An evaluation of commercially available biological filters for recirculating aquaculture systems

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ABSTRACT

Three different commercially available biological filters were evaluated in triplicate on a 60 m³ tank-based Tilapia system under commercial warmwater growout conditions. The study was performed at the North Carolina State University Fish Barn—a commercial scale research and demonstration recirculating aquaculture facility operated by the department of Biological and Agricultural Engineering. Total ammoniacal nitrogen (TAN) removal rates were determined for the three types of biofilters for a range of concentrations ranging from 0.13 to 1.20 g TAN m^{-3}. TAN concentrations were varied by feed rates and ammonium chloride additions, and limited by fish feeding response. Maximum feed rates were 65 kg feed d^{-1} using a 40% protein diet at a maximum biomass of 5500 kg. Average observed TAN removal rates (in g TAN m^{-3} of unexpanded media d^{-1} ± standard deviation) for the three filters were 267 ± 123, 586 ± 284, and 667 ± 344 for the moving bed bioreactor, floating bead filter, and fluidized sand filter, respectively. These results are considerably lower than results previously published at the laboratory scale using artificial waste nutrients. This study highlights the need for future biofilter evaluations at the commercial scale using real aquaculture waste nutrients.

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1. Introduction

Aquaculture production accounts for almost 50% of the seafood consumed worldwide (FAO, 2005). As the aquaculture industry continues to grow in response to demand for increased seafood production, the need for environmentally conscious operational practices and facility designs becomes more important (Abey-singhe et al., 1996; Timmons et al., 1998; Peachey, 2008). Reducing the volume of the effluent stream and reusing more water within the culture system are primary issues for recirculating aquaculture systems (RAS) as stocking densities and facility size increase. Recirculating aquaculture systems rely heavily on biological filtration as a mechanism to reduce the effluent stream volume and make existing system water suitable for the cultured organisms (Losordo and Hobbs, 2000; Satoh et al., 2000; Chen et al., 2006). To develop environmentally conscious operational practices, a realistic understanding of how biological filters are affected by an increase in production intensity is required.

As a result of metabolism, ammonia is directly excreted by the cultured species. In solution, ammonia maintains equilibrium between an ionized (NH_4^+) and unionized (NH_3) form. Unionized ammonia-nitrogen (NH_3) is toxic to most aquacultured aquatic organisms and must be controlled within the production system (Meade, 1985). Biological filters are used to reduce the TAN concentration (the sum of the ionized (NH_4^+) and unionized (NH_3) forms of ammonia in solution) through nitrification, the biological process oxidizing ammonia to nitrate with nitrite as an intermediate component. Recirculating system designs must maximize the TAN removal rate to maximize system water reuse and minimize the impact of TAN on the cultured product. Biological filters with high TAN removal rates are able to effectively reduce the impact of TAN in RAS.

In order to meet the increasing intensity of aquaculture production facilities, biological filters with superior performance characteristics are required. To date, the majority of biological filter performance evaluations have been performed at the small, laboratory scale under conditions not adequately representative of actual production conditions (Losordo et al., 2000; Eding et al., 2006). Biofilter evaluations at the larger pilot or commercial scales using actual waste nutrients will yield results more pertinent to actual aquaculture production conditions (Ester et al., 1994; Losordo et al., 2000; Brazil, 2006; Chen et al., 2006). TAN and organic carbon concentrations in commercial scale systems are typically considerably different from those in lab scale studies. TAN

concentrations in commercial scale RAS are usually limited by the cultured species' tolerance and lower than those used in lab scale studies (Zhu and Chen, 1999; Losordo et al., 2000; Ling and Chen, 2005). Organic carbon concentrations in the production environment are generally higher than those used in lab scale studies due to increased biomass system-wide and feed inputs. The concentration of organic carbon is related to feed assimilation and solids removal efficiency. The inhibitory effect of organic carbon on nitrification has been studied at the small scale and is well accepted (Zhu and Chen, 2001; Nogueira et al., 2002; Ling and Chen, 2005; Michaud et al., 2006). The impact of organics on biofilter performance at the large production scale has not been studied as extensively. A comparison of organic carbon concentrations and the associated TAN removal rates between lab scale studies and actual production conditions is needed. Additionally, large-scale evaluations of filter performance will provide actual maintenance and operational characteristics unique to individual filter types. Understanding the associated operational and required maintenance characteristics of different filters is an important part of the selection process for facility operators and designers alike.

Several recent publications have stressed the need for developing reporting standards for biofilter evaluations. Reporting biological filter performance characteristics in a standardized form is important in order to provide information to the industry for biofilter sizing and selection (Colt et al., 2006; Drennan et al., 2006). Standards such as TAN and nitrite conversion rates based on media volume will facilitate a simple and effective means for direct comparison. Volumetric TAN conversion rate (VTR) and volumetric nitrite conversion rate (VNR) have been proposed recently in the literature (Malone and Beecher, 2000; Colt et al., 2006; Drennan et al., 2006) and hold great value in terms of creating reporting standards. Biofilter media is currently evaluated and compared according to its theoretical specific surface area (SSA)—a measure of the total surface area of the media per unit volume with greatest value placed on the highest SSA. The reason for such valuation is based on the fact that bacteria create a visco-elastic layer, or biofilm on the surface of the media. The theory behind such valuation is that the greater the SSA, the more bacteria are supported and the more TAN removed. In practice, the bacteria create a stratified biofilm with the faster growing heterotrophic bacteria layering over the slower growing autotrophic nitrifying bacteria (Nogueira et al., 2002). Stratification reduces mass flux of substrate through the biofilm, creating an oxygen diffusion gradient thus creating favorable conditions for anoxic processes (Schramm et al., 1996; Wik, 1999; Zhu and Chen, 2002). The environment below a thick biofilm layer may be totally anaerobic and no nitrification will occur (Schramm et al., 1996). This stratified biofilm effectively covers the media, layering over structural and topographical features of the media designed to increase surface area. This covering of the media topography essentially creates a new usable media surface area, reducing the actual media surface area used by the nitrifying bacteria. Estimating nitrification rates based on theoretical surface area can often be misleading. Substrate conversion rates based on the unexpanded volume of biofilter media will provide a more realistic rate of conversion by the same media.

This study was conducted to investigate performance characteristics of three different types of commercially available biological filters under commercial warmwater aquaculture growout (eutrophic) conditions. Performance characteristics in this study are reported with the proposed volumetric reporting standards. A statistical model was developed in this study for estimating volumetric TAN removal rate (VTR). The model is based on the better of two different predictor variables typically used in predicting and reporting VTR at TAN concentrations typical of commercial scale aquaculture.

2. Materials and methods

2.1. Experimental setup

This study was conducted at a scale and under conditions similar to commercial finfish production. It should be noted that conditions in the production system environment are in constant flux and steady state conditions are difficult to attain. Such variability in operating conditions is mainly due to variation in feed rates based on fish feeding and management efforts to meet and maintain good water quality to promote optimum growth and fish health. Samples were taken over a 180-day period and data used for this study were selected from the dataset based on pseudo-steady state conditions defined as constant feed rates for at least 7 days and relatively stable TAN concentrations for at least 3 days. A return to pseudo-steady state conditions required time intervals as long as 2 weeks after changes in system operating conditions were implemented.

2.1.1. Culture system and biomass loading

The North Carolina State University Fish Barn was the site for this study. This facility is a commercial scale indoor recirculating aquaculture research and demonstration facility operated by the North Carolina State University department of Biological and Agricultural Engineering (Losordo et al., 2000). One of two culture tanks was used as the main system in which Tilapia were grown as a source of waste nutrients. The single 60 m³ culture tank was stocked with an average of approximately 5000 fish during the study period. A total of three Tilapia cohorts were grown in the system during the time the filters were in operation, though only two cohorts were grown during the data collection period. The first cohort was used to condition the filters. The initial biomass for the first cohort was 1115 kg, and the final biomass was 3050 kg. The second cohort was grown for approximately 2 months once data collection began with a starting biomass of 2470 kg and harvested at a biomass of 2764 kg. The second cohort was affected by an illness which impacted the final biomass as fish were lost to disease. The third cohort was moved into the system immediately after harvesting of the second with a starting biomass of 2531 kg and a final biomass of 5500 kg.

The fish were fed once per hour, 24 times per day using a broadcast feeder controlled by a programmable logic controller (PLC). Feeding the fish 24 times per day produced a relatively constant oxygen demand and kept dissolved oxygen (DO) concentrations and other water quality parameters relatively steady over a 24-h period. Daily feed rates were based on average fish biomass and increased incrementally to support maximum growth rates. A floating feed with 40% protein content was used throughout the entire study.

2.1.2. Filter system

As described by Losordo et al. (2000), water flowed out of the culture tank and was mechanically filtered through a drum screen filter with 40 micron screens. The water flowed from the drum screen filter to a pumping sump (sump #1) that held approximately 9 m³ of water from which nine biological filters were supplied (Fig. 1). Filter size for this study was based on the estimated volumetric nitrification capacity. The original design feed rates for a two tank system at the NCSU Fish Barn was approximately 100 kg d⁻¹ of 38% protein feed. The TAN excretion rate is typically estimated as 3% of the daily feed rate by weight (Wheaton et al., 1994). Filter size was determined using manufacturers' reported TAN removal rates and assuming equal TAN loading rates to all of the nine filters. The TAN concentration was increased over the study period to provide TAN removal rates over a range of TAN loading rates.
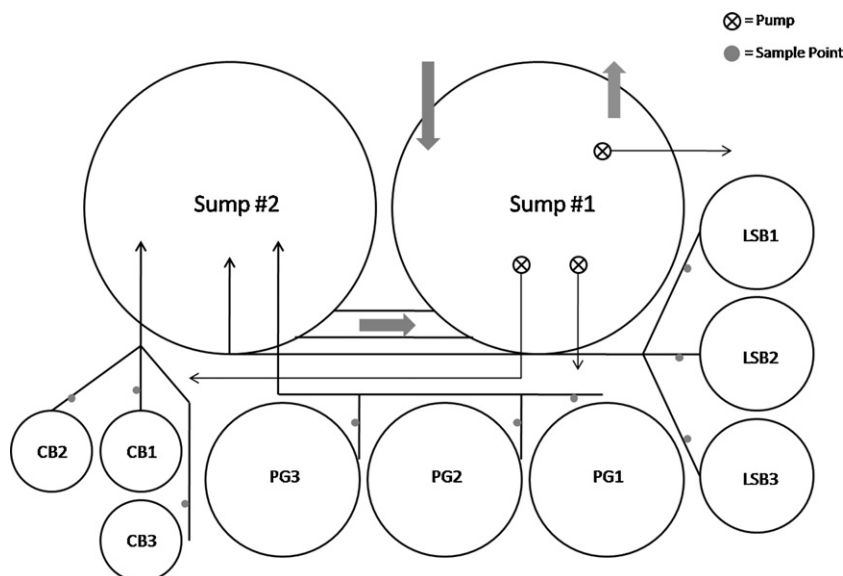
Fig. 1. A schematic of the experimental filter system layout. The system contained three filter types: fluidized sand (CB), floating bead (PG), and moving bed (LSB). All filters were evaluated in triplicate.

This study evaluated the operational characteristics of three different types of biological filters, each type tested in triplicate. The filter types that were evaluated are listed below:

1. The Clearwater™ Low Space Bioreactor, model LSB25 (LSB; Aquatic Eco-Systems, Inc., FL, USA). Each filter was filled with 0.71 m³ of previously unused Kaldnes biofilter media (Kaldnes North America Inc., RI, USA). Each reactor was 1.22 m³. Air was supplied to the filters through an octopus diffuser system at an average rate of 100.5 L min⁻¹ (4.9 m³ air m⁻³ reactor volume h⁻¹). The low pressure air served as a source for aeration and mixing, effectively operating each filter as a completely stirred-tank reactor (CSTR).
2. The PolyGeyser® Floating Bead filter, model DF15 (PG; Aquaculture Systems Technologies, LA, USA). Each filter contained 0.425 m³ of floating polyethylene dimpled beads, with a slightly elongated shape similar to that of a grain of rice. Each reactor was 3.5 m³ and contained within it a 0.75 m³ air charge chamber. Air was supplied to each of the three filter air chambers at an average rate of 6.6 L min⁻¹. This rate of air flow provided for approximately 13 backwash cycles per day for each filter.
3. The CycloBio® Fluidized Bed Biological filter: 61 cm (24 in.) diameter (CB; Marine Biotech, Inc., MA, USA). Each reactor was 0.71 m³ in volume and contained 0.34 m³ of silica sand as the biofilter media with an effective size of 0.53 mm and a uniformity coefficient of 1.3. Sand properties were appropriate according to specifications provided by the manufacturer. The average flow rate per unit area was 0.87 m³ m⁻² min⁻¹.

Water leaving the filters was returned to sump #2 (Fig. 1). The two sumps were connected to allow water from sump #2 to flow back to sump #1. A check valve prevented flow from sump #1 to sump #2. Water returned to the culture system was pumped from sump #1 at an average rate of 950 L min⁻¹. The flow from the drum screen filter was equal to the flow pumped into the culture system.

2.1.3. Pumps

Three pumps were used to supply effluent to each group of filters. All three filter systems were individually supplied by Goulds model series 3656 pumps (ITT – Goulds Pumps, NY, USA). At the beginning of data collection the LSB and CB systems were each supplied by a 1.5 kW pump (pumps #1 and #3, respectively; model 53BF1G4D0). On day 59 of the sampling period a larger 2.24 kW pump (model 52BF1H4C0) was installed on the CB system to increase available pressure head and provide a strainer basket to capture debris. Ultimately, the strainer basket was removed (as it reduced flow too much), leaving the replacement pump in place. The PG system was supplied by a 1.12 kW pump (pump #2; model 55BF2F4B0) as the model DF15 filters required less pumping head and higher flow rates than the other two types of filters. All pumps received water from sump #1 at a level approximately 3.5 m below the surface to allow for uniform water quality supply to all filters. Mixing of sump #1 was achieved by the constant flow of effluent from sump #2 and flow from the drum screen filter. This was a combined average flow rate of almost 2.1 m³ min⁻¹ which effectively replaced the entire volume of sump #1 every 4.25 min.

2.1.4. Plumbing and flow rates

The filters were supplied by way of one manifold system for each group of filters. Flow measurements were taken at the inlet pipe to each filter individually using a Dynasonics® Transit Time Ultrasonic Flow Meter (TFXP Series; Racine Federated Inc., WI, USA). The inlet pipes from the manifold to the filters were lengthened to a minimum of 1.5 m to accommodate the requirement of a fully developed flow profile within the pipe and provide for more accurate flow measurements in this study. Inlet pipes for all three filter groups were of the same diameter for flow measurement purposes. Flow to each filter was individually controlled using a ball valve directly in front of the filter inlet. Flow rates within each group were equalized with the total variation in flow rates between all three filters being no greater than 10%. Flow rates were recorded at the time of sampling and adjusted after sampling was complete, if required.

2.1.5. Dosing system

A dosing system was installed to periodically supplement the wastewater with ammonium chloride (NH₄Cl) using a Hannah Blackstone BL20-1 (HANNA Instruments® Inc., RI, USA) positive displacement dosing pump. The pump was located directly above a 625 L polyethylene storage tank. Substrate was dosed into the effluent pipe of the drum screen filter to ensure proper mixing within the sump. Adjustment of the amount of substrate dosed was determined by the substrate concentration that was desired within

the culture system and ultimately feeding the biofilters. The pump flow rate was easily controlled using the adjustment knob. Adjustment was required when higher TAN concentrations were required for the study and fish feeding rates could not provide the necessary ammonia concentration. Similarly, when feed was withdrawn for harvesting periods, ammonia was added to maintain biofilter activity as the culture system water was not circulated through the biofilter system.

Dosing of ammonium chloride (NH_4Cl) began on day 30, and was maintained for the remainder of the study to ensure TAN concentrations were sufficiently high. A solution of 80 kg NH_4Cl m^{-3} water was mixed in the storage tank. Changes in the dosing regimen were followed by 10–14-day acclimation periods to allow filters ample time to compensate for the change in concentration. During the acclimation periods, samples were collected to monitor water quality conditions.

2.2. Operation of biological filters

All three filter groups were in full operation for a minimum of 194 days prior to the beginning of the data collection period. TAN loading rates were varied throughout the study to obtain volumetric TAN removal rates (VTRs) for a range of TAN concentrations. Feeding rates and ammonia additions were adjusted to regulate the TAN concentration within the culture system. Samples were taken when conditions met the pseudo-steady state requirements of constant feed rates for at least 7 days and relatively stable TAN concentrations for at least 3 days. Maintenance of the filters was performed as needed. The PG filters required periodic flushing of accumulated material in the bottom of the filters through a valve. Since these filters were utilized for nitrification purposes only and not loaded with waste solids, they were flushed as necessary and not on a regular or routine schedule to minimize system water loss. As with the startup of any new large-scale water treatment system, some problems were encountered and adjustments and modifications made. The CB and LSB systems primarily required maintenance to ensure that proper flow into the filters was maintained. Beads from the PG filters were found in the inlet screens and annuli of the LSB and CB filters, respectively, throughout the duration of the study. There were several instances where both the LSB and CB filter system inlets were found to be almost completely blocked by beads from the PG filters and were consequently shut down for cleaning. There was also one instance where the inlets for the LSB and CB filters were blocked by feces from the culture system when the drum screen filter stopped functioning properly and allowed solids laden water to bypass the filter. As a modification, all possible locations for bead loss in the PG filters were sealed with 100% silicone or EPDM and foam gaskets to minimize bead loss. It was also noted that beads or parts of beads were also lodged in the holes of the containment screen that is located directly above the air trigger backwash mechanism of the bead filters. Some beads were also noted to have been forced through the screen due to the intensity of the backwash event. To reduce further bead loss or destruction due to the backwash mechanism, a small area of the screen was blocked with a solid sheet of high density polyethylene (HDPE) approximately 10 cm square. The HDPE sheet was spaced 2 cm from the screen so as not to prevent water flow through a portion of the screen. These modifications appeared to reduce bead fracturing and loss; however some beads were still observed in the system throughout the study.

During the acclimation process, two of the three CB filters required maintenance as the annulus for each was blocked by debris. Sand was removed and the annuli of the two CB filters were flushed to remove debris. The debris blocking the annuli was mainly beads from the PG filters and small tree parts from

the trees surrounding the filter system. The sand from both CB filters also had pieces of cellophane which had to be removed. The cellophane was likely left over from the manufacturing process of the filters as the sand was carefully inspected before being added to the filters during the initial measurement. The sumps were consequently sealed more tightly so as to prevent further blocking of the annuli. Consequently the sand was measured as it was removed from the filters to clear the blockages and it was found that approximately 12% of the initial sand volume had washed out of each filter and had settled in sump #2. Removal rate calculations based on the volume of sand were based on the measured volumes for the remainder of the study thereafter. Bacterial biomass control mechanisms were not used with the CB filters for this study. A biomass control mechanism could capture both biomass and sand being washed out of the filters and return it to the bottom of the filter. As TAN concentration increases, additional biomass attaches to the sand media. This effectively increases the diameter of the particle and reduces the overall particle density, thus making the particle more buoyant. Increased buoyancy causes sand to wash out from the filter if a biomass control mechanism is not in place or if flow rates are not adjusted as growth occurs. A 5 cm port was provided on each CB filter body approximately 25 cm below the top of the filter weir. Final sand volumes for the three CB filters were 30%, 15%, and 26% less than the initial volume for CB1, CB2, and CB3, respectively.

2.3. Sampling and analysis

During the conditioning period prior to data collection, occasional samples were taken to monitor water quality. Twelve days prior to the start of data collection, regular samples were taken to identify stable water quality conditions within the system. Sampling and water quality analysis were conducted as described below.

2.3.1. Water quality

Grab samples were taken at the pump #1 outflow sample port and each filter's respective sample port located directly after the filter exit (Fig. 1). Inlet pipes for the three pumps were located within 1 m of one another, and approximately 4 m below the water surface to insure identical water quality was delivered to each of the filter systems. Inlet dissolved oxygen (DO) concentrations taken at the pump outflows of the three pumps for each of the filter systems were compared in preliminary tests. The DO concentrations were nearly identical and it was decided based on these comparisons to use samples taken from the outflow of pump #1 as the inlet data for each of the filter systems. To eliminate possible introduction of any settled material in the sample port into the water sample, water was wasted at the sampling port for 10 s prior to any sampling. Sample bottles were then rinsed three times with water from the sample ports before being filled. Full sample bottles were immediately placed in an ice bath within an insulated container to stabilize the samples for transport to the Environmental Analysis Lab in the Department of Biological and Agriculture Engineering at North Carolina State University (approximately 9 km from study site).

All water samples were analyzed for TAN, nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), alkalinity (as mg $CaCO_3$ L^{-1}), pH, DO, temperature, chemical oxygen demand (COD), and total organic carbon (TOC). Samples were analyzed by automated analysis (Bran & Luebbe Digital Autoanalyzer III) for TAN by the salicylate method, NO_2-N by the cadmium reduction method, and nitrite-nitrogen plus nitrate-nitrogen (NO_2-N + NO_3-N) by the copper–cadmium reduction method (EPA, 1984). Chemical oxygen demand (COD) was analyzed by potassium dichromate–sulfuric

acid digestion and colorimetric analysis using a HACH Dr/2010 spectrophotometer (Hach Method 8000. EPA Approved-Federal register, 1980). Analysis of TOC was conducted using a Teledyne Tekmar Apollo 9000 combustion TOC analyzer with auto-sampler via oxidation by combustion and IR detection (EPA, 1984).

Dissolved oxygen concentrations and temperatures were measured at the pump #1 outflow and outflow fitting of each filter using a portable oxygen meter (Yellow Springs Instruments, Model 55, OH, USA). Alkalinity and pH measurements were made using a bench-top pH meter (Thermo Fisher Scientific Inc., Accumet Basic with model 13-620-530 pH/ATC electrode, MA, USA). Alkalinity measurements were performed on site by potentiometric titration to end point pH 4.8 (EPA, 1984).

2.3.2. Volumetric TAN conversion rate

The volumetric TAN conversion rate (VTR) is defined as the daily amount of TAN converted to nitrite per unit volume of unexpanded media. VTR was calculated using the filter flow rates, non-expanded volume of media inside the filter, and the difference in TAN concentrations between the influent and effluent water for each filter. For this study, a modified form of the VTR equation from Colt et al. (2006) was used:

$$VTR = 1.44(Q_f)\frac{TAN_I - TAN_E}{V_m} \qquad (1)$$

where VTR is measured as g TAN converted m^{-3} filter media (unexpanded) d^{-1}, Q_f is the flow rate through the filter (L min^{-1}); V_m is the total unexpanded volume of the filter media (m^3); and TAN_I and TAN_E are influent and effluent TAN concentration (g m^{-3}), respectively.

2.3.3. Volumetric nitrite conversion rate

The volumetric nitrite conversion rate (VNR) is the overall daily amount of nitrite-nitrogen (NO_2-N) converted to nitrate-nitrogen (NO_3-N) per unit volume of unexpanded media volume. VNR is a function of both VTR as well as the apparent volumetric nitrite conversion rate (VNRA) within the filter (Malone and Beecher, 2000), and are defined as:

$$VNRA = 1.44(Q_f)\frac{(NO_2 - N)_I - (NO_2 - N)_E}{V_m} \qquad (2)$$

$$VNR = VTR + VNRA \qquad (3)$$

where VNR and VNRA are measured as g NO_2-N removed m^{-3} filter media (unexpanded) d^{-1}, and $(NO_2-N)_I$ and $(NO_2-N)_E$ are the influent and effluent nitrite-nitrogen concentrations (g m^{-3}), respectively. Eq. (3) describes the nitrite conversion rate as nitrite is being produced when ammonia is converted to nitrite in the filters. Because of this, the apparent nitrite conversion may be estimated as near zero, while the filter may actually be removing nitrite (Malone and Beecher, 2000).

2.4. Statistics

For the statistical analysis of data from this study, various linear models for VTR were considered. These models included parameters for effects such as filter type and TAN concentration or loading rate, as described in Section 3. Some degree of variable selection among these candidate factors was undertaken, either via F-tests comparing nested models, or via graphical assessment, to obtain the most descriptive and best fitting model, while striving to maintain parsimony. Errors of VTR about the model-based predictions were assumed normally distributed. The SAS software package (SAS, 2004), specifically PROC MIXED, was used to obtain the output needed for inference about the effects of the experimental factors. A significance level of 5% was used for all tests of significance. The graphical figures in this manuscript were generated using SAS (SAS, 2004) and JMP (JMP, 2007) statistical software packages.

3. Results and discussion

3.1. Summary statistics and model development

Data was collected over a period of 180 days. From that dataset, 24 days were chosen based on pseudo-steady state conditions. A summary of the filter specific operating characteristics for the 24 days is provided in Table 1. Substrate loading rates differ between filters due to design flow parameters specific to each filter. The substrate loading rate was calculated as:

$$LRS = 1.44(Q_f)\frac{S_I}{V_m} \qquad (4)$$

where LR_S = substrate loading rate (g m^{-3} (media, unexpanded) d^{-1}), S_I = influent substrate concentration (g m^{-3}), Q_f = flow into filter (L min^{-1}), and V_m = unexpanded volume of filter media (m^3). This equation effectively normalizes the substrate available to the bacteria contained within the filters for a more realistic comparison between filter types.

Water quality parameters monitored for the length of the study are summarized in Table 2. Variations in TAN and NO_2-N concentrations were expected per experimental design. Maximum TAN concentrations were limited by the feeding response. This was perhaps due to limitation by CO_2 as there was no CO_2 removal device installed on the system. To maintain a system pH above 6.8, the minimum pH required for maximum nitrification in RAS (Groeneweg et al., 1994), sodium bicarbonate additions were significant. Increased alkalinity was used as a pH buffer in addition

Table 1
Summary statistics of filter specific parameters for the CycloBio (CB), Low Space Bioreactor (LSB), and PolyGeyser (PG) filters. Statistics are based on observations during the 24 days of pseudo-steady state conditions.

Parameter	Mean ± standard deviation (minimum, maximum)		
	CB	LSB	PG
Hydraulic load rate (L min^{-1})	253 ± 38 (180, 345)	359 ± 15 (327, 383)	505 ± 27 (457, 570)
Hydraulic retention time (min^{-1})	2.8 ± 0.4 (2.1, 3.9)	3.4 ± 0.1 (3.2, 3.7)	6.8 ± 0.2 (6.1, 7.7)
TAN load rate (g m^{-3} (media) d^{-1})	777 ± 388 (85, 1710)	507 ± 206 (86, 943)	1163 ± 493 (194, 2345)
Nitrite-N loading rate (g m^{-3} (media) d^{-1})	2646 ± 2120 (354, 7365)	1536 ± 1174 (227, 5234)	3569 ± 2869 (484, 13015)
VTR (g m^{-3} (media) d^{-1})	667 ± 345 (85, 1600)	267 ± 123 (66, 542)	586 ± 284 (48, 1231)
VNR (g m^{-3} (media) d^{-1})	1295 ± 567 (448, 2501)	353 ± 208 (7, 699)	352 ± 260 (−51, 879)
Efficiency (% TAN removed)	87 ± 16 (43, 100)	53 ± 13 (29, 100)	49 ± 16 (10, 100)
Influent DO (g m^{-3})	5.8 ± 1 (5.0, 8.1)	5.8 ± 1 (5.0, 8.1)	5.8 ± 1 (5.0, 8.1)
Effluent DO (g m^{-3})	2.0 ± 1 (0.5, 4.9)	5.2 ± 1 (3.1, 7.7)	4.4 ± 1 (3.0, 7.7)
ΔO_2 ($DO_{OUT} - DO_{IN}$) (g m^{-3})	−3.9 ± 1 (−1.9, −5.8)	−0.7 ± 0 (0.0, −2.0)	−1.4 ± 1 (−0.4, −2.2)
ΔpH ($pH_{OUT} - pH_{IN}$)	−0.03 ± 0.1 (−0.18, 0.08)	0.13 ± 0.1 (−0.06, 0.29)	0.02 ± 0.1 (−0.26, 0.20)
Expanded bed height (CB only) (cm)	192 ± 13 (175, 215)		

to providing a source of inorganic carbon for the autotrophic nitrifying bacteria in the form of sodium bicarbonate ($NaHCO_3$). Variations in pH and alkalinity were due to balancing the effects of feed rates, CO_2 production, and $NaHCO_3$ additions.

Various linear and non-linear statistical models were examined. Using the Akaike's Information Criterion (AIC) as a basis for model selection, the AIC number may be derived using a mixed analysis in SAS and penalizes for adding predictor variables into models (Akaike, 1974). Smaller AIC numbers represent models more appropriate for response prediction. Only the models which best predicted biofilter performance were selected and are presented in the following subsections. Temperature, pH, alkalinity, influent DO, TOC, and COD were not found to be statistically significant for predicting VTR or VNR. The best fit models for predicting VTR performance were based on TAN concentration or TAN loading rate (LR_{TAN}). Similarly, the best fit model predicting VNR relied only on log-transforms of nitrite-N loading rate (LR_{NO_2}) and LR_{TAN}. These results suggest that for this study, TAN and NO_2-N concentration and loading rates were the significant limiting factors for nitrification.

3.2. TAN and VTR

VTR was calculated each day for each of the nine individual filters. For statistical analysis a mixed model appropriate to the experimental design was used. Two statistical models were compared testing for linear dependence by VTR on TAN concentration or LR_{TAN}, respectively. These models included fixed effects for linear dependence on the bulk solution TAN concentration (Eq. (5)) or TAN substrate loading rate (LR_{TAN}) (Eq. (6)), with possible filter type-specific slopes, and independent, normally distributed random effects for day, individual filter and experimental error. The variance components for the random day effect were also type-specific, as there was considerably more variability in VTR for the CB filters. A least squares means analysis was used for each model to estimate the mean VTR for each filter type over the range of filter type-specific operating conditions and to compare the standard error associated with such estimates associated with each model. The least squares means analysis uses an average of the predictor variable specific to each filter type to estimate an average VTR for the three filter types. The least squares means analysis was used to show the difference in mean VTR values generated from the proposed linear model estimates and the observations of this study. The VTR under these two models and the estimates to each respective model are then given by the following expressions:

$$VTR = \beta_0 + TAN(\beta_1 + CB(\beta_2) + LSB(\beta_3)) + CB(\beta_4) + LSB(\beta_5) + D + F_I + E \tag{5}$$

$$\widehat{VTR} = -108.0 + TAN(1007.3 + CB(824.2) + LSB(431.3)) + CB(244.5) + LSB(77.9) \tag{5.1}$$

Table 2
Summary statistics for the culture system water quality parameters. The summary is based on the 24 days of pseudo-steady state conditions.

Variable	Mean (Std. Dev.)	Minimum	Maximum
TAN concentration ($g\,m^{-3}$)	0.69 (0.3)	0.13	1.20
Nitrite-N concentration ($g\,m^{-3}$)	2.1 (1.5)	0.31	6.66
DO concentration ($g\,m^{-3}$)	5.8 (0.8)	5.0	8.1
Temperature (°C)	28.9 (2.3)	24.0	31.6
pH	7.16 (0.14)	6.85	7.48
Alkalinity (as g $CaCO_3$ m^{-3})	261 (70)	100	422
TOC ($g\,m^{-3}$)	25.1 (16)	7.1	58.5
COD ($g\,m^{-3}$)	80 (12)	51	98

$$VTR = \beta_0 + LR_{TAN}(\beta_1 + CB(\beta_2) + LSB(\beta_3)) + CB(\beta_4) + LSB(\beta_5) + D + F_I + E \tag{6}$$

$$\widehat{VTR} = -86.6 + LR_{TAN}(0.58 + CB(0.86) + LSB(0.54)) + CB(123.5) + LSB(78.9) \tag{6.1}$$

where VTR = volumetric TAN removal rate ($g\,m^{-3}$ (media, unexpanded) d^{-1}), $\widehat{VTR}$ = predicted VTR based on least squares estimates to Eqs. (5) and (6) as shown in Eqs. (5.1) and (6.1), respectively, β_0 = Y-intercept for the PG filter type ($g\,m^{-3}$ (media, unexpanded) d^{-1}), TAN = TAN concentration ($g\,m^{-3}$), LR_{TAN} = TAN loading rate ($g\,m^{-3}$ (media, unexpanded) d^{-1}), β_1, β_2, β_3 = slope parameters for the PG, CB, and LSB filter types, respectively, β_4 and β_5 = the difference in the Y-intercepts from PG for the CB and LSB filter types, respectively, the CB, LSB, and PG coefficients are chosen as either the numerals 0 or 1 to indicate which model is of interest, and, D, F_I, and E are the random error components for day, individual filter, and experimental effects, respectively.

3.2.1. TAN concentration and VTR

The linear dependence of VTR on TAN concentration is shown in Fig. 2. The relationships between TAN concentration and VTR for the CB and PG filters were very similar. The PG filters showed the possibility of outperforming the CB filters at higher TAN concentrations as the slope of the line for PG TAN removal was greater than the slope of the line for CB TAN removal in Fig. 2. The CB filters demonstrated variability in VTR and problems maintaining fluidization levels of the sand at higher TAN concentrations (>0.8 g TAN m^{-3}). The LSB filters showed steady VTR performance, though achieved the lowest TAN removal rates of the three filter types in this study. The linear relationship between VTR and TAN concentration was strong for the LSB and PG filter types (R^2 = 0.92 and 0.93, respectively), and was more variable for the CB filters (R^2 = 0.48). VTR for the LSB filters was significantly lower than the CB and PG filters using Eq. (5). Additionally, the CB and PG filters were not significantly different in terms of TAN removal using Eq. (5). Analysis using the least squares means method provided mean VTR estimates for each of the filter types based on Eq. (5.1) (Table 3). These estimates were the same as the observed means for the LSB and PG filters, and slightly higher than the observed mean for the CB filters. The estimate for the CB filters was based on 20 days of data as there were 4 days when they were out of service and resuming pseudo-steady state operation. While the CB filters produced a higher mean VTR, the PG filters performed more consistently over the entire range of TAN concentrations for this study. The PG filters were able to close the gap between the CB filters at higher TAN concentrations, effectively eliminating any significant differences in VTR performance based on TAN concentration. Such consistency in VTR is very important in aquaculture production. Consistency in VTR performance may be seen in Fig. 2. The majority of data points for the PG and LSB filters were tightly grouped about the respective regression lines, as were the respective confidence intervals (Fig. 2). However, the data for the CB filters showed an increase in variability in VTR at TAN concentrations >0.8 g m^{-3} (see Fig. 2 and standard error in Table 3).

The variability in VTR at higher TAN concentrations and lower R^2 value for the CB filters may be attributed to the lack of a bacterial biomass control mechanism on these filters. As noted previously in Section 2.2, the CB filters were found to have lost sand over the course of the study. The loss of sand and biomass will create unbalanced results as the overall mass balance would have to account for the loss of biomass and media in the filter effluent. When a particular CB filter appeared to have flow problems, the filter was emptied to clean the annulus. Flow problems were noted
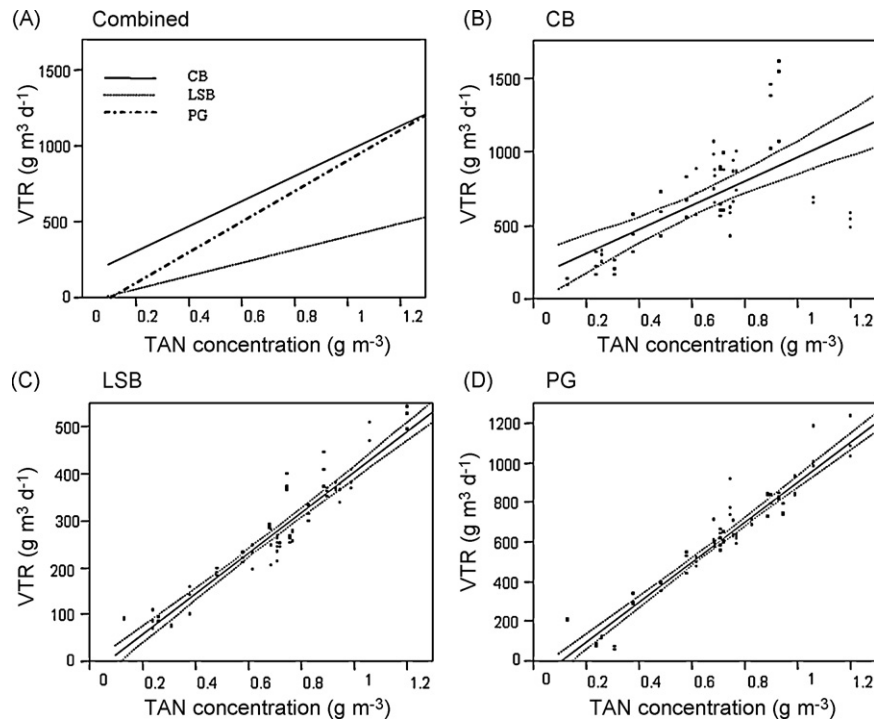
Fig. 2. Effect of TAN concentration ($g\,m^{-3}$) on VTR ($g\,m^{-3}$ media d^{-1}). A graph of all three filters (A) compares the three filter types directly and corresponding regression lines. Graphs with 95% confidence intervals about the regression lines for filter types CB (B), LSB (C), and PG (D) are also shown.

when reduced bed expansion and dead spots caused by unexpanded media accumulated in one part of the filter were observed.

Analysis of several higher order relationships suggested possible non-linearity in the relationship between TAN concentration and VTR. The possibility of non-linearity is most likely due to either increasing variation or possible filter failure at TAN concentrations $>0.8\,g\,m^{-3}$. The linear relationship is visible for all three filters, with variation in VTR increasing for the CB filters above 0.8 TAN m^{-3} (Fig. 2). The linearity results for all three filter types are similar to previous studies evaluating biofilter performance at low TAN concentrations commonly found in aquaculture production systems. Ester et al. (1994) found that TAN removal rates for rotating biological contactors (RBC) were linear under commercial scale aquaculture conditions. Zhu and Chen (1999) modeled biofilter nitrification for a single-rate limiting substrate at a wide range of TAN concentrations in moving bed bioreactors. They demonstrated linear TAN removal at low input TAN concentrations similar to conditions found in RAS. The same study developed a modified Michaelis–Menten model for TAN concentrations below 3 g TAN m^{-3} as:

$$R = \frac{R_{max}}{K_s}(S - S_{min}) \tag{7}$$

where R = areal or volumetric TAN removal rate ($mg\,m^{-x}\,d^{-1}$; areal ($x = 2$) or volumetric ($x = 3$)), R_{max} = maximum TAN removal rate ($mg\,m^{-x}\,d^{-1}$), S is the TAN concentration ($mg\,L^{-1}$), S_{min} is the

minimum concentration required for nitrification as determined in the study ($mg\,L^{-1}$), and K_s is the half saturation constant for TAN ($mg\,L^{-1}$). Nitrification is limited by a minimum TAN concentration ($S_{min} = 0.07\,mg\,L^{-1}$) (Zhu and Chen, 1999). Modifying the Michaelis–Menten equation to account for S_{min} produces better TAN removal predictions. However, Eq. (7) is based on a single limiting substrate and does not account for nitrification inhibition within the filter at high organic loading rates (Malone et al., 2006). The same equation also assumes that adequate flow is provided to the filter and bacteria contained therein. Comparisons using only the bulk solution substrate concentrations to predict biofilter performance typically assume ideal conditions inside the filters. The potential for low substrate availability to the bacteria in a filter due to inadequate flow is possible in "real-world" applications and must be taken into consideration. By accounting for both the hydraulic loading rate as well as the influent substrate concentration, predictions made using the substrate loading rate provide a composite understanding of the impacts of both parameters and normalize comparisons between different filter types. Evaluating the relationship between LR_{TAN} and VTR allows for more accurate comparisons between filter types by accounting for individual hydraulic loading rates as well as TAN concentration.

3.2.2. TAN loading rate and VTR

By accounting for the influent flow rate, LR_{TAN} provides a different visualization of VTR for the three filter types from that of TAN concentration (Fig. 3). The CB filters continued to show the highest TAN removal rates of the three filters. Interestingly, the PG filters showed a considerable difference, comparatively. At $LR_{TAN} < 1000\,g\,m^{-3}\,d^{-1}$ the PG filters actually produced the lowest VTR of the three biofilters in this study, though there was no significant difference between the VTR for the LSB and PG filters ($p = 0.97$). In sizing the biofilters used in this study, the authors relied on the manufacturer's nitrification capacity estimates. The authors endeavored to match nitrification capacity without trying to match flow rates. The manufacturer's suggested flow rate for the PG filters was 65% greater than for the LSB filters. To try to make up

Table 3
Model based estimates of mean VTR based on average TAN concentration using the Eq. (5) solution, Eq. (5.1). The average TAN concentration of $0.69\,g\,m^{-3}$ was used for the LSB and PG filters, and $0.64\,g\,m^{-3}$ for the CB filters.

Filter type	VTR estimate ($g\,m^{-3}\,d^{-1}$)	Standard error ($g\,m^{-3}\,d^{-1}$)
CB	704.6	67.16
LSB	267.2	39.72
PG	586.3	41.60
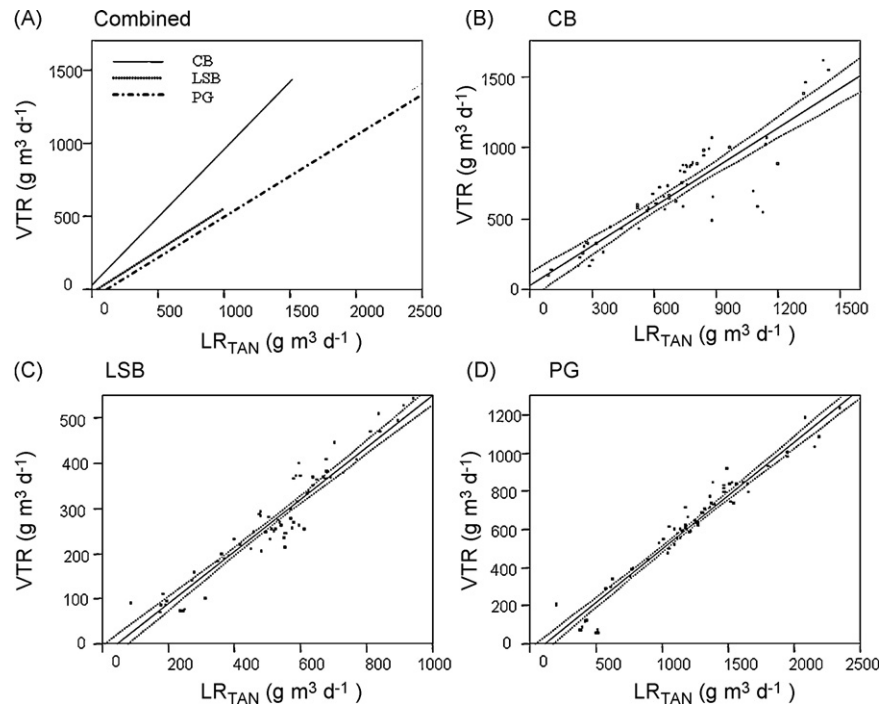
Fig. 3. Effect of TAN substrate loading rate on VTR. A graph of all three filters (A) compares the three filter types directly. Graphs with 95% confidence intervals about the regression line for filter types CB (B), LSB (C), and PG (D) are also shown.

for this difference, the PG filters were operated below the manufacturers recommendation. As such, maximum VTR for the PG filters may not have been achieved at the lower TAN concentrations.

Another important performance characteristic of note is substrate removal efficiency. The greater the percentage of substrate removed from solution per pass through a filter, the greater the substrate removal efficiency. Substrate removal efficiency may be increased by reducing flow to the reactor, though volumetric substrate removal rates, e.g. VTR, may be decreased. Additionally, some species require a low culture system TAN concentration. Filters with greater removal efficiency will provide lower TAN concentrations returning to the culture system. The CB filters showed the highest removal efficiency followed by the LSB and PG filters, respectively (Table 1). Conversely, the hydraulic loading rates and reactor volumes for the three filter types ranked from high to low were PG, LSB, and CB, respectively. The CB and LSB filters were operated at or above the manufacturer suggested maximum flow rates while the PG filters were operated at approximately half the maximum suggested flow rate. Increasing the flow will decrease the efficiency per pass through the filters.

Using a statistical model with influent TAN concentration as the sole predictor (Eq. (5)) does not predict VTR as well as one using LR_{TAN} as the predictor (Eq. (6)). Eq. (6) is a better fit for the linear representation of biological filter performance for all three filter types (R^2 = 0.84, 0.92, and 0.94 for CB, LSB, and PG, respectively). VTR for the CB filters differed significantly from the PG and LSB filters, and there was no significant difference between the VTR for the PG and LSB filters. Mean VTR estimates based on Eq. (6) solution (Eq. (6.1)) using the least squares means analysis showed estimates similar to Eq. (5.1) for all the filters (Table 4). The standard errors for the VTR estimates in Table 4 were considerably lower than those in Table 3. This further supports Eq. (6) as the more effective model for estimating mean VTR performance for the filters. Data points for all three filter types are more tightly grouped about the respective regression lines, as are the respective

confidence intervals as compared to Fig. 2 (see Fig. 3). The narrower confidence intervals show that using TAN loading rate as the sole predictor leads to more precise prediction of biofilter VTR performance. As a result, LR_{TAN} provided a more realistic understanding and prediction of VTR performance for this study.

3.2.3. Previous TAN removal studies

Monod style kinetics are typically used to describe the TAN conversion rate as the TAN loading rates increase (Malone et al., 2006). Analysis of this relationship shows a first order, linear relationship at low TAN loading rates shifting to a half-order relationship at moderate to high TAN loading rates, and finally resulting in a zero-order relationship at high TAN loading rates. Analysis of Fig. 3 shows that a linear relationship, not a Monod relationship existed for all of the operating conditions for this study. This demonstrated that these filters were capable of operating at higher TAN loading rates than evaluated as an approach toward either half-order or zero-order relationships was not achieved. Evaluations at higher TAN concentrations would provide additional performance data. However, the lower range of TAN concentrations used in this study were more representative of limits for safe operation of RAS production with more sensitive species of fish. For this study, TAN concentrations were limited by fish feeding response. TAN concentrations higher than 1.5 g m^{-3} are generally not recommended in recirculating aquaculture, though Tilapia systems with concentrations as high as 2.5 mg TAN L^{-1} have been observed with good performance given proper

Table 4
Model based estimates of mean VTR based on average LR_{TAN} values specific to each filter type from Table 1 and based on Eq. (6) least squares estimate, Eq. (6.1).

Filter type	VTR estimate (g m^{-3} d^{-1})	Standard error (g m^{-3} d^{-1})
CB	701.0	22.61
LSB	267.2	7.62
PG	586.3	14.03

system design and maintenance (Timmons et al., 2006). TAN loading rates could be achieved at the same TAN concentrations by increasing the flow rates to each filter. Higher flow rates would be possible in the LSB and PG filters but not in the CB filters due to the fluidization height of the sand bed unless media size is altered. Any increase in flow rate risks loss of sand from the filter into the production system.

TAN conversion rates reported in this study show considerable difference from previous studies. Zhu and Chen (1999) reported TAN removal rates for a moving bed bioreactor at the lab scale using TAN as the sole substrate at concentrations substantially higher than this study. The resulting average VTR (374 g m^{-3} d^{-1}) was 40% greater than the VTR reported for the LSB in this study. In a follow-up study evaluating the effects of organic carbon on TAN removal rate, the VTR reported at a ratio of C/N = 1.0 and 2.0 for the moving bed filter was 17% (312 g m^{-3} d^{-1}) and 9% (291 g m^{-3} d^{-1}) higher, respectively than that of the LSB filters in this study with a C/N ratio of 36.9 (Zhu and Chen, 2001). The same study showed that organic carbon inhibits the rate of TAN removal by 70%, and reported no significant difference in TAN removal rates between C/N = 1.0 and 2.0. Ling and Chen (2005) reported TAN removal rates for fluidized sand filters as well as floating bead filters at C/N = 0, 0.5, 1.0, and 2.0. Comparing the reported TAN removal rates for their study at the highest C/N ratio (2.0), VTR values were 63% (956 g m^{-3} d^{-1}) and 637% (4917 g m^{-3} d^{-1}) higher than those reported for this study comparing the floating bead and fluidized sand filters, respectively. All of the previous studies were conducted at the lab scale using artificial waste nutrients. Results from the current study highlight the considerable differences between nitrification rates measured in large-scale filters using water from fish culture systems and small lab scale filters using only artificial nutrients.

A direct comparison of the C/N ratios from this study and the previous studies described above is not possible. TOC analysis was used for this study. The majority of previous studies using artificial waste nutrients did not incorporate refractory organics, thus TOC measures for the studies were the same as the amount of biologically degradable organic carbon (BDOC) in the systems. TOC, like COD, measures both the biologically and the chemically degradable organic carbon in the water. C/N ratios described in the previous studies to relate biofilter performance to water quality parameters were based on BDOC analysis, a measure of only biologically degradable organic carbon.

Studies reporting TAN removal rates conducted at a scale more similar to that of commercial RAS have been lacking. A review of floating bead filter performance evaluations by Malone and Beecher (2000) showed that the VTRs demonstrated by the PG filters in this study were considerably higher than other previously reported studies. In a study performed at the pilot scale, VTR reported for a propeller washed floating bead filter and a fluidized sand filter were 78% (127 g m^{-3} d^{-1}) and 82% (117 g m^{-3} d^{-1}) less, respectively, than those reported in the study presented here (Pfeiffer and Malone, 2006). The primary difference in the system design from the study presented herein was the pre-filtration of the effluent prior to biofiltration. The influent water in the current study was mechanically filtered through a drum filter with 40 micron screens. The propeller washed bead filter served as the sole means of mechanical filtration for their study. As such, the TAN removal provided by the bead filter was largely secondary to solids removal. Failure to remove particulate organic matter (POM) from the influent water to the biofilter increases the heterotrophic activity in the filter and results in reduced TAN removal rates (Leonard et al., 2002; Michaud et al., 2006). Greiner and Timmons (1998) reviewed microbead biofilter TAN removal rates (1100 g m^{-3} d^{-1}) under pilot scale production conditions removing only settleable solids while allowing suspended solids to travel to the filter. The TAN removal rates reported in the same range of TAN concentrations for this study were 18%, 34%, and 195% higher than those reported in this study for the fluidized sand, floating bead, and moving bed filters, respectively. The same study reported average TAN removal efficiency for the microbead filter of 9%, considerably lower than the efficiency reported in this study. It is also important to note that Timmons et al. (2006) recommends areal hydraulic loading rates (m^3 d^{-1} m^{-2} filter cross-section) for the microbead filters 3%, 191%, and 162% higher than the equivalent flow rates used in this study for the CB, LSB, and PG filters, respectively. Timmons et al. (2006) also reported microbead TAN removal efficiencies between 11% and 29% for a variety of commercial scale operations. These lower efficiencies are most likely due to higher hydraulic loading rates. Standards in terms of operating conditions and subsequent reporting of results are necessary to provide accurate and actual comparison of biofilter performance. A direct comparison of the results from this study with the two studies mentioned above is difficult as differences in the methods of solids removal may yield different TAN removal rates.

Previous studies performed at the lab scale have also emphasized steady state conditions. Conditions whereby environmental variables as well as substrate loading and conversion are constant are considered steady state. In the production environment, true steady state conditions are nearly impossible to attain and maintain, nor are they necessarily desirable. In production, feed applied to the systems should theoretically be increased each day, in keeping with the practice of feeding according to biomass, which is constantly increasing. Daily variation in feed rates, chemical additions, and equipment operation are only a few factors responsible for reducing the stability of the system. The purpose of production is to maximize yield, and maximum yields are achieved by re-evaluating the rate of production on a regular basis. Making changes to improve and increase the rate of production are part of the daily operation in the production environment. Such changes are therefore responsible for the difficulty in attaining steady state conditions by any "text book" definition. This study defined pseudo-steady state conditions as constant feed rates for at least 7 days and relatively stable TAN concentrations for at least 3 days prior to sampling. Pseudo-steady state conditions were used as an alternative to previously defined steady state conditions in an attempt to qualify samples as appropriate for analysis. Colt et al. (2006) proposes either graphical or statistical approaches toward defining steady state conditions, but recognizes the difficulty of such a definition for the larger production scale environments as well. Agreement within the research community on a definition for steady state or pseudo-steady state conditions is needed for future large commercial scale biofilter evaluations.

3.3. Nitrite

3.3.1. Summary statistics

The main focus of this study was on TAN conversion under normal operating conditions at the commercial scale. As such, nitrite-N (NO$_2$-N) was not monitored in this study until day 68. Samples were analyzed for NO$_2$-N on day 68 and for the remainder of the days sampled during the 180-day study period. NO$_2$-N concentrations were monitored as a means to ensure proper biofilter functioning and to provide comparison with the water quality tests performed at the study site. VNR was calculated every day for each of the nine individual filters for each of the 12 days in this study when the filters were determined to be at pseudo-steady state operating conditions. Overall, NO$_2$-N concentrations in the culture tank were within acceptable levels throughout the study (see Table 2) except for day 99 when the NO$_2$-N concentration was

6.66 g m^{-3}. Similarly, day 99 had the highest TAN concentration (1.2 g m^{-3}) for the 24 useable days. A comparison of the apparent VNR (VNRA) and the natural log of NO_2-N loading rates ($\log(LR_{NO_2})$) showed considerable differences between the three filter types. The CB filters showed a great deal of variability in VNRA, though the trend was positively increasing as $\log(LR_{NO_2})$ increased with no negative VNRA values. The LSB filters also showed a substantial amount of variability in VNRA and there were 2 days of net nitrite production observed (negative VNRA values). The linearity of the relationship between VNRA and $\log(LR_{NO_2})$ was low for the CB and LSB filters ($R^2 = 0.19$ and 0.12, respectively). The comparison between $\log(LR_{NO_2})$ and VNRA is most notable for the PG filters as there was a consistent net nitrite production. A negative linear dependence of VNRA on $\log(LR_{NO_2})$ alone by the PG filters was markedly higher than the CB and LSB filters ($R^2 = 0.48$). Even when accounting for the production of nitrite by VTR, VNRA still should not be negative, though VNRA values near or slightly above zero are plausible (Malone and Beecher, 2006). When filters are operated at conditions similar to steady state, negative VNRA values would imply a net nitrite production.

3.3.2. VNR statistical model development

Several statistical models were considered for obtaining the best predictions of mean VNR values for the filters. The best fit model chosen included fixed effects for linear dependence on log transformations of LR_{TAN} and LR_{NO_2}, with possible filter type-specific slopes, and independent, normally distributed random effects for day, individual filter and experimental error. The mean VNR prediction under this model is then given by the following expression:

$$VNR = \beta_0 + \log(LR_{TAN})(\beta_1 + CB(\beta_2) + LSB(\beta_3))$$
$$+ \log(LR_{NO2})(\beta_6 + CB(\beta_7) + LSB(\beta_8)) + CB(\beta_4)$$
$$+ LSB(\beta_5) + D + F_I + E \qquad (8)$$

$$\widehat{VNR} = -1748.3 + \log(LR_{TAN})(267.6 + CB(876.7)$$
$$+ LSB(216.7)) + \log(LR_{NO_2})(31.5 + CB(275.2)$$
$$+ LSB(69.4)) + CB(-4624.4) + LSB(290.1) \qquad (8.1)$$

where VNR = volumetric nitrite removal rate (g m^{-3} (media, unexpanded) d^{-1}), $\widehat{VNR}$ = predicted VNR based on model solution for Eq. (8) as shown in Eq. (8.1), $\log(LR_{TAN})$ = natural log of LR_{TAN} (g m^{-3} (media, unexpanded) d^{-1}), β_0 = Y-intercept for the PG filter type (g m^{-3} (media, unexpanded) d^{-1}), $\log(LR_{NO_2})$ = natural log of LR_{NO_2} (g m^{-3} (media, unexpanded) d^{-1}), β_1, β_2, β_3 = $\log(LR_{TAN})$ slope parameters for the PG, CB, and LSB filter types, respectively, β_6, β_7, β_8 = $\log(LR_{NO_2})$ slope parameters for the PG, CB, and LSB filter types, respectively, β_4 and β_5 = the difference in the Y-intercepts from PG for the CB and LSB filter types, respectively, the CB, LSB, and PG coefficients are chosen as either the numerals 0 or 1 to indicate which model is of interest, and D, F_I, and E are the random error components for day, individual filter, and experimental effects, respectively.

Comparing VNR to $\log(LR_{NO_2})$ and $\log(LR_{TAN})$ separately, all three filter types showed positive VNR values. VNR was variable for all three filter types (Table 1). The CB filters produced the highest nitrite removal rates in this study, followed by the LSB and PG filters, respectively. Linear dependence of VNR on $\log(LR_{NO_2})$ alone was moderate for all three filter types ($R^2 = 0.54$, 0.59, and 0.37, for the CB, LSB, and PG filters, respectively). Linear dependence of VNR on $\log(LR_{TAN})$ alone was also moderate for all three filter types ($R^2 = 0.46$, 0.81, and 0.61, for the CB, LSB, and PG filters, respectively). Using a model combining both of these effects

provided more realistic estimation and a better fit to the data of the VNR performance for the filters.

For Eq. (8), the solution to which is Eq. (8.1), the VNR for the CB filters differed significantly from the PG and LSB filters. While the LSB filters produced the lowest mean VTR values for this study, there was no significant difference in VNR between the PG and LSB filters showing the need for improved VNR/VNRA performance by the PG filters.

Visualizing Eq. (8.1) is best achieved three-dimensionally (Fig. 4). A 3D analysis, generated using a smoothing spline interpolation (SAS, 2004), of the combined effects of $\log(LR_{NO_2})$ and $\log(LR_{TAN})$ on VNR demonstrates how both affected the VNR performance for each filter type. For all three filters there was a generally increasing trend in VNR as both variables increased. The variability was more readily visible in the 3D analysis for the CB filters, and the decline in VNR at higher LR_{TAN} and LR_{NO_2} in the PG filters was also apparent. The LSB filters showed the most consistent VNR performance as seen in the surface plot for these units.

3.3.3. Nitrite production in biofilters

The apparent production of nitrite by the PG filters is most likely due either to the capturing and degradation of suspended organic solids in the media bed or by the release of nitrite from the settled solids at the bottom of the filter prior to removal. Floating bead filters have been designed to serve as both biological filters and mechanical filtration devices (Malone et al., 1993). As the fish culture system water is circulated through a floating bead filter, suspended solids are captured in the media bed where biofiltration processes are occurring. Periodic perturbation (backwashing) of the media bed is required to release captured solids and restore hydraulic conductivity. The captured solids are degraded in the media bed between backwashing events creating a source of ammonia production. Retention times of the suspended waste solids in the media bed due to the frequency of backwash events is a possible underlying reason for the observed increase in nitrite production in the PG filters. Given the strong TAN removal rates demonstrated by the PG filters, it is possible that the ammonia produced through organic waste solids degradation is converted to nitrite quickly in the media bed. For this study, the filters were backwashed at a frequency slightly less than every 2 h. Manufacturer suggested times between backwash events ranged between 2 and 6 h. Operating the filters with more time between backwash events would have increased the retention time, possibly increasing nitrite production. Pfeiffer and Malone (2006) showed a slight increase in VTR by increasing the backwash frequency in a propeller washed bead filter, though no report on VNR was made. In order to determine if VNRA may be improved by changing the backwash frequency in the PG filters, further investigation will be required.

Another possible source of nitrite production in the PG filters is from the settled solids in the bottom of the filters. The bottom area in the PG filters, where captured solids settle after a backwash event, may have a very low oxygen concentration. Anoxic conditions in which no available free oxygen exists may support denitrification. Most denitrifying bacteria are facultative anaerobes, performing dissimilative nitrate reduction in the absence of free oxygen. Incomplete reduction of nitrate to nitrite may occur at low oxygen concentrations due to oxygen repression of enzymes involved in the nitrate reduction pathway (Betlach and Tiedje, 1981; van Rijn et al., 2006). With virtually no flow in the bottom region of the PG filters, settled solids remaining in the filter may contribute to the incomplete reduction of nitrate to nitrogen gas, resulting in an accumulation of nitrite. Nitrate was not monitored for this study, thus a complete nitrogenous mass balance was not possible. Performing a mass balance on the nitrogen for the filters
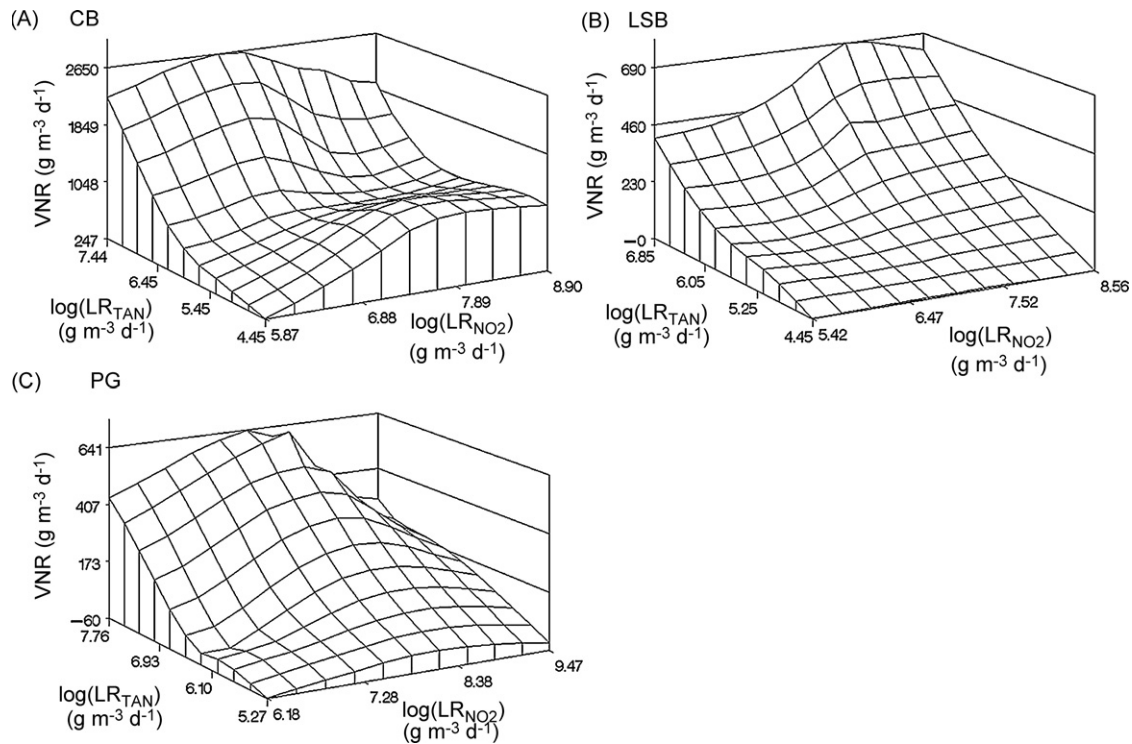
Fig. 4. A three-dimensional analysis of the combined effects of $\log(LR_{TAN})$ and $\log(LR_{NO_2})$ on the nitrite removal rate (VNR) with a smoothing spline interpolation. The graphs for all three filters are based on the least squares estimate to Eq. (8) (Eq. (8.1)): CB (A), LSB (B), and PG (C).

would provide more data for identifying the source of the nitrite production within the PG filters. Further investigation is required to determine the effect on water quality of the settled solids in the filter.

Positive VNR values are expected once VTR is taken into account based on Eq. (3). The realized nitrite removal rates of the filters are apparent in the VNRA data. VNRA is a measurement of what is experienced by the culture organisms within the system. Negative VNRA values imply a net production of nitrite, though nitrite may still be removed by the filters. Negative VNR and VNRA values should be cause for alarm, implying a limitation of the filter.

Only half of the usable sample days contained nitrite data. More data is needed to construct a suitable analysis of VNR performance related to each biofilter. VNR values are not widely published. VTR has been the primary focus of the majority of biofilter evaluations. As such, there is little information in the literature from which to draw reasonable VNR expectations and comparisons for each of the three filter types evaluated in this study. Additionally, the importance of VNRA should not be discounted. VNRA provides a measurement of the total NO_2-N removed per pass through the filter. Negative VNRA values imply net nitrite production. Such implications have possible far-reaching effects on aquaculture production systems. The PG filters demonstrated negative VNRA values over the course of the study once regular NO_2-N measurements were taken. Proportionately, VNRA was 48% and 24% of VNR for the CB and LSB filters, while VNRA for the PG filters was negative. The CB filters also showed the highest VNR as well as VTR of the three filter types. It is likely that the excess nitrite concentration in the influent generated by the PG filters elicited a positive response by the nitrite oxidizing bacteria (NOB) in the CB filters. The limitation of the PG filters to remove nitrite effectively created an excess nitrite concentration in the influent for all the filters. Eq. (8) demonstrates the dependence of LR_{NO_2} on VNR, showing that as influent nitrite concentrations increase, VNR will also increase. Thus, increasing the nitrite concentration in the influent to the CB filters provided more substrate for the NOB

within the filter if the NOB were not limited. The lower proportion of VNRA to VNR for the LSB filters may suggest that the NOB were limited in responding to the increase in the nitrite concentration. Limitation of the NOB in the LSB filters requires further investigation, though may be due to limitation of substrate diffusion.

3.4. Individual filter performance effects

In an effort to make sure that each filter was supplied with similar water, all filters in this study were supplied with water from a common sump. The filter system was operated as a recirculating loop separate from the culture system and it recirculated on itself rather than directing filter effluent immediately back into the culture system. The authors recognize that using the same water source for all of the different filter types in the evaluation study could have skewed performance results for an individual filter. Water quality parameters of the sump water may be modified based on different filter removal characteristics. However, based on data from this study and hands-on operational experience with the filters, the authors feel that individual filter design played a more important role in determining substrate removal rates than any influences from the use of a single-sump water source. In order to make conclusions about biofilter performance with a suitable level of certainty, an evaluation must either supply filters from the same water source, or run each filter on independent systems for extended periods of time and use a long term averages from performance data.

4. Conclusions

Evaluations at the large-scale using actual waste nutrients provide a more suitable basis for sizing and selection of biofilters for RAS. TAN removal rates at the commercial scale using real fish waste were determined in this study to be considerably lower than those previously determined in lab scale studies. Conversely, the

variability in operating conditions at the large production scale caused considerably higher variability in VTR than previously observed in smaller lab scale studies. Reporting standards for biofilter performance studies should be followed for more realistic comparison and application in RAS. The relationship between VTR and influent TAN concentration provides an easy, though less appropriate comparison of biofilter performance as it relates to normal, commercial scale operating conditions per manufacturer operating guidelines. Statistical analysis of this relationship is markedly different from the relationship between VTR and TAN substrate loading rate. Using substrate loading rate as the predictor for substrate removal offers a greater understanding of substrate removal capacity for each filter type. When DO is not limiting, the TAN substrate loading rate allows for better VTR prediction based on the amount of TAN available to the bacteria within the biofilter. Future biofilter evaluation studies should focus equally on VTR as well as VNR as a means of more wholly understanding biofilter performance at the large commercial scale under actual production conditions.

Acknowledgments

References

Abeysinghe, D.H., Shanableh, A., Rigden, B., 1996. Biofilters for water reuse in aquaculture. Water Sci. Technol. 34 (11 pt 7), 253–260.

Akaike, H., 1974. A new look at the statistical model identification. In: IEEE Transaction on Automatic Control, AC, vol. 19. pp. 716–723.

Betlach, M.R., Tiedje, J.M., 1981. Kinetic explanation for accumulation of nitrite, nitric oxide and nitrous oxide during bacterial denitrification. Appl. Environ. Microbiol. 42, 1074–1084.

Brazil, B.L., 2006. Performance and operation of a rotating biological contactor in a tilapia recirculating aquaculture system. Aquacult. Eng. 34 (3), 261.

Chen, S., Ling, J., Blancheton, J., 2006. Nitrification kinetics of biofilm as affected by water quality factors. Aquacult. Eng. 34 (3), 179–197.

Colt, J., Lamoureux, J., Patterson, R., Rogers, G., 2006. Reporting standards for biofilter performance studies. Aquacult. Eng. 34 (3), 377–388.

Drennan@@II, D.G., Hosler, K.C., Francis, M., Weaver, D., Aneshansley, E., Beckman, G., Johnson, C.H., Cristina, C.M., 2006. Standardized evaluation and rating of biofilters II. Manufacturer's and user's perspective. Aquacult. Eng. 34 (3), 403–416.

Eding, E.H., Kamstra, A., Verreth, J.A.J., Huisman, E.A., Klapwijk, A., 2006. Design and operation of nitrifying trickling filters in recirculating aquaculture: a review. Aquacult. Eng. 34 (3), 234–260.

Ester, C.C., Novak, J.T., Libey, G.S., Boardman, G.R., 1994. Rotating Biological Contactor Performance in Recirculating Aquaculture Systems. (cited in Wheaton et al., 1994).

FAO, 2005. Review of the State of the World Marine Fishery Resources. FAO, Rome.

Greiner, A.D., Timmons, M.B., 1998. Evaluation of the nitrification rates of microbead and trickling filters in an intensive recirculating tilapia production facility. Aquacult. Eng. 18 (3), 189–200.

Groeneweg, J., Sellner, B., Tappe, W., 1994. Ammonia oxidation in nitrosomonas at NH_3 concentrations near K_m: effects of pH and temperature. Water Res. 28 (12), 2561–2566.

SAS Institute Inc., Cary, NC, USA.

Leonard, N., Guiraud, J.P., Gasset, E., Cailleres, J.P., Blancheton, J.P., 2002. Bacteria and nutrients - Nitrogen and carbon - In a recirculating system for sea bass production. Aquacult. Eng. 26 (2), 111–127.

Ling, J., Chen, S.L., 2005. Impact of organic carbon on nitrification performance of different biofilters. Aquacult. Eng. 33 (2), 150.

Losordo, T.M., Hobbs, A.O., 2000. Using computer spreadsheets for water flow and biofilter sizing in recirculating aquaculture production systems. Aquacult. Eng. 23 (1), 95–102.

Losordo, T.M., Hobbs, A.O., DeLong, D.P., 2000. The design and operational characteristics of the CP&L/EPRI fish barn: a demonstration of recirculating aquaculture technology. Aquacult. Eng. 22 (1), 3–16.

Malone, R.F., Chitta, B.S., Drennan, D.G., 1993. Optimizing nitrification in bead filters for warmwater recirculating aquaculture systems. In: Jaw-Kai Wang (Ed.), Techniques for Modern Aquaculture. ASAE Publication 02-93 (ISBN 0-9293355-40-7; LCCN 93-71584).

Malone, R.F., Beecher, L.E., 2000. Use of floating bead filters to recondition recirculating waters in warmwater aquaculture production systems. Aquacult. Eng. 22 (1), 57–73.

Malone, R.F., Bergeron, J., Cristina, C.M., 2006. Linear versus Monod representation of ammonia oxidation rates in oligotrophic recirculating aquaculture systems. Aquacult. Eng. 34 (3), 214–223.

Meade, J.W., 1985. Allowable ammonia for fish culture. Prog. Fish-Cult. 47, 135–145.

Michaud, L., Blancheton, J.P., Bruni, V., Piedrahita, R., 2006. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. Aquacult. Eng. 34 (3), 224–233.

Nogueira, R., Melo, L.F., Purkhold, U., Wuertz, S., Wagner, M., 2002. Nitrifying and heterotrophic population dynamics in biofilm reactors: Effects of hydraulic retention time and the presence of organic carbon. Water Res. 36 (2), 469–481.

Peachey, B., 2008. Environmental stewardship—What does it mean? Process Saf. Environ. Protect. 86 (4), 227–236.

Pfeiffer, T., Malone, R., 2006. Nitrification performance of a propeller-washed bead clarifier supporting a fluidized sand biofilter in a recirculating warmwater fish system. Aquacult. Eng. 34 (3), 311–321.

SAS Institute Inc, 2004. SAS 9.1.3 Help and Documentation. SAS Institute Inc., Cary, NC, pp. 2000–2004.

Satoh, H., Okabe, S., Norimatsu, N., Watanabe, Y., 2000. Significance of substrate C/N ratio on structure and activity of nitrifying biofilms determined by in situ hybridization and the use of microelectrodes. Water Sci. Technol. 41, 317–321.

Schramm, A., Larsen, L.H., Revsbech, N.P., Ramsing, N.B., Amann, R., Scheifer, K.H., 1996. Structure and function of a nitrifying biofilm as determined by in situ hybridization and the use of microelectrodes. Appl. Environ. Microbiol. 62, 4641–4647.

Timmons, M.B., Summerfelt, S.T., Vinci, B.J., 1998. Review of circular tank technology and management. Aquacult. Eng. 18 (1), 51–69.

Timmons, M.B., Holder, J.L., Ebeling, J.M., 2006. Application of microbead biological filters. Aquacult. Eng. 34 (3), 332–343.

United States Environmental Protection Agency (US-EPA), 1984. Methods for the Chemical Analysis of Water and Wastewater, EPA-600/4-79-020. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

van Rijn, J., Tal, Y., Schreier, H.J., 2006. Denitrification in recirculating systems: theory and applications. Aquacult. Eng. 34 (3), 364–376.

Wheaton, F.W., Hochheimer, J.N., Kaiser, G.E., Malone, R.F., Krones, M.J., Libey, G.S., Easter, C.C., 1994. Nitrification filter design methods. In: Timmons, M.B., Losordo, T.M. (Eds.), Aquaculture Water Reuse Systems: Engineering Design and Management. Elsevier, Amsterdam, pp. 101–171.

Wik, T., 1999. Adsorption and denitrification in nitrifying trickling filters. Water Res. 33 (6), 1500–1508.

Zhu, S., Chen, S., 1999. An experimental study on nitrification biofilm performances using a series reactor system. Aquacult. Eng. 20 (4), 245–259.

Zhu, S., Chen, S., 2001. Effects of organic carbon on nitrification rate in fixed film biofilters. Aquacult. Eng. 25 (1), 1–11.

Zhu, S., Chen, S., 2002. The impact of temperature on nitrification rate in fixed film biofilters. Aquacult. Eng. 26 (4), 221–237.