

# **Effects of Chloramine-T and Hydrogen Peroxide on Nitrification in Fluidized-Sand Biofilters for Cold Water Fish Production**

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## **Introduction**

In a previous study using fluidized-sand biofilters it was determined that formalin treatments, at levels commonly used in fish culture, caused no apparent significant effect on biofilter performance when tested under ambient conditions (Heinen et al. 1995). Given that most commonly used therapeutants are biocides it was assumed that they must have some effect on the microbial community within 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 do 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 chloramine-T and hydrogen peroxide on biofilter efficiency was undertaken.

## MATERIALS AND METHODS

All tests were conducted using two identical recirculating systems. Each system contained: 1500-L culture tank; drum filter; pump sump; two degassers with sumps; and six identical biofilters operating in parallel.

Before a given chemotherapeutant test the ambient biofilter water chemistry was analyzed first, then the biofilters were challenged with a spike of ammonium chloride solution ( $\text{NH}_4\text{Cl}$ ) approximately four times that of the ambient influent TAN concentration, and then the chemotherapeutant was added to the system immediately after the challenge. Twenty-four hours after each chemotherapeutant treatment, ambient biofilter performance was measured and then the biofilters were again challenged with a spike of ammonium chloride. Parameters measured during these tests were: temperature, pH, dissolved oxygen, TAN, and nitrite-nitrogen. Water quality parameters were all analyzed according to standard methods (APHA, 1989). At least 4-6 weeks were allowed to elapse between tests with a given chemotherapeutant to allow the biofilters time to stabilize from any perturbations caused by previous treatments. Two months were allowed to elapse between the conclusion of one set of chemotherapeutant tests and the onset of tests with the next chemotherapeutant.

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.

Recycle bath treatments were conducted by leaving all 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.

Single static bath and recycle bath treatments with 9 ppm of chloramine-T were conducted first and then a multiple static bath treatment at 12 ppm consisting of three treatments given on alternate days. The hydrogen peroxide treatment consisted of one static bath treatment at 100 ppm.

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 are in compliance with Animal Welfare Act (9CFR) requirements and were approved by the Freshwater Institute Institutional Animal Care and Use Committee.

## **Results and Discussion**

After the 9 ppm single chloramine-T static bath treatment, ambient ammonia removal (AAR) increased 20% and challenged ammonia removal (CAR) decreased 5%. 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 100 ppm single hydrogen peroxide static bath treatment caused almost total impairment of nitrification. Twenty-four hours after treatment the AAR was reduced by 84% and the CAR by 57%.

The primary goal in this research was to determine which of the chemotherapeutants evaluated affect biofilter performance, and how acutely; 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 they felt 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, within the particular recirculating system used in this experiment, can be illustrated using Liao and Mayo's (1972) equation for calculating steady-state concentrations in recirculating systems. For example: assuming an initial TAN 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%.

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 the Glenwood State Fish Hatchery, Utah.

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 data for comparison. Bullock and others at the Freshwater Institute observed hydrogen peroxide treatments at 100 ppm to cause a major impairment of biofilter efficiency (unpublished data).

Only the single 9 ppm chloramine-T recycle bath, and the single 100 ppm hydrogen peroxide static bath had a highly significant impact on both AAR and CAR. These treatments should be avoided as they could cause major changes in water quality.

The multiple 12 ppm chloramine-T static bath treatment caused a highly significant effect on CAR only. This treatment could also cause significant impairment of water quality.

Irregardless 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 before resuming normal operations. Chloramine-T could possibly be used with caution in a static bath treatment at the lowest concentration possible.

The results 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.

### **Acknowledgements**

This research was funded by the U.S. Dept. of Agriculture's Agriculture Research Service under agreement 59-1931-3-012 and any opinions, findings, conclusions, or recommendations expressed in this publication are those of The Conservation Fund or the authors and do not necessarily represent those of the USDA.

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