Effects of Selected Chemotherapeutants on Nitrification in Fluidized-Sand Biofilters for Coldwater Fish Production

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ABSTRACT

Four fish chemotherapeutants, formalin, benzalkonium chloride, chloramine-T, and hydrogen peroxide were evaluated for their effect on the nitrification efficiency of fluidized-sand biofilters. The chemotherapeutants were added at conventional concentrations to two small-scale (2,200 L) coldwater recirculating rainbow trout (*Oncorhynchus mykiss*) culture systems each containing six fluidized-sand biofilters operating in parallel. Nitrification efficiency of biofilters was calculated before and after chemotherapeutant treatments by determining ammonia removal efficiency at ambient conditions, and also when challenged with a sudden increase of ammonium chloride at a concentration four times that of the ambient total ammonia-nitrogen (TAN). Two formalin treatments in recycle bath mode at 167 and 300 ppm were conducted with only the 300 ppm treatment having a significant negative effect on biofilter nitrification efficiency. Four single benzalkonium chloride treatments of one and 2 ppm were conducted; two static bath treatments and two recycle bath treatments. Of these four tests, only the recycle bath treatments caused biofilter nitrification efficiency to be significantly impaired. Two multiple treatments with benzalkonium chloride were conducted: one static bath treatment and one recycle bath treatment. These treatments caused ammonia removal efficiency to decrease by 18% in the static bath treatment and by 63% in the recycle bath treatment. Of these two tests, only the recycle bath treatment caused a significant impairment of nitrification. Single static bath and recycle bath treatments with 9 ppm of chloramine-T both resulted in significant impairment of nitrification, as did a 12 ppm multiple static bath treatment. A single static bath treatment with 100

ppm of hydrogen peroxide caused almost total failure of nitrification within 24 h of treatment but biofilters were able to remove 23% of TAN within 48 h of treatment.

INTRODUCTION

As land and water resources become increasingly limited, interest in recirculating aquaculture systems as a sustainable form of food production is growing. In order to be economical, recirculating systems must maintain high densities of fish, a condition that provides favorable conditions for the outbreak and spread of disease (Noble and Summerfelt 1996). Typical disease treatment protocols often require the availability of large volumes of water (Noga 1996). Given that one of the primary reasons for operating recirculating systems is to conserve water, conducting disease treatments without flushing the system with fresh water after the treatment would be preferable. In addition, in some cases there is not enough water available for the complete water exchange necessary to flush the chemotherapeutant from the system.

A typical recirculating system generally possesses two separate flows: the system flow, and the make-up flow. The system flow is the internal flow rate of the water passing through the tanks and other system components, while the make-up flow is the flow rate of fresh water entering and leaving the system. In coldwater aquaculture the make-up flow rate typically ranges from 1-20% of the system flow rate and is used for the control of temperature and water quality.

The typical methods of disease treatment within recirculating systems are either a static bath treatment or a flow-through treatment (Noga 1996). Static bath treatments are conducted by treating the culture organism in a static volume of water followed by flushing. Flow-through treatments are conducted by allowing water to flow one way through the system in a single pass and constantly adding the chemotherapeutant to maintain the desired concentration. Another option, unique to recirculating systems, is a recycle bath treatment where the chemotherapeutant is added to the culture system under normal operating conditions. From a disease management perspective, disease treatment using a recycle bath treatment might be desirable in order to decrease the possibility that the biofilter could become a reservoir for pathogens

(Noble and Summerfelt 1996). Therefore, recycle bath treatments would be preferred from the standpoint of efficacy and system management if the chemotherapeutant did not impair nitrification.

Most tank based aquaculture systems rely on flow and fresh water inputs to remove toxic metabolites and add oxygen. Treatments that require static volumes are difficult or impossible to sustain unless special design considerations, such as in-tank oxygenation and plumbing are made. Disease treatment using the static bath method will result in a lower concentration of chemotherapeutant exposure to the biofilters, because the chemotherapeutant that reaches the biofilters is diluted by the water volume residing in other compartments of the system once normal flows are resumed. If static bath treatments are not an option, then the recirculating aquaculturist must use a recycle bath treatment. A recycle bath treatment can maintain flow within the culture tank but also results in a continual exposure of the biofilter to the chemotherapeutant, which could result in impairment or failure of nitrification.

An important component of recirculating systems, biofilters support living populations of nitrifying bacteria that transform ammonia and nitrite, which are toxic to fish, into nitrate, which is relatively non-toxic. It is important that biofilters operate at peak efficiency during disease outbreaks because any impairment of biofiltration will serve to increase the stress on the fish through the reduction of water quality (Klontz 1993). Because of the biological nature of biofilters, they are often presumed to be sensitive to the biocidal agents added to recirculating systems for the control of pathogens. For these reasons it is important that aquaculturists know the effects of commonly used chemotherapeutants on biofilters, and how extensive these effects may be. In a previous study using fluidized-sand biofilters it was determined that formalin treatments at levels commonly used in fish culture caused no apparent effect on biofilter performance when tested under ambient conditions (Heinen et al. 1995). Given that most commonly used chemotherapeutants in aquaculture are biocides, it was assumed that they must have some effect on the microbial community associated with biofilters.

Fluidized-sand biofilters are typically designed with excess nitrification capacity (Summerfelt 1996; Summerfelt and Cleasby 1996) in the form of surface area available for microbial colonization. This

excess capacity allows fluidized-sand biofilters to nitrify more ammonia and nitrite than they are exposed to under normal operating concentrations. Because of this property it was hypothesized that a change in the microbial community caused by a chemotherapeutant treatment that was not evident when a biofilter was tested under ambient conditions would become evident when the biofilter was "challenged" with a spike of higher than normal ammonia concentration. Challenging the biofilters under normal conditions should allow for the determination of their maximum instantaneous capacity, which could then be used as a benchmark to compare biofilter performance after exposure to a chemotherapeutant. If a chemotherapeutant treatment caused an impairment of maximum biofilter nitrification capacity that was not apparent under ambient conditions, it should become apparent when the biofilters are challenged. Hence, it was thought that the effect of chemotherapeutants on biofilter nitrification capability might be ascertained through the determination of diminished maximum capacity.

With this in mind, an investigation into the effect of formalin, benzalkonium chloride, chloramine-T, and hydrogen peroxide on biofilter efficiency was undertaken. The goal of this study was to determine what effect the method of treatment might have on biofilter efficiency, and to prescribe modifications of these methods to minimize the effect of a given therapeutant on biofiltration. The four chemicals tested were chosen because of their widespread historical use in coldwater aquaculture (Noble and Summerfelt 1996). Formaldehyde, benzalkonium chloride and chloramine-T are in use in the countries of the European Union and Iceland (Schlotfeldt 1990). However, within the United States, only formalin is approved by the U.S. Food and Drug Administration (FDA) for use on food fish. Benzalkonium chloride is approved for use only as a disinfectant in aquaculture. Hydrogen peroxide is not approved, but is considered of low regulatory priority and the FDA is unlikely to object to its use. Attempts to register chloramine-T for treatment of bacterial gill disease are presently underway (J. Bowker, personal communication).

MATERIALS AND METHODS

Recirculating System

All tests were conducted using two identical recirculating systems (Figure 1). Each system contained one 1,500-L culture tank; one drum filter; one pump sump; two degassers with sumps; and six identical biofilters operating in parallel. Each 15 cm inside diameter fluidized-sand biofilter contained 4,700 cm^3 of silica sand and treated a flow of nine liters/min (L/min) for a total system flow rate of 54 L/min. The average diameter of the sand used in the biofilters was 0.17 mm and the static height of each sand bed was 30.5 cm. The system was stocked with rainbow trout (*Oncorhynchus mykiss*) maintained at a density that ranged from 23-38 kg/m^3. Fish were fed continually using mechanical feeders at a rate of approximately 2% of their body weight per day with Southern States 3/32" 40% Protein Trout Grower Feed[1] (Southern States Cooperative, Richmond, VA, USA). The make-up flow, a hard spring water (300 ppm as $CaCO_3$) at 11.5°C, was added at a rate of 5% of the system flow to provide approximately two system volume turnovers per day. Temperatures within the culture system ranged from 14-16°C. Biofilter influent samples were collected from sampling ports in the common influent line for each set of three biofilters while biofilter effluent samples were collected from sampling ports at the top of each individual biofilter.

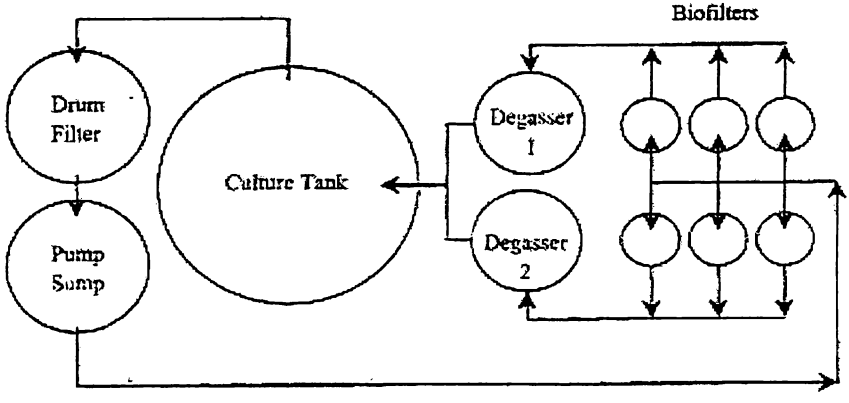

Figure 1. Diagram of recirculating systems used for chemotherapeutant experiments

Chemotherapeutant Treatments

Formalin is typically used for the treatment of external parasites at a concentration of 167 ppm for one hour followed by flushing (Noga 1996). Formalin treatments (37% solution of Formalin-F®[1] (formaldehyde), Natchez Animal Supply, Natchez, MS, USA) were conducted at 167 and 300 ppm in recycle bath mode with single treatments.

A historical treatment for bacterial gill disease with benzalkonium chloride is to treat with 1-2 ppm for one hour followed by flushing (Bullock 1990; Noga 1996). Noble and Summerfelt (1996) reported that an effective treatment technique for benzalkonium chloride was three 2 ppm treatments 48 hours apart. Single treatments of benzalkonium chloride (50% solution of benzalkonium chloride (dimethyl benzyl ammonium chloride), Argent Chemical Laboratories[1], Redmond, WA, USA) were conducted using both static bath and recycle bath treatments at 1 and 2 ppm. Multiple treatments, consisting of three treatments 48 h apart, with benzalkonium chloride at 2 ppm were also conducted in both static bath and recycle bath mode.

Chloramine-T is used as a bactericide at concentrations ranging from 9-12 ppm for one-hour static bath treatments either singly, or in a series of three treatments given on alternate days (Bills et al. 1988; Bullock et al. 1991). Single static bath and recycle bath treatments with 9 ppm of chloramine-T (N-chloro-p-toluene sulfonamide sodium salt, Sigma Chemical Co.[1], St. Louis, MO, USA) were conducted first, and then a multiple static bath treatment consisting of three treatments at 12 ppm was performed on alternate days.

Hydrogen peroxide is used as a bactericide and fungicide with a one hour static bath treatment at concentrations ranging from 100-500 ppm (Arndt and Wagner 1997; Rach et al. 1997). The hydrogen peroxide treatment (35% solution of Peroxyclear®[1] (hydrogen peroxide), EKA Chemicals, Marietta, GA, USA) consisted of one static bath treatment at 100 ppm. This concentration was chosen because previous unpublished work indicated that the peroxide treatment would significantly impair biofilter performance. Hence, a concentration at the lower end of the reported range was chosen.

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[1]Use of trade names or specific manufacturers or suppliers does not indicate endorsement.

Before a given chemotherapeutant test the ambient biofilter water chemistry was analyzed, then the biofilters were challenged. The chemotherapeutant was added to the system immediately after the challenge. Chemotherapeutant concentration was determined every twenty minutes in the culture tank and in the biofilter influent and effluent lines. Formalin concentrations were measured directly using the Purpald colorimetric method (Chemetrics[1], Calverton, VA, USA). The concentration of benzalkonium chloride was determined by analyzing the quaternary ammonium compounds (QAC) present in the water using the Direct Binary Complex colorimetric method (Hach Chemical Co.[1], Loveland, CO, USA) and establishing the relationship between benzalkonium chloride concentration and measured QAC. The concentration of chloramine-T was determined by analyzing the concentration of total chlorine present in the water using the DPD (N, N-diethyl-p-phenylenediamine) method (Hach Chemical Co., Loveland, CO, USA) and establishing the relationship between calculated chloramine-T concentration and measured free chlorine. Hydrogen peroxide concentrations were measured directly using the thiocyanate method (Chemetrics, Calverton, VA, USA).

At least 4 weeks were allowed to elapse between tests with a given chemotherapeutant to allow the microbial flora of the biofilters time to stabilize from any perturbations caused by previous treatments. The maximum time that elapsed between the conclusion of one set of chemotherapeutant tests and the onset of tests with the next chemotherapeutant was two months.

Static Bath Treatments

Static bath treatments were conducted by turning off the make-up flow to prevent dilution of the chemotherapeutant, and isolating the biofilters in a separate recirculating loop to maintain fluidization. The chemotherapeutant was then added to the static culture tank and the above conditions were maintained for an hour after which normal operating conditions were resumed. In this type of treatment, biofilters were exposed to the chemotherapeutant only after normal operations were resumed, at which time the chemotherapeutant would have been diluted by water volume residing in other compartments of the system. In the case of this experiment, chemotherapeutants in the culture tank were diluted by 40% once normal operations were resumed.

Recycle Bath Treatments

Recycle bath treatments were conducted by leaving all flow processes in their normal mode with the only difference being that the make-up flow was turned off to prevent dilution of the chemotherapeutant. The chemotherapeutant was then added in aliquots throughout the system. Normal make-up flow operating conditions were resumed after one hour. During recycle bath treatments the biofilters were left connected to the main flow and as such were continually exposed to the chemotherapeutant during treatment.

Ammonia Challenge Tests

As a preliminary step the biofilters were challenged at various total ammonia nitrogen (TAN) concentrations up to five times higher than ambient in order to determine the maximum TAN concentration that could be assimilated without a significant drop in biofilter efficiency. A TAN concentration five times higher than ambient resulted in a significant reduction in ammonia removal (30%), whereas at concentrations approximately four times higher than ambient, there was very little difference in biofilter efficiency under ambient and challenge conditions.

The fact that a recirculating system was used required two issues to be addressed by the experimental design in order for the challenge test to be successful: time of sample collection, and prevention of contamination by the recirculating spike. The time when the peak concentration of injected TAN occurred was determined by proportionally metering a concentrated solution of ammonium chloride into the system pump intake. Samples were then collected at the site of biofilter influence every 30 sec and analyzed for TAN. The time required for injected material to recirculate back to the point of injection was determined by injecting a 10 mL aliquot of dye(red food coloring) into the pump intake and collecting samples at regular intervals. The absorbance of these samples was recorded at 500 nm with a Hach DR2000 spectrophotometer. It typically took 9-10 min for the dye to return to the point of injection at the pump intake. The hydraulic retention time of the biofilters was approximately 20 sec. The TAN concentration reached a peak at 5 min after injection. To achieve a consistent TAN spike concentration across tests, the biofilter influent and effluent samples were collected at precisely six min after the onset of the ammonium

chloride solution injection. Collecting the samples at six min was long enough to ensure that the ammonia concentration was at its peak level but was still short enough to prevent the ammonia spike from recirculating through the system.

Before and 24 h after each chemotherapeutant treatment, ambient biofilter performance was measured and then the biofilters were challenged with a spike of ammonium chloride solution approximately four times that of the ambient influent TAN concentration. The ammonium chloride solution (8.93 g NH_4Cl/L) was metered directly into the pump intake for six minutes with samples collected from the biofilter influent and effluent at the end of this time period. Parameters measured were: temperature, pH, dissolved oxygen, TAN, and nitrite-nitrogen. Water quality parameters were all analyzed according to standard methods (APHA 1989).

Biofilter nitrification efficiency was calculated by subtracting the outlet concentration from the inlet concentration and dividing the difference by the inlet concentration. The statistical significance of differences between removal efficiencies was determined using a one-tailed Wilcoxon paired-sample test (Zar 1974) on the mean of six biofilters. A non-parametric test was chosen because the data was not distributed normally.

The experimental protocol and methods described were in compliance with Animal Welfare Act (9CFR) requirements and were approved by the Freshwater Institute Institutional Animal Care and Use Committee.

RESULTS

Under ambient conditions, influent concentrations for TAN and nitrite ranged from 0.18-0.52 mg/L and 0.009-0.086 mg/L, respectively (after treatment with hydrogen peroxide the ambient TAN peaked at 1.8 mg/L and dropped back to normal levels after three days). During the biofilter challenges influent concentrations of TAN and nitrite ranged from 1.07 - 1.52 mg/L and 0.014-0.105 mg/L, respectively. Total suspended solids measurements during the testing period averaged 2.9 mg/L with little difference between biofilter influent and effluent being observed.

Table 1. Summary of effects of formalin recycle bath treatments on biofilter nitrification efficiency. Values are means of six biofilters with the standard error. Double asterisks indicate a highly significant difference (p<0.01 between before and after values for one particular test.

Treatment		Removal Efficiency	
		Ambient TAN	Challenged TAN
167 ppm Formalin			
	Before	0.91 ± 0.02	0.69 ± 0.04
	After (24 h)	0.90 ± 0.04	0.81 ± 0.06
300 ppm Formalin			
	Before	0.79 ± 0.02	0.68 ± 0.05**
	After (24 h)	0.76 ± 0.03	0.55 ± 0.06

Table 2. Summary of single benzalkonium chloride treatments at different concentrations and treatment modes and their effect on biofilter nitrification efficiency. Values are means of six biofilters with the standard error. Single asterisks indicate a significant difference (p<0.05) between before and after values for one particular test, while double asterisks indicate a highly significant difference (p<0.01).

Treatment		Removal Efficiency	
		Ambient TAN	Challenged TAN
1 ppm Benzalkonium Chloride *Static Bath*			
	Before	0.76 ± 0.02	0.79 ± 0.04
	After (24 h)	0.95 ± 0.03	0.82 ± 0.05
1 ppm Benzalkonium Chloride *Recycle Bath*			
	Before	0.79 ± 0.02	0.78 ± 0.05**
	After (24 h)	0.82 ± 0.02	0.68 ± 0.06
2 ppm Benzalkonium Chloride *Static Bath*			
	Before	0.35 ± 0.03	0.61 ± 0.03
	After (24 h)	0.85 ± 0.04	0.68 ± 0.05
2 ppm Benzalkonium Chloride *Recycle Bath*			
	Before	0.96 ± 0.03*	0.83 ± 0.06**
	After (24 h)	0.74 ± 0.10	0.56 ± 0.11

The concentration of formalin remained relatively constant during the tests while the concentration of benzalkonium chloride decreased by about 50% over the space of an hour. During static bath treatments the concentration of chloramine-T decreased on average by 18%. The decrease in chloramine-T concentrations during recycle bath treatments averaged 43%. The concentration of hydrogen peroxide declined by 25% during the last 30 min of treatment after staying constant for the first 30 min.

The 167 ppm formalin test produced a slight reduction in ambient TAN removal (AAR) and a 12% increase in challenged TAN removal (CAR) (Table 1). After the 300 ppm formalin treatment there was a slight decrease of both AAR and CAR.

A 1 ppm benzalkonium chloride single static bath treatment resulted in a slight increase of both AAR and CAR (Table 2). The single 1 ppm benzalkonium chloride recycle bath treatment resulted in a slight increase of AAR and a 10% decrease in CAR. The single 2 ppm benzalkonium chloride static bath treatment caused an increase in both AAR and CAR. Of the single benzalkonium chloride treatments, the most dramatic effect was observed in the 2 ppm recycle bath treatment. Both AAR and CAR were reduced by over 20%. There was an 18% reduction of both AAR and CAR after the end of the multiple benzalkonium chloride static bath treatment. At the end of the multiple recycle bath treatment AAR had decreased by 63% and CAR by 46% (Table 3). The comparative effects of the multiple benzalkonium chloride treatments are displayed in Figure 2.

After the 9 ppm single chloramine-T static bath treatment AAR increased 20% and CAR decreased 5% (Table 4). The AAR decreased 10% and the CAR decreased 9% after the 9 ppm single chloramine-T recycle bath treatment. After the set of multiple 12 ppm chloramine-T static bath treatments there was only a slight decrease in AAR while CAR decreased by 8%. The longer term effects of the chloramine-T treatments are displayed in Figure 3.

The 100 ppm single hydrogen peroxide static bath treatment caused almost total impairment of nitrification (Table 5). Twenty-four hours after treatment the AAR was reduced by 84% and the CAR by 57%. From Figure 4, it can be seen that the biofilters had significantly recovered 11 days after treatment.

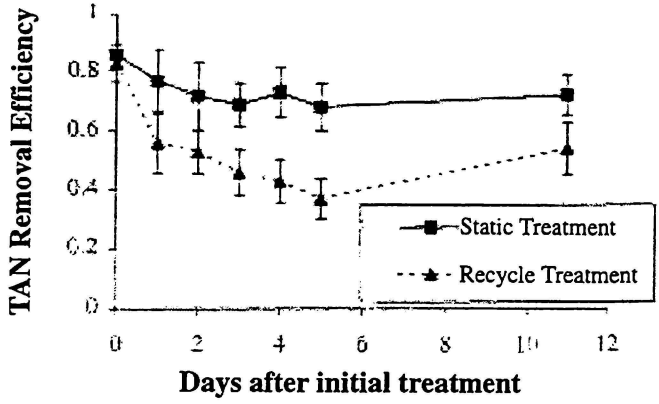

Figure 2. Effect of multiple benzalkonium chloride treatments (static bath and recycle bath) on challenged biofilter TAN removal efficiency. Values are means ± standard errors of six biofilters.

Table 3. Summary of effects of three consecutive benzalkonium chloride treatments on biofilter efficiency under two different treatment modes. Values are means of six biofilters with the standard error. Double asterisks indicate a highly significant difference (p<0.01) between initial and final values for one particular test.

	Removal Efficiency	
Treatment	**Ambient TAN**	**Challenged TAN**
2 ppm Benzalkonium Chloride		
(Three Static Bath Treatments/Alternate Days)		
Before	0.86 ± 0.04	0.86 ± 0.04
After (24 h)	0.87 ± 0.05	0.77 ± 0.11
Before	0.64 ± 0.04	0.72 ± 0.12
After (24 h)	0.78 ± 0.06	0.69 ± 0.08
Before	0.59 ± 0.06	0.73 ± 0.08
After (24 h)	0.68 ± 0.08	0.68 ± 0.08
2 ppm Benzalkonium Chloride		
(Three Recycle Bath Treatments/Alternate Days)		
Before	0.96 ± 0.03**	0.83 ± 0.06
After (24 h)	0.74 ± 0.10	0.56 ± 0.11
Before	0.71 ± 0.10	0.53 ± 0.07
After (24 h)	0.54 ± 0.15	0.46 ± 0.08
Before	0.52 ± 0.15	0.43 ± 0.08
After (24 h)	0.33 ± 0.12	0.37 ± 0.07

Table 4. Summary of chloramine-T treatments at different concentrations and treatment modes and their effect on biofilter nitrification efficiency. Values are means of six biofilters with the standard error. Single asterisks indicate a significant difference (p<0.05) between before and after values for one particular test, while double asterisks indicate a highly significant difference (p<0.01). For the 12 ppm chloramine-T tests asterisks indicate significant difference between initial and final values.

Treatment		Removal Efficiency	
		Ambient TAN	Challenged TAN
9 ppm Chloramine-T	***Static Bath***		
	Before	0.71 ± 0.03	0.65 ± 0.06*
	After (24 h)	0.91 ± 0.02	0.60 ± 0.05
9 ppm Chloramine-T	***Recycle Bath***		
	Before	0.80 ± 0.03**	0.89 ± 0.03**
	After (24 h)	0.70 ± 0.01	0.80 ± 0.05
12 ppm Chloramine-T			
(Three Static Bath Treatments/Alternate Days)			
	Before	0.85 ± 0.03	0.81 ± 0.06**
	After (24 h)	0.94 ± 0.01	0.79 ± 0.07
	Before	0.85 ± 0.01	0.82 ± 0.08
	After (24 h)	0.77 ± 0.04	0.83 ± 0.08
	Before	0.99 ± 0.01	0.74 ± 0.09
	After (24 h)	0.84 ± 0.02	0.73 ± 0.08

Table 5. Summary of effects of static bath treatment with hydrogen peroxide on biofilter nitrification efficiency. Values are means of six biofilters with the standard error. Double asterisks indicate a highly significant difference (p<0.01) between before and after values for one particular test.

Treatment		Removal Efficiency	
		Ambient TAN	Challenged TAN
100 ppm Hydrogen Peroxide			
	Before	0.85 ± 0.03**	0.61 ± 0.08**
	After (24 h)	0.01 ± 0.01	0.04 ± 0.06

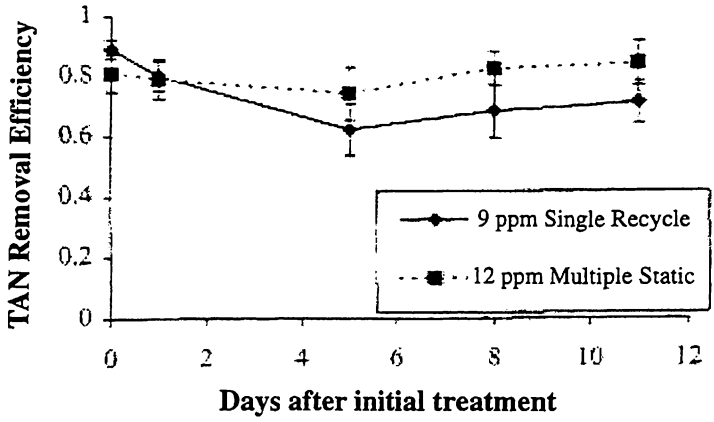

Figure 3. Effect of single and multiple chloramine-T treatments on challenged biofilter TAN removal efficiency. The multiple treatments ended on day 5. Values are means ± standard errors of six biofilters.

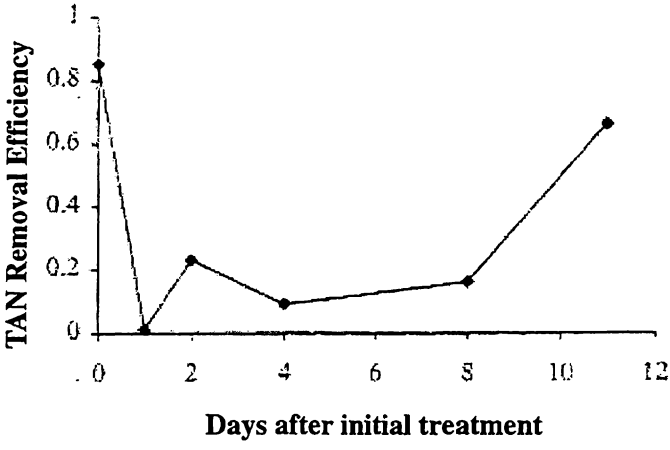

Figure 4. Effect of single 100 ppm hydrogen peroxide static bath treatment on ambient biofilter TAN removal efficiency. Values are means of six biofilters. Ambient values were used because ambient TAN values rose high enough after the treatment to render biofilter challenge superfluous.

DISCUSSION

The primary goal in this research was to determine which of the chemotherapeutants evaluated affect biofilter performance, and to what effect, with the overall concern being the preservation of adequate water quality for fish rearing. As long as adequate water quality can be maintained, minor drops in biofilter efficiency can be tolerated. Within the recirculating aquaculture system used in these experiments, biofilter nitrification efficiency often fluctuates from 5-10% over a period of several days without significant effects on water quality (unpublished data). The authors chose to make the distinction between significant ($p<0.05$) and highly significant ($p<0.01$) statistical differences in biofilter efficiency because it was assumed that only highly significant differences would have a biologically significant effect on biofiltration and the resulting water quality. The effect of changes in nitrification efficiency on TAN concentrations can be illustrated using Liao and Mayo's (1972) equation for calculating steady-state concentrations in recirculating systems. For example: assuming an initial removal efficiency of 90%, a 10% decrease in TAN removal efficiency will increase the tank TAN concentration by 12%; a 20% decrease will increase it by 27%; while a 60% decrease will increase it by 170%.

$$TAN = \left\{ \frac{1}{1 - R + (R \cdot f_{rem})} \right\} \cdot \frac{P_{TAN}}{Q}$$

TAN= total ammonia-nitrogen, mg/L
R= recycle fraction, decimal
f_{rem}= TAN removal efficiency, decimal
P_{TAN}= daily production of TAN, kg/d
Q= system flow, L/min

Of the two formalin treatments, only the single 300 ppm formalin recycle bath treatment caused significant impairment of TAN removal and only within the CAR component. In comparison, Weinbeck and Koops (1990) found that indefinite treatments with 149 ppm formalin had an adverse affect on nitrification in their biofilters, while Heinen et al. (1995) found indefinite treatments at 120 ppm, and one hour treatments at 167 ppm, had no effect on nitrification.

Both of the single benzalkonium chloride recycle bath treatments (1 and 2 ppm) caused significant impairment of CAR. Of these two tests only the 2 ppm test caused a significant reduction of AAR. This impairment of nitrification would be expected, since this test resulted in the highest concentration of benzalkonium chloride that the biofilters were exposed to. With multiple benzalkonium chloride treatments, only the 2 ppm recycle bath treatments significantly impaired TAN removal. Similarly, Noble and Summerfelt (1996) reported that 2 ppm benzalkonium chloride treatments had a negative impact on biofilters at a coldwater hatchery.

All of the chloramine-T treatments caused a significant reduction of CAR, while only the single 9 ppm chloramine-T recycle bath treatment caused a significant reduction of both AAR and CAR. In contrast, Noble and Summerfelt (1996) reported that treatment with 12 ppm of chloramine-T had no effect on biofilters at a coldwater hatchery.

The single 100 ppm hydrogen peroxide treatment caused significant reduction of both AAR and CAR. As there was limited literature available on the effect of hydrogen peroxide treatments on biofilters, the authors had to rely on anecdotal information for comparison. As such, Bullock and others at the Freshwater Institute have observed hydrogen peroxide treatments at 100 ppm to cause a major impairment of biofilter efficiency (unpublished data).

The tests having a highly significant impact on both AAR and CAR were: multiple 2 ppm benzalkonium chloride recycle bath; single 9 ppm chloramine-T recycle bath; and single 100 ppm hydrogen peroxide static bath. These treatments should be avoided in recirculation systems as they could cause major changes in water quality.

Treatments having a highly significant effect on CAR only were: single 300 ppm formalin recycle bath; single 2 ppm benzalkonium chloride recycle bath; single 1 ppm benzalkonium chloride recycle bath; and multiple 12 ppm chloramine-T static bath. These treatments could also cause significant impairment of water quality in a recirculating system.

Regardless of treatment type or concentration, chloramine-T and hydrogen peroxide consistently impaired nitrification. The severe impact

of hydrogen peroxide would make it suitable for use as a chemotherapeutant in recirculating systems only if completely flushed out of the system or completely inactivated before resuming normal operations. Chloramine-T could possibly be used with caution in a static bath treatment at the lowest concentration possible. Both formalin and benzalkonium chloride did not cause impairment of nitrification at lower concentrations, hence they could probably be used safely at these concentrations with a static bath treatment being the preferred mode of application. Aquaculturists considering the use of chemotherapeutants in recirculating systems should exercise caution as it is difficult to predict the potential effects of these chemotherapeutants on other types of recirculating systems. They should also be aware that the effect of a given chemotherapeutant on water quality will depend on the type of biofilter and ambient water quality. Our experience has been that fluidized-sand biofilters are particularly resilient to perturbations. The maintenance of good water quality should provide an adequate buffer in the event of a low-level, temporary biofilter impairment caused by a chemotherapeutant treatment.

The results of this research support the hypothesis that impairment of nitrification in fluidized-sand biofilters can be determined through challenging the biofilters with high concentrations of TAN. In all cases where AAR was significantly impaired, CAR was also significantly impaired. In two of the tests only CAR was significantly impaired, indicating that the nitrifiers within the biofilter were affected by the chemotherapeutant without any apparent effect on AAR. Excluding hydrogen peroxide, the results of this research show that while biofilters were all impaired to a certain extent by the chemicals used, the effect was primarily related to concentration. It should be kept in mind that the static bath treatments with the same concentration as recycle bath treatments resulted in lower biofilter exposure concentrations. This brings up the need for further research to test the efficacy of longer duration disease treatments at lower concentrations and the effect of these long duration treatments on biofilters.

The fact that chemotherapeutants are effective at reducing or eliminating fish pathogens and relatively ineffective at eliminating microbes within a biofilter is not surprising. In order to become permanently established within a biofilter nitrifying bacteria must form biofilms (Hagopian 1998). Bacteria can protect itself from antimicrobial

agents through the production of a film of hydrated exopolysaccharides (EPS) and are inherently more resistant to antimicrobials than their planktonic forms (Costerton et al. 1995). Since the concentrations required for disinfection with benzalkonium chloride and formalin are 200 ppm and 10,000 ppm respectively (Stoskopf 1993), it is not surprising that the concentrations used in these experiments did not have a greater effect on the biofilters with their well-established populations of nitrifiers. Such high concentrations are necessary because disinfection is generally directed against biofilms (Block 1991). LeChevallier (1991) reports that it generally requires 150 times the CT factor (concentration x time) of hypochlorous acid to achieve the same reduction in activity in a biofilm as against planktonic forms. In addition, microbes use calcium and magnesium ions in the production of EPS (Costerton et al. 1995). Anderson (1985) reported that Roccal®, a quaternary ammonium compound analogous to benzalkonium chloride, was more toxic to biofilters in soft waters than in hard waters. The water used in this experiment was hard, which may explain why benzalkonium chloride was not observed to cause greater impairment of the biofilter. While the resistance of bacteria within biofilms to antimicrobials allows a margin of safety, the aquaculturist who uses antimicrobials should still exercise caution, as it appears that repeated use may have serious consequences on biofilter performance.

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