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ARTICLE

Low-Dose Hydrogen Peroxide Application in Closed Recirculating Aquaculture Systems

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Abstract

The aim of the present work was to simulate water treatment practices with hydrogen peroxide (HP) in recirculating aquaculture systems (RAS). Six identical 1,700-L pilot-scale RAS were divided into two experimental groups based on daily feed allocation and operated under constant conditions for a period of 3 months. The organic and nitrogenous loadings of the systems differed fourfold between the two groups and were achieved by predefined constant daily feed loads and constant additions of water. The fixed cumulative feed burden was 1.6×10^3 mg feed/L in the low-intensity RAS and 6.3×10^3 mg/L in the high-intensity RAS. The decay of HP in rearing tanks and disconnected biofilter units was investigated by means of HP spiking experiments. The decay in high-intensity RAS rearing units and biofilters was orders of magnitude faster than that in low-intensity units. The application of HP impaired biofilter nitrite oxidation in low-intensity RAS but not in high-intensity RAS. The impact of HP exposure time on biofilter nitrification capacity was then assessed in biofilter bench-scale experiments with nitrite spiking. Exposure time was found to significantly affect nitrite oxidation. Compared with unexposed biofilter elements, nitrite oxidation was reduced more than 90% following 3 h of exposure to 15 mg HP/L, whereas 30 min of exposure had only minor negative effects on nitrite oxidation. The findings of this study demonstrate the potential for developing HP water treatment practices for RAS and contradict prevailing notions that HP cannot be used safely in RAS that employ biofiltration. The development of effective new HP treatment protocols for recirculating aquaculture could reduce the current dependence on formalin to improve water quality and control parasitic loads.

In most aquaculture systems, permanent or periodic water disinfection is employed to improve fish rearing conditions through the control or elimination of pathogenic organisms. Such water hygiene strategies range from the occasional addition of a chemical agent to constant disinfection through ultraviolet (UV) irradiation and ozone generation in advanced recirculating aquaculture systems (RAS).

Numerous partially and fully recirculating aquaculture systems use common technology, such as mechanical and microbiological filtration, without relying on UV or ozone disinfection, as the latter treatments are at times neither logistically nor economically feasible. Such systems, however, can be challenged by pathogens such as *Ichthyophthirius multifiliis* (the parasite that causes white spot disease, or "Ich") and must then rely

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TABLE 1. Controlled conditions for the two treatment groups based on fixed quantities of daily added feed in the respective recirculating aquaculture systems (RAS; $n = 3$ for each treatment group). Abbreviations are as follows: COD = chemical oxygen demand and TSS = total suspended solids.

RAS type	Make-up water (L/d)	Feed quantity (g/d)	Cumulative feed burden (mg/L)	Biomass (kg/m^3)	COD (mg O_2/L)	NO_3-N (mg/L)	TSS (mg/L)	Alkalinity (meq)
Low intensity	80	125	$1.6 \cdot 10^3$	24 ± 1	27 ± 5	54 ± 5	8 ± 3	1.2 ± 0.3
High intensity	80	500	$6.3 \cdot 10^3$	92 ± 1	102 ± 9	196 ± 10	17 ± 4	4.1 ± 0.5

[1]From Colt et al, (2006), corresponding to 640 and 160 L of make-up water per kilogram of feed.

on efficient water treatment strategies to avoid severe losses (Jørgensen et al. 2009). Formalin is an example of a commonly applied therapeutic agent with a relatively high therapeutic index that has been considered safe to use. However, owing to worker safety issues and the chemical's potential for negative environmental effects in systems with low retention times, the use of formalin may be banned or considerably restricted (Pedersen et al. 2010).

Easily degradable oxidative disinfectants such as hydrogen peroxide (HP) and HP-liberating sodium percarbonate (SPC) have documented antimicrobial properties that can be useful for aquaculture purposes (Rach et al. 2000; Buchmann et al. 2003; Schmidt et al. 2006; Heinecke and Buchmann 2009). Heinecke and Buchmann (2009) conducted temperature-related dose–response studies of SPC's effectiveness against *I. multifiliis* theronts in vitro and found that exposure to 32 mg SPC/L (equivalent to 11 mg HP/L) for 1 h was sufficient to destroy the infective parasitic stage. Hydrogen peroxide does not present any food safety issues and degrades quickly into harmless substances. These beneficial attributes can support environmentally sustainable management practices (Boyd et al. 2005; Clay 2008) and are in line with the concept of green chemistry (Anastas and Warner 1998). From a management point of view, however, HP has some drawbacks. For example, its application in RAS entails the risk of impairing biofilter performance significantly (Schwartz et al. 2000). This risk can be minimized by applying lower doses for prolonged periods of time, but this may create the risk of underdosing. The effects of HP and HP-releasing products in biofilters have previously been investigated, and substantial variation between types of systems has been observed (Pedersen et al. 2006; Møller et al. 2010).

The purpose of this study was to investigate the fate and effect of simulated low-dose (10–20-mg/L) HP applications in RAS. The degradation of HP in rearing units and in biofilter compartments was investigated by employing two triplicate groups of closed pilot-scale RAS differing in loading level over a period of 3 months. The effects on biofilter nitrification performance were evaluated in pilot-scale closed RAS and bench-scale experiments in which inoculated biofilter elements from operating systems were transferred to a standardized setup, exposed to a fixed HP concentration for a set period of time, and subsequently spiked with nitrite to assess nitrite oxidation performance.

METHODS

RAS and rearing conditions.—Six identical 1,700-L fully recirculating aquaculture systems, each with a rearing tank connected to an up-flow, submerged, fixed-bed biofilter, trickling filter, and swirl separator (Pedersen et al. 2009), were randomly divided into two experimental groups (Table 1). The feeding load (the overall fixed experimental factor) was held constant for 3 months at daily quantities of either 125 g (low intensity) or 500 g of feed (high intensity) per RAS. Water renewal was fixed at 80 L/d in each RAS, corresponding to cumulative feed burdens of 1.6×10^3 mg/L and 6.3×10^3 mg/L in the low- and high-intensity RAS, respectively (Colt et al. 2006). A quasi-steady state was achieved after a 3-month acclimation period with constant nitrate levels and rainbow trout *Oncorhynchus mykiss* densities kept at approximately 24 and 92 kg/m^3, respectively, by regular biomass removal. Nitrification was stable, with total ammonia nitrogen (TAN) concentrations averaging 0.14 and 0.41 mg/L in the low- and high-intensity systems, respectively, with only minor daily fluctuations. Nitrite levels were also stable at 0.14 mg NO_2^--N/L in the low-intensity RAS and 0.55 mg/L in the high-intensity RAS.

Temperature was maintained at approximately 18.0°C (17.8–18.1°C) by the use of heating elements, and oxygen saturation was maintained above 70% (6.8–8.5 mg O_2/L) by applying pure oxygen and aeration in the respective rearing tanks. Regulation of pH (to keep it in the range 7.0–7.3) was done on a daily basis by the addition of sodium bicarbonate equivalent to 20% of the added feed quantity to compensate for the decrease in alkalinity due to nitrification (Loyless and Malone 1997).

The day following the full-scale HP application experiments, all six RAS were allocated even amounts of fish feed (500 g/tank), corresponding to the high-intensity level. This was maintained over a 2-week period during which the potential impact of HP application on biofilter performance was assessed. The TAN and NO_2^--N concentrations were monitored daily using color test kits (Merck) to evaluate the nitrification process.

Application of hydrogen peroxide.—The removal of HP in the water phase was preliminarily investigated in batch experiments with water samples from both groups of RAS. Hydrogen peroxide was added to 500-mL samples (while being stirred) at a dose equivalent to 20 mg/L and subsampled regularly to determine time-related HP removal at room temperature.

Hydrogen peroxide was added to the experimental RAS in two different ways. Prior to HP application, the inflows to and

outflows from the rearing tank were discontinued, resulting in two separate closed systems: a rearing unit holding the fish and a biofilter unit. Hydrogen peroxide was then added either to the rearing unit (deliberately and transiently without water exchange and with the supply of additional oxygenation) or the bypassed biofilter compartments, as described by Pedersen et al. (2009). In both types of experiments, the time after HP addition was noted when water samples were collected and analyzed for HP concentration over a period of 4 hours.

Bench-scale biofilter performance test.—Biofilter elements from the submerged biofilter (www.expo-net.dk) were removed from a high-intensity RAS and transferred to temperature-adjusted, aerated system water, as described by Møller et al. (2010). Individual Bioblok cylinders were then placed in an experimental setup with an HP sensor and dosage feedback mechanism (ElectroCell; Tarm, Denmark) that allowed automatic regulation of the HP concentration in the water. Biofilter elements were exposed to a fixed concentration of 15 mg HP/L for 30 min, 1 h, or 3 h in triplicate and transferred to separate bench-scale setups, as described in Møller et al. (2010). Concomitantly, sodium nitrite was added to each reactor at a level of approximately 3 mg NO_2^--N/L, and periodic water sampling was performed to assess nitrite oxidation performance. Unexposed biofilter elements underwent the same protocol (transfer and nitrite spiking) and served as controls.

Analysis.—Water samples were fixed with addition of a fourfold strength HP reagents (Tanner and Wong 1998) which stopped further HP decay before analysis. Total ammonia nitrogen and nitrite were analyzed spectrophotometrically as in Suhr and Pedersen (2010). Total suspended solids, chemical oxygen demand (COD), and alkalinity were determined as in Pedersen et al. (2009). The decomposition of HP was calculated either by applying a constant rate of decay (i.e., the rate during the first hour) or by means of the first-order exponential equation $C_t = C_0 e^{-kt}$, where C_t denotes the concentration of HP at time t, C_0 denotes the initial concentration, and k denotes the constant rate of decay in 1/h. The time needed for the concentration to drop by a factor of 2—the half life—was calculated as $t_{1/2} = \log_e(2)/k$.

RESULTS

Water Phase Degradation

An instant, exponential decay of HP was observed in both types of water. Hydrogen peroxide was removed significantly faster from the water in the high-intensity systems ($12.6 \pm 2.0\ mg \cdot L^{-1} \cdot h^{-1}$ [mean ± SD]) than from that in the low-intensity systems ($2.2 \pm 0.5\ mg \cdot L^{-1} \cdot h^{-1}$). The corresponding average half-lives were found to be 36 min and approximately 6 h.

HP Decay in Rearing Tanks

When HP was added to the rearing tanks, significant decay was observed (Figure 1). The exponential decay was significantly faster in the high-intensity tanks, where 95% of the added HP was removed within 1.5 h. In contrast, HP was reduced by only 25% after 1.5 h in the low-intensity RAS, and after 4 h the HP concentration leveled out to approximately 10 mg/L. The

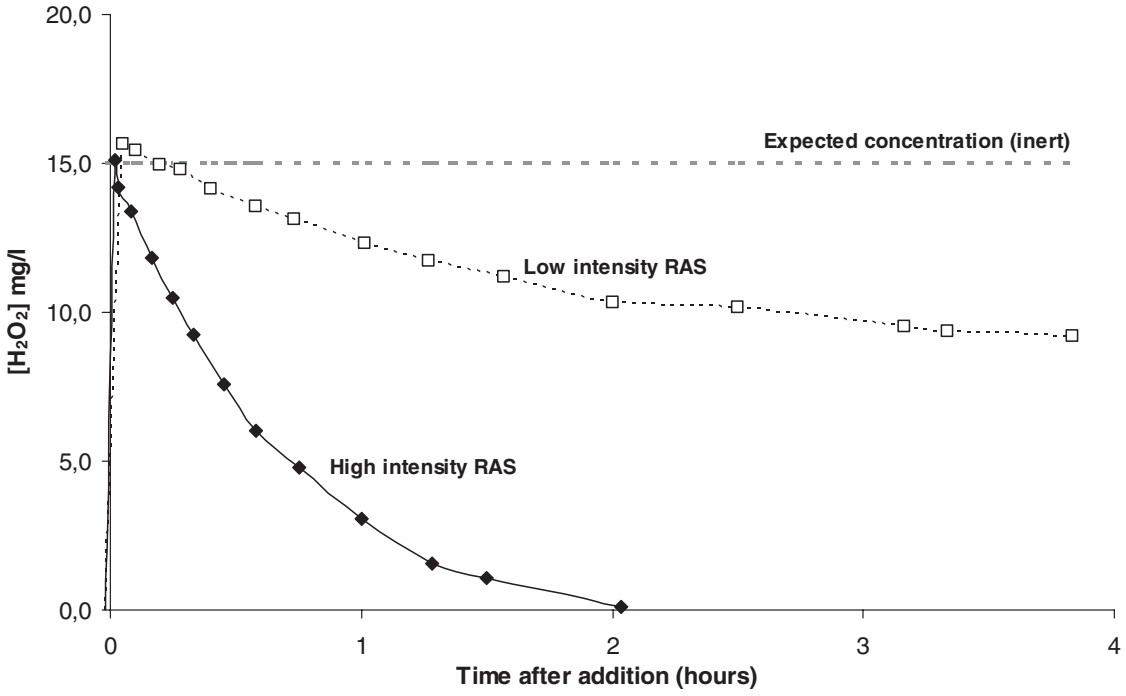

FIGURE 1. Fate of HP added to closed rearing tanks from two groups of RAS. Fish in low-intensity RAS were fed 125 g feed/d, fish in high-intensity RAS 500 g/d. The constant rate of decay (k) in the high-intensity systems was estimated to be 2.04/h, that in the low-intensity systems 0.144/h.

active HP concentrations over a 4-hour treatment period were 25% and 80% in the high- and low-intensity RAS, respectively, compared with a theoretical, nondegradable therapeutic agent. Fish did not appear to change their behavior during treatment, and no mortalities were recorded during the experiment or the subsequent 2 weeks.

Biofilter Removal of HP

Figure 2, which shows the addition of HP to the closed biofilter loop, reveals a delayed mixing (approximately 15 min) and subsequent HP removal. Despite the addition of identical quantities of HP, the intended initial concentration of 15 mg/L was not achieved throughout the loop in the high-intensity systems, as it was in the low-intensity systems. There was low variability within both types of system. The elimination rates (k) differed significantly between the low- and high-intensity groups (0.66 ± 0.11 versus 3.51 ± 0.60) and hence the half-lives did as well (63 ± 8 min versus 13 ± 2.3 min). Liberation of oxygen after the addition of HP was observed to increase the dissolved oxygen concentration in the water by 1.5 mg/L in the low-intensity RAS, compared with 3.5 mg/L in the high-intensity RAS (Figure 3). Simultaneously, a minor increase in pH (<0.3 pH units) was found in both types of system.

Nitrification Performance

Following the addition of HP and the fourfold increase in daily feed quantity, a significant increase in nitrite concentration was observed in all three (formerly) low-intensity RAS. Nitrite levels rose to more than 1 mg NO_2^--N/L after 2 d, and feeding was therefore reduced. After 8–10 d, nitrite levels were again low and stable. In the other three RAS (high intensity), no significant nitrite accumulation was observed. Ammonia levels were not found to be affected in either of the two types of RAS.

Nitrite Oxidation Performance (Bench Scale)

The nitrite spiking experiments with separate, detached biofilter elements showed constant, substrate-independent removal of nitrite at rates depending on the HP exposure time (Figure 4). The unexposed biofilter elements removed nitrite at a rate of 0.69 mg NO_2^--N/L, equivalent to a biofilter surface-specific 0-order rate of 0.19 $g \cdot m^{-2} \cdot d^{-1}$. The nitrite oxidation rate was inversely correlated with the exposure time. The

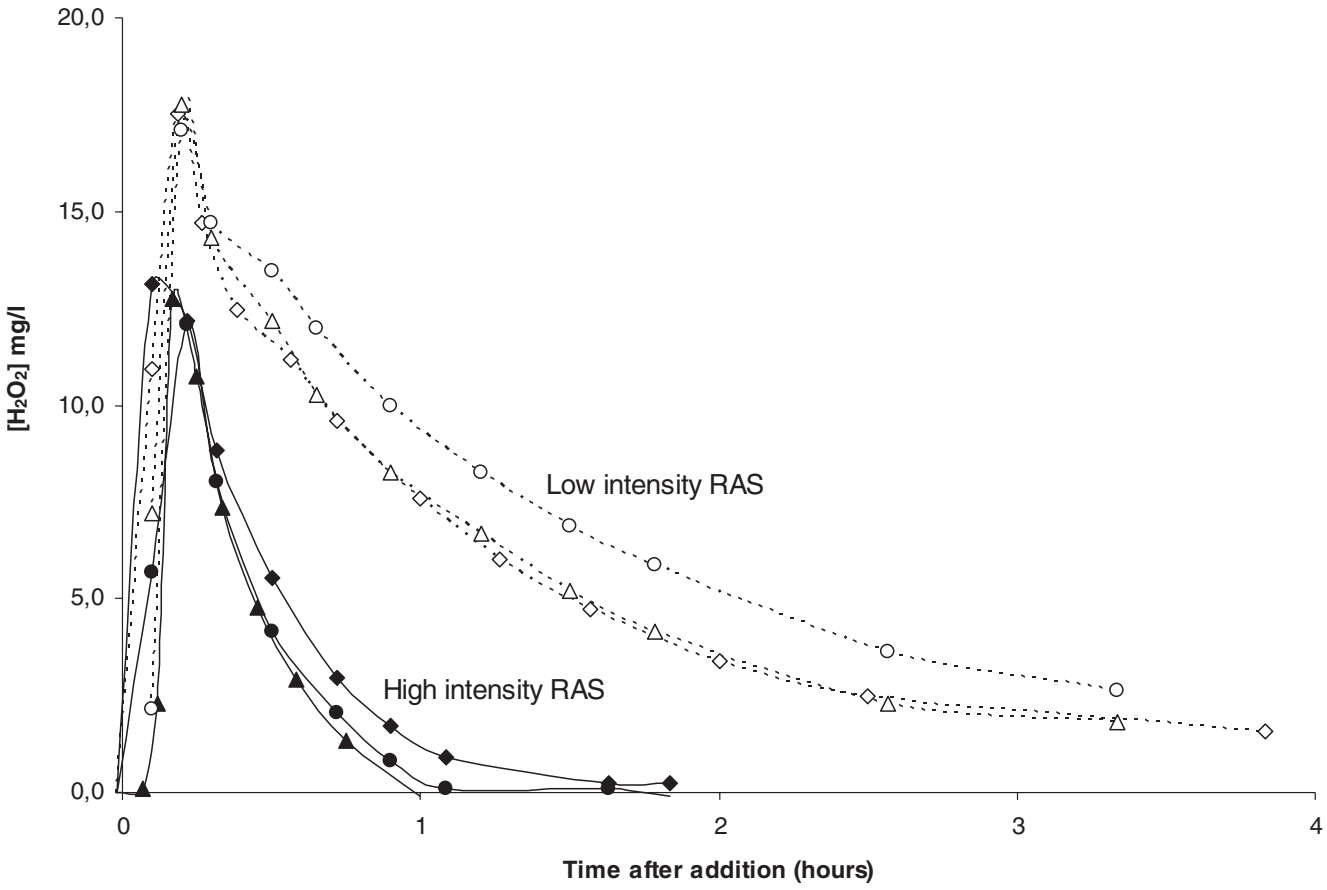

FIGURE 2. Fate of HP added to bypassed biofilter sections in two groups of RAS. The average constant rates of decay (k) were estimated to 3.51/h and 0.66/h based on triplicate experiments in the high- and low-intensity systems, respectively, which are equivalent to half-lives of 12 and 63 min.

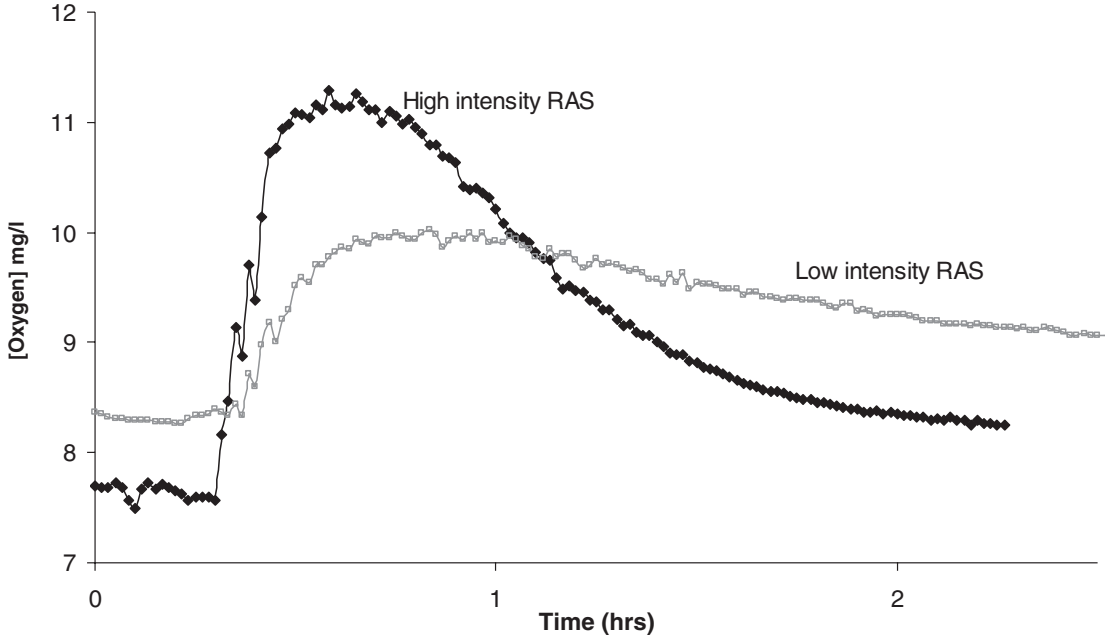

FIGURE 3. Time profile of dissolved oxygen levels from biofilter systems exposed to 15 mg/L H_2O_2.

biofilters with the longest HP exposure time showed the most pronounced inhibition, with their performance being reduced more than 90% compared with control levels. Except for some variability in decay rates within the 0.5-h exposure treatment group, very similar decay patterns were observed among the triplicate groups.

DISCUSSION

The results reveal a significant degradation of HP in both the high- and low-intensity RAS. Adding HP to the high-intensity RAS at the low doses tested led to rapid degradation (half-life, <15 min). This decay pattern is in line with previous findings (Rach and Ramsey 2000; Saez and Bowser 2001), in which the

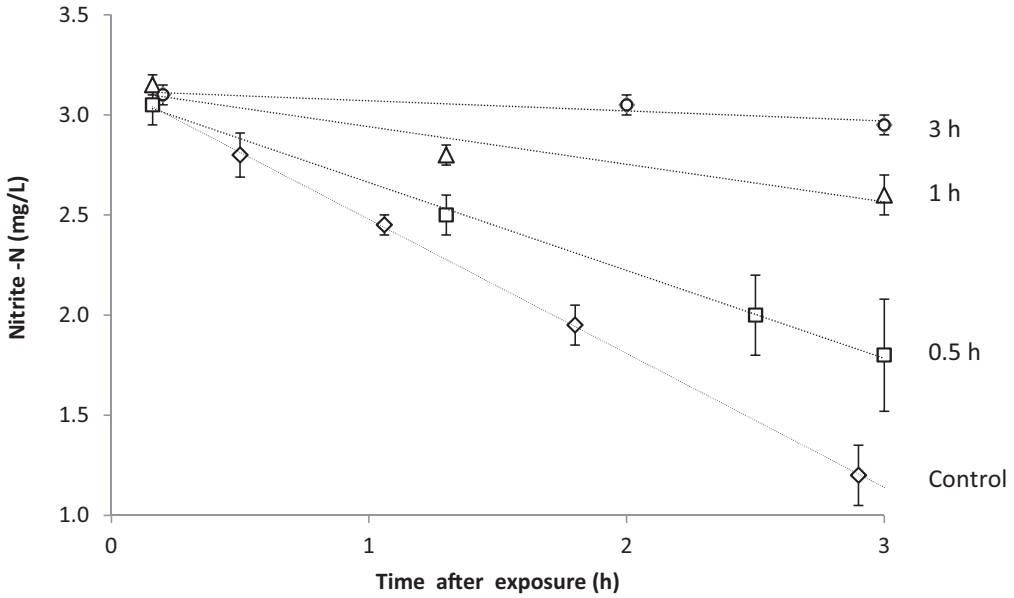

FIGURE 4. Results of nitrite spiking triplicate experiments (mean ± SD) with biofilters exposed to 15 mg H_2O_2/L with different contact times. Control refers to unexposed biofilter elements.

effects of dilution could not be assessed. These decay rates, similar to those obtained by Pedersen et al. (2006) and Møller et al. (2010) are orders of magnitude faster than those reported by Tort et al. (2003). Tort et al. (2003) investigated low-dose HP degradation in a number of water tanks without fish and observed half-lives ranging from 20 h to several days. They found a positive correlation between organic matter content and the decay rate; however, decay rates reflected a modest microbial abundance and activity was artificially low. Hydrogen peroxide is predominately degraded by microbial activity (Liu et al. 1998), as opposed to chemical oxidation. Pedersen (2010) similarly observed that HP decay rates differed significantly between pasteurized and unpasteurized RAS water samples with the same COD. Despite high COD levels in the pasteurized samples, the HP decay rates were not significantly different than those in Milli-Q water (water with no microbial activity), whereas HP decay in the untreated aquaculture water was pronounced (>1 mg $HP{\cdot}L^{-1}{\cdot}h^{-1}$; Pedersen 2010). In aquaculture systems, the microbial abundance is positively correlated with organic matter content and hence attenuated in the presence of biofilms. This is reflected in the present study, in which the prolonged fixed feed intensity caused differences in water quality parameters and conditions for suspended as well as immobilized microorganisms. Water disinfection thus depends on the microbial abundance in the water phase (which, in general, is correlated with COD level) as well as other pools (e.g., active, colonized surfaces) of microorganisms.

The present study also reveals the potential negative side effects of HP application in terms of impaired biofilter function, specifically, with respect to the nitrification process. If biofilter systems have a lower cumulative feed burden (and hence a low hydraulic nitrogenous load [g N feed/m^2 biofilter surface per day]) or biofilters are exposed to elevated HP concentrations for prolonged periods of time, the most sensitive nitrification step—nitrite oxidation—can be transiently inhibited (Hagopian and Riley 1998; Pedersen et al. 2009).

In the full-scale experiments, nitrite levels increased following HP application in all low-intensity RAS. This phenomenon, however, was not observed in the high-intensity RAS. In high-intensity RAS, both nitrite and TAN remained unaffected by HP treatment at the therapeutic concentrations applied. This finding indicates the potential for using low-dose (10–20-mg/L) HP in commercial RAS. Earlier, Schwartz et al. (2000) observed a severe biofilter collapse following application of 100 mg/L to a fluidized sand bed filter. Only limited work and effort has since been published on the potential use of HP in RAS.

With an antimicrobial agent, high therapeutic doses are likely to affect the beneficial nitrifying bacterial communities in the fixed biofilm. To avoid this, water treatment practices in RAS have to be considered as "reverse biofilm control," in the sense that the nitrifying microorganisms have to be protected to some extent. This can be achieved by (1) bypassing the biofilter unit(s) during water treatment (e.g., applying HP to rearing units only and letting the chemical decay therein), (2) bypassing the biofilter section(s) to the degree that they only receive low-residual HP, or (3) deliberately letting only one of many biofilter sections receive the residual HP. Bypassing biofilter units has a potential disadvantage in that it might serve to protect a parasite reservoir and thus render treatments ineffective. In any case, HP decay is likely to be system specific, and the development of new treatment practices using it therefore requires a caution.

The overall rapid decay of HP in RAS calls for methods of monitoring actual HP concentrations and feedback mechanisms that maintain an effective dose. Otherwise, there is a risk of underdosing and hence reduced treatment efficiency, as documented by Heinecke and Buchmann (2009). This can be circumvented by using either HP sticks (Merck 1.1011), monitoring changes in the oxygen concentration as HP degrades, or analytically measuring the HP concentration during treatment to verify the active HP concentration. In case of substantial HP demand or decay, HP needs to be added repeatedly to ensure an effective level (Møller et al. 2010). Alternatively, existing swimming pool technology can be applied to control HP concentrations where HP monitoring via online sensors and continuous dosage through feedback systems are employed.

Our investigations were performed under controlled conditions mimicking commercial conditions; however, the potential differences between pilot-scale and full-scale RAS need to be considered (Colt et al. 2006). Our preliminary studies of HP decay in water and biofilters from commercial, intensive model trout farms in Denmark indicate that HP is degraded even faster in these systems. Systems with a high loading rate typically have thicker biofilms and more robust biofilters. Such commercial systems are expected to cause even higher rates of HP decay. Further studies employing full-scale commercial systems, and the subsequent compilation of practical experiences, are therefore required before treatment practices and safe treatment regimes can be established.

The applied low-dose HP treatment with an exposure time of a few hours is orders of magnitude lower than the safe levels of 70–100 mg H_2O_2/L up to two hours, as reported for salmonids (Rach et al. 1997; Gaikowski et al. 1999) and was not found to negatively affect the gills.

The present study suggests that HP can be applied at low doses in RAS and thus replace formalin as a therapeutic agent. The results also demonstrate the intricacy of HP decomposition and provide an interesting perspective for developing and refining improved water treatment practices.

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