Water Quality Impacts of Three Biofilter Designs in Recirculating Aquaculture Systems

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ABSTRACT

Nine recirculating aquaculture systems utilizing three biofilter types were placed on line and stocked with yellow perch, *Perca flavescens*, fingerlings. Biofilter type differed among systems, and included upflow pulsed bed bead filter, packed tower trickling filter, and rotating biological contactor. Following filter acclimation, a comparative analysis of biofilter performance was conducted, involving measurement of temperature, pH, dissolved oxygen, total ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, alkalinity, total hardness, carbonaceous biochemical oxygen demand, dissolved organic carbon, and total suspended solids. Filter bed emergence promoted effective carbon dioxide stripping, pH maintenance, and consistent nitrification performance in trickling filters and rotating biological contactors. Higher total ammonia nitrogen mass removal rates were observed in trickling and rotating biological contactor filters than in bead filters. Low total ammonia nitrogen mass removal rates and nitrification efficiencies for all filters resulted from relatively high carbonaceous biological oxygen demand loadings. Analysis of areas under mass removal curves showed that RBC filters were surface area limited. Foam formation in trickling filters effectively removed total suspended solids from the culture water. Filter type did not have a significant effect on median
organic water quality parameter values in the production tanks. Although differences in nitrification performance and certain water quality parameters were observed between filter types, the data set did not indicate that one filter type should be considered generally most effective at treating wastewater produced in a recirculating aquaculture system.

INTRODUCTION

Effective biofiltration is a key part of recirculating aquaculture systems (Libey and Miller 1985; Wheaton et al. 1991). Biofilters maintain chemoaustrophic bacteria, including nitrifiers which biochemically oxidize total ammonia ($\text{NH}_4^+$-N and $\text{NH}_3$-N) to nitrate, thereby allowing recirculation of culture water. Although nitrification occurs throughout the culture system (Rogers and Klemetson 1985; Losordo 1991), high levels of sustained nitrification cannot be attained without use of a biofilter. Organic degradation within the culture environment can significantly deteriorate system water quality and increase biofilter clogging (Lucchetti and Gray 1988). The majority of organic wastes stem from uneaten feed, sloughed biofilm, and fecal matter (Libey 1993; Piedrahita et al. 1996).

Biofilters used in production aquaculture include submerged bead reactors, fluidized sand reactors, trickling filters, rotating biological contactors, and rotating drums (Miller and Libey 1985; Rogers and Klemetson 1985; Malone et al. 1993; Honeyfield and Watten 1996; Summerfelt 1996; Westerman et al. 1996). This raises the question of which configuration expresses the greatest number of positive attributes regarding treatment effectiveness, filter operational characteristics and filter management needs under waste loading conditions characteristic of production aquaculture. This study evaluated three types of biofilters used for production of yellow perch (Perca flavescens) in recirculating aquaculture systems. The biofilter designs evaluated were upflow pulsed bed bead filter, packed tower trickling filter, and rotating biological contactor (RBC).
Specific objectives of this study were:

1. To evaluate acclimation times of the respective filter types,
2. To evaluate system water quality as a function of filter type,
3. To relate treatment efficiencies for each filter type (as a function of filter waste loading rates in g/m²/d), and
4. To evaluate filter performance as a function of filter design and operational characteristics.

**MATERIALS AND METHODS**

**Culture Methods**

_Stocking and System Characterization_ — Nine recirculating systems at the Virginia Tech Aquaculture Center were placed on line and stocked with yellow perch at a density of approximately 455 fish m⁻³ (Schmitz, 1999). Fingerlings measured approximately 9 cm total length, with a mean weight 5.0 g.

Each system consisted of an 8,330 L rectangular culture tank (6.1m x 1.5m x 1.2m), micro-screen drum filter (Aqua-Manna, Ladoga, IN, USA), biofilter, U-tube with pure oxygen injection, and three 0.75 kW pumps (Figure 1). The drum filter employed a 120-micron mesh screen and a vacuum device for solid waste removal, and was the site for new water additions to the system. Biofilter type (Figure 1 a,b,c) differed among systems. Degassing chambers were employed before bead and trickling filters. Three replicates were used for each filter type. Biofilters were randomly assigned to culture systems to avoid any bias of position effects within the culture facility. System flow rates were adjusted to obtain approximately two system turnovers per hour.

The systems were located in an aluminum frame building (33.5m x 15.2m x 4.8m). Lighting was low to minimize algal growth and stress responses of fish to activity around the tanks. An automatic timer produced a 16-hour light: 8-hour dark photoperiod. An exhaust fan and four propane gas heaters were used to regulate ambient air temperature.
Biofilter Characterization — Media characteristics for the upflow pulsed bed bead filter, packed tower trickling filter, and rotating biological contactor are given in Table 1.

The upflow pulsed bed bead filters (Figure 1a) included three stages, each column (0.74 m diameter x 2.11 m height) comprising one stage. Each stage employed a bed of 2 x 3 mm ABS (acrylonitrile, butadiene and styrene) plastic beads with a specific gravity of 1.04 (International Polymer Corp., Allentown, PA, USA). Water was pumped upward through the stages to expand the beds. Expansion promoted bed turnover and agitation of the biofilm on the beads. Each bed was expanded for approximately 1 minute, and allowed to settle for 2 minutes (Honeyfield and Watten 1996). Water flow was controlled with a timed electric ball valve assembly.

Packed tower trickling filters (Aqua-Manna, Inc., Ladoga, IN, USA) consisted of a cylindrical vessel packed with a single-face corrugated plastic medium (0.76 m diameter x 0.76 m height) positioned parallel to water flow (Figure 1b). Water was pumped approximately 2.4 m through a center pipe to the top of the medium and was distributed by a rotating spray bar. As water trickled downward throughout the medium, it was aerated and CO$_2$ was stripped.

| Media | Media Surface Area (m$^2$) | Media Volume (m$^3$) | Specific Surface Area (m$^2$/m$^3$) | Median Flow Rate (L/min) |
|-------|---------------------------|---------------------|------------------------------------|-------------------------|
| Bead  | 1044                      | 0.379               | 2757                               | 269 (223-329)           |
| Trickling | 465                  | 0.277               | 1681                               | 327 (303-394)           |
| RBC   | 325                       | 1.78                | 184                                | 340 (318-390)           |
Rotating biological contactor filters (Fresh-Culture Systems, Inc., Breinigsville, PA, USA) consisted of a cylindrical drum (1.22 m diameter x 1.52 m length) rotated at approximately 1 rpm by air injected below a series of louvers located around the center of the drum (Figure 1c). Rotation of the filter resulted in emergence of the biofilm from the water column, meeting the biofilm’s oxygen requirements and stripping CO₂.

**Biofilter Acclimation** — After stocking, concentrations of total ammonia-nitrogen (TAN) and nitrite-nitrogen (NO₂⁻-N) were monitored daily to assess nitrification activity. Feeding rates through this period rose from 500 g initially to 1000 g/system/day. Water exchanges were used as necessary to prevent prolonged exposure of fish to elevated TAN and NO₂⁻-N concentrations. Biofilters were considered fully acclimated when TAN and NO₂⁻-N levels consistently remained below 0.5 mg/L. Following acclimation, studies on biofilter performance began.

**Daily Operations and Water Quality Parameters** — All systems were initially filled with well water. Municipal water was utilized for daily water replacements. New water was introduced into the systems each morning following water sampling. Well water also was used for emergency water exchanges. Targeted ranges for basic water quality parameters were chosen to optimize environmental conditions for both fish and nitrifiers: NH₃-N < 0.05 mg/L (Colt and Armstrong 1981) NO₂⁻-N < 1.0 mg/L (Losordo 1991), NO₃⁻-N < 100 mg/L (Losordo 1991), dissolved oxygen > 5 mg/L (Kaiser and Wheaton 1983; Losordo 1991), pH 6.5-8.0 (Meade 1989), temperature 22-23°C (Schmitz 1999), alkalinity > 100 mg/L (Meade 1989; Losordo 1991), and hardness > 100mg/L (Meade 1989). NaHCO₃ additions were made to a system when pH and alkalinity levels dropped below 7.0 and 100 mg/l (as CaCO₃), respectively. Surface agitators were added as needed to bead filter systems to maintain targeted pH levels to maintain fish.

**Feed Administration** — Fish were fed a 42% crude protein, 12% fat, 3% crude fiber and 13% moisture floating pellet diet (Rangen, Inc., Buhl, ID, USA) two to three times daily. Rations were recorded to track system feed input (Figure 2). Schmitz (1999) reported data on fish production.
Table 2. Median values (95% CI) for basic water quality parameters. Parameters in each column with same superscript are not significantly different at the p < 0.05 level.

| Filter Type | TAN\(^1\) (mg/L) | NH\(_3\)-N\(_2\) (mg/L) | NO\(_2\)-N (mg/L) | NO\(_3\)-N (mg/L) | DO (mg/L) | pH | Temp. (\(^\circ\)C) | Alkalinity (mg/L) | Hardness (mg/L) | Feed (kg/d) |
|-------------|-----------------|-----------------|-----------------|-----------------|-----------|----|----------------|-----------------|----------------|-------------|
| Bead        | 1.06\(^a\) \((1.00-1.09)\) | 0.006\(^a\) \((0.0051-0.0063)\) | 0.390\(^a\) \((0.340-0.435)\) | 66\(^a\) \((62-71)\) | 9.5\(^a\) \((9.3-9.7)\) | 7.12\(^a\) \((7.09-7.14)\) | 23.2\(^a\) \((23.2-23.4)\) | 136\(^a\) \((124-148)\) | 217\(^a\) \((192-253)\) | 1.95\(^a\) |
| Trickling   | 0.90\(^b\) \((0.85-0.94)\) | 0.008\(^b\) \((0.0077-0.0084)\) | 0.400\(^a\) \((0.345-0.472)\) | 77\(^b\) \((69-83)\) | 9.2\(^a\) \((9.0-9.4)\) | 7.28\(^b\) \((7.26-7.33)\) | 23.2\(^a\) \((23.1-23.3)\) | 112\(^b\) \((104-123)\) | 202\(^a\) \((178-237)\) | 1.99\(^a\) |
| RBC         | 0.85\(^b\) \((0.82-0.90)\) | 0.006\(^a\) \((0.0055-0.0061)\) | 0.381\(^a\) \((0.355-0.405)\) | 77\(^ab\) \((67-85)\) | 9.5\(^a\) \((9.2-9.8)\) | 7.19\(^c\) \((7.16-7.23)\) | 22.3\(^b\) \((22.1-22.4)\) | 128\(^a\) \((118-137)\) | 223\(^a\) \((192-253)\) | 2.22\(^a\) |

\(^1\) Maximum TAN values for systems with each filter type: bead, 1.89; trickling, 1.82; RBC, 1.74 mg/L.

\(^2\) Maximum NH\(_3\)-N values for systems with each filter type: bead, 0.015; trickling, 0.018; RBC, 0.071 mg/L.

\(^3\) as CaCO\(_3\).
Water Quality Monitoring

**Nitrogenous Wastes and Physical Characteristics** — Daily water sampling commenced at 8 AM, prior to the first fish feeding. Samples were taken from the production tank prior to mechanical and biofilter treatment (sample point 1) (Figure 1). Grab samples were taken periodically from biofilter influents and effluents (sample points 2 and 3) to monitor filter performance. Filter performance also was monitored at 4-hour intervals during analysis of diurnal system dynamics.

Temperature (°C), pH, dissolved oxygen (DO) and TAN were measured daily. Nitrite-nitrogen (NO$_2^-$.N), nitrate-nitrogen (NO$_3^-$.N) and alkalinity (as CaCO$_3$) were measured weekly. Total hardness (as CaCO$_3$) was tested periodically. All tests followed protocols presented in the Standard Methods handbook (APHA et al. 1995). A YSI Model 58 dissolved oxygen meter (YSI Co., Yellow Springs, OH, USA) was used for temperature and DO measurements, and a Hanna Instruments Model HI 1270 pH probe (Hanna Instruments, Woonsocket, RI, USA) was used to monitor pH. TAN, NO$_2^-$.N and NO$_3^-$.N were analyzed using a Hach DR/2000 spectrophotometer (Hach Co., Loveland, CO, USA). Total alkalinity and total hardness both were analyzed via Hach titrations. Calculations of NH$_3$-N were made using equations presented by Emmerson et al. (1975).

**Organic Wastes** — Monitoring of carbonaceous biochemical oxygen demand (cBOD$_5$), dissolved organic carbon (DOC), and total suspended solids (TSS) analysis began on days 126, 259, and 108 of the study, respectively, and continued for the remainder of the production cycle. cBOD$_5$ samples were drawn from sample points 1 and 3 for each system. Samples were drawn in triplicate and immediately analyzed for initial DO concentrations. Final DO concentrations were measured following a 5-day incubation period (APHA et al. 1995). A YSI model 5905 BOD probe (YSI Co., Yellow Springs, OH, USA) was used to obtain both initial and final DO concentrations. DOC samples were drawn from sample points 1 and 3 for each system. Samples were immediately filtered through 0.45 micron membrane filters (Gelman Sciences Inc., Ann Arbor, MI, USA) and stored at 4°C until analysis (APHA et al. 1995). A Dohrmann Model DC-80 TOC Analyzer (Rosemount Analytical Inc., Lansdowne, PA, USA) and Horiba Model PIR-2000 Infrared Gas Analyzer (Horiba Instruments Inc., Irvine, CA, USA) were used for analysis. TSS were estimated using the filtration method (APHA et al. 1995).
Grab samples were collected from all system sample points and stored at 4°C until analysis within 7 days of sampling (APHA et al. 1995).

Statistical Analysis

All statistical tests were performed using the Minitab statistical software package, release 10 Xtra (Minitab 1995). Data for all test parameters were tested for normality. Because the majority of test parameters were not normally distributed, nonparametric statistical analyses were applied to the data. Mood’s median analysis tested for equality of the medians between all filter types for a given test parameter. If a significant difference ($p \leq 0.05$) was detected, a Mann-Whitney two-sample rank test was applied to the data to determine which data representing filter types were significantly different ($p \leq 0.05$).

Data for all water quality test parameters were analyzed by filter type for systems that proved viable throughout the entire 292-day study. Data from systems 3 (RBC), 7 (trickling), and 8 (bead) were not included in the analysis. In system 3, all fish died following a break in the aquaculture facility’s main water distribution pipe, when cold, chlorinated water entered the culture tank. Data from system 7 (trickling) was excluded from final analysis due to low fish numbers, resulting either from initial understocking or high rates of perch cannibalism (Schmitz 1999). In system 8, mortalities resulted from an unknown cause, resulting in a > 60 % population reduction within the system (Schmitz 1999). Hence, statistical analysis included data from two replicates for each filter type.

Table 3. Median values (95% CI) for organic water quality parameters. None of the values in a given column are significantly different at the $p < 0.05$ level.

| Filter Type | $\text{cBOD}_5$(mg/L) | DOC(mg/L) | TSS(mg/L) |
|-------------|-----------------------|-----------|-----------|
| Bead        | 48 (32-64)            | 14 (12-20)| 13 (10-19)|
| Trickling   | 45 (31-58)            | 13 (10-14)| 11 (8-16)|
| RBC         | 44 (33-55)            | 15 (14-19)| 8 (5-13)|
Table 4. Median influent and mass loading values for TAN and organic water quality parameters (95% CI). Values in same row with different superscripts are significantly different at p < 0.05.

| Test Parameter | BEAD | TRICKLING | RBC |
|----------------|------|-----------|-----|
|                | Influent (mg/L) | Mass Loading (g/m²·d⁻¹) | Influent (mg/L) | Mass Loading (g/m²·d⁻¹) | Influent (mg/L) | Mass Loading (g/m²·d⁻¹) |
| TAN            | 1.10 a (0.10-1.23) | 0.14 a (0.13-0.15) | 0.91 b (0.83-0.99) | 0.96 b (0.87-1.04) | 0.88 b (0.79-1.02) | 8.17 c (7.41-9.48) |
| cBOD₅          | 48 a (32-64) | 6 a (4-8) | 45 a (31-58) | 48 b (35-58) | 44 a (33-55) | 409 c (298-560) |
| DOC            | 14 a (12-20) | 1.8 a (1.5-2.5) | 13 a (10-14) | 13.6 b (10.4-15.0) | 15 a (14-19) | 136.5 c (126.8-174.7) |
| TSS            | 17 a (10-21) | 2.1 a (1.3-2.7) | 13 a (9-25) | 13.3 b (9.0-26.5) | 10 a (6-15) | 96.9 c (54.4-138.2) |
Figure 1a.

Figure 1b.

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RESULTS

Biofilter Acclimation

For all biofilter types, TAN and NO$_2$-N levels increased to a peak, and then decreased to steady state conditions (Figures 3 a,b). The troughs in curves before the peaks result from episodes of water flushing used to reduce TAN concentrations before filters were fully acclimated. TAN concentrations for all filter types peaked between days 22 and 25. Bead and RBC filters peaked at TAN concentrations of 3.68 and 2.92 mg/L, respectively, with trickling filters peaking at a lower concentration of 1.60 mg/L. For NO$_2$-N concentrations, peaks were observed for all filter types between days 40 and 43. The RBC filters peaked at 4.06 mg/L, while the bead and trickling filters peaked at 2.41 and 2.03 mg/L, respectively. The rate of decline to steady state conditions in nitrogenous waste levels was similar among all filter types. All filters reached steady state conditions for TAN around day 42, and for NO$_2$-N around day 52. Times to TAN and NO$_2$-N acclimation were somewhat longer than the 20-35 days reported by Wheaton et al. (1991).
Basic Water Quality Analysis — TAN, NH$_3$-N, NO$_2$-N (Figure 4a), and NO$_3$-N (Figure 4b) concentrations increased through the study in all systems. TAN and NO$_3$-N steadily increased until roughly days 98 and 182, respectively; water flushing then was practiced to manage their concentrations. NO$_3$-N concentrations were directly reduced via water exchanges. TAN fluctuations were probably a function of NO$_2$-N control, and also were directly affected via water exchanges when microbial oxidation was not sufficient for nitrite reduction. Median TAN and NH$_3$-N values (Table 2) were greater for systems with trickling filters than for those with RBC or bead filters ($p < 0.001$). All median values were well below the upper limit of the target range set for this study. Maximum TAN and NH$_3$-N values experienced by the filters (Table 2, footnotes 1 and 2) rarely reached levels considered harmful to fish health.

NO$_2$-N concentrations rose through the study for all filter types (Figure 4a). This lag between loading and *Nitrobacter* community response is typical of biofilters in recirculating aquaculture systems (Lucchetti and Gray 1988). The greatest NO$_2$-N fluctuations were observed in trickling filter systems, where a maximum value of 2.24 mg/L was observed on day 201 of the study. These large fluctuations may have resulted from the high rate of feed administration to the trickling filter systems (Figure 2). The median daily rate of feed administrated to trickling filter systems was significantly higher than those to bead ($p < 0.001$) or RBC ($p = 0.01$) systems, which were not significantly different ($p = 0.10$). NO$_3$-N concentrations also were greatest in the trickling filter systems, with a maximum value of 143 mg/L, while bead and RBC filter systems both had maximum values of 118 mg/L. Median nitrate levels in bead filter systems were lower than those in trickling filter and RBC systems, although significantly lower only for those in trickling filter systems ($p = 0.04$). NO$_3$-N levels were considered non-problematic until levels surpassed 100 mg/L at approximately day 182 of the study (Figure 4b). Thereafter, water exchanges were used for reduction of nitrate concentration.

The pH values for all systems ranged from 7.0-7.4. The pH levels in trickling and RBC systems were typically higher than those in bead systems. Higher pH values most likely resulted from the carbon dioxide
Table 5. Median mass removal rate and percent removed values (95% CI) for TAN and organic parameters over the course of study. Values in same row with different superscripts are significantly different at p < 0.05.

| Parameter | BEAD | TRICKLING | RBC |
|-----------|------|-----------|-----|
|           | Mass Removal (g/m²-d⁻¹) | Percent Removed (%) | Mass Removal (g/m²-d⁻¹) | Percent Removed (%) | Mass Removal (g/m²-d⁻¹) | Percent Removed (%) |
| --- | --- | --- | --- | --- | --- | --- |
| TAN | 0.004 a (0.003-0.009) | 4.3 a (2.4-10.2) | 0.037 b (0.006-0.062) | 5.2 a (0.6-9.4) | 0.14 b (-0.18-0.33) | 1.9 a (-1.7-5.5) |
| cBOD₅ | 0.5 a (0.2-1.0) | 9.7 a (4.4-12.8) | 1.4 a b (-2.9-7.7) | 5.6 a (-6.5-15.2) | 8.3 b (-2.9-127.7) | 3.3 a (-0.9-30.1) |
| DOC | -0.02 a (-1.02-0.41) | -0.9 a (-69.0-16.4) | 0.33 a (-0.40-1.11) | 2.7 a (-3.8-8.6) | 2.68 a (-53.28-22.27) | 1.7 a (-43.7-11.8) |
| TSS | 0.4 a (0.1-1.1) | 22.5 a (4.4-41.5) | 8.3 a (2.1-15.4) | 54.6 a (19.6-66.9) | 12.5 a b (-15.3-54.0) | 9.6 a (-14.8-51.9) |
stripping capabilities of the trickling and RBC filters. Elevated dissolved CO$_2$ also can suppress pH below optimal levels for nitrifiers (Grace and Piedrahita 1994), potentially decreasing nitrification rates. Higher mean alkalinity values in the bead systems (Table 2) were the consequence of more frequent sodium bicarbonate additions. In response to continued pH suppression, at day 156 of the study, surface agitators were placed in all bead filter systems after passage of the water through the filter beds to effect carbon dioxide stripping and maintenance of pH above 7. Higher NH$_3$-N observed in trickling filter systems than in RBC and bead filter systems may be attributed to elevated pH levels due to the effective carbon dioxide stripping abilities of the trickling filters.

DO typically ranged from 8.5-10.5 mg/L, with 5.2 mg/L being the lowest concentration observed among all systems. DO was not a limiting factor for fish or biofilter performance at these concentrations (Kaiser and Wheaton 1983; Losordo 1991).

Hardness values typically ranged from 150-280 mg/L in all systems. Hardness remained above levels considered critical for fish growth.

**Organic Water Quality Analysis** — Median organic water quality parameter values did not differ significantly among systems with different filter types (Table 3). Trend lines were fitted to the data to track organic parameter levels during the course of the study.

The greatest cBOD$_5$ increase was observed in bead filter systems, where levels increased by approximately 34 mg/L. cBOD$_5$ values in trickling and RBC systems increased approximately 28 and 29 mg/L, respectively. Since function of nitrifiers was inhibited chemically during cBOD$_5$ analysis, heterotrophic bacteria exerted all of the measured oxygen demand. These high values indicated that heterotrophs were consuming considerable dissolved oxygen, and likely were impacting the activity of nitrifying bacteria.

Bead and RBC systems displayed increases in TSS levels of approximately 7 mg/L, while trickling systems showed almost no increase (1 mg/L). This suggests that trickling filters were effective in suspended solids removal. This was unexpected, since trickling filters are not designed specifically for solids removal. The high specific surface area of the trickling filter media corresponds to a low void ratio, which
may explain why these filters removed suspended solids and became clogged halfway through the study. This particular media may have been a poor choice for use in a trickling filter since long-term performance would be expected to degrade as a result of solids accumulation in the filter. Bead filters were expected to be most efficient for suspended solids control. In a filter comparison study (Westerman et al. 1996), floating-bead biofilters were the only filter type that significantly reduced suspended solids levels (5-6 kg SS/m³ day⁻¹). Characterizing performance of a floating-bead filter and an RBC operating in series, Delos Reyes and Lawson (1996) also found that the bead filter captured a large portion of the solids in the filter influent.

DOC levels decreased through the study period for all filter types. Bead filter systems showed a DOC reduction of approximately 5 mg/L. Levels in trickling and RBC systems decreased approximately 1 and 2 mg/L, respectively.

Mass Removal Analysis

**TAN Mass Removal** — Influent TAN in bead filter systems was significantly higher than in trickling (p = 0.004) and RBC (p = 0.03) filter units (Table 4), and did not differ among RBC and trickling filter systems (p = 0.48). Higher influent TAN to bead filters was most likely a function of lower nitrification rates in bead than in trickling or RBC filters. Median mass loading values (g/m²/d) were greatest in RBC systems (Table 4). The highest TAN mass removal rate (g/m²/d) was observed in RBC systems, followed by trickling filter systems (Table 5). Bead filters exhibited the lowest removal rate, significantly less than RBC (p = 0.05) and trickling (p = 0.01) filters. TAN removal rates in trickling and RBC filters were not significantly different (p = 0.13). TAN removal efficiencies did not differ among filter types (p = 0.82).

**Organic Mass Removal** — Influent organic levels were not significantly different among filter types (Table 4). Organic mass loading was highest in RBC filters, lowest in bead filters, and significantly different among filter types.

The RBC filters were found to have the highest organic mass removal rates among all filters for all parameters tested (Table 5). $cBOD_5$ removal rate in RBC systems was approximately 17 times greater than in bead
systems (p = 0.03), with total grams removed approximately 5 times greater. cBOD₅ removal rate in trickling filters did not differ from those in bead (p = 0.09) or RBC (p = 0.09) filters.

The TSS removal rate in the trickling filters was approximately 21 times that in bead filters (p = 0.003). Total grams of TSS removed in trickling filters were approximately 9 times greater than that in bead filters. TSS removal rate in RBC filters did not differ from those in bead (p = 0.13) or trickling (p = 0.33) filters. Although trickling filters are not intended for solids removal, the data showed effective solids removal from the culture effluent. In one particular system, solids removal was so great that the trickling filter clogged two months before the end of the study. After being taken offline and pressure washed, removing excess solids and biofloc, the filter was placed back online and operated normally through the remainder of the study.

A net increase in DOC was observed across bead filter beds, although DOC levels decreased in all systems for all filter types over the course of the study. Organic matter was observed to accumulate in the bead beds throughout the study. Degradation of this matter was most likely responsible for the net increase in DOC concentrations across the filter beds. Dissolution and degradation processes may not have occurred

Figure 2. Average weekly feed additions
Figure 3a.

Figure 3b. Biofilter microbial acclimation, showing concentrations of: a) total ammonia nitrogen, and b) nitrite nitrogen (NO$_2$-N) as indicators of nitrifier population function.
quickly enough to increase system concentrations over the course of the study.

Percent removal values were not significantly different ($p = 0.35$) among filter types for any organic parameter monitored.

**Diurnal TAN Analysis**

Fish normally were fed in the morning between 9 and 10 AM and again in the evening between 5 and 6 PM. Because nitrification rates vary over the course of a day, due to fish feedings and associated ammonia production, we investigated diurnal fluctuations in TAN mass removal rate and percent removal.

TAN mass removal rates increased for all filter types between hours 0 and 4 (Figure 5a). TAN mass removal rates were not significantly different among filter types for hr 0 ($p = 0.51$) or hr 4 ($p = 0.51$). At hr 8, bead systems exhibited lower TAN mass removal rate than trickling ($p = 0.03$) and RBC ($p = 0.03$) filters, among which removal rates did not differ ($p = 0.94$). These relationships also were observed at hr 12. After peaking between hr 12 and 16, mass removal rates declined for all filter types, but did not decline to the levels observed at hr 0. Other diurnal analyses of various biofilters (Twaroska et al. 1997, Westerman et al.)

![Figure 4a.](image)

Figure 4a.
1996) showed that TAN removal increased with increasing TAN concentrations before peaking and declining, and also that TAN mass removal rates at hr 24 did not declined to levels observed at hr 0. Nitrification efficiencies increased once adequate feeding-induced ammonia was present (Figure 5b).

Analysis of the area under the concentration curves showed that TAN mass removal per unit surface area of 0.04, 0.11, and 0.10 g/m²/day for bead, trickling and RBC filters, respectively. Total mass removal for the 24-hr sampling periods was 40, 50, and 33 g for bead, trickling and RBC filters, respectively. TAN mass removed was higher for bead filters than for RBC filters, but mass removed per unit surface area was higher for RBC filters, suggesting that surface area of RBCs was limiting. We estimated the additional RBC surface area (SA) needed to compensate for this removal difference as:

\[
SA \ (m^2) = \frac{(Bead_{TMR} - RBC_{TMR})}{RBC_{MRSA}}
\]

where: TMR = total TAN mass removed (g), and MRSA = mass removed per unit surface area (g/m²/day). We estimated that the RBC filters would have needed an additional 70 m² of surface area to remove as much TAN as the bead filters.

![Figure 4b. Median weekly a) nitrite (NO₂-N), and b) nitrate (NO₃-N) concentrations during the course of the study.](image)

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Figure 5a.

**DISCUSSION**

**TAN Mass Removal as a Function of Filter Design**

The biofilters employed in this study were commercially available (trickling filter, RBC) or an experimental prototype (bead filter). Flow rates were equalized at two system turnovers per hour, and filters were not sized to provide similar amounts of surface area. To a certain extent, TAN mass removal rates are a function of TAN mass loading rates. Hence, differences in surface area of the filters may have affected filter performance. We found that RBC filters yielded the highest TAN mass removal rate, followed by trickling filters, with the lowest removal rate in bead filters. Other filtration studies also found that RBC filters provided better or more consistent nitrification performance than other filter types. Miller and Libey (1985) found that RBCs yielded the greater TAN mass removal rates than packed tower trickling filters or fluidized bed reactor filters at three fish stocking densities. Rogers and Klemetson (1985) found TAN removal efficiency of more than 90% for an RBC and 50% for a trickling filter. Westerman et al. (1996) reported higher TAN removal rates for bead filters (120-160 g/m³/d) than for RBCs (101 g/m³/
d); however, converting these rates into grams removed per unit filter surface area yielded 0.10-0.13 g/m²/d and 0.27 g/m²/d, respectively. Malone et al. (1993) found that TAN mass removal rate of a mechanical washed bead filter (0.291 g/m²/d) slightly exceeded that of a RBC (0.280 g/m²/d). However, Delos Reyes and Lawson (1996) found that a RBC yielded much higher nitrification performance than a mechanical washed bead filter when operated in series. TAN mass removal rate for the bead filter was 0.056 g/m²/d, and for the RBC, 0.257 g/m²/d. Removal efficiencies were 5 and 52% for the bead filter and RBC, respectively. Our results regarding trickling filter performance were similar to those of Singh et al. (1999), where trickling and bead filter configurations were compared. Systems employing trickling filters maintained lower TAN and NO₂-N than those with bead filters over the course of their study.

One reason why TAN removal in RBCs and trickling filters exceeded that in bead filters is that RBC and trickling filter beds are exposed to air, where atmospheric oxygen is capable of satisfying some of the oxygen demands of the exposed biofilms. Bacterial oxygen demand in bead filters can be met only by oxygen available within the water column. Lower TAN mass removal rates in bead filters than in trickling and RBC filters also may have been due to unintended retention of solids in bead filters. During each upwelling cycle, aggregated solids were to be released from the filter bed to the water flow, allowing the mechanical filter to intercept the solids and discharge them from the system. However, as organic waste loading to the filters increased through the course of the study, upwelling failed to control build-up of organic material in the bead filter beds. Water channelization occurred in the bead filter columns as a result of filter bed clogging.

TAN mass removal rates in our study were somewhat lower than removal rates in the filtration studies cited. We offer two explanations. First, our data were collected using samples drawn at 8 AM, before the fish were fed; TAN concentrations were relatively low prior to first feeding, leading to low estimates of removal rates and nitrification efficiencies. Second, nitrification efficiency decreases with increasing organic concentrations (Bovendeur et al. 1990; Figueroa and Silverstein 1992; Manem and Rittman 1992; Malone et al. 1993). cBOD₅ levels in our study ranged from 12-75 mg/L, with median values around 45 mg/L (Table 5). Nitrification rates decrease at cBOD₅ levels > 20 mg/L (Figueroa and Silverstein 1992). Nitrification efficiency drops below
10% once total organic carbon (TOC) levels reaches 12 mg/L (Abeysinge et al. 1996), corresponding to a BOD level of about 20 mg/L. Hence, high cBOD$_3$ levels in this study may have contributed to the relatively low TAN removal rates and nitrification efficiencies exhibited by all filter types.

Solids removal in trickling filters

During diurnal sampling, analysis of areas under removal curves showed that trickling filters removed the greatest amount of TAN mass over the 24 hr sampling periods. TSS removal and intermittent foam fractionation observed in the trickling filters are believed responsible for high nitrification rates. Foam condensate was first observed around day 217 of the study and was observed intermittently thereafter. Condensate would emerge from the water column in the degassing chambers (Figure 1a) following discharge from the trickling filters. Fine solids < 30 mm, which predominate in aquaculture effluent particle size distributions (Chen et al. 1991; Easter 1992), are not effectively removed by conventional solids removal devices (e.g., settling tanks and microscreen filters) and accumulate with time. Chen et al. (1993) showed that foam fractionation provided effective solids removal from aquaculture effluents, including organic solids < 30 mm in diameter. The high percentage of TSS removed by trickling filters (Table 7) confirms that these filters were effectively removing solids from the culture water. The small opening sizes (approx. 16 mm$^2$) of vertical passages in the corrugated plastic filter medium used in this study most likely contributed to foam fractionation and TSS removal.

Commercial application

This study was performed on a pilot-scale. However, trickling and RBC filters of the designs used in this study have been employed in commercial aquaculture facilities, adding insights regarding filter performance. In one particular facility, three trickling filters were employed for each culture tank, with two operated simultaneously, and the third off-line. Filter operation was rotated every 24 hours; an operating filter would be taken offline for cleaning, and the newly-cleaned filter would be placed back on-line. Filter cleaning was considered the major drawback to operation of these filters (J. Bradley, Aqua-Manna, Inc., Ladoga, IN, personal communication). RBC filters
have proven relatively maintenance free, less prone to mechanical failure, and capable of sustaining TAN at or below 3 mg/L when feeding up to 272 kg feed/system/day (D. Prillaman, Blue Ridge Aquaculture, Martinsville, VA, USA, personal communication). The bead filters evaluated in this study have not been used on a commercial scale (B. Watten, U.S. Fish and Wildlife Service, Kearneysville, WV, USA, personal communication). Other upflow bead filtration designs have been used with success in commercial operations (S. Abernathy, TilTech Aquafarm, Robert, LA, USA, personal communication).

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