Hydrogen peroxide application to a commercial recirculating aquaculture system

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• Full scale test and application of H₂O₂ on a commercial model trout farm
• Step-by-step approach including characterization of biofilter nitrification capacity before and after H₂O₂ application (analytically verified)
• Beneficial environmental and hygiene aspects of the reported H₂O₂ application
Hydrogen peroxide application to a commercial recirculating aquaculture system

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Running title: “Hydrogen peroxide application to commercial RAS”
Hydrogen peroxide application to a commercial recirculating aquaculture system

Abstract.
An important part of the management of recirculating aquacultural systems is to ensure proper rearing conditions in terms of optimal water quality. Besides biofiltration, current methods include use of use of micro-screens, UV irradiance and use of various chemical therapeutics and water borne disinfectants. Here we present a low dose hydrogen peroxide (H₂O₂) water hygiene practice tested on a commercial Model Trout Farm. The study included application of H₂O₂ in a separate biofilter section and in the raceways with trout. Peroxide addition to the biofilter (C₀=64 mg H₂O₂/L) significantly reduced ammonium removal efficiency (0.13 vs. 0.60 g N·m⁻²·d⁻¹) and nitrification partly recuperated within 7 days. Nitrite removal after H₂O₂ addition was only slightly impaired and no build-up of either ammonia/ammonium or nitrite was observed in the system. Application of H₂O₂ was rapidly degraded and caused substantial release of organic matter from the biofilter and hence increased the water flow and improved the hydraulic distribution through the biofilter. Low concentration H₂O₂ of about 15 mg/L was obtained in the raceways for three hours with temporarily disconnected biofilter sections, until H₂O₂ levels were < 5 mg/L and considered safe to re-introduce to the biofilter sections. H₂O₂ addition in the raceways appeared to improve the water quality and did not affect the fish negatively. The study illustrates the options of using an environmental benign, easily degradable disinfectant and challenge the dogma that hydrogen peroxide is not suitable to recirculating aquaculture systems due to the risk of a biofilter collapse.

Key words: management practice, water quality, hygiene, disinfection, biofilter nitrification, model trout farm, environmental impact
I. INTRODUCTION

In order to achieve proper fish rearing conditions, the occasional use of chemical disinfectants such as formalin, copper sulphate, Chloramine-T, peracetic acid, or hydrogen peroxide are commonly used (Boyd and Massaut, 1999, Rintimäki et al., 2005). The applications range from egg disinfection (Wagner et al., 2008) to system sanitization (Waldrop et al., 2009) and are often used to control fungal and bacterial growth and to suppress parasitic load in systems where preventive biosecurity measures are insufficient (Rach et al., 2000; Schmidt et al., 2006; Kristensen & Buchman 2009).

Numerous considerations must be made when administering disinfection treatments. For example, a high treatment efficacy against the target organisms has to be achieved while fish health, food, worker and environmental safety are not compromised. An additional concern that relates to recirculating aquaculture systems (RAS) is the risk of impairing communities of nitrifying bacteria in the biofilters, potentially causing substantial ammonia and/or nitrite accumulation (Noble and Summerfelt, 1996; Pedersen et al, 2009).

Pressure from external parasites can be controlled, either preventively or curatively, by regular water treatment practices over a prolonged period of time by applying either formalin or sodium chloride or a combination thereof (Mifsud & Rowland, 2008). Both agents can suppress pathogen levels and decrease fish mortality (N.H. Henriksen, Danish Aquaculture Organisation, pers. Comm) but the treatment regimens used have drawbacks, which leaves room for further improvement. Beside a worker safety issue (Lee and Radtke, 1998), formalin in systems with short retention time and without biofilters can potentially result in a concomitant discharge of formaldehyde exceeding the values set by national authorities (The Environmental Protection Agency under Danish Ministry of the Environment (Pedersen et al, 2007). Sodium chloride is typically applied to raise the salinity to 5-15 % which require substantial amounts of salt (5-15 kg per m³), potentially impacting the receiving water body. Non-chemical mechanical control (Shinn et al, 2009) or UV irradiation (Sharrer et al, 2005) are other options that have been documented to control important parasite infections, but these measures are presently not economically feasible to the majority of commercial, outdoor aquaculture operations.

Hydrogen peroxide (H₂O₂) fulfills the requirements as an alternative candidate for aquaculture disinfection (Schmidt et al., 2006), and is an example of an environmentally benign chemical (Block, 2001). Hydrogen peroxide is easily degradable and does not create harmful disinfection by-products and hence, it is not expected to cause environmental concerns. Hydrogen peroxide complies with most principles of green chemistry, defined as “the utilisation of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemical products” (Anastas & Warner, 1998). Nevertheless, formalin is still a preferred chemical, and in order to change common practice, further documentation on the safety and efficacy of H₂O₂ is therefore needed.

Different studies have focused on various aspects of H₂O₂ application in aquaculture (reviewed in Schmidt et al., 2006). Treatment efficacy studies with H₂O₂ have been reported (e.g. Rach et al., 1997; Gaikowski et al., 2000) as well as analytical verification of
H$_2$O$_2$ concentration during treatment (Rach et al., 1997; Rach & Ramsey, 2000, Pedersen et al., 2011) environmental issues (Saez and Bowser, 2001) and studies related to H$_2$O$_2$ application in aquaculture systems with biofilters (Schwartz et al., 2000, Møller et al., 2010, Pedersen et al., 2011).

Heinecke & Buchmann (2009) documented the antiparasitic effects of the H$_2$O$_2$ releasing compound sodium percarbonate against *Ichthyophthirius multifiliis* in a laboratory study. These dose-response correlations allow aquaculturists to adapt their own system-specific water treatment routines. In case of implementing prolonged low dose H$_2$O$_2$ (≤ 15 mg/L H2O2) exposure it has to be considered thought that the laboratory data was obtain under conditions not directly comparable to practical farming operation. To implement this lab-based suggestion, effective on-farm treatment regimens have to be practical and realistic.

Therefore, reliable sets of guidelines tested at real farming conditions are needed to accelerate the generation of a new, alternative water treatment management practice.

The goal of this study was to investigate the potential of H$_2$O$_2$ as a viable water treatment procedure in a commercial, freshwater trout farm. The study mimicked water treatment regimens in full scale, by including analytical verification of H$_2$O$_2$ concentrations and an assessment of the potential impairment of the nitrifying activity in the biofilters. Issues of water treatment management practice, present limitations and future perspectives are presented and discussed.

2. MATERIALS AND METHODS

2.1. Description of aquaculture facility

The experiments were carried out at Tingkærø Fiskerfælles (Randbøldal, Denmark), a commercial freshwater recirculating aquaculture system. The particular aquaculture system (Model Troutfarm concept) consisted of 12 interconnected raceways (each 150 m$^3$), four airlifts, two side blowers, a 70 μm drum filter and a biofilter section consisting of 6 separate biofilters in parallel (Fig. 1; Table 1). Make up water (groundwater) was approximately 20 l/s with an internal flow of 600 l/s (velocity 10 cm/s) circulated by 4 airlifts each connected to a side blower. The farm produced rainbow trout Oncorhynchus *mykiss* (250-400g) and had an approximate standing stock ranging from 30 to 35 metric tonnes during experiments. Fish feed (Biomar, Denmark) equivalent to approximately 1 % body mass/day were administered during the period from 6 a.m. to 6 p.m.

Three separate experiments were sequentially carried out at the trout farm during a summer period: i) High dose single point H$_2$O$_2$ addition to a closed biofilter section, ii) Single point H$_2$O$_2$ addition to the raceways, and iii) Multiple H$_2$O$_2$ addition to the raceways and evaluation of associated biofilter performance.
2.2. Experiment I: High dose single point \( \text{H}_2\text{O}_2 \) addition to a closed biofilter section

Two identical biofilter sections were randomly selected for this experiment. One biofilter section was acutely exposed to \( \text{H}_2\text{O}_2 \). In connection with \( \text{H}_2\text{O}_2 \) application, water inlet to the test biofilter section was shortly sealed off as a common management routine and to avoid any leakage. From this biofilter section duplicate samples of biofilter elements were collected just prior to \( \text{H}_2\text{O}_2 \) exposure and at three other occasions (1 hr., 18 hrs. and 7 days after exposure). A neighbouring biofilter sections served as a control and biofilter elements not exposed to \( \text{H}_2\text{O}_2 \) were samples as control.

The \( \text{H}_2\text{O}_2 \) exposed biofilter section was fitted with Hach Lange online sensors (pH, \text{Oxygen}, and conductivity) connected to HQ40D multimeters® (Hach Lange, Loveland, Co.USA) to monitor potential changes related to \( \text{H}_2\text{O}_2 \) addition and degradation. A total of 10 kg 35 w/w % \( \text{H}_2\text{O}_2 \), equivalent to 3500 g \( \text{H}_2\text{O}_2 \), with a nominal \( \text{H}_2\text{O}_2 \) concentration equivalent to 64 mg/L was added and distributed evenly to the test biofilter section, and water samples were collected and fixed at regular intervals. Biofilter performances were evaluated in terms of standardised ammonia/ammonium and nitrite spiking experiments with representative subsamples of biofilter elements. Biofilter elements of equal volume (0.90 l) were transferred (duplicate subsampling and performance test) to aerated batch reactors and each supplied with 2.3 liter system water (Møller et al., 2010). After 0.5 hours of acclimatization, stock solutions of either NHCl or NaNO were added. Water samples were collected and filtered (0.2 \( \mu \text{m} \) Sartorius®) every 5 minutes until almost complete N-oxidation was achieved.

2.3. Experiment II: Single point \( \text{H}_2\text{O}_2 \) addition to raceways

This experiment was a preliminary test to investigate distribution and hydraulic patterns as well as to determine the magnitude of \( \text{H}_2\text{O}_2 \) degradation rate. A total of 20 L of 35 % \( \text{H}_2\text{O}_2 \) was quickly added to the airlift located at the inlet to rearing section 1 (Fig. 1). Based on predicted mixing and water velocity as well as the fish behaviour in front of the \( \text{H}_2\text{O}_2 \) pulse, different consecutive sampling locations were identified for collecting water samples for the analytical verification of \( \text{H}_2\text{O}_2 \) concentration. Each section was 25 meter long, resulting in a total linear distance of 300 meter from biofilter outlet to inlet. Concurrently, the farm manager used \( \text{H}_2\text{O}_2 \) sticks (Merckoquant® 110011 [range:0-25 mg/L \( \text{H}_2\text{O}_2 \)]) to follow the chemical pulse and to ensure that corresponding actions could be taken in a timely manner, in case \( \text{H}_2\text{O}_2 \) concentration level became critical for the biological filters. As a precautionary action bulkheads were removed between ends of raceways, thereby bypassing the biofilters (Fig.1)

2.4. Experiment III: Multiple and prolonged \( \text{H}_2\text{O}_2 \) addition to the raceways and evaluation of implications on biofilter activity

The purpose of this experiment was to test a \( \text{H}_2\text{O}_2 \) treatment regimen averaging 10 mg \( \text{H}_2\text{O}_2 \) /L for 3 hours, based on Henicke and Buchmann (2009) and recommended by veterinarian (N. H. Henriksen, Danish Aquaculture Association, pers. comm.). Prior to the application, the entire biofilter (all 6 sections) was bypassed by removing wood bulkheads in the
raceway sections and aeration was ceased in the biofilter sections to minimize water flow into the biofilter sections. Doing this, water was redirected from raceway 6 and 12 back to raceway 1 and 7, respectively, creating two closed recirculation loops (as shown in Fig. 1). Representative subsamples of biofilter elements were collected from a biofilter section and served as a control for the baseline nitrification performance.

The total application of H₂O₂ was 80 litre 35% H₂O₂, equivalent to c. 31.6 kg H₂O₂ with a theoretical nominal concentration around 20 mg H₂O₂/L in the rearing units. To ensure ideal mixing and an even distribution of H₂O₂, 20 liter of H₂O₂ were concurrently added into each of the four airlifts. Unlike Experiment 2, H₂O₂ was added over a prolonged period of time of 15 minutes, corresponding to the theoretical retention time in the four rearing units, by use of 25 liter barrels with a 5 mm hole at the bottom. Water samples were collected at the outlet of raceway 6 and 12 during the experiment. Three hours after to experimental commencement, it was decided to reopen the biofilter flow to two of the six biofilter sections, as H₂O₂ concentration was sufficiently low (< 5 mg H₂O₂/L according to sticks). Forty-five minutes later, all biofilters were in normal operation.

Similar to Experiment I, biofilter nitrification performance of unexposed and H₂O₂ exposed biofilter elements were evaluated in bench scale reactors with NH₄Cl spiking. Three samples of biofilter elements were tested: control (prior to H₂O₂ exposure); minimally exposed (three hours after H₂O₂ exposure and by-passed from the raceway); and biofilter elements exposed to residual H₂O₂ (sampled additional 45 minutes after reopening the biofilter, corresponding to 3¾ hours after H₂O₂ exposure in the raceway).

2.5. Analysis

Water samples for total ammonia/ammonium-nitrogen (TAN), nitrite-N and nitrate-N were analysed immediately, or kept refrigerated at 5° C for later analysis. Samples for determination of organic matter content as chemical oxygen demand (COD) were fixed with 2 ml 4 M HCL/L sample and kept frozen for subsequent analysis. Chemical analysis of total ammonia/ammonium-N (TAN), nitrite-N and COD were made as described by Pedersen et al., 2009; H₂O₂ analysis were made according to Tanner and Wong (1998) modified by four-fold stronger fixating reagents, made with 1.2 g NH₄VO₃, 5.2 g dipicolinic acid and 60 ml conc. H₂SO₄.
3. RESULTS

3.1. Single point H$_2$O$_2$ addition to a closed biofilter section

The theoretical initial H$_2$O$_2$ concentration of 64 mg/L was reached shortly after addition, only to exponentially decrease to baseline during the following 30 minutes (Fig. 2). After mixing, H$_2$O$_2$ concentration decayed exponentially according to the equation Ct = C₀e$^{-kt}$, (Ct being the concentration at time=t; C₀ the nominal concentration at time=0 and k the exponential reaction rate) with a half-life of ∼5 minutes. The first three measurement of H$_2$O$_2$ in the biofilter (all above 45 mg/LH$_2$O$_2$ (Fig.2) might be underestimated and connected with a some analytical variation due to the high absorbance in undiluted water samples.

The H$_2$O$_2$ application in the closed biofilter section led to significant fluctuations of oxygen and redox, whereas pH and conductivity did not change (Fig. 3). After H$_2$O$_2$ application, oxygen concentration reached an increased plateau approximately 2.5 mg O$_2$/L higher than prior to H$_2$O$_2$ application, indicating an instant inhibition of heterotrophic bacteria and autotrophic nitrifying bacteria. In association with the H$_2$O$_2$ addition, the biofilter section was vigorously aerated (submerged nozzles) following the common backwash protocol; as a result, excessive amounts of organic matter were shed into the water phase and directed to the sludge compartment.

The H$_2$O$_2$ application significantly inhibited biofilter nitrification in terms of reduced ammonia oxidation rates. Baseline ammonia oxidation rates (0° order) of unexposed biofilter elements were measured to be 0.59 g N/m$^2$/d. Test of H$_2$O$_2$ exposed biofilter elements at three different recovery times revealed significantly reduced ammonia oxidation rates of 0.24 N/m$^2$/d (1 hr), 0.13 N/m$^2$/d (18 hrs.) and 0.31 N/m$^2$/d (7 days) (Fig. 4; Table 2).

Comparative measures of TAN removal in biofilters from a neighbouring biofilter section revealed a rate of 0.61 N/m$^2$/d. Nitrite oxidation performance was evaluated similarly, and was found to be only marginally negatively affected compared to unexposed groups (Fig. 5; Table 2). The H$_2$O$_2$ procedure caused liberation of organic matter from the biofilter elements (COD values in the biofilter section after H$_2$O$_2$ application was measured to approx. 800 mg O$_2$/L, more than a forty-fold increase compared to the raceway water COD) and reduced the hydraulic resistance through the biofilter section.

3.2. Single point H$_2$O$_2$ addition to production unit

The fate of H$_2$O$_2$ throughout the rearing units when added to the airlift system at the inlet is shown in Fig. 6. Sampling at various positions revealed the consequences of dilution and decomposition, in terms of flattened and extended concentration peaks. The results from sampling point 12 showed that a substantial quantity of H$_2$O$_2$ was still present at the rear end of the production unit just prior to the inlet to the biofilter sections. At rearing unit 9, approximately 85 % of the total added H$_2$O$_2$ was measured as a plug flow pulse.
3.3. Multiple H₂O₂ addition in production unit and biofilter evaluation

The precautionary setup that allowed bypassing of the biofilter sections led to two identical loops within the production unit. Figure 7 shows the resulting H₂O₂ concentration in these two loops during a time span of 4 hours. In both loops, the application procedure led to initial fluctuations in H₂O₂ concentration during the first hour after addition, after which a steady decay occurred. Continuous exponentially decomposition of H₂O₂ occurred throughout the monitoring period with an approximate rate constant k of 0.45/h, corresponding to half-lives of 1.5 hours.

Evaluation of ammonia oxidation performance showed that the biofilter elements from the biofilter section (disconnected from the rearing units with H₂O₂ for three hours and then exposed to residual H₂O₂ for 45 minutes) had slightly reduced TAN removal rates of 0.56 gN/m²/d compared to unexposed (control) biofilter elements with TAN removal rates of 0.69 g N/m²/d (Table 2).

3.4. Associated management issues

All three experiments combined normal aquaculture operational practices with new therapeutic measures. Addition of H₂O₂ directly to the biofilter caused considerable liberation of organic matter. This was controlled by enclosing the biofilter section and redirecting the COD-enriched water to the sludge compartment. The applications of H₂O₂ in Experiments II and III were similar to normal practice with formalin using a simple dosage regulation in terms of prolonged application using a barrel/reservoir with a hole. The visual response of the trout to the chemical treatment was an aggregation downstream of the concentration pulse. This reaction was similar to reactions associated with formalin application, but much less pronounced compared to fish reaction when peracetic acid compounds are applied (Jens Grøn, Farm manager; Personal comm.). The safety measures of isolating the production units from the biofilter sections was not common practice but was possible due to the system design and associated with some extra effort (< half an hour). During the experiments, the fish farmer successively used Merckoquant H₂O₂ sticks around the production unit and was able to obtain very reliable readings when compared with values from the chemical analysis. This monitoring allowed the fish farmer to potentially adjust the H₂O₂ concentration and to notice when the H₂O₂ level was sufficiently low (H₂O₂ < 5 mg/L) to let the water pass through the biofilter again.
4. DISCUSSION

This step-by-step test of H$_2$O$_2$ in a commercial operation provides new information to the fish farmer on how to implement a safer and more environmentally friendly water treatment practice. The actions taken were found not to harm the fish, and - though not quantified - the farm manager reported reduced fish mortality and improved water quality afterwards. Additionally, the altered treatment protocol was easily adopted, and the concomitant sanitation of the biofilter section (moderate biofilm control) was found to improve the biofilter hydraulics by removing particulate organic matter and loosen immobilized biofilter elements. The potential effects of impaired nitrification could, in this particular case, be circumvented by an alternating hygiene routine, e.g. sanitizing one of the six biofilter sections every second week.

Despite obvious beneficial attributes of H$_2$O$_2$ and well-known effects in North American hatcheries (Schmidt et al, 2006), H$_2$O$_2$ still remains relatively unproven in outdoor semi-recirculating aquaculture systems. Instead, the use of and experience with formaldehyde exceed by far the use of H$_2$O$_2$. Until recently, there has been little incentive for farmers to replace formaldehyde (Pedersen 2007). Recent Danish certified organic aquaculture requirements obligate farmers seeking this certification to operate their fish farm without using formaldehyde despite its known broad therapeutic range to control most common or important parasites in commercial conditions. Formaldehyde is known to have a broad therapeutic range and a high treatment efficacy against most common/important parasites under commercial conditions, except at low temperature conditions. Hands-on experience of using H$_2$O$_2$ by fish farmers is presently being gained. Recent investigations with application of low dose H$_2$O$_2$ in commercial fish farms have documented the ability of low dose H$_2$O$_2$ in eliminating a number of parasites (Pedersen & Henriksen, 2011). However, low dose H$_2$O$_2$ apparently has a limited effect against gill amoeba and Ichthyobodo necator (Costia) infections. Therefore, more potent treatment regimens are required to replace formaldehyde for these infections.

Increasing the H$_2$O$_2$ dose could potentially have detrimental effects on biofilter performance as observed in the present Experiment I and as reported by Schwartz et al. (2000). The study by Schwartz et al. (2000) was conducted with quantities of H$_2$O$_2$ equivalent to 100 mg H$_2$O$_2$/L and they observed an 80% reduction in ammonium removal in a fluidized sand bed filter. Both nitrification processes can be affected (Hagopian and Riley, 1998), but in the present experiment primarily ammonia oxidation was impaired. The immediate reduction in TAN removal rate was more pronounced than the nitrite oxidation, which is in contrast to other studies (Pedersen et al, 2009). The 3-4 fold decrease in TAN removal rate after one week suggests that the nitrifiers were inhibited and partially able to recover, considering the doubling time of several days (Hagopian and Riley, 1998). The water temperature was approximately 16.5°C at the day of experimentation; at this temperature, a two- to three-fold faster H$_2$O$_2$ decay would be expected compared to situations with water temperature at 6°C due to microbial activity (Unpubl. data). The relative high water temperature (ranging from 16 to 18°C) the following week also affected the recuperation of the nitrifiers, which expectedly would be significantly slower during colder conditions.
Møller et al. (2010) and Pedersen et al. (in press) found that transient low-dose H₂O₂ did not affect the nitrification process substantially, when tested in a pilot scale RAS with low organic and nitrogenous loading and a thin biofilm. Measures could be taken to avoid any biofilter impairment when using H₂O₂. The present results combined with the recommendations provided by Heinecke & Buchmann (2009) opens up for the option of treating water with low concentration of H₂O₂ also in commercial RAS with nitrifying biofilters.

There are certain additional hygiene aspects regarding the use of H₂O₂. Besides antiparasitic abilities (Block, 2001), recent studies have also documented the potential of H₂O₂ in combination with UV to improve water quality and control geosmine and -2-methylisoborneol (Klausen & Grønborg, 2010). Hydrogen peroxide products (high dose technical H₂O₂ or sodium percarbonate) appear to be compatible candidates to hypochlorite (Waldrop et al., 2009), when disinfection practices have to be fully implemented to RAS; this possibility deserves further attention.

In conclusion, the present study challenges the current paradigm of H₂O₂ being incompatible with RAS due to the risk of biofilter collapse. It was possible to maintain and control low dose H₂O₂ concentrations in a large, full scale RAS in commercial operation. Though not quantified, water quality was reported improved following H₂O₂ application and empirical observations indicate that a number of parasites were efficiently eliminated. It still remains untested whether H₂O₂ application in full scale systems can fully replace the use of formaldehyde, as low dose H₂O₂ application presently seems insufficient to fully control gill amoeba and I. necator (Costia) infections.

Acknowledgement
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REFERENCES


Figures (7) Tables (2)

Fig. 1. Schematics of the fish farm, with 6 biofilter section and 12 raceway rearing units (numbered). Long arrows show flow direction under normal operation; dotted lines indicate alternative flow pattern when biofilters are bypassed and the two sets of bulkheads are removed (not to scale).

Fig. 2. Concentration of hydrogen peroxide measured in the water of a 55 m³ biofilter section exposed to 10 kg H₂O₂. Theoretical nominal H₂O₂ concentration was ~64 mg/l.
Fig. 3. Logging data of oxygen, pH and Redox (ORP) from a trial where 10 kg 35% H₂O₂ was applied to a closed, disconnected biofilter section at t=0.

Fig. 4. Removal of ammonia/ammonium (TAN concentration; mean± std. dev) from batch experiments with biofilter elements collected at Tingkæravad Trout farm. Experiments were made in a duplicates based on five sampling occasion: Biofilter elements were collected before H₂O₂ exposure (Unexposed), and again 1 hour, 18 hours and 1 week after H₂O₂ exposure. Biofilter elements from an identical biofilter section not exposed to H₂O₂ were collected at day 7 (Cont.)
Fig. 5. Nitrite-N concentration data (mean ± std. dev.) from batch experiments with biofilter elements. Experiments were made in a duplicate set-up with biofilter elements from two identical biofilter sections. One biofilter section was exposed to H₂O₂ (Test) whereas the other was unexposed (control). Experiments were made on two occasions (Day 1 and day 7 after exposure).

Fig. 6. Concentration of H₂O₂ in the raceways after H₂O₂ addition at the inlet to raceway 1
Fig. 7. Concentration of hydrogen peroxide after addition of 4*20 L 35% H₂O₂ to rearing units at Tingkæravad Trout farm. Loop 1 included raceway 1 to 6; loop 2 included raceway 7 to 12. Water samples were collected at two identical positions at the outlet from the two loops, sterile filtered, quenched and measured with a spectrophotometer. The nominal concentration equals 20 mg H₂O₂/L assuming ideal mixing and no internal degradation.
Table 1: Fish farm data

<table>
<thead>
<tr>
<th>Tingkjærvad Troutfarm</th>
<th>Specifications</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rearing units (total)</td>
<td>1500 m³</td>
<td>12 identical, serial units</td>
</tr>
<tr>
<td>Biofilter (total)</td>
<td>300 m³</td>
<td>6 identical, parallel sections</td>
</tr>
<tr>
<td>Makeup flow (Qₘ)</td>
<td>20 l/s</td>
<td>Ground water</td>
</tr>
<tr>
<td>Internal flow (Qₑₑₑₑₑₑ)</td>
<td>650 l/s</td>
<td>Circulated via airlift systems</td>
</tr>
<tr>
<td>Circulation time</td>
<td>50 min</td>
<td></td>
</tr>
</tbody>
</table>

*Biofilter characteristics*
- Filter volume (without media) V₀: 60 m³ Per biofilter section
- Cross sectional area of filter A_c: 20 m² Per biofilter section
- Filter volume (with media) V_F: 50 m³ Per biofilter section, adjusted for media and void space

*Biofilter media characteristics*
- Submerged upflow, fixed bed (lower layer): 14 m³ BioBlok HD 150 (ExpoNet®); 150 m²/m³
- Moving bed (upper layer): 14 m³ Penta Plast; 800 m²/m³ according to manufacturer
- Total active surface area of media (A_media): 13300 m²

* Data on airlifts; sludge cones, drum filter etc. not included

# Double layer compartment; data on air nozzles and void space below media layers are not provided

Table 2: Evaluation of biofilter performance measured in batch reactors with biofilter elements from Tingkærvad Trout Farm. Removal of total ammonia/ammonium nitrogen (TAN) were assessed in time series and calculated according to biofilter volume and surface/volume specifications. Representative sub-samples of biofilter elements were taken out: before H₂O₂ application; at the end of the treatment period from the bypassed biofilters; and 1 hour after reopening into the biofilter section.

<table>
<thead>
<tr>
<th>Test groups of biofilter elements</th>
<th>Max TAN removal (0⁰) g N/m²/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before H₂O₂ addition</td>
<td>0.69 ± 0.13</td>
</tr>
<tr>
<td>End of treatment and before reopening the biofilter section</td>
<td>0.71 ± 0.05</td>
</tr>
<tr>
<td>One-hour after reopening the biofilter</td>
<td>0.56 ± 0.12</td>
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