.

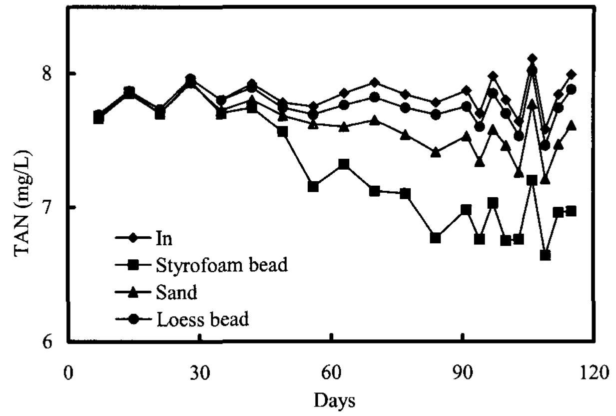

Fig. 2. TAN concentrations in the inlet and outlet water of sand filter, loess bead filter, and styrofoam bead filter measured over the experiment period.

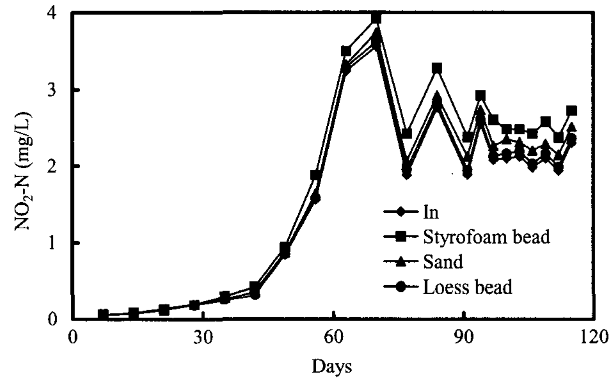

Fig. 3. Nitrite nitrogen concentrations in the inlet and outlet water of sand filter, loess bead filter, and styrofoam bead filter measured over the experiment period.

Following the decreasing level of TAN, NO_2-N was produced in all the three biofilters. Corresponding to the maximum reduction of TAN in the styrofoam bead filter, the NO_2-N levels in its outlet water were also high (Fig. 3). Incomplete nitrification occurred in all these three biofilters and resulted in the accumulation of nitrite in the culture tank. This phenomenon lasted throughout the experimental period. Hence water had to be changed periodically to ensure relatively low nitrite levels in the biofilter columns.

Increases in nitrate levels were recorded in the outlet water of these three biofilters after 30~60 days of conditioning (Fig. 4). The nitrate levels were the highest in the outlet water from styrofoam bead filter and lowest in the loess bead filter. The mean inlet nitrate level was 9.4±0.7 mg/L.

Decrease in pH was noted in the outlet water in all the three biofilters (Fig. 5). It was also greater in styrofoam bead than in the other two biofilters. pH in the inlet water averaged 7.7±0.1. Decrease in total alkalinity showed a trend similar to that of pH. The average DO concentration in the inlet water to each biofilter was 8.2±0.2 mg/L (Fig. 6). The maximum decrease was also recorded in the styrofoam bead filter and the level of DO was 4.1±0.2 mg/L.

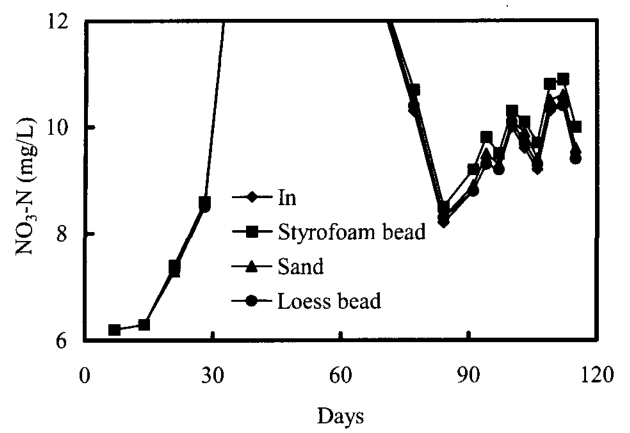

Fig. 4. Nitrate nitrogen concentrations in the inlet and outlet water of sand filter, loess bead filter, and styrofoam bead filter measured over the experiment period.

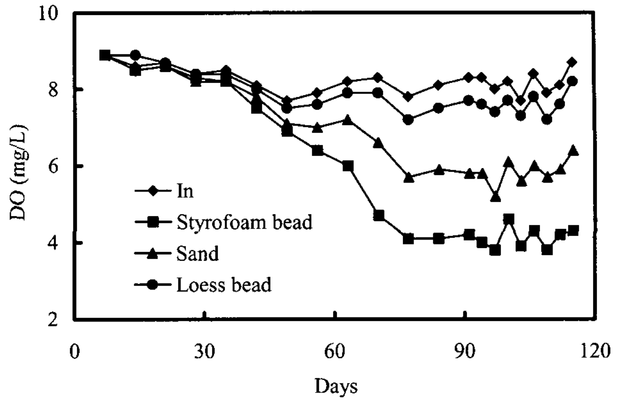

Fig. 6. The DO concentrations in the inlet and outlet water of sand filter, loess bead filter, and styrofoam bead filter measured over the experiment period.

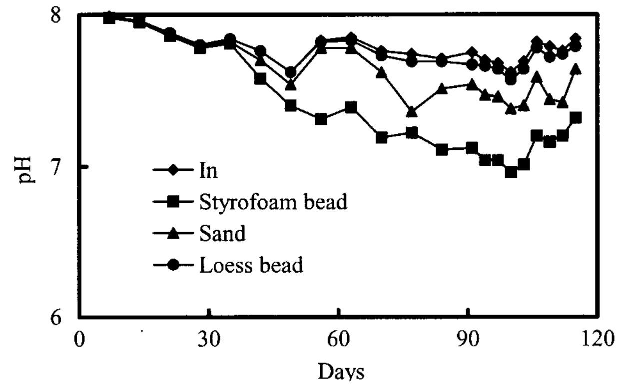

Fig. 5. pH values in the inlet and outlet water of sand filter, loess bead filter, and styrofoam bead filter measured over the experiment period.

Production of TAN, NO_2-N, NO_3-N, total alkalinity and O_2

Total nitrification refers to the difference of TAN levels between inlet water and outlet water of each biofilter. In styrofoam bead filter, it averaged 0.95 mg/L. Sand filter showed a lower degree of nitrification and in loess bead filter the lowest (Table 3). On the volumetric basis, styrofoam bead filter showed significantly higher TAN conversion rates than the other two filters, and sand filter converted significantly more TAN than loess bead filter ($P<0.05$). However, on the areal basis, no significant difference in the rates of TAN conversion was found between styrofoam bead filter and sand filter or between sand filter and loess bead filter, though loess bead filter did exhibit a significantly higher TAN removal rates than those in styrofoam bead filter ($P<0.05$). Gravity and void ratio of the filter media contributed partly for this difference. Usually, high void ratio is attributed to media with least contact with each other. Loess beads had a higher void ratio than the other media, implying that loess beads had relatively more available surface for nitrifiers. As the styrofoam bead filter worked in the downflow mode, more beads would contact with each other and thereby reduce the surfaces available for nitrifiers. Hence, the calculated surface area for a given volume of medium was not the actual surface that was available for nitrifiers. Secondly, the calculation also included the surface of the medium and passive surface area of the filter column and connecting PVC pipes.

Table 3. Average nitrification, nitrite production, total alkalinity and oxygen consumption of three biofilter media tested in present experiment

	Styrofoam bead	Sand	Loess bead
Total nitrification (mg/L)	0.95±0.05^a	0.37±0.03^b	0.11±0.01^c
VTR (g TAN-N/m^3/day)	682±35^a	269±8^b	79±5^c
ATR (g TAN-N/m^2/day)	0.24±0.01^b	0.27±0.01^{ab}	0.31±0.02^a
Nitrite production (mg/L)	0.44±0.02^a	0.20±0.01^b	0.06±0.01^c
ANR (g NO_2-N/m^2/day)	0.13±0.01	0.16±0.01	0.15±0.01
VNR (g NO_2-N/m^3/day)	384±32^a	154±13^b	41±4^c
VAR (g $CaCO_3$/m^3/day)	4343±361^a	1772±48^b	534±45^c
Oxygen consumption (mg/L)	4.1±0.2^a	2.2±0.2^b	0.6±0.1^c
OCR (g O_2/m^3/day)	2978±128^a	1608±32^b	320±7^c

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

Thus the passive surface contributed an additional 14% to the total surface area in the loess bead filter but only 4% to sand filter and 1.3% to styrofoam bead filter (Table 2).

No significant differences of NO_2-N conversion rates were found among these three biofilters on the areal basis. On the volumetric basis, the highest NO_2-N conversion rate was recorded in styrofoam bead filter and was significantly higher than those in the other two biofilters. The lowest value was obtained in the loess bead filter. Consumption of oxygen and total alkalinity was the highest in styrofoam bead filter. On the volumetric basis, styrofoam bead filter showed significantly higher oxygen and total alkalinity consumption rates than the other two biofilters ($P<0.05$).

Discussion

TAN

This study has shown that the calculation for the TAN removal rate must be based on area rather than volume. Secondly, the actual available passive surface area must also be estimated and included in the calculation. The importance of the passive surface area for adhesion of nitrifiers was reported by others as well. For instance, Hargrove et al. (1996) estimated that the passive surface area contributed up to 10~30% TAN removal. Checking the TAN removal rate without medium in the filter column provided the required information for accurate evaluation of the areal TAN removal rates of different media. Since the filters tested in present experiment will continuously be used for other experiment, no efforts were made on this.

Nitrite production

Net productions of NO_2-N were recorded in all these three biofilters, regardless of which kind of filter media. This resulted in the accumulation of NO_2-N in culture water with the maximum values up to 3.8 mg/L, which was above that recommended for aquaculture (Liao and Mayor, 1974; Russi et al, 1974).

Many factors including pH (Alleman, 1985), substrate inhibition, DO (Alleman, 1985; Liu and Capdeville, 1994), chlorinity (Hill and Gelman, 1977), and light (Horrigan et al., 1981; Olson, 1981) are known to affect ammonia and nitrite oxidizers, and to cause nitrite accumulation. In 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, as all media were kept under darkness.

In fresh water system, van Rijn and Rivera (1990) found that the high inflow ammonia caused a relatively large production of nitrite, which might not be directly available to nitrite oxidizers. They also found that nitrite removal by the trickling filter took place, when ambient ammonia concentrations were lower than 1 mg/L, but nitrite accumulated at a higher ambient ammonia level. Observing the accumulation of nitrite in submerged-bed system, Hao and Chen (1994) attributed it to an irreversible inhibitory effect of hydroxylamine on the nitrite oxidizers. These findings may partially explain the accumulation of nitrite in present experiment.

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

After having assessed the performance of trickling filters on 14 eel farms, Kamstra et al. (1998) noted that nitrite oxidation capacity in biofilms seemed to be variable and sensitive to environmental disturbances. Comparing the nitrification of freshwater and seawater biofilms, Nijhof and Bovendeur (1990) concluded that the high accumulation of nitrite in seawater system was due to the slow development of oxidation capacity of nitrite during the start-up periods. This also might be true in the present experiment, as nitrite accumulation could be detected just after the start of TAN reduction, perhaps indicating a higher growth rate of ammonia oxidation bacteria than nitrite oxidation bacteria (Grommen et al., 2001).

Briefly, the accumulation of nitrite in present experiment, might be due to incomplete nitrification caused by the short period of conditioning, high ammonia and water loading rate, and shallow depth of media.

Oxygen and total alkalinity consumption

Lekang and Kleppe (2000) noted that media with high void ratios usually have a reduced need for consuming oxygen from the water in trickling filters; similar results were also obtained in loess bead filter, which resembled the trickling model of the present experiment. However, the oxygen consumption rate should be mainly related to the nitrification capacity of the biofilter, since biofilm with great nitrification capacity consumed greater amount of oxygen. The total alkalinity consumption rates showed the same trend, as TAN conversion rates. It was not reduced in the sand filter, when compared to the calculated consumption rate based on the TAN conversion, indicating the production of alkalinity by sand and shell was too small to be detectable.

Comparison of the nitrification rate

The values for nitrification rate of the three media ranged between 0.24 and 0.31 g/m^2/day. Reported value for the seawater trickling filter was 0.28 g/m^2/day (Nijhof and Bovendeur, 1990). In freshwater systems, the reported values ranged between 0.5 and 2 g/m^2/day (Anderson et al., 1994; Boller et al., 1994; Parker et al., 1997). Hence the nitrification rates in seawater system were quite lower than those in freshwater systems. This may 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 in synthetic seawater, when it was used in an air-lift contactor containing bacteria immobilized in a cellulose carrier. Rosa et al. (1998) also observed that the presence of NaCl seriously affected the biofilm development and induced a two-fold reduction of nitrification in aerated biological filters.

Start-up period

Kawai et al. (1964) and Hirayama (1974) found that a period of 60 days was required for a marine biofilter to reach its full nitrifying capacity at temperatures of 20~22°C. In aquaculture systems, the required start-up period ranged from 28 to 60 days (Forster, 1974; Carmignani and Bennett, 1977; Perfettini and Bianchi, 1990). However, Nijhof and Bovendeur (1990) observed that a relatively longer start-up period of more than 90 days was required for the biofilters to achieve the maximum ammonia removal rate. In present experiment, the TAN conversion rate became relatively stable after 90 days of conditioning. No obvious differences in the start-up periods were found among these biofilters. However, the incomplete nitrification indicated that a longer conditioning period would be required.

Conclusions and design implication

Under the present experiment conditions, all the three biofilter media showed nitrification capacity. On the volumetric basis, styrofoam beads exhibited the fastest TAN conversion rate. The TAN conversion rates averaged 682, 269 and 79 g TAN / m^3/day in styrofoam bead, sand, and loess bead filters, respectively. Nitrite accumulations were recorded in the outlet water in all three biofilters. The nitrite nitrogen removal rates averaged to 384, 154, and 41 g/m^3/day in styrofoam bead, sand and loess bead, respectively. Based on the TAN and nitrite conversion rates, styrofoam bead showed the best performance among the tested filter media. Also, the low gravity and cost of styrofoam bead make handling easier and more cost-effective for commercial application. Especially, the head loss in styrofoam bead filter was the lowest among the tested biofilters. However, the accumulation of nitrite may be a problem for practical application. Decrease of the water loading rate or increase of the medium depth may be helpful. Nevertheless, the use of synthetic water in present experiment should be questioned. For design and application, it must be noted that suspended solids and high levels of COD encountered in the actual aquaculture systems may decrease the TAN conversion rate and cause clogging problems. Flooded conditions found in sand filter column already indicated the tendency towards clogging. Presently, testing of these media under different loading rates of water and organic matter is under progress to verify the capacity of nitrification and organic matter removal of these media.

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Manuscript Received: March 4, 2003
Revision Accepted: July 18, 2003
Responsible Editorial Member: Jeong-Yeol Lee