Aquacultural Engineering 92 (2021) 102140

Contents lists available at ScienceDirect

Aquacultural Engineering

journal homepage: www.elsevier.com/locate/aque

Hydrogen peroxide oxygenation and disinfection capacity in recirculating aquaculture systems

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A R T I C L E I N F O

Keywords:
Hydrogen peroxide
RAS
Dicentrarchus labrax

A B S T R A C T

Hydrogen peroxide (H_2O_2) treatment is an alternative for disinfection in aquaculture, which may be advantageous as it dissociates and disinfects while increasing water oxygen concentration. Yet, accurate dosing remains undeveloped in Recirculating Aquaculture Systems (RAS). Dosage requirements can depend on organic burden, stocking density, feeding frequency, salinity, temperature and biofilter performance. The present case study investigated the dual effect of H_2O_2 application for oxygen enrichment and disinfection when continuously applied to a RAS rearing European seabass. H_2O_2 addition equivalent to 2.4 and 15.8 H_2O_2 mg L^{-1} were applied for 4 h per day in three 5-days experiments. H_2O_2 was injected at the inlet of protein skimmer and/or the rearing tanks in or without combination with traditional disinfection methods. Water microbial load and oxygen saturation were determined, along with stress markers glucose and cortisol in blood plasma of fish. Doses of 15.8 mg L^{-1} H_2O_2 steadily increased oxygen levels in holding tank water from ~50 % to over 100 % saturation while reducing microbial load (from 604.4 CFU ml^{-1} in the rearing tanks before dosing to 159.8 CFU ml^{-1} after application), achieving suitable conditions for commercial fish densities in RAS. The doses used had negligible impact on biofilter performance and did not affect the fish in terms of stress. Overall results indicate H_2O_2 is effective for disinfection and oxygenation of RAS systems when applied at appropriate dosage and we recommend the protein skimmer as the safest position in order to protect the bacterial community of the biofilters and the reared fish.

1. Introduction

Intensive aquaculture is increasingly dependent on Recirculating Aquaculture Systems (RAS) technology, which is extremely efficient in terms of water use. Massive market investment is expected globally for optimization of RAS, mostly in terms of wastewater treatment (Jostrom et al., 2015). Water quality, achieved by technical water filtration, is key in RAS and improvements in filtration function and efficiency are essential to reduce future costs.

Nitrification and denitrification filters in RAS reduce the levels of potentially toxic nitrogen compounds (Pedersen et al., 2015). Mechanical filtration additionally eliminates solid waste from the feed and excretion of the animals before disinfection, with ozone injected into protein skimmers to eliminate bacteria and diluted protein complexes from the water (Timmons and Ebeling, 2013). Ozone increases production costs and requires control mechanisms to avoid overdosing reaching bacterial communities of the biofilters, the tanks or the farm staff. Moreover, ozonation may lead to the formation of organic and inorganic by-products such as aldehydes, ketones and bromate (Schroeder et al., 2015), some of which may be recalcitrant to subsequent biological filtration (Aljundi, 2011). Continued use of hazardous substances such as ozone and another disinfectants (e.g. iodophors, glutaraldehyde, formalin, sodium hypochlorite and sodium hydroxide) drives the search for new, non-toxic yet effective treatment and disinfection methods (Pedersen and Pedersen, 2012).

A possible alternative disinfectant is hydrogen peroxide (H_2O_2), a strong oxidant considered “green” as it breaks down into nontoxic, environmentally benign by-products (Hâncu et al., 2002; Pedersen and Pedersen, 2012). The dissociation of H_2O_2 in water occurs via dismutation reaction leading to the formation of molecular oxygen, two water molecules and one hydroxyl radical (Pardieck et al., 1992). Accordingly, 0.47 g of molecular oxygen will be formed from 1 g H_2O_2.

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https://doi.org/10.1016/j.aquaeng.2020.102140
Received 15 May 2020; Received in revised form 11 November 2020; Accepted 16 November 2020
Available online 26 November 2020

Pardieck et al. (1992) referred that the transference of four electrons is required for the dissociation which occurs in two- or one- transfer steps with the concomitant transient formation of intermediates including H_2O_2 as well as hydroxyl and superoxide radicals. Radicals give H_2O_2 its disinfection potential (Linley et al., 2012). Hydroxyl radicals are short-lived and extremely strong oxidizing agents leading to biomolecules oxidation via hydrogen abstraction and hydroxyl addition, to further organic and peroxyl radicals formation and finally, to protein peroxidation (Koppenol et al., 2010; Legrini et al., 1993). H_2O_2 has been used as a disinfectant instead of antibiotics, antimicrobials and other biocides, which can lead to resistant bacterial strains (Helgesen et al., 2017; Pedersen et al., 2009). The growth of pathogens in RAS biofilms may be inhibited with the application of the organic peroxide peracetic acid (PAA) (Fredricks, 2015). Additionally, the use of H_2O_2 enriches the system water with oxygen whilst disinfecting, meaning potential savings in term of oxygen expenses in aquaculture farms. The oxygenation capacity has been successfully used to mitigate low dissolved oxygen levels originated in rivers and basins in Brazil due to domestic water discharge and intense rainfall (Dos Santos and Teixeira, 2019). H_2O_2 is also used to eradicate harmful algal blooms from lakes and coastal bays, as well as for in situ bioremediation of contaminated soils and water bodies (Matthijs et al., 2012; Pardieck et al., 1992; Randhawa et al., 2012). Dosage levels are critical when used in systems containing animals. Treatment with high doses of H_2O_2 ranging from 1500−2000 mg L^{-1} is also a common practice against ectoparasites in aquaculture but, if incorrectly applied, causes acute toxicity, stress and detoxification responses in fish (Gaikowski et al., 1999; Vera and Migaud, 2016). Safe levels between 70−100 mg L^{-1} with exposure up to two hours have been reported for salmonids (Pedersen et al., 2012a; Rach et al., 1997), while safe concentrations for shrimps are in the order of 14.3 μL L^{-1} (using 29 μL L^{-1} equivalent to 100 ppm using H_2O_2 29 %) (Furtado et al., 2014). Melanization and erratic swimming behaviour as hallmarks for acute toxicity have been reported for shrimps reared in biofloc systems using rapidly administered H_2O_2 to overcome low oxygen levels due to the absence of aeration (Furtado et al., 2014).

Constraints for the use of H_2O_2 are transportation, storage, handling of concentrated H_2O_2 solutions, which are considered dangerous, and decay after long periods of incorrect storage (e.g. if not shielded from light and transition metals) reducing its quality as disinfectant (Yao et al., 2017). H_2O_2 can also impacts the microbial community of RAS biofilters (Fredricks, 2015; Pedersen et al., 2012a, Pedersen et al., 2009; Pedersen et al., 2012b). Pedersen et al. (2012a) applied low dose H_2O_2 to the rearing tanks of a RAS and found a reduction in nitrite oxidation probably related to elimination of biofilter bacteria. The observed impairments were only in low intensity RAS while high-intensity RAS remained unaffected, assumedly due to the higher organic burden observed in intensive culture systems, which allows faster degradation or decay of radicals before they reach the biofilter. These authors found a 90 % reduction of nitrite oxidation in bench-scale experiments after 3 h exposure to 15 mg L^{-1} and minor negative effects after 30 min exposure.

There is a dearth of data on the application of H_2O_2 for simultaneous disinfection and oxygenation in aquaculture. Such an approach requires balancing oxygen demand (including bacterial activity), permissible H_2O_2 concentrations according to the systems organic burden, bacterial load of specific culture systems and impact on the reared fish. The latter is assessed by measurements of standard (representing energy cost for metabolic maintenance) and active (representing maximum oxygen consumption during high swimming activity) metabolic rates revealing oxygen needs of individual organisms as hallmarks for their performance in terms of energy (Luna-Acosta et al., 2011).

The present study aims to explore oxygenation and disinfection capacity of H_2O_2 in a research system rearing juvenile European seabass *Dicentrarchus labrax* at high commercial stocking densities (21.6 kg m^{-3}) (FAO, 2005-2020). Different application points are compared using very low H_2O_2 concentrations in a RAS. Measurements take into account the possible effects on fish response, biofilter performance and decay period. The performance of the system is measured when two disinfection methods, ozone and H_2O_2, are simultaneously applied and when a higher dose of H_2O_2 is applied as the only source for disinfection and oxygenation.

2. Materials and methods

2.1. Experimental set-up and analysis of water parameters

Experiments were conducted at the Centre for Aquaculture Research in Bremerhaven, Germany. A RAS with a total working volume of 5 m^3 water and flow rate of 7.5 m^3 h^{-1} was used. The system water was spread over three holding tanks (1 m^3 volume containing 21.6 kg fish each), a drum filter, an ozone driven protein skimmer, a degasser tank, a nitrification filter (SUBMERS 850 × 1650 Erwin Sander Moving Bed Biofilm Reactor, 587 L working volume, 14.2 m^3 h^{-1} flow rate, using Kaldnes K3 Filter, hereafter called biofilter (BF)) and a collector tank (sump) connected to the skimmer in by-pass (Fig. 1). Oxygen was delivered to the biofilter by an air-pump connected to the nitrification unit and by injection of technical oxygen to the inlet to the tanks. Approximately 1−1.5 L min^{-1} pure oxygen was supplied. Temperature was maintained with a heat exchanger. Automated control units constantly measured the key water parameters of the system in different positions (at tanks: pH, salinity, temperature, oxygen saturation and redox potential; at protein skimmer: redox potential; at sump: redox potential). European seabass (602.6 ± 139.6 g) were daily fed to a 1.2 % body mass (three times per day) with a commercial feed Supreme 22, 4.5 mm pellets by Coppens International and the water level was adjusted on a daily basis when necessary to account for evaporation. System disinfection was achieved with ozone in the protein skimmer, except during the experimental periods (Erwin Sander Ozonerzeuger Multizon 5 g h^{-1}; 20 g m^{-3} Ozone).

In order to determine H_2O_2 requirements and according to the proposed models of Claireaux and Lagardère (1999), we assumed a standard metabolic rate (SMR) of 75 mg O_2 kg^{-1} h^{-1} as the minimum oxygen demand and an active metabolic rate (AMR) of 250 mg O_2 kg^{-1} h^{-1} as the maximum oxygen demand assuming a rearing temperature of 17−18 °C. Oxygen demand from *D. labrax* SMR and AMR ranges from 40−117 mg O_2 kg^{-1} h^{-1} to 300−408 mg O_2 kg^{-1} h^{-1} depending on temperature and individuals weight and length (Claireaux and Lagardère, 1999; Luna-Acosta et al., 2011). The bacterial oxygen consumption of the filter was incorporated to the oxygen demand of the system based on the authors' own experience, calculating that a small RAS of 5 m^3 water capacity rearing 60 kg fish requires around 330 mg O_2 kg^{-1} fish h^{-1}. These performance data and the dissociation equations of H_2O_2 were used as reference to estimate the stoichiometric amounts of H_2O_2 required to support the production of *D. labrax* in terms of oxygen consumption and disinfection of the system. Values were calculated conservatively based on tolerance thresholds reported in the literature for salmonids and bacteria in the biofilters and the features of the system predicting different accumulation of H_2O_2 in the different compartments (Fredricks, 2015; Gaikowski et al., 1999; Pedersen and Pedersen, 2012; Roque et al., 2010; Vera and Migaud, 2016).

A Smart Digital S-DDA (Grundfos, Germany) H_2O_2 dosage pump with a suction pipe that includes a filling level sensor was adapted to a 5 L 35 % H_2O_2 container (Evonik-dedicated grade) via 4/6 mm PVC pipes. The pump outlet was connected to different points of the system according to the performed experiment (Experiments I-III). The pump had a minimum dosing capacity of 2.5 ml h^{-1} and a maximum of 7.5 L h^{-1} and was adjusted for automatic continuous flow control and auto-degassing. The pump flow rates ranged from 30 ml h^{-1} to achieve low nominal concentrations of 2.4 mg L^{-1} h^{-1} up to a flow rate of 200 ml h^{-1} to test the highest nominal concentration of 15.8 mg L^{-1} h^{-1} on the total system volume. During dosage and after shutdown of the dosage units, H_2O_2 concentration was measured in the different compartments of the

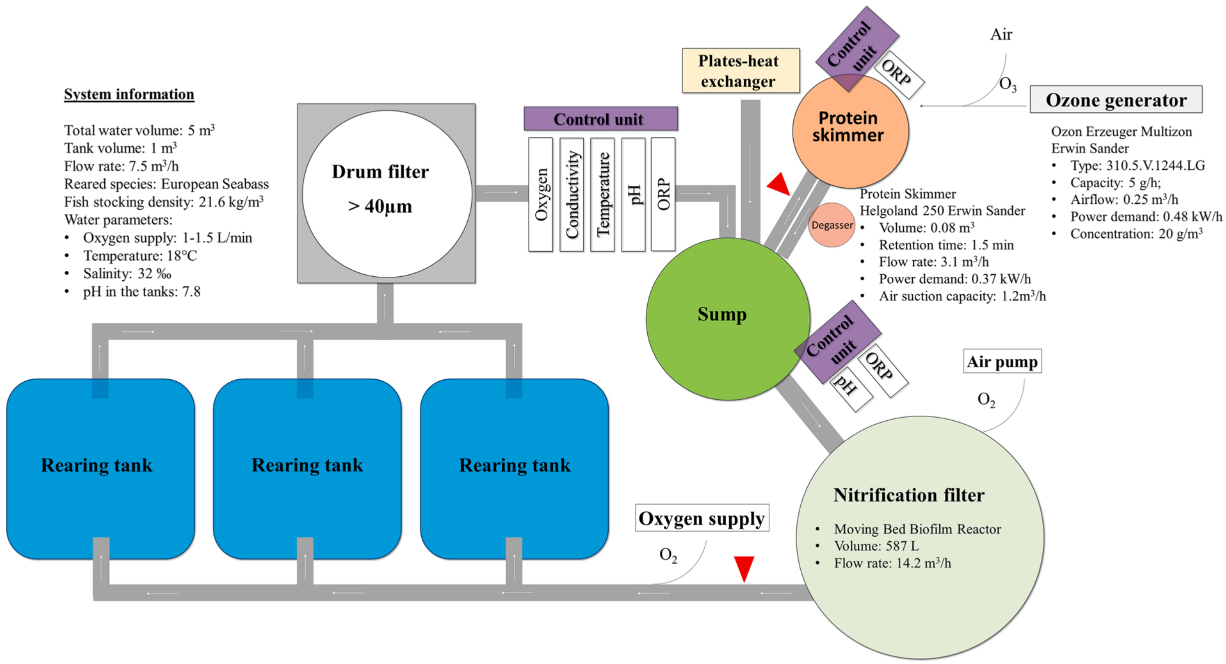

Fig. 1. Schematic representation of the RAS, sampling points (rearing tanks, sump, protein skimmer and nitrification filter) and injection sites (represented with a red arrow heads).

systems with Quantofix Peroxide test sticks (Sigma Aldrich 0−25 mg L^{-1} and 0−100 mg L^{-1}) to assess achievement of steady concentrations and H_2O_2 decay.

Water samples were collected daily at the same time from holding tanks, sump, degasser outlet of the protein skimmer and occasionally, the biofilter, before and after dosage for analysis of the main nutrients present in the system (nitrate, nitrite, ammonium), total dissolved organic carbon (TOC), turbidity and microbial load. Ammonium-N (NH_4-N) and Nitrite-N (NO_2-N) determinations were carried out on subsamples with a DR2800 Spectrophotometer (Hach Lange, Germany) and the Salicylate (for ammonium Method 8155, in the range 0.01−0.5 mg L^{-1}) and Diazotization (for nitrite Method 8507, in the range of 0.002−0.3 mg L^{-1}) methods, respectively. Subsamples were not previously filtered and only when the values exceeded the measurements range of the method, dilutions with Millipore filtered water were performed. Turbidity was also photometrically determined (Method 747 for Turbidity measurements at 860 nm given in FAU, Hach Lange). Nitrate-N (NO_3-N) was measured with a DIQ/S-182 WTW Universal Transmitter with IQ-Sensor Net for Nitrate-N (WTW, Germany). TOC was determined for samples of Experiment II and III with a Shimadzu TOC-VCSH + TNM-1 Analyser equipped with an ASI-V Auto-sampler. Total microbial count (also referred in the manuscript as microbial burden or microbial load) was determined as colony forming units (CFU) per ml sample in triplicates with certified chromogenic Compact Dry TC plates (R-Biopharm AG, Germany) incubated for 48 h by 35 °C in an incubator VWR INCU-Line IL-10 following producer's instructions. These plates (50 mm diameter) contain a detection specific nutrient pad (in this case a standard nutrient medium for the detection of total microbial count-specifically, of aerobic mesophilic counts in form CFUs, with integrated tetrazolium salts as redox indicator die). Once in contact with the sample, the pad is transformed evenly into a gel. According to previous test of these plates in our system the samples were not diluted. Colonies were counted under a binocular microscope and not stained as well as fungi colonies were included in the counts.

2.2. Experiment I: suitability of different application points for very low (2.4 mg L^{-1} h^{-1}) H_2O_2 dosage

H_2O_2 was continuously applied for a period of 4 h at a system nominal concentration of 2.4 mg L^{-1} h^{-1} (pump flow rate of 30 ml h^{-1}) at:

1. the inlet to the rearing tanks,
2. the inlet to the protein skimmer
3. simultaneously at both positions

Each injection position was tested for 5 days during which ozone generators were switched off. As reference, system parameters were measured in the same RAS while under usual operation practices (with continuous ozone disinfection) over a period of 5 days before H_2O_2 dosage.

2.3. Experiment II: hydrogen peroxide application at tank inlet combined with ozone disinfection

In the second experiment, the 4 h H_2O_2 application was performed continuously in the inlet to the tanks as above. Ozone disinfection in the protein skimmer was, however, maintained in combination with H_2O_2 testing. This combination was tested for 5 consecutive days at a system nominal H_2O_2 concentration of 2.4 mg L^{-1} h^{-1}. As the application of H_2O_2 led to a net increase in the oxidation-reduction potential (ORP) of the water of the system, adjustments were necessary in order to allow for correctly controlled ozone dosage into the protein skimmer. The regulation levels of the system controllers based on ORP in the protein skimmer and in the sump were changed as follow: protein skimmer changed from min-max 350−520 mV to 400−550 mV; sump changed from 210−260 mV to 250−300 mV.

2.4. Experiment III: hydrogen peroxide based oxygenation

To test if the oxygen supply of the RAS could be replaced by administering H_2O_2, the oxygen supply bringing oxygen to the tanks was switched off during the daily morning feeding and the system was left to reach minimum levels of oxygen (ca. 50 % DO; mild hypoxia between

40–70 % DO, Makridis et al. (2017)). Feeding fish immediately after cutting the oxygen supply to the tanks rapidly exacerbated oxygen depletion. The critical level recommended for *D. labrax* is above 60 % (Panagiotis et al., 2016). Thereafter H_2O_2 was continuously applied at a pump delivery flow of 200 ml h^{-1} (corresponding to a system nominal H_2O_2 concentration of 15.8 mg L^{-1}) to the inlet of the protein skimmer for 4 h per day during 5 days in which no additional disinfection method was used. This concentration was based on previous H_2O_2 determinations in the biofilter and the tanks taking into account recommended values referred in the literature and recirculation features of the system. The system was continuously controlled for oxygen saturation and well-being of the reared fish until suitable oxygen levels in the tanks were reached (>85 % DO). As the H_2O_2 concentration used could lead to steady concentrations in the nitrification filter close to the 5 mg L^{-1} threshold published by Pedersen and Pedersen (2012) as safe-level for biofilter performance, additional water samples from this compartment were also analysed. Reference system parameters were measured while working under usual operation practices (with continuous ozone disinfection and no H_2O_2 dosage).

2.5. Fish stress/metabolic response

The health status, long-term metabolic response of the seabass and their behavioural responses during dosage were evaluated. Behaviour was observed and blood samples were taken from the caudal vein at the end of each week from five individuals. Blood sampling was performed after the first feeding and during H_2O_2 application. Fish were sedated with MS222, blood samples were taken and centrifuged at 2000 *g* for 15 min at 4 °C to separate blood cells from the plasma. Plasma subsamples (100 µL) were transferred to 1.5 ml Eppendorf safe tubes and stored at −80 °C for further cortisol measurements and the remaining plasma was used for the determination of Glucose content. Measurements of Cortisol (using Cortisol Saliva ELISA tests, RE52611, IBL International; Germany) and Glucose (with a Fuji Dry Chem NX500i blood autoanalyser and Fuji Dri-Chem Slides GLU-PIII, Fujifilm Europe), following previous published methodology (Bögner et al., 2018).

2.6. Statistical analysis

An exploratory data analysis was performed and data analysed for normality and homoscedasticity (Shapiro-Wilk's Test/QQ-plot and Levene's Test/Fligner Killeen Test respectively). As response variables, measured water parameters and physiological and growth parameters of the fish were used: dissolved oxygen (DO in %); total microbial count (TC in CFU); Nitrate (NO_3-N), Nitrite (NO_2-N), Ammonium (NH_4-N), TOC (all in mg L^{-1}); Turbidity (in FAU); Glucose (in mg dl^{-1}); Cortisol (in ng ml^{-1}); Length (in cm); Weight (in g). The covariate was measurement time (with 2–3 possible levels according to the response variable: reference for data collected during usual operation practices; before; and after application with daily variations as replicates). For continuous response variables following a normal distribution and accomplishing the homoscedasticity assumption, the treatment means were compared using repeated measures ANOVA . Non-normal distributed or heteroscedastic data were analysed using a Kruskal Wallis H Test (for more than two groups) and Wilcoxon Test (for two groups). Significant differences between conditions were located using posthoc tests (Tukey's HSD test, Dunnett's C Test/Scheffée Test depending on statistical assumptions). All statistical analyses were performed using R Version 3.5.0 (R_Core_Team, 2018). A confidence interval of 95 % was applied.

3. Results

3.1. Effect of different application points at very low H_2O_2 dosage

The water in the tanks, as main oxygen consumption site in the system showed the lowest oxygen saturation levels compared to the sump and protein skimmer (Table 1) across all applications. H_2O_2 application steadily increased oxygen saturation in all compartments (Fig. 2) independent of the application position.

The highest H_2O_2 concentration measured during application to the inlet of the tanks was 3.5 mg L^{-1} in the water column and 0.8 mg L^{-1} in the biofilter. After one hour of shutting off the dosage units, the levels of H_2O_2 measured in the tanks were between 0.1−1 mg L^{-1}.

H_2O_2 application at the inlet to the protein skimmer had a poorly impact on the microbial community of the tanks while it reduced the microbial burden in the water of the sump and the skimmer. On the contrary, applying H_2O_2 at the inlet to the tanks reduced the microbial load in the water of the rearing tank while the communities present in the skimmer and the sump were not strongly affected (Fig. 3A, B, C). The combined application at both skimmer and tank inlet resulted in lowest counts in the tank water, a high reduction in the sump and similar number of colonies in the skimmer as observed in the reference week, while achieving steady H_2O_2 concentrations in the biofilter ranging between 1−3 mg L^{-1}. Ozone disinfection was not applied during the experimental period resulting in a cumulative increment in the microbial load reflected in high variability in the microbiological analysis, especially compared to the reference period. There were no significant changes in nutrient concentrations, turbidity or TOC due to H_2O_2 application and all compartments of the system were under the 5 mg L^{-1} H_2O_2 limit (Table 2).

3.2. Hydrogen peroxide application in tank inlet combined with ozone disinfection

Combined application of H_2O_2 and ozone led to an increase in mean oxygen concentration in the system from 79 % before application to over 90 % after 4 h application (Table 3).

The microbial load was reduced in the water column of the tanks after application of H_2O_2 (Fig. 4). The number of CFUs assessed from water samples from the biofilter, protein skimmer and sump increased. The type, colour and size of the colonies changed during this experiment suggesting that different microbial groups could be present.

There were no significant changes in dissolved nitrogen compounds, but turbidity was improved and the treatment reduced TOC levels in the water column during the combination of H_2O_2 and ozone (Table 3).

3.3. Hydrogen peroxide-based oxygenation

Under artificially created oxygen depletion conditions, H_2O_2 application led to a consistent and stable increase of the oxygen level of the tanks (Fig. 5). Mean oxygen saturation increased from an artificial minimum of 53.1 ± 10.0 % (n = 5 days) achieved 150 min after switching off the oxygen supply and before dosage to 101.7 ± 5.6 % (n = 5 days) after 4 h H_2O_2 application. After 30 min application, the levels of oxygen of the system were above 60 %, the critical level recommended for *D. labrax* and by 1 h, the levels were near the oxygen saturation before switching off the oxygen supply. Temporal maxima of 3−4 mg L^{-1} H_2O_2 were measured in the biofilter during application and no changes nitrate or ammonia concentration were observed. Nitrite, on the other hand, was significantly reduced ($\chi^2 = 8.08$, df = 1, p = 0.01, n = 5 days). Turbidity was significantly improved during this experiment ($F_{(1,38)} = 8.02$, p = 0.01, n = 5 days) while TOC increased, but not significantly differed between before and after application (Table 4).

The greatest reduction in microbial CFUs was observed at the protein skimmer (Fig. 6C) followed by the rearing tanks (Fig. 6A) and the biofilter (Fig. 6D). Minimal reduction was found in the sump (Fig. 6B). Each compartment reached different equilibration levels of H_2O_2 during the dosage period leading to variation in the achieved oxygen saturation. In general, the H_2O_2 concentration increased within the first 30 min dosage and remained at stable levels within each compartment thereafter until stopping dosage. No H_2O_2 remained in the system 2 h after application

Table 1
Daily dissolved oxygen (%) variation measured in the tanks, the sump and the protein skimmer sections of the system while: i) applying ozone as usual operation practice for disinfection (reference), ii) before H_2O_2 application and iii) after H_2O_2 dosage to a nominal concentration of 2.4 mg L^{-1} at different application points. Values are mean ± SD, n = 5 days.

Dissolved oxygen (%)									
Sampling point→	Tanks			Sump			Protein skimmer		
Application point ↓	reference	before	after	reference	before	after	reference	before	after
Inlet tanks	75.4 ± 3.1	73.6 ± 0.9	79.1 ± 12.8	90.8 ± 6.1	86.7 ± 12.8	90.7 ± 13.8	90.3 ± 7.6	85.8 ± 3.6	94.1 ± 12.1
Inlet protein skimmer	84.9 ± 5.9	82.0 ± 3.6	86.6 ± 6.7	89.7 ± 5.9	89.0 ± 1.9	91.1 ± 4.0	97.0 ± 4.4	93.2 ± 3.3	93.1 ± 1.5
Inlet tanks and protein skimmer	75.5 ± 4.0	77.6 ± 3.4	82.8 ± 13.3	83.3 ± 3.2	82.7 ± 9.0	89.8 ± 8.5	80.8 ± 9.3	88.3 ± 6.2	88.4 ± 9.5

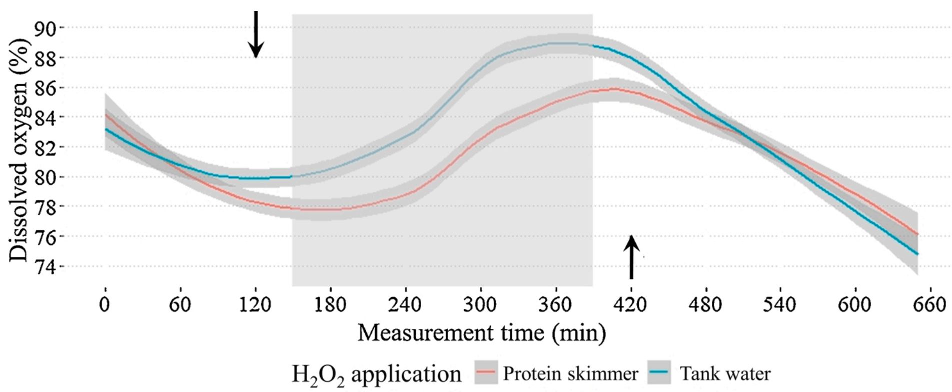

Fig. 2. Dissolved oxygen measured in the tanks before, during and after hydrogen peroxide application to a nominal concentration of 2.4 mg L^{-1} h^{-1} in the inlet to the protein skimmer or to the tanks. Arrows represent feeding events, grey regions around the lines represent the daily variation on measurements performed each 10 min within the tanks and central grey region represents H_2O_2 application period.

was stopped.

The most efficient oxygenation levels were obtained in this experiment. Under this concentration there was a reduction in the number of colonies to levels comparable to those obtained under ozone disinfection in the reference periods. Notably, under normal operational practices with ozone disinfection, most CFU were medium to small in size and varied in colour from brown to red. During H_2O_2 application there was a reduction in size of the colonies and an increase in their number. This trend was positively correlated with increasing H_2O_2 concentrations. Occasionally a reddish shroud grew and fungi appeared in the plates.

3.4. Stress response of the rearing fish

Feeding was not affected during the experimental period and fish gained weight as expected according to the feeding ratio used (Table 5). No mortalities were recorded and no external abnormalities in gills and skin were found. The stress parameters used showed no significant differences with respect to the reference samples taken during common system operation. High variability in main stress response (cortisol) was observed in all experiments with significant differences at the individual level ($\chi^2 = 41.599$, df = 20, p = 0.003) but no effect due to the treatment or the tank. Higher cortisol levels were expected during application periods in comparison to reference weeks. This was the case during experiment I but not during the combination with ozone disinfection. The highest cortisol levels were observed during the application of 15.8 mg L^{-1} h^{-1} (experiment III), but were not significantly different from the cortisol levels measured during the application of the lower doses in the previous experiments.

4. Discussion

Novel, practical and economical methods of disinfection and oxygenation can potentially make large-scale RAS even more economically viable. Closed RAS mainly apply UV and ozone to disinfect water. The application of UV radiation involves requirements such as clear water (low suspended solids in the water column) and high energy costs and it is constraint due to the amount of water that can be treated and the flow rate of the system (Glaze et al., 1987). In the case of ozone, even when currently proved as the most effective solution for RAS, implies risks and a controlled application with technologies, which are not always affordable to all farms. The search for a variety of disinfections methods is therefore still running and the enrichment of the aquaculture disinfection portfolio is not only necessary but also desired. Moreover, the use of a single disinfection method, even when effective, might lead to establishment of bacterial communities, the “winners”, which reduce the variability of a healthy microbiome, necessary for the water cleaning process and as probiotic element required for fish health.

The application of H_2O_2 has been mostly reduced in aquaculture to the use of PAA, which rapidly becomes hydrolysed into H_2O_2 and acetic acid (Liu, 2016). However, PAA and H_2O_2 have different antimicrobial properties with the first being more effective as a bactericidal while the second is stronger as sporicide (Baldry, 1983). In most cases, PAA has been studied in detail and has been therapeutically used in freshwater systems (Pedersen et al., 2013, Pedersen et al., 2009; Russo et al., 2007; Straus et al., 2012). Few references refer the use of H_2O_2 in fresh or salt water for bioremediation (Matthijs et al., 2012; Randhawa et al., 2012), even if naturally produced H_2O_2 is present in many aquatic environments (Rusak et al., 2011).

Direct application of H_2O_2 solutions is limited nowadays. Yet, even reduced concentrations may be useful and effective as disinfectant and its potential for a fast oxygenation may be crucial for systems such as ponds, with localized and sometimes temperature-dependent reductions on dissolved oxygen levels. Therefore, a better understanding of its action mechanisms in different water matrices are worth to be published and pioneers in the field had already shed light on the kinetics of the process in aquaculture systems (Arvin and Pedersen, 2015).

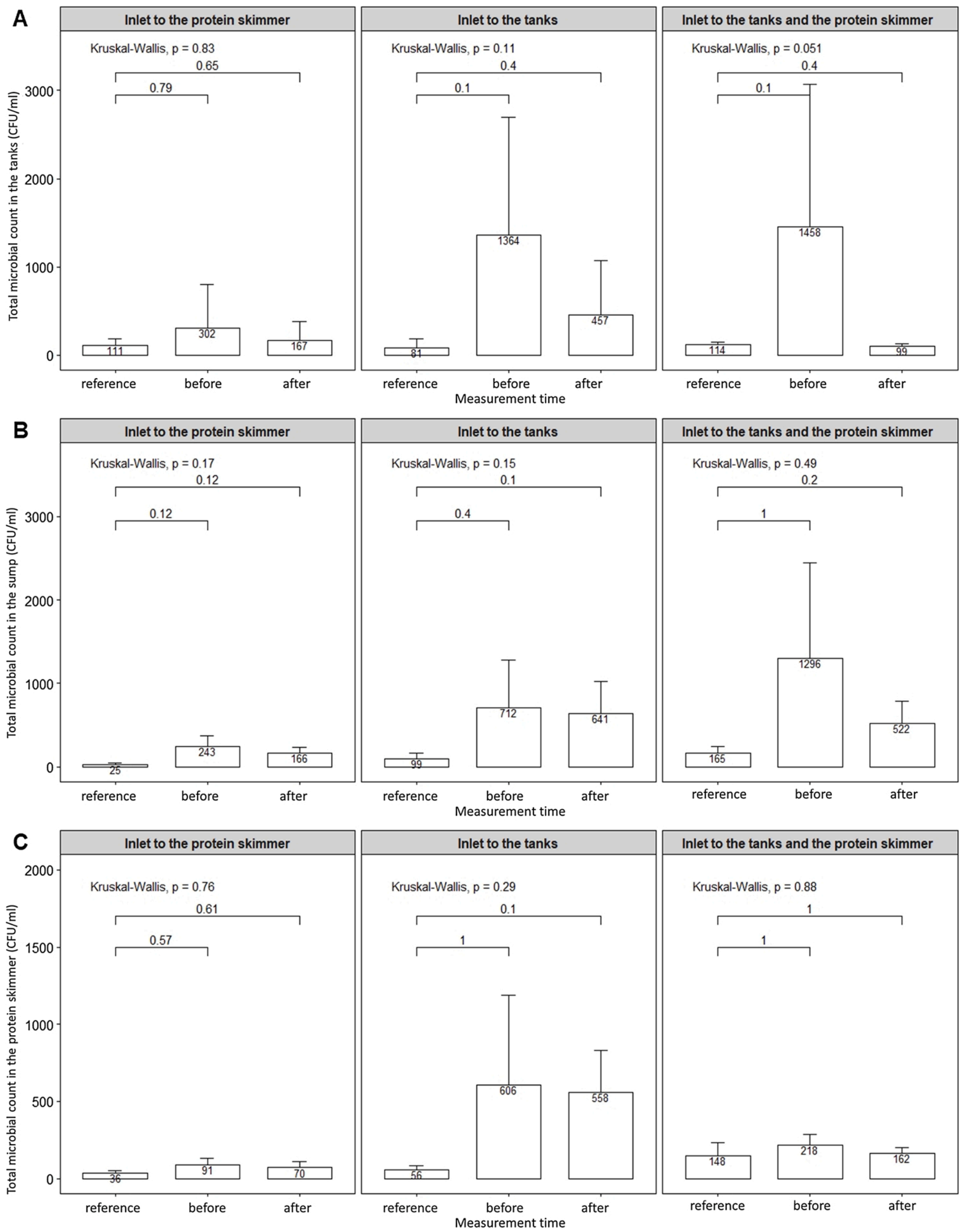

Fig. 3. Total microbial count (CFU ml $^{-1}$) measured in water samples from: A) the tanks, B) the sump, and C) the protein skimmer before and after the application of 2.4 mg L^{-1} h^{-1} hydrogen peroxide for 4 h in different positions in the system. Reference refers to water samples from periods in which the system was using ozone for disinfection. Left panels = application to the inlet of the protein skimmer; central panels = application in the inlet to the tanks; right panels = application on both sites. Bars represent mean values ± SD, n = 5 days, each including technical replicates.

4.1. *Effect of H_2O_2 application on RAS water parameters*

In the current study, the application of H_2O_2 to a RAS system proved effective for oxygenation of the system and reduction of microbial burden. The H_2O_2 concentrations tested herein varied from very low (2.4 mg L^{-1} h^{-1}) to low (15.8 mg L^{-1} h^{-1}) according to previous studies (Pedersen et al., 2012a; Pedersen and Pedersen, 2012; Pedersen et al., 2009). Yet, these concentrations were enough to keep the system at levels, optimal for health status of the fish and performance of the nitrification filter while effectively increasing oxygen concentration. Any shift in disinfection methodology is likely to lead to a mid-term shift in the microbiome of the system until it reaches a new steady state, as observed in this study. However, the effects of such a drift in microbial composition due to H_2O_2 replacement of, for example, ozone, still needs to be assessed. Low concentrations selectively worked for the control of cyanobacterial blooms in a lake using 2.0 mg L^{-1} H_2O_2 homogeneously distributed, without disrupting attendant phytoplankton, zooplankton and macrofauna and the controlling effect remained for 7 weeks (Matthijs et al., 2012).

An important aspect of application of H_2O_2 as oxidant in aquaculture is the position in the system and its combination with other oxidation processes (e.g. ozone). Based on our results, the safest position for application in our system, even when not yielding the highest oxygen saturation, remains the protein skimmer. This application point reduces risks, as it has high organic burden running off from the drum filter and is not close to sensitive compartments such as filters and rearing tanks. Moreover, the combination with ozone improved the turbidity and reduced the levels of TOC in the water as we expected and evidenced the buffering capacity of the different compartments.

Organic matter plays a crucial role in the dissociation of H_2O_2 and PAA. Indeed salinity and temperature enhance PAA and H_2O_2 decay in media not restricted to freshwater, iron and sodium chloride are known H_2O_2 catalysts while phosphate (e.g. sodium phosphate) acts as inhibitor of the H_2O_2 dissociation reaction and, depending on the proportions in which PAA and H_2O_2 are present in the matrix, the formed quantity of brominated or chlorinated by-products relevant for aquaculture systems varies (Bielski and Allen, 1977; Pedersen et al., 2013; Pedersen and Lazado, 2020; Shah et al., 2015). Furthermore, only 3 % of the H_2O_2

Table 2
Daily variation in nitrite, nitrate, ammonia, applied H_2O_2 and turbidity measured in the tanks, the sump and the protein skimmer sections of the system: i) before H_2O_2 application and ii) after H_2O_2 dosage to a nominal concentration of 2.4 mg L^{-1} at different application points. Values are mean ± SD, n = 5 days, each (day includes the values of measured technical replicates).

Experiment I - H_2O_2 application to different points of the system (2.4 mg L^{-1} h^{-1})								
Sampling point →	Tanks		Sump		Protein skimmer		Biofilter	
H_2O_2 Application point ↓	before	after	before	after	before	after	before	after
NO_2-N (mg L^{-1})								
Inlet tanks	0.26 ± 0.10	0.25 ± 0.05	0.26 ± 0.10	0.25 ± 0.04	0.25 ± 0.14	0.25 ± 0.04	¥	¥
Inlet protein skimmer	0.21 ± 0.10	0.20 ± 0.11	0.21 ± 0.11	0.21 ± 0.11	0.21 ± 0.13	0.21 ± 0.11	¥	¥
Inlet tanks and skimmer	0.22 ± 0.08	0.21 ± 0.03	0.22 ± 0.08	0.22 ± 0.03	0.21 ± 0.11	0.22 ± 0.03	¥	¥
NO_3-N (mg L^{-1})								
Inlet tanks	21.5 ± 3.6	21.3 ± 2.9	21.4 ± 3.7	21.4 ± 3.2	21.5 ± 3.7	21.4 ± 3.1	¥	¥
Inlet protein skimmer	45.6 ± 6.0	44.9 ± 5.8	45.6 ± 6.1	44.6 ± 5.4	45.6 ± 6.0	44.6 ± 5.4	¥	¥
Inlet tanks and skimmer	40.5 ± 2.4	40.1 ± 2.7	40.7 ± 2.5	40.1 ± 2.7	40.6 ± 2.5	40.0 ± 2.7	¥	¥
NH_4-N (mg L^{-1})								
Inlet tanks	0.11 ± 0.08	0.06 ± 0.08	0.07 ± 0.07	0.09 ± 0.09	0.02 ± 0.06	0.02 ± 0.02	¥	¥
Inlet protein skimmer	0.28 ± 0.22	0.20 ± 0.18	0.35 ± 0.28	0.24 ± 0.16	0.27 ± 0.13	0.23 ± 0.16	¥	¥
Inlet tanks and skimmer	0.05 ± 0.05	0.04 ± 0.04	0.05 ± 0.02	0.07 ± 0.06	0.02 ± 0.03	0.04 ± 0.03	¥	¥
H_2O_2 (mg L^{-1})								
Inlet tanks	0.00 ± 0.00	2.43 ± 1.28	0.00 ± 0.00	1.01 ± 0.45	0.00 ± 0.00	1.06 ± 0.57	0.00 ± 0.00	0.72 ± 0.97
Inlet protein skimmer	0.00 ± 0.00	0.78 ± 1.22	0.00 ± 0.00	1.28 ± 0.92	0.00 ± 0.00	2.71 ± 1.93	0.00 ± 0.00	0.36 ± 0.40
Inlet tanks and skimmer	0.00 ± 0.00	1.47 ± 0.56	0.00 ± 0.00	0.93 ± 0.44	0.00 ± 0.00	1.51 ± 1.20	0.00 ± 0.00	0.50 ± 0.30
Turbidity (FAU)								
Inlet tanks	−4.57 ± 0.52	−4.87 ± 0.40	¥	¥	¥	¥	¥	¥
Inlet protein skimmer	−4.89 ± 1.04	−4.39 ± 0.74	¥	¥	¥	¥	¥	¥
Inlet tanks and skimmer	−5.20 ± 1.08	−4.81 ± 0.23	¥	¥	¥	¥	¥	¥

Table 3
Daily variation in water parameters measured in the tanks, the sump, the protein skimmer and the biofilter sections of the system i) before H_2O_2 application and ii) after H_2O_2 dosage to a nominal concentration of 2.4 mg L^{-1} applied to the inlet of the tanks with additional ozone application in the protein skimmer. Values are mean ± SD, n = 5 days (except dissolved oxygen, each day includes the values of measured technical replicates).

Experiment II - H_2O_2 application to the tank (2.4 mg L^{-1} h^{-1}) with additional ozone								
Sampling point →	Tanks		Sump		Protein skimmer		Biofilter	
Water parameter ↓	before	after	before	after	before	after	before	after
Dissolved oxygen (%)	79.6 ± 5.7	91.6 ± 8.6	91.4 ± 5.8	97.3 ± 2.9	98.7 ± 5.1	101.2 ± 4.7	94.3 ± 12.8	96.2 ± 7.3
Turbidity (FAU)	−4.67 ± 0.41	−5.03 ± 0.47	¥	¥	¥	¥	¥	¥
H_2O_2 (mg L^{-1})	0.00 ± 0.00	2.14 ± 1.53	0.00 ± 0.00	0.82 ± 0.43	0.00 ± 0.00	1.04 ± 0.30	0.00 ± 0.00	0.74 ± 1.02
TOC (mg L^{-1})	12.51 ± 1.93	10.48 ± 2.96	12.94 ± 2.53	11.87 ± 2.49	12.12 ± 1.97	11.90 ± 1.87	12.07 ± 2.85	10.93 ± 3.21
NO_2-N (mg L^{-1})	0.07 ± 0.01	0.06 ± 0.04	0.05 ± 0.00	0.05 ± 0.05	0.02 ± 0.02	0.04 ± 0.06	¥	¥
NO_3-N (mg L^{-1})	39.9 ± 2.3	39.6 ± 2.0	40.0 ± 2.5	39.8 ± 2.2	39.9 ± 2.7	39.8 ± 2.2	40.0 ± 2.5	39.8 ± 2.3
NH_4-N (mg L^{-1})	0.08 ± 0.05	0.10 ± 0.05	0.02 ± 0.03	0.06 ± 0.03	0.04 ± 0.03	0.04 ± 0.02	¥	¥

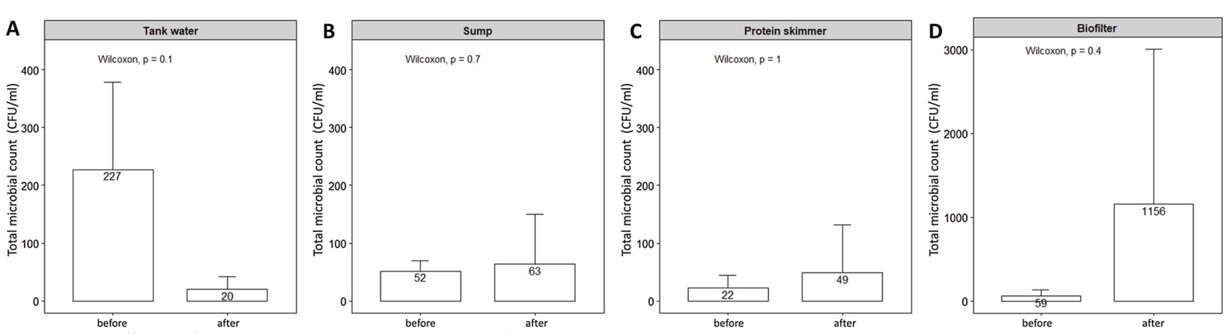

Fig. 4. Total microbial count (CFU ml^{-1}) before and after 4 h application of 2.4 mg L^{-1} h^{-1} hydrogen peroxide at the inlet to the tanks in combination with ozone disinfection. Water samples taken from: A) Tank water; B) Sump; C) Protein skimmer; D) Biofilter. Bars represent mean values ± SD, n = 5 days, each including technical replicates.

dissociation has been related to abiotic process while planktonic bacteria and bacterial aggregates account for 16 % and 81 % of its decomposition (Pedersen et al., 2019). The threshold for H_2O_2 application relies much more on the robustness of the bacterial communities of the filters than in the tolerance levels of the fish. Schwartz et al. (2000) tested the effects of 100 mg L^{-1} H_2O_2 on the performance of Fluidized-Sand Biofilters and found that even when a single static bad treatment by this concentration caused nitrification failure within 24 h of treatment, bacteria could recover very fast and became able to remove 23 % of total ammonia nitrogen within the following 48 h. For these reasons we think that the organic and microbial load of the nitrification filter in our study was high enough to support H_2O_2

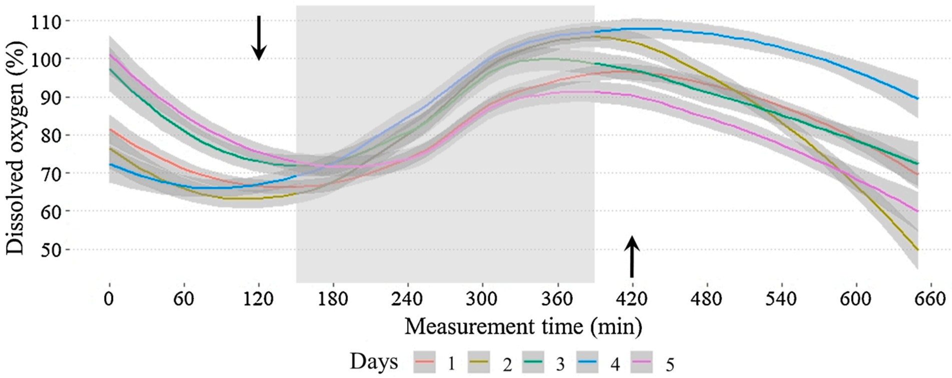

Fig. 5. Dissolved oxygen measured in the tanks before, during and after hydrogen peroxide application to a nominal concentration of 15.8 mg L^{-1} h^{-1} in the inlet to the protein skimmer without further oxygen supply or disinfection in the system. Initial oxygen depletion levels were artificially created by switching off the oxygen supply while feeding. Arrows represent feeding events, grey regions around the lines represent the daily variation on measurements performed each 10 min within the tanks and central grey region represents H_2O_2 application period.

Table 4
Daily variation in water parameters measured in the tanks, the sump, the protein skimmer and the biofilter sections of the system i) before H_2O_2 application and ii) after H_2O_2 dosage to a nominal concentration of 15.8 mg L^{-1} applied in the protein skimmer after artificially depleting oxygen in the system. Values are mean ± SD, n = 5 days (except dissolved oxygen, each day includes the values of measured technical replicates).

Experiment III - H_2O_2 application to the protein skimmer (15.8 mg L^{-1} h^{-1}) without additional oxygenation								
Sampling point →	Tanks		Sump		Protein skimmer		Biofilter	
Water parameter ↓	before	after	before	after	before	after	before	after
Dissolved oxygen (%)	53.1 ± 10.0	101.7 ± 5.6	56.6 ± 16.3	76.4 ± 24.5	56.6 ± 15.8	78.2 ± 27.5	53.5 ± 13.1	78.0 ± 29.7
Turbidity (FAU)	−3.83 ± 0.53	−4.70 ± 0.55	¥	¥	¥	¥	¥	¥
H_2O_2 (mg L^{-1})	0.00 ± 0.00	3.73 ± 1.17	0.00 ± 0.00	4.00 ± 3.03	0.00 ± 0.00	5.54 ± 4.35	0.00 ± 0.00	1.73 ± 1.25
TOC (mg L^{-1})	18.00 ± 6.26	20.50 ± 7.37	20.65 ± 8.14	21.00 ± 8.55	21.01 ± 8.29	21.27 ± 7.33	22.35 ± 7.66	22.89 ± 8.60
NO_2-N (mg L^{-1})	0.19 ± 0.05	0.15 ± 0.03	0.20 ± 0.06	0.15 ± 0.03	0.19 ± 0.09	0.16 ± 0.03	¥	¥
NO_3-N (mg L^{-1})	47.6 ± 2.5	47.2 ± 1.8	47.6 ± 2.5	47.3 ± 1.8	47.7 ± 2.5	47.3 ± 1.8	47.6 ± 2.4	47.3 ± 1.8
NH_4-N (mg L^{-1})	0.11 ± 0.01	0.11 ± 0.06	0.07 ± 0.06	0.06 ± 0.03	0.09 ± 0.09	0.05 ± 0.03	¥	¥

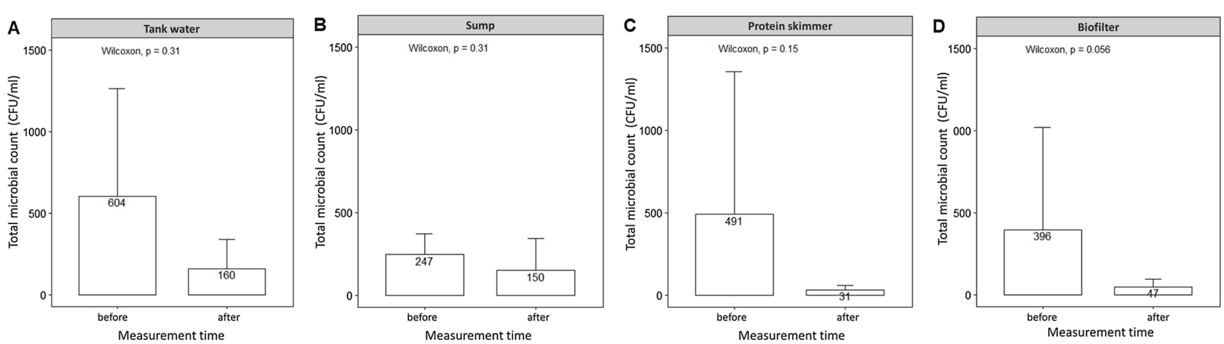

Fig. 6. Total microbial count (CFU ml^{-1}) before and after 4 h application of 15.8 mg L^{-1} h^{-1} hydrogen peroxide at the inlet to the to the protein skimmer without further ozone disinfection or oxygenation. Water samples taken from: A) Tank water; B) Sump; C) Protein skimmer; D) Biofilter. Bars represent mean values ± SD, n = 5 days, each including technical replicates.

dissociation even at the highest concentration tested as the levels measured in the filter were below the 5 mg L^{-1} reported in the literature for inhibition of the nitrification process (Pedersen and Pedersen, 2012). According to the results of Pedersen and Lazado (2020) with a RAS rearing *Salmo salar*, probably higher concentrations might had been used in our system under the stocking density and the temperature at which the RAS was tested. Yet, prolonged exposure might deplete the buffering capacity against oxidative equivalents of the system. We clearly improved the turbidity of the water of the system by 15.8 mg L^{-1}. The increase in TOC might had been the result of the cleaning effect of H_2O_2 on the pipes of the system and could have contributed to the fast decay measured, even taking into account the inaccuracy of H_2O_2 determination via peroxide sticks.

Although H_2O_2 is known to be toxic at cellular concentrations of 0.1 mM (3.4 mg L^{-1}) or lower (Halliwell et al., 2000; Ohguro et al., 1999), some microorganisms may be more susceptible to low (< 3 mM or 10.2 mg L^{-1}) than to intermediate (5–20 mM or 170–680 mg L^{-1}) concentrations (Linley et al., 2012) and many of them have developed strategies to overcome increasing and dangerous environmental concentrations of H_2O_2 using it as a significant part of their metabolism (Bond et al., 2020; Pardieck et al., 1992). These strategies are mostly based on enzymes such as catalase, superoxide dismutase and alkyl hydroperoxidases which are strong scavengers for cellular H_2O_2 residues and are also present in higher organisms allowing them to adapt and resist treatments (Agusti-Ridaura et al., 2020; Chapman, 2003; Pardieck et al., 1992). Moreover, many bacterial groups create extracellular polymeric substances with antioxidant capacity, mainly polysaccharides and proteins, which actively contribute as non-enzymatic quenchers to H_2O_2 decomposition (Gao et al., 2015).

The microbiome of a RAS is diverse and characterization studies are expensive (Schreier et al., 2010). Therefore, it is important to make use of markers, e.g. changes in nitrogen species concentrations in the system, before molecular markers for the expression of protective enzymes are chosen. A net nitrite increase is an indication that bacteria in the

Table 5
Body measurements and stress response of *D. labrax* during hydrogen peroxide exposure to doses between 2.4 - 15.8 mg L^{-1} h^{-1}. Values represent mean ± SD, n = 5 fishes per sampling point.

Treatment ↓	Weight (g)	Length (cm)	Glucose (mg/dl)	Cortisol (ng/mL)	Sampling date
Reference - common operational practice (with ozone disinfection)	505.2 ± 113.9	34.7 ± 2.8	133.8 ± 30.1	38.8 ± 38.7	01.06.2018
Experiment I - H_2O_2 application to the inlet of the tanks and protein skimmer (2.4 mg L^{-1} h^{-1})	530.3 ± 107.7	34.9 ± 2.1	145.6 ± 26.1	48.5 ± 38.3	29.06.2018
Experiment I - H_2O_2 application to the inlet of the tanks (2.4 mg L^{-1} h^{-1})	565.8 ± 151.1	35.5 ± 2.7	111.8 ± 10.3	48.6 ± 35.5	13.07.2018
Experiment I - H_2O_2 application to the inlet of the protein skimmer (2.4 mg L^{-1} h^{-1})	605.4 ± 130.5	36.0 ± 2.4	115.6 ± 31.8	56.7 ± 53.4	25.07.2018
Experiment II - H_2O_2 application to the inlet of tanks (2.4 mg L^{-1} h^{-1}) with additional ozone	615.4 ± 128.3	36.1 ± 2.1	118.2 ± 34.2	34.0 ± 16.2	03.08.2018
Experiment III - H_2O_2 application to the inlet of the protein skimmer (15.8 mg L^{-1} h^{-1}) without additional oxygenation	708.1 ± 159.8	37.8 ± 2.5	169.2 ± 31.5	65.6 ± 64.2	17.08.2018

nitrification filter might be affected due to disruptions to the genre *Nitrobacter* (Pedersen et al., 2012a; Pedersen and Pedersen, 2012). A concomitant increase on ammonium signals a stronger effect, affecting also *Nitrosomonas,* known to be more resilient to environmentally adverse conditions (Timmons and Ebeling, 2013). In the current experiments, nitrifying groups with enzymatic protection mechanisms appear to have been supported, as after a microbial reduction in all compartments, no significant changes in the main nitrogen species were found and the oxidative capacity of H_2O_2 might had even contributed to a reduction of nitrite at the highest concentration tested. Yet, we used a conservative application mode, over a short period, in a simple case study without replication and in which sequential sampling might had an impact on the determined microbial load present in the system even when recovery periods were ensured between experiments and the selected application concentrations were supported by the buffered capacity of the organic and microbial burden of the system. For conclusive results on microbiome shifts, future more detailed, bacterial composition studies under altered sterilisation are necessary in order to assess whether specific groups are favoured by a specific dosage, if these groups influence the functionality of the system, and if variations alter likelihoods of opportunistic growth of pathogenic microorganisms due to the elimination of concurrent interspecific interactions. The development, for example, of preferential groups able to cope with applied H_2O_2 concentrations, but being inefficient nitrifiers, could strongly affect the effectiveness of water treatment and should be included in further studies with long-term analyses of the bacterial communities of the system. In addition, for application in other system designs, the toxicological impact of the planed concentration, the main physico-chemical characteristics of the system and the oxygen demand according to the stocking density and size of the filters needs to be estimated.

Finally, assuming operation budgets for water oxygenation (0.2−0.3 € per day), biofilter oxygenation (0.78 € per day), and ozone disinfection (small ozone generator: 7.67 € per day; large generator: 15.07€ per day) for a standard small-scale RAS as used in the present study, a simple calculation results in oxygenation and disinfection costs ranging between 8.65 € and 16.46 € per day not accounting for power demand, ozone control units and biosecurity related issues. Using H_2O_2 and assuming full replacement of ozone generation results in costs between 1.28 € and 4.14 € per day (Table 6) for the lowest (2.4 mg L^{-1} h^{-1}) and highest (15.8 mg L^{-1} h^{-1}) H_2O_2 concentrations tested in this study (with additional expenses for pump acquisition, power demand and H_2O_2 storage) to achieve similar oxygenation and disinfection standards. These numbers underline the affordability and potential of application of H_2O_2 in future aquaculture.

4.2. Effect of H_2O_2 application on fish stress parameters

In terms of stress, a highly variable glucose and cortisol response appears related to inherent individual variability of fish. High glucose levels is related to stress, especially if heightened cortisol levels in blood are also found but may also be associated to the feeding status of the fish at the moment of sampling. Glucose levels between 100−150 mg dl^{-1} have been reported in the literature for *D. labrax* in a study testing different feedings (Gutierrez et al., 1984). Daily and annual rhythms for these blood parameters have also been identified (Gutiérrez et al., 1987). European seabass is considered a species with high cortisol levels, and within a population, individuals with genetically driven consistent low or high cortisol concentrations can be found (Samaras et al., 2016). These authors stated free and total post-stress plasma cortisol values determined by ELISA Kits far higher than those found in the present study ranging from 129.1 ± 43.9–217.2 ± 58.6 ng ml^{-1} for low cortisol response and 247 ± 85.1–439.2 ± 31.1 ng ml^{-1} for high cortisol response in around 90 g individuals. The levels of cortisol 15 days after exposure to the stress protocol (recovered) in their study ranged from 93.4 ± 63.4 ng ml^{-1} for low-level fish and 203.3 ± 121.1 ng ml^{-1} for high-level fish.

Variations in cortisol responses referred in the literature may arise from different blood sampling methodologies (e.g. sedation with ethylene glycol monophenyl ether vs. clove oil) and individual fish age/size. For instance, ontogenetic profiles of basal cortisol content in four

Table 6
Estimation of the economic improvements implied in the application of H_2O_2 at the minimum and maximum nominal concentrations tested in the present study. Costs per day based on real budgets from the used RAS: i) 1-1.5 L min^{-1} technical oxygen represent 0.2-0.3 € day^{-1}; ii) Air compressor pump for biofilter aeration producing 120 L h^{-1} with a power demand of 130 W h^{-1} represents 0.78 € day^{-1}; iii) Ozone generator to produce 5 g h^{-1} Ozone (required for 1.25-2.5 kg feed day^{-1}) with costs in terms of investment and maintenance of 7.67 € day^{-1}. Production costs assumed to be: 1 L 50 % H_2O_2 = 1€; or 1 L 35 % H_2O_2 = 0.70€ (Source: EVONIK). Power supply cost assumed as 0.25 € kW^{-1}. Budget for power demand of ozone generator or H_2O_2 application pump not included.

Cost item for oxygenation and disinfection	Cost estimation (€)		
	per day	per week	per year
Biofilter oxygenation 130 W h^{-1} 0.25 € kW^{-1}	0.78	5.46	284.70
Water oxygenation 1 L min^{-1}	0.20	1.40	73.00
Ozone disinfection (Investment and maintenance)	7.67	53.69	2799.55
Water oxygenation-disinfection 30 ml h^{-1} 35% H_2O_2	0.50	3.50	182.50
Water oxygenation-disinfection 200 ml h^{-1} 35% H_2O_2	3.36	23.52	1226.40
System under common practice (with oxygen and ozone)	**8.65**	**60.55**	**3157.25**
Oxygenation and disinfection with H_2O_2 with 2.4 mg L^{-1} h^{-1}	**1.28**	**8.96**	**467.20**
Oxygenation and disinfection with H_2O_2 with 15.8 mg L^{-1} h^{-1}	**4.14**	**28.98**	**1511.10**

developmental larval and postlarval stages showed variations ranging from 0.12–47.91 ng g^{-1} between hatching and flexion stages (Pavlidis et al., 2011). High variability is therefore also expected to occur within juveniles and adult fish cohorts. Other disparities may arise from the method used to determine the cortisol level in plasma or as "body content". Planas et al. (1990) studied the stress response and showed well-defined daily and annual variations. For fish in the size of the individuals used in the present study a range in plasma cortisol response between 10–60 μg dl^{-1} has been reported using a direct RIA method for determination (Planas et al., 1990). Except for the great variability observed in both markers in the present study, these did not correlate with each other or with the treatment during the whole experimental period and no inferences of negative impact associated to the application of H_2O_2 can be elucidated. Another aspect to take into account is the fish adaption capacity to oxidants, which led to less cortisol release by Rainbow trout challenged with 1 mg L^{-1} PAA (Liu, 2016). For further studies, other less variable stress/health markers should be included, especially if long-term experiments are planned. These may include histological analysis of skin epithelium and gills and markers for ionic balance and oxidative stress (Reiser et al., 2010; Straus et al., 2012; Vera and Migaud, 2016). Moreover, the application strategy needs to be revised, as short term high doses (pulse application) or continuous low doses imply completely different scenarios for the development of putative pathogen resistance in the system and for adaption mechanisms of the fish against oxidative stress to be up-regulated (Liu, 2016).

5. Conclusion

Very low concentrations of H_2O_2 can be used in RAS as a promising tool in terms of oxygenation and disinfection. For its application, basic knowledge on system design, flow/accumulation of organic material, physiochemical parameters and stocking density may be used for the determination of system specific required concentrations and easy-to-record parameters may serve as marker for system performance during application. The present results obtained under culture conditions similar to common aquaculture commercial production systems offer realistic insights for adaption of H_2O_2 dosage routines to commercial RAS and could help making disinfection practices more environmentally friendly and sustainable by offering further knowledge on safe application, efficacy and potential alternative benefits of H_2O_2.

Funding

This study was financed and technically supported by EVONIK as part of the industry project named "RAS H_2O_2 Trail". EVONIK had no involvement in study design, collection, analysis and interpretation of data or in the writing process.

CRediT authorship contribution statement

Desislava Bögner: Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization, Writing - review & editing. **Mirko Bögner:** Conceptualization, Writing - review & editing, Project administration, Funding acquisition. **Frederike Schmachtl:** Investigation, Writing - review & editing. **Nicolas Bill:** Investigation, Writing - review & editing. **Jörn Halfer:** Investigation, Writing - review & editing. **Matthew J. Slater:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

None.

Acknowledgments

There is no conflict of interest related to this study. EVONIK financed the study but did not influence the performance of the experiments or the results. The authors wish to thank Prof Boris Koch for access to laboratory facilities, to Jana Geuer, Claudia Burau und Dieter Jansen, for their invaluable support in DOC measurements and their expert technical assistance.

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