

Tolerance to Formalin by a Fluidized-Bed Biofilter and Rainbow Trout *Oncorhynchus mykiss* in a Recirculating Culture System

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Abstract

Formalin tolerances of a fluidized-bed sand biofilter and rainbow trout *Oncorhynchus mykiss* were tested in a semi-closed freshwater recirculating culture system. Progressively higher formalin levels were tested until fish mortality ($\leq 1.9\%$) occurred. A 1-h exposure to 167 ppm formalin, followed by flushing, was safe for trout (no mortality) at 15.0 C but not at 16.5 C. A 110-ppm indefinite treatment (no flushing) was safe at 17.3 C, but 120 ppm at 17.3 C and 100 ppm at 17.8 C were not. Biofilter nitrification was not impaired by 1-h formalin treatments up to 167 ppm at 16.5 C, and not, usually, by indefinite treatments up to 120 ppm at 17.3 C. However, a final indefinite treatment of 70 ppm was followed by abnormally high nitrite levels for 9 d. Formaldehyde remained detectable in the system for 11 h during indefinite treatment at 120 ppm formalin. Tests on the system's CO₂ stripper did not indicate that it removed formaldehyde.

Formalin, usually purchased as a 37% solution of formaldehyde by weight, is often used to treat fish for ectoparasites, but in recirculating fish culture systems it may kill or impair the nitrifying bacteria of biofilters. Biofilters can be protected by isolation from the rest of a system during treatment, followed by flushing of the rest of the system with clean water before reconnection. However, an untreated biofilter may serve as a reservoir for parasites, and unnecessary flushing wastes water and energy. To avoid these problems, safe levels of formalin for biofilters need to be known, both for short-term (1-h) treatments followed by flushing and for indefinite treatments, i.e., ones without subsequent flushing. The maximum safe dose in a system may be determined by the tolerance of either the biofilter or the fish.

The major purpose of this study was to determine approximate maximum safe levels of formalin for the biofilter or rainbow trout *Oncorhynchus mykiss*, depending on which had the lower tolerance, for both 1-h and indefinite treatments in a recirculating

culture system. A minor purpose was to determine whether an air stripper in the system removed much formaldehyde during treatments.

Materials and Methods

The recirculating culture system was a semi-closed freshwater system containing: 1) two cross-flow fish tanks with water volumes of 10.0 m³ apiece; 2) an oxygenator for incoming water for each tank; 3) an 80- μ m microscreen filter for effluent from each tank; 4) a fluidized-bed biological filter with sand as a medium (fluidized volume 3.1–3.4 m³); and 5) a combination oxygenator and CO₂ stripping unit which followed the biofilter. Water flowed sequentially through an oxygenator, fish tank, and microscreen filter at 314 L/min and then fell into a sub-floor sump. A guide tube directed this water to the intakes of pumps which pumped water through the biofilter at 631 L/min. Water then fell through the oxygenator/CO₂ stripper and into the subfloor sump and was pumped back to the fish-tank oxygenators. Water in the CO₂ stripper fell a total distance of 133 cm, including 104 cm through 5.1-cm Nor-Pak polypropylene packing material (Jaeger Products, Inc., Spring, Tex-

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as), at a rate of 591 L/min while air was blown up through it at 5,097 L/min (gas/liquid ratio 8.6). The system was operated with a water volume of 28.0 m³ without fish and about 25.3 m³ with fish, after correction for fish volume using a specific gravity of 1.02 for trout (Leitritz and Lewis 1976). Water exchange rate was 39 L/min (220% of system volume, with fish, per day), giving a retention time (turnover time) of 10.9 h, calculated by dividing the system water volume by the exchange flow rate. New water was spring water at about 12 C, and system water temperature varied with air temperature. The system contained 10,838–17,465 “all-female” Kamloop strain rainbow trout with a size range of approximately 30–340 g and a biomass range of 1,602–2,372 kg during the experimental period. Feeding was ad libitum from pendulum demand feeders, and fish were not fasted before or during treatments.

Treatment concentrations are given as parts per million (ppm = $\mu\text{L/L}$) of 37% formaldehyde. Amounts of formalin added were based on system water volume without fish, as is the usual practice. Formaldehyde concentrations were measured with CHEMetrics ampoules (VACUettes Kit K-4605D, from CHEMetrics, Inc., Calverton, Virginia), which are visually compared to color standards. Total ammonia-nitrogen (TAN) and nitrite-nitrogen ($\text{NO}_2\text{-N}$) concentrations in biofilter effluent and in fish tanks were used as indicators of biofilter function and were measured by the Nessler and diazotization methods, respectively, with a Hach DR/2000 spectrophotometer before treatment and 20–24 h after treatment began. Normal maximum levels of TAN and $\text{NO}_2\text{-N}$ were 0.4 and 0.20 mg/L, respectively, for biofilter effluent, and 2.0 and 0.20 mg/L, respectively, for fish tanks. Typical water quality parameters in fish tanks were: temperature 13–18 C; dissolved oxygen 8–10 mg/L; TAN 1.1–1.5 mg/L (unionized ammonia-nitrogen <0.01 mg/L); $\text{NO}_2\text{-N}$ 0.02–0.19 mg/L; and pH 7.0–7.3. Dissolved oxygen in the biofilter effluent was

1–3 mg/L. Effects of formaldehyde on TAN measurement by the Nessler method, the salicylate method (using the Hach spectrophotometer), and an ammonia probe (Orion model 95-12) were also tested, by addition of 100 ppm formalin to one of a pair of samples of fish tank water before analysis.

One-h formalin tolerances were tested by exposing the entire system to progressively higher initial formalin levels of 50, 80, 110, 140, and 167 ppm for 1 h and then flushing (exchange rate 440 L/min) for 1 h after each exposure (Table 1). Formalin was first diluted in buckets and then simultaneously added to each fish tank and the sub-floor sump to ensure rapid attainment of a uniform concentration. Dilution by normal water exchange was estimated to have reduced average 1-h concentrations by 4%. Treatments were spaced at least 2 d apart, based on the fact that trout can be treated daily with formalin (Piper et al. 1982) and on the observation that our fish appeared to behave normally the day after a treatment. Water temperature ranged from 13 to 15 C except for the 167-ppm treatment, which was tested at both 15.0 C and 16.5 C. Before each trial, 50 L of biofilter bed material were transferred to an aerated container, to serve as an inoculum for the biofilter in case treatment seriously reduced nitrification.

The entire system was also exposed to indefinite treatments of progressively higher initial formalin levels of 15, 30, 40, 60, 80, 100, 110, and 120 ppm at temperatures of 16–18 C, beginning 11 d after the last 1-h treatment and with at least 3 d between indefinite treatments (Table 1). Biofilter bed material was again set aside each time. An exponential curve was fitted (quasi-Newton method in STATISTICA/W 4.3; StatSoft, Tulsa, Oklahoma) to a plot of formaldehyde concentration in one fish tank over time at 17 ± 1 C for the highest indefinite formalin concentration (120 ppm). The initial point was calculated, not measured, because mixing was incomplete then. The theoretical loss curve expected if formaldehyde were

lost only because of water exchange was also plotted, using the following equation for a mixed-flow reactor (Levenspiel 1989):

$$C_t = C_0 \times e^{-(t/\bar{t})}$$

where: C_t = concentration at any time t ; C_0 = concentration at time 0; and \bar{t} = retention time. In addition, a continuous-flow stirred-tank reactor model (Tchobanoglous and Schroeder 1985) was used to derive a relationship for C_0 , C_t , the first order rate constant (K) for loss of formaldehyde at 17 ± 1 C, and the system's hydraulic exchange rate (Q/V , where Q = exchange rate and V = water volume):

$$C_t = C_0 \times e^{-(Q/V + K)t}$$

The rate constant was then found from the slope, $-(Q/V + K)$, of the regression of $\ln C_t$ versus t . The percentage of formaldehyde loss due simply to water exchange was estimated by dividing the rate of loss due to water exchange, $-(Q/V)$, by the total rate of loss, $-(Q/V + K)$.

Subsequently, the system was exposed to two indefinite 100-ppm treatments and one indefinite 70-ppm treatment at temperatures of 17–18 C, to test for formaldehyde removal by the CO₂ stripper (Table 1). We had been unable to detect differences in formaldehyde concentrations in water entering and leaving the stripper, so the stripper was turned off for 2-h periods to see if this caused changes in loss curves. During the two 100-ppm treatments, the stripper was on during the first 2 h, off for the next 2 h, and on thereafter. During the 70-ppm treatment, the stripper was off for the first 2 h and on after that. Biofilter bed material was not set aside during these last three tests, since the biofilter had already successfully tolerated higher formalin concentrations.

Results

The Nessler method was found to give an intense yellow color and erroneously high ammonia values in the presence of formaldehyde. Measurement of paired water samples with and without addition of 100

TABLE 1. Formalin treatments in a recirculating culture system containing rainbow trout. Treatments were done in the order given.

Initial formalin concentration (ppm) ^a	Temperature (C)	Time since previous treatment (d)	Status after treatment	
			Biofilter	Trout
1-h treatments				
50	14.7	—	ok	ok
80	13.4	2	ok	ok
110	13.4	2	ok	ok
140	14.5	4	ok	ok
167	15.0	2	ok	ok
167	16.5	116	ok	1.9% dead
Indefinite ^b treatments				
15	18.3	11	ok	ok
30	18.3	3	ok	ok
40	18.2	8	ok	ok
60	18.2	7	ok	ok
80	17.3	3	ok	ok
100	16.4	4	ok	ok
110	17.3	3	ok	ok
120	17.3	4	ok	0.4% dead
Indefinite ^b treatments for CO ₂ stripper tests				
100	17.3	14	ok	ok
100	17.8	2	ok	0.2% dead
70	18.3	4	NO ₂ -N up	ok

^a $\mu\text{L/L}$ of 37% formaldehyde.

^b No flushing was done; formaldehyde was removed by degradation and normal water exchange.

ppm formalin yielded 17.6 and 1.14 mg/L TAN, respectively. The salicylate method failed to detect TAN in the presence of formaldehyde, giving readings of 0.0 and 1.0 mg/L TAN for paired samples with and without addition of 100 ppm formalin, respectively. However, ammonia probe measurements were not affected by 100 ppm formalin, indicating that ammonia and formaldehyde did not react under the conditions in the system.

Table 1 lists treatment effects on fish and biofilter. The 1-h 167-ppm treatment at 16.5 C was followed within 24 h by 1.9% fish mortality, although no fish had been killed earlier by the same treatment at 15.0 C or by lower 1-h treatments. The indefinite 120-ppm treatment at 17.3 C was followed by

0.4% fish mortality, but no fish had been killed by the indefinite 110-ppm treatment at 17.3 C or by lower indefinite treatments. The last indefinite 100-ppm treatment, at 17.8 C, was followed by 0.2% fish mortality.

The biofilter did not exhibit obvious damage from 1-h treatments of 50–167 ppm at 13.4–16.5 C; TAN and NO₂-N levels remained within normal ranges. The 1-h 140- and 167-ppm treatments caused large numbers of small (4–5 mm) oligochaetes to wash out from the biofilter; the worms were still active at the 140-ppm concentration but were non-motile and presumed dead at 167 ppm. However, these oligochaetes were once again abundant in the biofilter bed a month later. The worms were identified as *Pristina plumaseta* (Brinkhurst 1986).

The indefinite 15–120 ppm formalin treatments at 16–18 C did not cause obvious damage to the biofilter; TAN and NO₂-N levels again remained within normal ranges. Oligochaetes were washed out from the biofilter in large numbers at treatments ≥ 110 ppm, and small numbers were seen at lower concentrations, but no observations were made on their motility. Formalin at 120 ppm required about 11 h to disappear from the system at 17 ± 1 C, and the rate of loss was greater than if formaldehyde were lost only because of water exchange (Fig. 1).

The first order rate constant for formaldehyde loss at 17 ± 1 C was found from the slope, $-(Q/V + K)$, of the regression of $\ln C_1$ versus t ($n = 22$, adjusted $r^2 = 0.914$):

$$\begin{aligned} -(0.924/\text{h} + K) &= -0.354/\text{h} \\ K &= 0.354/\text{h} - 0.0924/\text{h} = 0.262/\text{h} \end{aligned}$$

These results indicate that 26% ($-0.0924/ -0.354 \times 100$) of the formaldehyde loss was due to water exchange, while, correspondingly, 74% was due to degradation in the system, assuming loss to the atmosphere was negligible.

During the two trials with the CO₂ stripper turned off from 2 until 4 h after treatment began, loss curves appeared to flatten out during the off periods. However, the trial with the stripper turned off until 2 h

after treatment began had a loss curve with a similar shape (Fig. 2). The data therefore provided no evidence that formaldehyde was removed by the CO₂ stripper.

After the last formalin treatment, NO₂-N in biofilter effluent and fish tanks rose to 0.5 mg/L, a stressful level for trout, and remained higher than the normal level of ≤ 0.20 mg/L for 9 d, except when reduced by flushing or cessation of feeding. This rise in NO₂-N was considered evidence of damage to the biofilter. TAN concentrations remained within normal ranges.

Discussion

Burrows and Combs (1968) stated that even low concentrations of formalin had a deleterious effect on nitrifying bacteria in recirculating systems for salmon, but Burrows later realized that formalin interference with measurement of ammonia had led to confusion about this matter (Collins et al. 1975). Our finding that the Nessler method yields false high values in the presence of formaldehyde suggests that this method was responsible for Burrow and Combs (1968) error. Lygren (1993) also reported high ammonia levels associated with formalin treatment in a recirculating system for turbot; but because the Nessler method was used (E. Lygren, personal communication), these high values were presumably erroneous. Collins et al. (1975) found that three indefinite 25-ppm applications of formalin, given on alternate days, had no effect on nitrification in 26-C freshwater recirculating systems containing channel catfish and submerged biofilters of quartz gravel and crushed oyster shell. Levine and Meade (1976) added formalin to 27-C cultures of nitrifying bacteria obtained from a freshwater fish culture system and found 72-h nitrification inhibitions ranging from 0% at 5 ppm to 100% at ≥ 50 ppm. Inhibition at 25 ppm was 31%, and the discrepancy between this and the lack of inhibition at this concentration reported by Collins et al. (1975) was explained by suggesting that in-

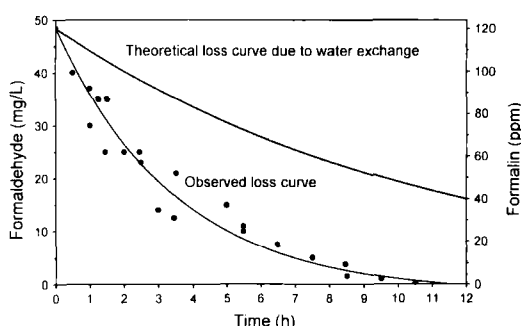


FIGURE 1. Loss of formaldehyde over time in fish tanks in a semi-closed recirculating trout culture system at 17 ± 1 °C. The observed loss curve is contrasted with the theoretical loss curve expected if formaldehyde were lost only because of normal water exchange. The right y axis gives equivalent concentrations of formalin (37% formaldehyde).

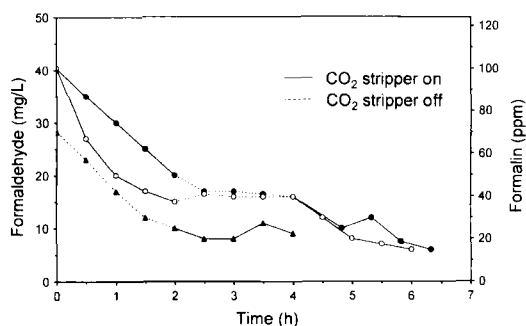


FIGURE 2. Loss of formaldehyde over time during three trials in fish tanks in a semi-closed recirculating trout culture system at 17–18 °C with the system's CO_2 stripper turned off for 2-h intervals. The right y axis gives equivalent concentrations of formalin (37% formaldehyde).

hibition might not have been found if testing had been done in a system also containing heterotrophic bacteria and organic matter. Wienbeck and Koops (1990) reported water quality changes associated with a routine indefinite treatment of about 60 mg/L formaldehyde (149 ppm formalin) in a semi-closed recirculating freshwater system for eels, with a submerged packing-medium biofilter. Biofilter nitrification ability was reduced for about a day after treatment, allowing nitrite concentrations to rise to 2–4 times pretreatment levels. Formaldehyde disappeared from the system in about 23 h at a water exchange rate of 20.5%/d.

Therapeutic concentrations of formalin for fish for 1-h and indefinite treatments have been 125–250 ppm and 15–25 ppm, respectively (Schnick 1973; Piper et al. 1982), except for Wienbeck and Koops's (1990) 149-ppm indefinite treatment. Effectiveness of treatments, as well as toxicity to fish, will vary with the rate of formaldehyde loss, which will vary with system design and management. Toxicity of formalin to fish can also vary with water temperature (greater toxicity at higher temperatures), with dissolved oxygen level (greater toxicity at low oxygen levels), and with fish species, strain, size, and condition (Piper and Smith 1973; Schnick 1973; Piper et al. 1982). Piper et

al. (1982) recommended that treatment levels for salmonids at temperatures above 10 °C not exceed 167 ppm formalin for 1 h; our results indicate that 167 ppm can be too high at 16.5 °C. Lower formalin doses can be used on successive days to avoid fish mortality at higher temperatures (Schnick 1973; Piper et al. 1982). Piper and Smith (1973) indicated that pretreatment fasting of fish reduced mortality. Wienbeck and Koops (1990) did not feed eels on treatment days, a prudent procedure in view of their finding that biofilter nitrification was impaired for about a day. However, we did not interrupt ad libitum demand feeding of our trout, for the sake of operational simplicity, nor did we check for impaired biofilter performance until the day after treatment.

The fact that the last treatment in the test series was followed by abnormally high nitrite levels for 9 d suggests that repeated formalin exposure resulted in enough cumulative mortality of nitrite nitrifying bacteria, *Nitrobacter* spp., to cause the nitrite rise. This possible carryover effect is probably not of practical concern for operating the system because formalin treatments are normally much less frequent. The three cases of fish mortality provided no evidence for a carryover effect of decreased fish tolerance to formalin, because formalin concentration or temperature had increased compared to

earlier trials. Mortality for the 1-h 167-ppm treatment is especially unlikely to have been influenced by previous treatments, because 116 d had passed and most treated fish had been harvested out of the system. Possible carryover effects causing increased tolerance to formalin have been mentioned for rainbow trout (McDaniel 1964, as cited by Piper and Smith 1973) and for biofilters (Wienbeck and Koops 1990), but without supporting data. The possibility of carryover effects was recognized from the beginning for both fish and biofilter, but testing of increasingly higher concentrations was considered the only simple, practical, and safe way to test tolerances in the system. Estimates of upper limits obtained in this manner can serve as guidelines for future treatments. A stepwise approach, but narrower in scope, is prudent even when small-scale tests or the literature have already given an indication of limits, especially for large systems in which overdosing can be disastrous and expensive.

Formaldehyde is readily biodegradable in aqueous solution (Pitter and Chudoba 1990; Krasner et al. 1993). Wienbeck and Koops (1990) conducted aquarium experiments supporting the idea of microbial degradation of formaldehyde: the presence of (microbially rich) activated sludge caused rapid loss of formalin, but water from an activated sludge system or the presence of fish did not. Formaldehyde loss in the commercial eel system they studied was largely attributed to microbial degradation, and the loss was allocated thus: 10% to water exchange, 20% to degradation in fish tanks, and 70% to degradation in the biofilter. Total estimated degradation in their system was 90%; this is greater than the 74% in our system, as would be expected on the basis of our greater water exchange rate.

A possible route of formaldehyde loss in fish culture systems is reaction with ammonia, according to suppositions by Levine and Meade (1976), citing Walker (1964), and by Wienbeck and Koops (1990), citing Hollemann and Richter (1961). However,

our ammonia probe data indicated that ammonia did not react with formaldehyde.

Wienbeck and Koops (1990) found that aeration did not cause formaldehyde loss in aquarium experiments. Similarly, our data show no evidence of removal of formaldehyde from our culture system water by air stripping. These results are in agreement with the low values of Henry's constant reported for formaldehyde in aqueous solution, $1.67\text{--}3.27 \times 10^{-7} \text{ atm}\cdot\text{m}^3/\text{mol}$, four orders of magnitude lower than for CO_2 (Cornwell 1990; Montgomery 1991). The value of Henry's constant is determined to a large extent by the intermolecular forces between solute and solvent. Formaldehyde reacts reversibly with water to form the monohydrate (methylene glycol, $\text{CH}_2(\text{OH})_2$) and a series of polymeric hydrates, although polymers are apparently not present at formaldehyde concentrations $\leq 2\%$ (Walker 1964). The percentage of monomeric unhydrated formaldehyde available for stripping at the temperatures and formalin levels of our study would have been $<0.001\%$ (Walker 1964).

The present study demonstrates biofilter tolerance of both 1-h and indefinite therapeutic levels of formalin. The method of testing progressively higher formalin levels can be applied to other recirculating aquaculture systems to determine tolerances of biofilters and/or fish. Upper permissible formalin concentrations in our system were determined by the sensitivity of the fish rather than the biofilter, until treatments carried out frequently for testing purposes apparently caused serious damage to nitrifying bacteria.

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