UP-SCALLING THE APPLICATION OF HYDROGEN PEROXIDE AS DESINFECTION METHOD IN A COMMERCIAL RAS REARING ATLANTIC SALMON *SALMO SALAR*: A CASE STUDY

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Introduction

Disinfection is a very important part of recirculation aquaculture systems (RAS). Common disinfection methods include chemical disinfectants, antibiotics, biocides, UV radiation and ozone.

Hydrogen peroxide (H_2O_2) has been on focus as a "green" alternative: High doses are associated with acute toxicity symptoms while low doses are harmless, offer additional system oxygenation and contribute to water quality improvement. The present study aims to describe the first case study up-scaling a continuous H_2O_2 application to commercial fish production in RAS.

Materials and Methods

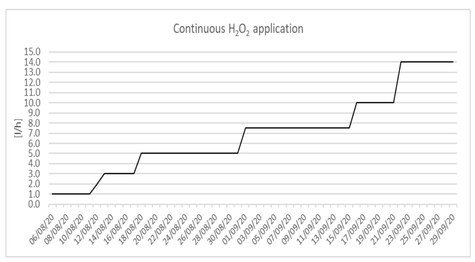
The present study was performed at RAS facilities of Danish Salmon A/S in Hirtshals, Denmark

Two identical Grow-out RAS with own water treatment elements and eight pre-grow tanks were used as treatment and control systems. Defined H_2O_2 quantities were applied with a dosing lance connected to a peristaltic pump and an International Bulk Container with 50% high purity hydrogen peroxide, (EVONIK Industries) to the system.

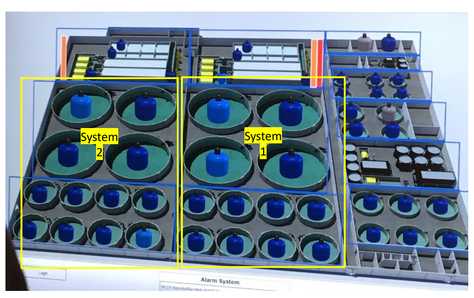
Determinations: Total microbial count, FISH, BacLight Viability, Water and production parameters

Results and Discussion

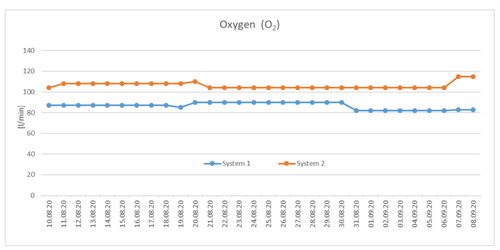
Application



Hydrogen peroxide application in the initial phase of the study.



Production facility of Danish Salmon. System1 (treatment) and System 2 (control). Application on the eight small pre-grow tanks of System 1



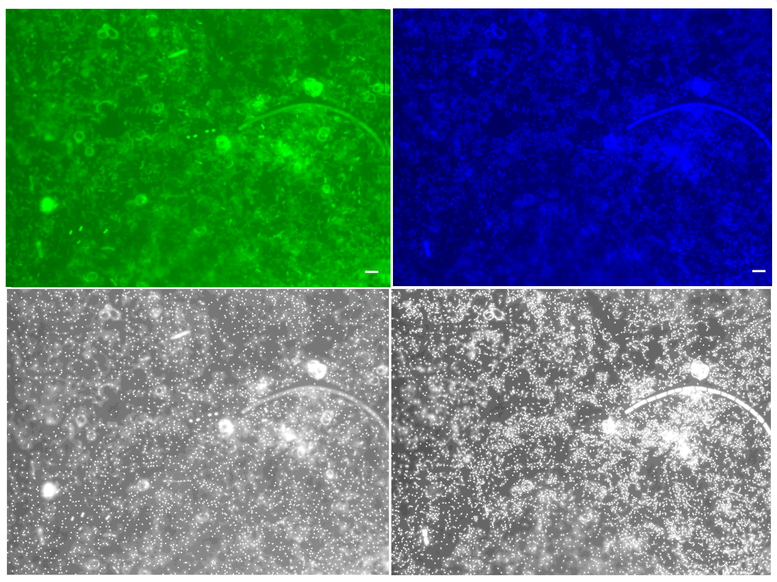
Oxygen demand System1 (treatment) and System 2 (control).

Application
- 4days-1L/h
- 2 days-increase to 3L/h
- 4days-3L/h
- 2 days-increase to 5L/h
- 10 days-5L/h
- 2 days-increase to 7.5L/h
- 15 days-7.5L/h
- 2 days-increase to 10L/h
- 5 days-10L/h
- 1 days-increase to 14L/h
- 6 days-14L/h

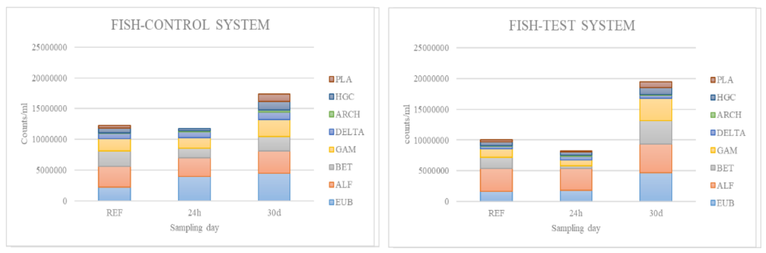
Sampling
- Reference sample from both system before starting the assays
- After 24h aaplication of 1L/h
- After 30 days application

Response of treated system:
- Initial feeding refusal, probably related to palatability of the feeds in an oxidative medium.
- No observed mortality or behavioural changes
- Higher Ozone demand until the 5L/h H_2O_2 was achieved. Thereafter this demand was lower than the required for control system
- Oxygen demand was lower than control
- After 14L/h inusual feeding behavour and incorrect measurements on redox probes detected.
- For application without ozone, the projected concentration would have been 20L/h, not achieved.
- Further experiments without combination with ozone are highle recommended
- Alternating the use of different disinfection methods might avoid accomodation of favorised microbial groups

FISH analysis



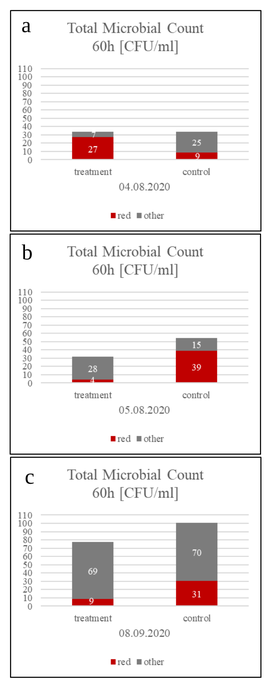
FISH assessment: Samples hybridized with DNA probes (in the picture-EUB) labelled with FAM fluorophore (in green) and counterstained with DAPI (in blue). In grey the corresponding counting analysis using ImageJ maxima function. Scale bar = 5µm.



FISH analysis of water samples from control and test system before starting application (REF), and after 24 h application by 1l/h and 30d application by 7.5 l/h combined with ozone

- The most represented bacterial group in the samples belonged to α-Proteobacteria (fam. Rhodobacteraceae) followed by β- and γ-Proteobacteria
- Already after 24h application some changes in bacterial community composition could be observed
- Changes in the abundance of the different bacterial groups analyzed could be attributed to variable vulnerability of members of these groups to the disinfection potential of H_2O_2 or its combination with ozone
- After 24 h application: Micobacterium (Actinobacteria-HGC) and α-Proteobacteria (most represented by Rhodobacteraceae -ALF), increased in the treated system
- The phylum Bacteroidota was highly represented by the fam. Flavobacteriaceae (data not shown)
- Among the members of the γ-Proteobacteria there was a reduction in members of the family Pseudoalteromonadaceae, Colwelliaceae, Nitrincolaceae while members of the families present at very low concentrations (Thiotrichaceae , Spongiibacteraceae, Legionellaceae) slightly increased. This selective impact need to be analysed in detail to determine potential outgrowing of opportunistic pathogens

BacLight and Total microbial counts analyses



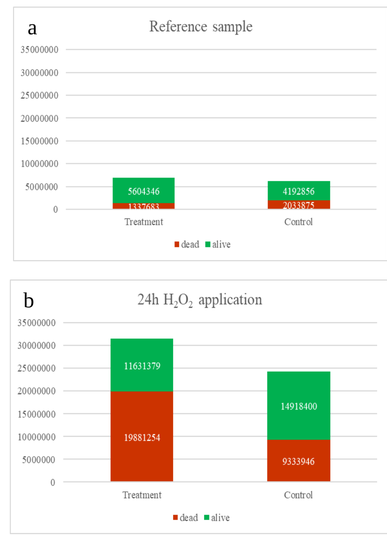
Total microbial counts (CFU/ml).

a) reference samples before starting;

b) sampling after 24 h by 1l/h application;

c) sampling after 30 days application by 7.5l/h

- Total microbial counts reflected a steady increase in the number of CFU/ml in both systems and evidenced microbial accommodation.
- Change over time on the kind and number of CFU/ml sample were used as initial marker for the determination of effects of the disinfection process on the microbial community of both systems
- The number of microbial colonies able to be detected via the chromogenic marker present in the Compact Dry TC plates was significantly reduced in the treated system



BacLight Viability results. a) Reference samples obtained before starting application from both systems while operating with ozone. b) Sampling after 24 h application by1l/h together with ozone disinfection in the test system.

- There was an increased rate of microbial mortality according to the viability results with higher values in the treated system reflecting the longer exposure of this system to oxidative stress

Acknowledgements

The authors would like to thank the support of the staff from Danish Salmon and EVONIK during the installations and sampling performed in this project. This study was part of the industrial project "Follow up test of hydrogen peroxide as disinfection method in aquaculture facilities rearing Atlantic Salmon" in cooperation with Danish Salmon and EVONIK

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ABSTRACTS

UP-SCALLING THE APPLICATION OF HYDROGEN PEROXIDE AS DESINFECTION METHOD IN A COMMERCIAL RAS REARING ATLANTIC SALMON (*Salmo salar*): A CASE STUDY

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Introduction

Disinfection is a very important part of recirculation aquaculture systems (RAS). RAS allows for controllable environments in which main variables relevant to animal welfare and a successful production can be manipulated as required to improve efficiency and profitability. Common disinfection methods include chemical disinfectants, antibiotics, biocides, UV radiation and ozone. They can be used to treat disease outbreaks, or to reduce the bacterial load of the system which otherwise could lead to the overgrowth of potential pathogens or opportunistic bacterial groups competing with biofilter bacteria for space and resources. Ozone is the most used disinfection method requiring expensive technology and trained stuff. Hydrogen peroxide (H_2O_2) has been on focus as a "green" alternative. High H_2O_2 doses are associated with acute toxicity symptoms for some species. Low doses are harmless, offer additional system oxygenation and contribute to water quality improvement. After testing the use of low doses in a small research RAS, the present study aims to describe the first case study up-scaling a continuous hydrogen peroxide application to commercial fish production in RAS with focus on the determination of required concentrations, application monitoring and variations on microbiome composition.

Material and Methods

The present study was performed at RAS facilities of Danish Salmon A/S in Hirtshals, Denmark, one of the European pioneers in rearing salmon in land based aquaculture facilities and producing about 1.200 metric tons/year of Atlantic salmon (*Salmo salar*). Two identical Grow-out RAS with own water treatment elements and eight pre-grow tanks were used as treatment and control systems to compare the effects of continuous H_2O_2 application in combination with ozone to common operational practice. Oxygen Cones and additional aeration stones ensured the basic Oxygen supply in the tanks. Defined H_2O_2 quantities were applied with a dosing lance connected to a peristaltic pump and an International Bulk Container with PERSYNT® 50, (EVONIK Industries) into the distribution pipe feeding a collection tank from where the water was evenly distributed to all tanks. Based on previous experiments, a final dosing of about 20 l/h was projected. Water samples were collected at the start (REF) and after slowly enhancing the dosage over time (24h and 30d), for the determination of the total microbial count (certified chromogenic Compact Dry TC plates from R-Biopharm), microbial viability (BacLight Viability Kit) and bacterial community composition (FISH). Water parameters (Ammonia, Nitrite, Nitrate, Phosphate, COD, Turbidity and H_2O_2 concentration) and production related information (feeding rate, fish biomass, oxygen consumption, ozone production) were also regularly evaluated.

Results and Discussion

H_2O_2 application started on August 06, 2020 with 1 l/h (0.51mg/L) and was increased over time up to 14 l/h (7.09 mg/L) (Fig1). On September 30, 2020 the application was stopped due to detected changes on feeding behavior of the fish as well as incorrect redox measurements in the treatment system which could probably be attributed to accumulation of oxidative species not having enough organic material to react. The test and control systems had similar biomass during the experimental period (test: 34.9-52.5 tones and control 36.4-51.4 tones) and the feed intake was accordingly adjusted (mean feed intake test: 496 kg/day; control 450 kg/day). The oxygen demand registered on the treated system (82 l/min - 90 l/min) was lower than the control (104 l/min - 115 l/min). In general, there was a reduction of turbidity and decreased nitrogen species and phosphate in the treated system. System maintenance (biofilter cleaning and backwashing) might had influenced the COD and total microbial counts measurements. Total microbial counts reflected a steady increase in the number of CFU/ml in both systems (Fig.2) and evidenced microbial accommodation. There was an increased rate of microbial mortality according to the viability results with higher values in the treated system reflecting the longer exposure of this system to oxidative stress (Fig 3). The community composition varied according to the treatment (Fig.4) and changes in the abundance of the different bacterial groups analyzed could be attributed to variable vulnerability of members of these groups to the disinfection potential of H_2O_2 or its combination with ozone.

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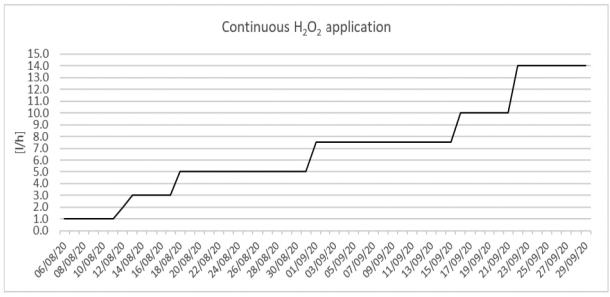

Fig.1 Hydrogen peroxide application in the initial phase of the study.

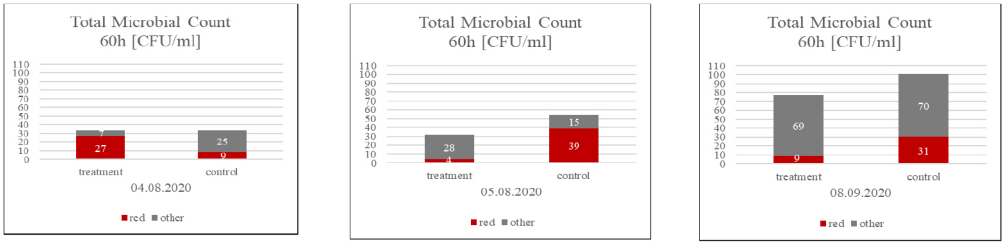

Fig.2 Total microbial counts (CFU/ml). a) reference samples before starting; b) sampling after 24 h by 1l/h application; c) sampling after 30 days application by 7.5l/h

Fig.3 BacLight Viability results. A) Reference samples obtained before starting application from both systems while operating with ozone. B) Sampling after 24 h application by1l/h together with ozone disinfection in the test

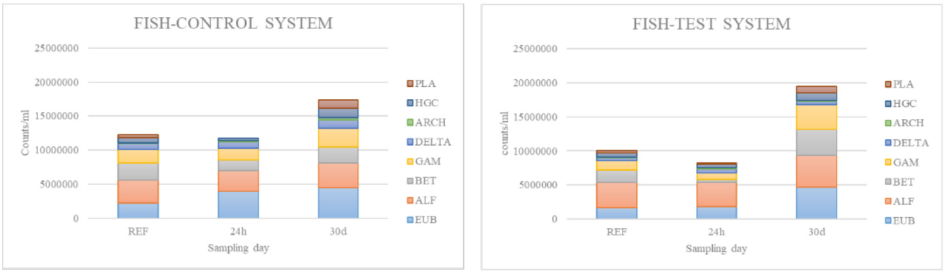

Fig.4 Fluorescence in situ hybridisation analysis of water samples from control and test system before starting application (REF), and after 24 h application by 1l/h and 30d application by 7.5 l/h combined with ozone.

This study was part of the industrial project “Follow up test of hydrogen peroxide as disinfection method in aquaculture facilities rearing Atlantic Salmon” in cooperation with Danish Salmon and EVONIK.