Received: 3 May 2021 | Revised: 5 September 2021 | Accepted: 3 February 2022

DOI: 10.1111/are.15804

Aquaculture Research WILEY

ORIGINAL ARTICLE

Removal of microparticles and bacterial inactivation in freshwater RAS by use of foam fractionation, H_2O_2 and NaCl

Leila Jafari[1] | Kim Joao de Jesus Gregersen[2] | Olav Vadstein[1] | Lars-Flemming Pedersen[2]

[1]Department of Biotechnology and Food Science, NTNU - Norwegian University of Science and Technology, Trondheim, Norway

[2]DTU Aqua, Section for Aquaculture, Technical University of Denmark, Hirtshals, Denmark

Correspondence
Leila Jafari, Department of Biotechnology and Food Science, NTNU - Norwegian University of Science and Technology, N-7491 Trondheim, Norway.
Emails: leilaj@stud.ntnu.no, ljaf@hvl.no

Funding information
Danish EPA Pesticide Research Program, Grant/Award Number: 667-00208

Abstract

Foam fractionation (FF) is an effective water treatment technology used to remove fine particles in seawater recirculating aquaculture systems (RAS). However, there is only limited available information on the operation and efficiency of FF in freshwater. This study investigated the treatment efficiency of FF by measuring changes in bacterial activity and microparticle densities in freshwater from RAS. Thirty-six separate batch tests were performed with FF separately and in combination with addition of hydrogen peroxide (H_2O_2) and salt (NaCl). Both chemicals are commonly used for water treatment in freshwater aquaculture. The experiment was a $2 \times 2 \times 3$ factorial design with 3 factors: FF (present or absent), H_2O_2 (0 or 10 mg L^{-1}) and NaCl (0, 3, or 10 ppt). FF reduced the concentration of microparticles and turbidity in freshwater by $58.7 \pm 5.4\%$ and $27.5 \pm 3.8\%$ respectively. H_2O_2 had a significant antimicrobial effect, and the combination of H_2O_2 and FF resulted in an 80% reduction in bacterial activity in freshwater. Addition of NaCl improved the efficiency of FF by further reducing particle concentration and turbidity two-fold at 10 ppt compared to 0 ppt. This study provides new knowledge on the potential use of FF to improve the water quality in freshwater RAS, and this was further enhanced by the addition of H_2O_2 or NaCl.

KEYWORDS
bacterial activity, foam fractionation, microparticle, protein skimming, RAS, water quality

1 | INTRODUCTION

Recirculating aquaculture systems (RAS) are characterized by limited water exchange, substantial feed input and associated needs for water treatment (Espinal & Matulić, 2019; Martins et al., 2010). Long retention time, accumulation of particulate matter and high levels of nutrients favour heterotrophic bacterial growth in RAS (Blancheton et al., 2013; Leonard et al., 2000; Rojas-Tirado et al., 2018). The heterotrophic bacteria colonize surfaces within the system and also constitute a substantial fraction of the microparticles in the water phase in the form of planktonic cells, multicellular bacterial aggregates or bio-flocs (Blancheton et al., 2013; de Jesus Gregersen et al., 2019; Leonard et al., 2000; Pedersen et al., 2017, 2019). Whereas large particles are easily removed from RAS (Cripps & Bergheim, 2000; Piedrahita, 2003), microparticles are difficult to remove by traditional mechanical processes, and they, therefore, accumulate (Fernandes et al., 2017; Patterson et al., 1999). Direct and indirect impacts of these particles on fish performance and RAS operation have recently been described (Becke et al., 2020; Schumann & Brinker, 2020). Recent aquaculture studies have demonstrated methods to control and remove the waterborne bacterial load by removing microparticles or inactivating bacteria (Bentzon-Tilia et al., 2016; Fossmark et al., 2020; Huyben et al., 2018;

de Jesus Gregersen et al., 2020; Wold et al., 2014). However, there is still a need to test and develop alternative, simple and cost-effective water treatment methods that can improve water quality in RAS.

One effective water treatment technology is foam fractionation (FF), a treatment that has been applied in various industries for decades (Burghoff, 2012; Timmons & Losordo, 1994). Foam fractionation or protein skimming is a physical-mechanical process that extracts dissolved and particulate matter from the water phase into foam. The treatment efficiency of FF depends on several factors, such as the air bubble diameter, the air-to-water ratio, the concentration and charge of dissolved compounds and solids, the retention time of the water in the reactor, the surface tension and the interaction of the surfactants to produce foam (Brambilla et al., 2008; Park et al., 2015; Wilson et al., 2001). The process of FF is well described (Lekang, 2020; Summerfelt, 1999; Timmons & Losordo, 1994), and the potential use of FF in aquaculture has been evaluated (Brambilla et al., 2008; Chen et al., 1994; French et al., 2000; Landau et al., 2002; Park et al., 2015; Peng et al., 2003). So far, FF is mainly applied in saltwater RAS (Colt & Huguenin, 2002), with limited information about FF application in freshwater aquaculture. The limited or absent use of FF in freshwater is primarily associated with a suboptimal removal efficiency due to larger bubble sizes and less surface tension compared to seawater. Recent studies have shown that ozone can improve FF treatment efficacy in seawater (Figueiras Guilherme et al., 2020; Park et al., 2015), although using ozone is challenging due to its toxicity and safety issues for fish and staff (Bregnballe, 2015). Hydrogen peroxide (H_2O_2) is a milder alternative to ozone with similar characteristics (i) can be used to oxidize organic matter (Cuerda-Correa et al., 2020) (ii) is easily degradable, (iii) do not form harmful by-products (Schmidt et al., 2006) and (iv) is commonly applied in aquaculture systems (Arndt & Wagner, 1997; Bögner et al., 2020; Pedersen & Pedersen, 2012; Rach et al., 1998; Saez & Bowser, 2001). Salt (NaCl) is also widely used in freshwater fish farms as an anti-parasitic treatment (Aihua & Buchmann, 2001; Jørgensen et al., 2009; Lahnsteiner & Weismann, 2007), and it increases the surface tension of the water.

The current study investigated the potential use of FF to improve water quality in freshwater RAS. The performance of FF in RAS water was tested by measuring bacterial activity (Pedersen et al., 2019) and the density of microparticles (de Jesus Gregersen et al., 2019) to evaluate its effects on water quality. This study also investigated whether the efficiency of FF could be improved by adding either H_2O_2 or NaCl, or combinations of the two.

2 | MATERIALS AND METHODS

The experiments were performed at the Section for Aquaculture, DTU Aqua, in Hirtshals, Denmark.

2.1 | RAS water

Water for the experiments was collected from a pilot-scale freshwater RAS operated for several months in steady-state at 16°C. The system included an 8.5 m^3 rearing tank stocked with approx. 200 kg of rainbow trout (*Oncorhynchus mykiss*) fed 1.0 kg of commercial feed daily and with a water renewal rate of 1.0 m^3/d. In addition, the system included two 0.40 m^3 fixed bed and two 0.40 m^3 submerged bio-filters, and a trickling and a drum filter (Pedersen et al., 2015). The water was collected from the outflow of the rearing tank early in the morning before the feeding commenced.

2.2 | Experimental design

Individual batch tests with 20 L RAS water were conducted in 30 L tanks (dimensions: Height = 37 cm, depth = 30 cm, width = 27 cm). Each tank was equipped with a pump (Tunze Silence 1073.008, Tunze Aquarientechnik GMBH, Germany) to ensure mixing of the water, a foam fractionator (Delaman® Protein Skimmer para Acuario Marino, size N°1, MN-27220-SE1, Amazon). The FF used was 28 cm high, and the air was supplied from the bottom using a wooden air stone (Sander No. 2, Erwin Sander Elektroapparatebau GmbH, Germany). The foam produced by the FF was collected by overflow into 2 L plastic bottles.

The experimental design included 3 factors (Figure 1): presence or absence of FF; H_2O_2 (0 or 10 mg H_2O_2 L^{-1}) and NaCl (salinity of 0, 3, or 10 ppt). Salinity was adjusted by adding sea salt (Sea Salt,

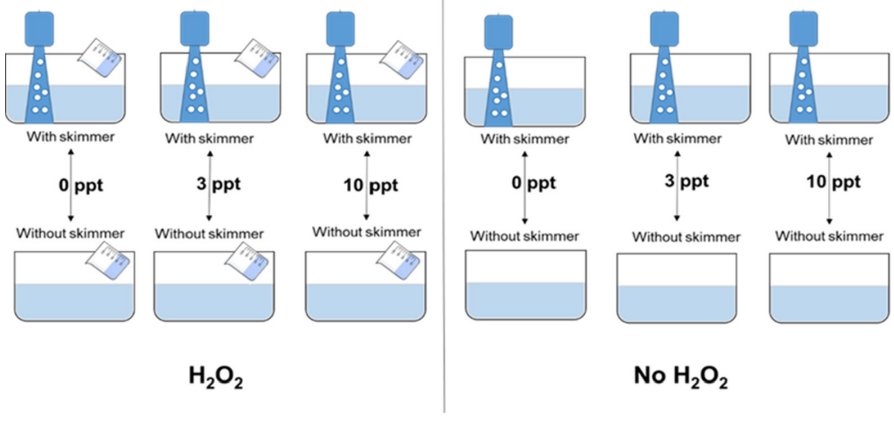

FIGURE 1 Conceptual sketch of the 3-factorial design experiment. Each treatment (n = 12) was conducted in triplicates in containers with 20 L RAS freshwater (N = 36). Samples were collected at time 0 and after 6 h

Aquaforest, Poland) in the RAS water before it was transferred into the experimental tanks (20 L in each). Each experimental trial lasted 6 h and samples were collected just prior to treatment (t = 0 h) and at the end of the trial based on preliminary trials. A total number of 36 batch experiments were performed over a period of three consecutive days.

2.3 | Sampling and analysis

Samples were collected and handled uniformly prior to the start and after 6 h of treatment. Turbidity was measured with a portable handheld turbidity meter (Hach 2100Q, Hach, United States). Particle concentration (# mL^{-1}) of particles ranging between 5.6 and 160 μm in diameter, was measured with a Coulter Counter (Multisizer 4e Coulter Counter, Beckman Coulter Life Science, US). The amounts of organic matter removed as foam (foamate) from the water by FF were analysed for total suspended solids (TSS) and total chemical oxygen demand (TCOD). TCOD (mg O_2 L^{-1}) was measured using two sets of test kits (LCK 314, LCK 1414; Hach, United States). TSS (mg L^{-1}) was quantified according to APHA standard method 2005.

The per cent removal efficiency measured as TSS and TCOD was calculated according to Eq. (1):

$$RE(\%) = \frac{C_f \times V_f}{C_o \times V_w} \times 100 \quad (1)$$

where RE is the removal efficiency (%), C_f is the concentration (TSS or TCOD) in the foamate, V_f is the total foamate volume (L), C_0 is the initial concentration (TSS or TCOD) in the water, and V_W is the initial water volume (L).

The bacterial activity in raw water samples was determined according to the hydrogen peroxide degradation method (Pedersen et al., 2019). This assay relies on the quantification of the enzymatic degradation of H_2O_2 under constant conditions, which is calculated as a degradation rate constant k (h^{-1}) based on Eq. (2):

$$C_t = C_0 \times e^{-kt} \quad (2)$$

where C_t and C_0 is the H_2O_2 concentration at time t and 0 respectively.

2.4 | Data analysis

The concentration of microparticles, turbidity and bacterial activity were normalized as per cent of measurements at time zero using formulae (3):

$$\%\ remaining = \frac{Value\ at\ time\ t}{Value\ at\ time\ 0} * 100 \quad (3)$$

The data were normalized to facilitate comparison between treatments at the end of the experiment and to correct for differences in start conditions for replicated experiments. A summary of the measured variable range at time zero is listed in Table 1. Data are presented as average ± SD. A three-way ANOVA was conducted to compare the main effects of foam fractionation, hydrogen peroxide and salinity and interaction effects. Differences in treatment means were tested by Tukey's post hoc test with a pre-defined significance level of $p < 0.05$. Statistical analyses were made using SPSS version 25.

TABLE 1 Range of selected RAS water quality parameters used for the batch experiments over three days. Measurements were done prior to the treatments (t = 0)

Variables	Range
Temperature	15.0–16.5
pH	7.4–7.8
Oxygen conc. (mg O_2 L^{-1})	>9
Bacterial Activity (k (h^{-1})	0.16–0.47
Particle numbers (# mL^{-1})	1.21–1.70·10^5
Turbidity (NTU)	4.4–5.8

2.5 | Ethics statement

The experiments conducted in this trial did not need any ethical approval, as no animals were used during the trial.

3 | RESULTS

3.1 | Bacterial activity

For the control treatment, the bacterial activity in the water increased after 6 h incubation (Figure 2a). In general, FF reduced bacterial activity and the addition of salt significantly lowered bacterial activity when comparing 10 and 0 ppt treatments (Figure 2a). The addition of H_2O_2 significantly reduced bacterial activity by 69.5 ± 5.3% at the three salinities tested ($p < 0.001$). When H_2O_2 was combined with FF, a further significant reduction in bacterial activity was observed. On average, at all three tested salinities the bacterial activity was 58% lower when H_2O_2 was combined with FF compared to H_2O_2 addition only (Figure 2a, Table 2).

3.2 | Microparticle concentration and turbidity

FF significantly reduced particle numbers and turbidity ($p < 0.001$, Figure 2b,c and Table 2). The average number of microparticles was 74.2 ± 4.1% lower and turbidity was 42.9 ± 3.7% lower in the FF treatments compared to control treatment without FF (Figure 2b,c). H_2O_2 addition did not affect particle numbers or turbidity ($p = 0.709$ and 0.184 respectively). Increased salinity significantly reduced both

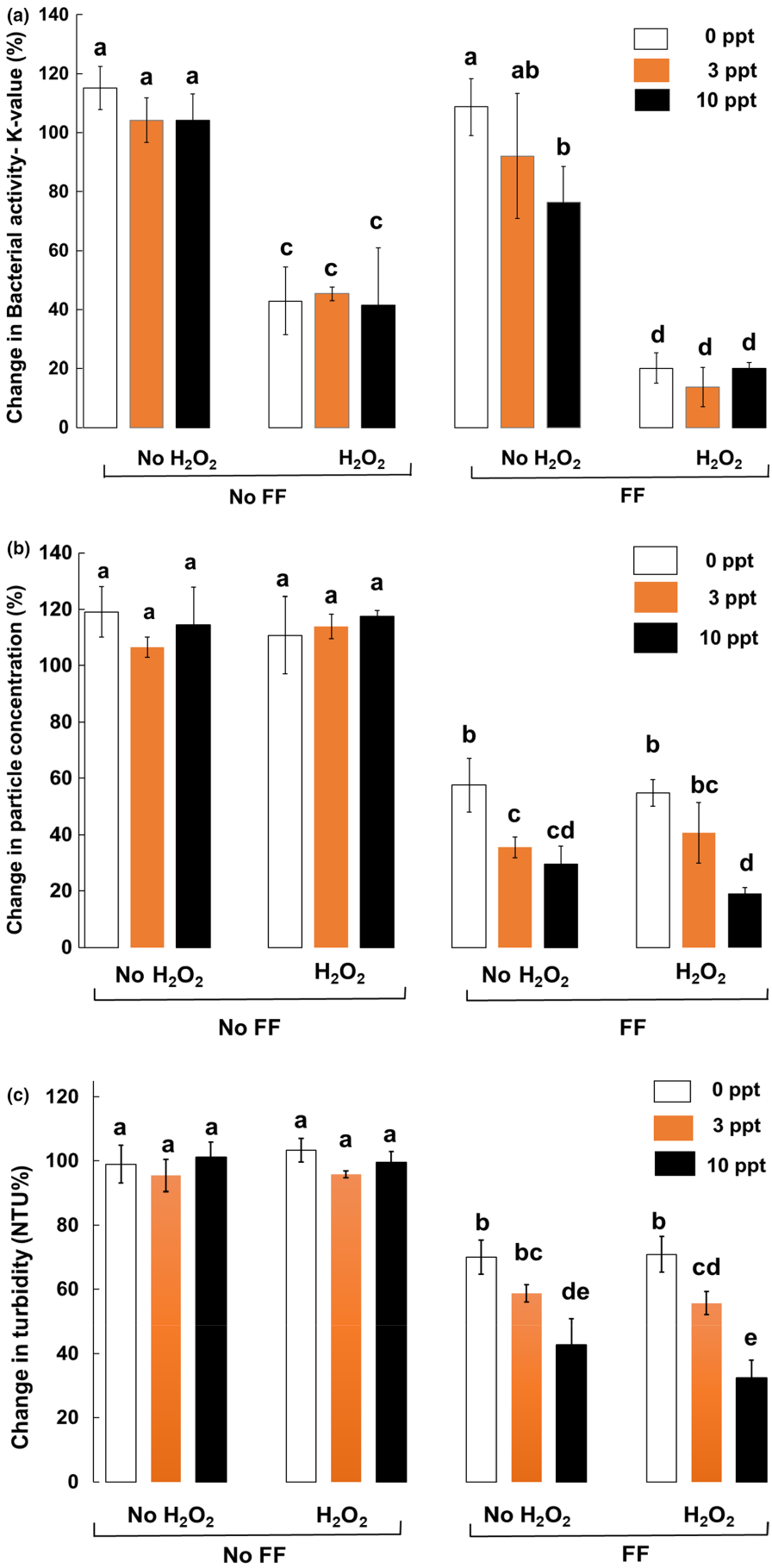

FIGURE 2 Relative changes in selected water quality variables after 6 h of treatment compared with start values; (a) bacterial activity, (b) particle concentration and (c) turbidity. The treatment combinations included foam fractionation (FF) vs. control (no FF); hydrogen peroxide (10 mg H_2O_2 L^{-1}) vs. control (no H_2O_2) and salinity (0, 3, or 10 ppt). The data presented (mean ± SD; n = 3) reflects normalized value calculated according to each group's initial values before treatment (t = 0). The different letter denotes significant differences between groups ($p < 0.05$)

TABLE 2 Three-way analysis of variance (ANOVA) of different water quality variables

Treatments	Statistical parameters	Bacterial activity	Particle concentration	Turbidity
FF[a]	F	30.392	766.40	494.84
	p-value	<0.001	<0.001	<0.001
	Effect size [b]	0.559	0.970	0.954
H_2O_2	F	354.886	0.709	1.871
	p-value	<0.001	0.142	0.184
	Effect size	0.937	0.006	0.072
Salinity	F	3.211	11.92	20.84
	p-value	0.058	<0.001	<0.001
	Effect size	0.211	0.498	0.635
Interaction(FF × H_2O_2 ×salinity)	F	1.22	1.063	0.774
	p-value	0.311	0.361	0.473
	Effect size	0.093	0.081	0.061
Interaction (FF ×salinity)	F	0.657	12.744	29.27
	p-value	0.528	< 0.001	<0.001
	Effect size	0.052	0.515	0.709
Interaction (FF × H_2O_2)	F	1.781	0.398	0.657
	p-value	0.195	0.534	0.426
	Effect size	0.069	0.016	0.027
Interaction (H_2O_2 ×salinity)	F	2.66	1.858	0.777
	p-value	0.09	0.178	0.474
	Effect size	0.182	0.134	0.060

[a]Foam Fractionation

[b]Partial Eta Squared from SPSS reported as Effect size.

particle number and turbidity when combined with FF ($p < 0.001$, Table 2).

3.3 | TSS and TCOD collected in the foamate

The organic matter removed from the water and collected as foamate ranged from 192–365 mg L^{-1} TSS and from 266 – 648 mg O_2 L^{-1} Total COD. This was also evident when calculated as the removal percentage of TSS and TCOD from the tanks (Figure 3). TCOD and TSS removal was improved with the addition of H_2O_2 compared to FF without H_2O_2 (Figure 3, Table 3). Salinity significantly improved the removal of TSS (from 29.3 ± 8.6% in 0 ppt to 60.8 ± 13.7% in 10 ppt, $p = 0.003$) and TCOD (from 19.7 ± 3.6% in 0 ppt to 32.1 ± 4.7% in 10 ppt, $p < 0.001$) regardless of the presence of H_2O_2 (Figure 3).

4 | DISCUSSION

The ability of FF to improve water quality by the removal of heterotrophic bacteria and particulate matter has been documented in seawater (Barrut et al., 2013; Brambilla et al., 2008; Peng & Jo, 2003; Peng et al., 2003) but not in freshwater. Performance of FF was examined in the freshwater, obtained from a pilot-scale RAS with rainbow trout, feed loading and retention time, reflecting commercial operation. The small-scale batch tests allowed quantification of the efficacy of FF with and without the addition of sodium chloride and hydrogen peroxide by measuring changes in several water quality variables.

4.1 | Foam fractionation removes microparticles and improves water transparency

FF significantly reduced the particle concentration by 58.7 ± 5.4% and improved turbidity in freshwater (27.5 ± 3.8%) compared to control treatments without FF. The microparticle concentration ($1.4{\cdot}10^5 \pm 0.2{\cdot}10^5$) included particles above 5 μm and concentrations are representative for what is found in RAS (Fernandes et al., 2017; de Jesus Gregersen et al., 2019). Microparticles accumulate in RAS and represent a significant source of bioavailable organic matter (de Jesus Gregersen et al., 2020; Pedersen et al., 2017), which FF potentially can harvest and remove from the water. Even though FF is considered less efficient in freshwater due to the lower density and surface tension of freshwater compared to seawater, the results of our experiment indicate that FF would be an effective way to remove particles and bacteria and thereby improve the water quality also of freshwater.

Bacterial activity in the water was reduced by FF treatment alone. However, the effectiveness was significantly increased by the

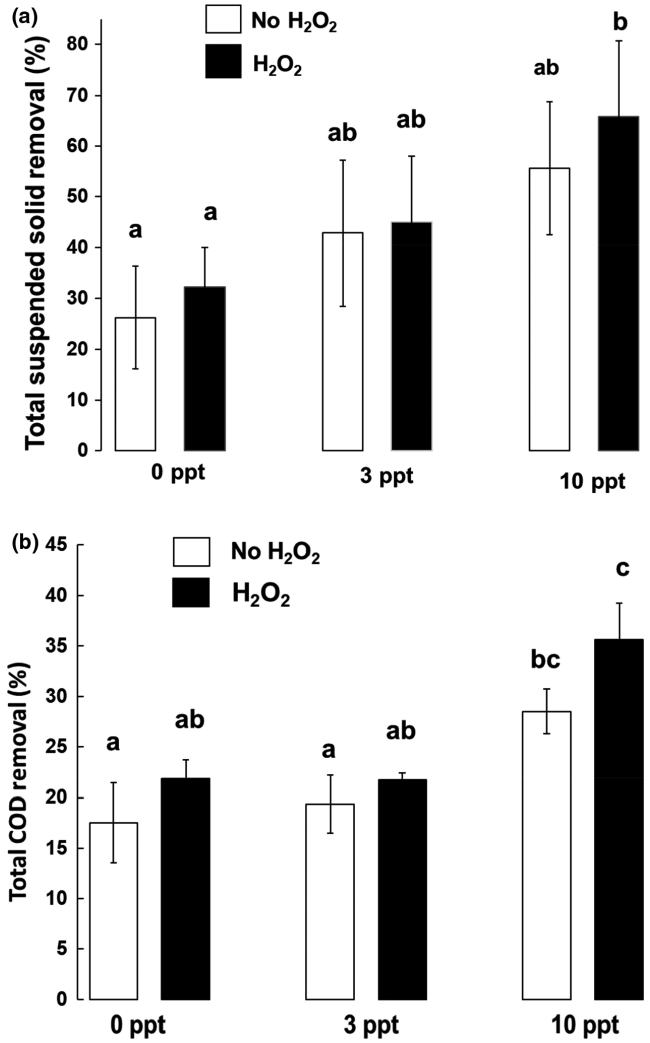

FIGURE 3 Percent removal efficiency of organic matter from the water as foamate after 6 h in the six treatments with FF; a) TSS and b) Total COD, (mean ± SD; n = 3). Treatments include salinity in three levels (0, 3 or 10 ppt) and the presence (H_2O_2) or absence of hydrogen peroxide (No H_2O_2). The different letters denote significant differences between groups ($p < 0.05$)

addition of H_2O_2 and salt. This can be explained by the ability of salt to improve FF removal efficiency of particles and, consequently, particle-associated and free-living bacteria from the water. This result agrees with the findings of Brambilla et al. (2008), showing that removal of heterotrophic bacteria (32 to 88%) was achieved by FF in seawater. Similarly, Rahman et al. (2012) who investigated the effect of FF on bacterial abundance and abalone growth, found a lower mean bacterial abundance in water treated with FF than in untreated water, thereby confirming the efficiency of FF for the removal of bacteria from seawater.

4.2 | Hydrogen peroxide reduces bacterial activity

Bacterial activity was significantly lower in water treated with H_2O_2 than in untreated water. This reduction most likely reflects the biostatic effects of H_2O_2 and the oxidative damage to relevant bacterial enzymes (Linley et al., 2012; Schmidt et al., 2006). The bacterial activity was further reduced by combining H_2O_2 and FF (average 58%) compared to the treatments without FF. This could reflect the combination of microparticle removal by FF, along with the oxidative and direct antimicrobial actions of H_2O_2 (Block, 2001). H_2O_2 addition apparently did not affect particle removal by FF. However, only microparticles with sizes above 5 μm were measured, excluding most of the free-living bacteria (de Jesus Gregersen et al., 2020). The results presented here indicate a beneficial effect of the combination of H_2O_2 and salinity (especially 10 ppt) for particle removal by the skimming process, even with only 6 h contact time. However, these findings and the interactions between FF, H_2O_2 and salinity were not statistically significant and require further research.

TABLE 3 Two-way analysis of variance (ANOVA) of different variables in the foamate

Treatments	Statistical parameters	TSS	Total COD
H_2O_2	F	1.105	12.63
	p-value	0.314	0.004
	Effect size [a]	0.084	0.513
Salinity	F	9.63	37.78
	p-value	0.003	<0.001
	Effect size	0.616	0.863
Interaction (H_2O_2 ×salinity)	F	0.159	1.095
	p-value	0.855	0.366
	Effect size	0.026	0.154

[a]Partial Eta Squared from SPSS reported as Effect size.

4.3 | Addition of sodium chloride improves foam fractionation

Increased salinity in the FF treatments significantly improved the FF removal efficiency in terms of reduction in particle concentration and turbidity. According to Colt and Huguenin (2002), foam fractionation performance depends on water composition, surface tension, salinity, bubble size among others. Salt addition improved FF removal efficiency by increasing the surface tension of the water, and smaller bubbles were observed during the trial. Chen et al. (1994) showed mathematically, that reducing bubble size increased the solids removal efficiency of foam fractionators. The effect of salt addition on bacterial activity depended on the presence of FF, as salinity alone did not significantly affect the bacterial activity. This suggests that the salt simply increased the efficiency of FF, resulting in an improvement in the removal of bacteria from the system and not killing them. FF treatments without H_2O_2 substantially lowered bacterial activities at 10 ppt salinity, indicating that salinity is an important factor for particle and particle-associated bacteria removal.

This result, an increase in the efficiency with increasing NaCl addition, further supports the theory of increased efficiency of FF at higher salinities.

5 | CONCLUSION

With a batch setup, it was demonstrated that FF reduced the fine particle concentration and improved the turbidity of freshwater RAS. The addition of hydrogen peroxide alone had a significant antimicrobial effect and reduced the bacterial activity, while the combination of FF with hydrogen peroxide further reduced the bacterial activity. The addition of a low concentration of salt (3 and 10 ppt) improved the particle removal process during foam fractionation, and the interaction effect of FF and salinity was statistically significant. The combination of hydrogen peroxide and 10 ppt salinity during FF further improved the particle removal.

The results presented in this study stressed that simple and low-tech solutions, such as foam formation, could be applied to circumvent the problem with accumulating bacteria and organic matter in freshwater RAS. These findings may encourage further testing and development of potential long-term operation and affordable solutions to remove dissolved and particulate organic matter by foam fractionation in freshwater RAS.

ACKNOWLEDGEMENTS

The study was funded by the Danish EPA Pesticide Research Program (J. no. 667-00208) as part of the project "Towards Biocide Free Land-based Aquaculture". The technical and analytical support by Kari Attramadal from NTNU and Brian Møller, Ulla Sproegel, Mang'era Samwel Mnyoro, Zhitao Huang at DTU Aqua Section for Aquaculture, Hirtshals, is highly appreciated.

AUTHORS' CONTRIBUTION

L.F.P. and K.J.J.G., conceived the research idea, L.F.P. K.J.J.G., O.V. and L.J. designed the experimental trial. L.J. conducted the experiments, collected the samples and performed the laboratory analyses. L.J., L.F.P. and K.J.J.G. processed and analysed the data. L.J wrote the first version of the manuscript, and all authors contributed to the writing and review of the final manuscript.

CONFLICT OF INTEREST

Authors have no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

All data utilized for this trial and to support the findings are available within the article.

ORCID

Leila Jafari https://orcid.org/0000-0002-3316-0145
Kim Joao de Jesus Gregersen https://orcid.org/0000-0003-3875-816X
Olav Vadstein https://orcid.org/0000-0001-8256-3939
Lars-Flemming Pedersen https://orcid.org/0000-0002-8361-9268

REFERENCES

Aihua, L., & Buchmann, K. (2001). Temperature-and salinity-dependent development of a Nordic strain of Ichthyophthirius multifiliis from rainbow trout. *Journal of Applied Ichthyology*, *17*(6), 273–276. https://doi.org/10.1046/j.1439-0426.2001.00279.x

Arndt, R. E., & Wagner, E. J. (1997). The toxicity of hydrogen peroxide to rainbow trout Oncorhynchus mykiss and cutthroat trout Oncorhynchus clarki fry and fingerlings. *Journal of the World Aquaculture Society*, *28*(2), 150–157. https://doi.org/10.1111/j.1749-7345.1997.tb00850.x

Barrut, B., Blancheton, J.-P., Callier, M., ChampagneJ, Y., & Grasmick, A. (2013). Foam fractionation efficiency of a vacuum airlift-Application to particulate matter removal in recirculating systems. *Aquaculture Engineering*, *54*, 16–21.

Becke, C., Schumann, M., Geist, J., & Brinker, A. (2020). Shape characteristics of suspended solids and implications in different salmonid aquaculture production systems. *Aquaculture*, *516*, 734631. https://doi.org/10.1016/j.aquaculture.2019.734631

Bentzon-Tilia, M., Sonnenschein, E. C., & Gram, L. (2016). Monitoring and managing microbes in aquaculture-Towards a sustainable industry. *Microbial Biotechnology*, *9*(5), 576–584. https://doi.org/10.1111/1751-7915.12392

Blancheton, J. P., Attramadal, K. J. K., Michaud, L., Roque, E., & Vadstein, O. (2013). Aquacultural Engineering Insight into bacterial population in aquaculture systems and its implication. *Aquaculture Engineering*, *53*, 30–39. https://doi.org/10.1016/j.aquaeng.2012.11.009

Block, S. S. (2001). *Disinfection, sterilization, and preservation*. , 5th. ed. Lippincott Williams & Wilkins

Bögner, D., Bögner, M., Schmachtl, F., Bill, N., Halfer, J., & Slater, M. J. (2020). Hydrogen peroxide oxygenation and disinfection capacity in recirculating aquaculture systems. *Aquacultural Engineering*, *92*, 11. https://doi.org/10.1016/j.aquaeng.2020.102140

Brambilla, F., Antonini, M., Ceccuzzi, P., Terova, G., & Saroglia, M. (2008). Foam fractionation efficiency in particulate matter and heterotrophic bacteria removal from a recirculating seabass (Dicentrarchus labrax) system. *Aquaculture Engineering*, *39*, 37–42. https://doi.org/10.1016/j.aquaeng.2008.05.004

Bregnballe, J. (2015). *A Guide to Recirculation Aquaculture*. FAO and Eurofish Report, 100.

Burghoff, B. (2012). Foam fractionation applications. *Journal of Biotechnology*, *161*(2), 126–137. https://doi.org/10.1016/j.jbiotec.2012.03.008

Chen, S., Timmons, M. B., Bisogni, J. J. Jr, & Aneshansley, D. J. (1994). Modeling surfactant removal in foam fractionation: I — Theoretical development. *Aquacultural Engineering*, *13*, 163–181. https://doi.org/10.1016/0144-8609(94)90001-9

Colt, J., & Huguenin, J. E. (2002). *Design and Operating Guide for Aquaculture Seawater Systems: Second Edition - Google Bøker*. Retrieved July 26, 2020, from Elsevier, 336 sider website: https://books.google.no/books/about/Design_and_Operating_Guide_for_Aquacultu.html?id=gWL61CChReEC&redir_esc=y

Cripps, S. J., & Bergheim, A. (2000). Solids management and removal for intensive land4 basedaquaculture production systems. *Aquaculture Engineering*, *22*, 33–56.

Cuerda-Correa, E. M., Alexandre-Franco, M. F., & Fernández-González, C. (2020). Advanced oxidation processes for the removal of antibiotics from water. An Overview. *Water (Switzerland)*, *12*, 102. https://doi.org/10.3390/w12010102

de Jesus Gregersen, K. J., Pedersen, P. B., Pedersen, L. F., & Dalsgaard, J. (2019). Micro particles and microbial activity in Danish

recirculating rainbow trout (Oncorhynchus mykiss) farms. *Aquacultural Engineering*, *84*, 60–66. https://doi.org/10.1016/j.aquaeng.2018.12.001

de Jesus Gregersen, K. J., Pedersen, P. B., Pedersen, L., Liu, D., & Dalsgaard, J. (2020). UV irradiation and micro fi ltration e ff ects on micro particle development and microbial water quality in recirculation aquaculture systems. *Aquaculture*, *518*, 734785. https://doi.org/10.1016/j.aquaculture.2019.734785

Espinal, C. A., & Matulić, D. (2019). *Recirculating Aquaculture Technologies. In Aquaponics Food Production Systems* (pp. 35–76). Springer International Publishing. https://doi.org/10.1007/978-3-030-15943-6_3

Fernandes, P. M., Pedersen, L. F., & Pedersen, P. B. (2017). Aquacultural Engineering Influence of fixed and moving bed biofilters on micro particle dynamics in a recirculating aquaculture system. *Aquaculture Engineering*, *78*, 32–41. https://doi.org/10.1016/j.aquaeng.2016.09.002

Figueiras Guilherme, M., de Jesus Gregersen, K. J., & Pedersen, L. F. (2020). Effects of foam fractionation and chemical disinfection on the removal of different microalgae cultures. *Aquaculture Research*, *65*, 1–10. https://doi.org/10.1111/are.14663

Fossmark, R. O., Vadstein, O., Rosten, T. W., Bakke, I., Košeto, D., Bugten, A. V., Helberg, G. A., Nesje, J., Jørgensen, N. O. G., Raspati, G., Azrague, K., Østerhus, S. W., & Attramadal, K. J. K. (2020). Effects of reduced organic matter loading through membrane filtration on the microbial community dynamics in recirculating aquaculture systems (RAS) with Atlantic salmon parr (Salmo salar). *Aquaculture*, *524*, 735268. https://doi.org/10.1016/j.aquaculture.2020.735268

French, K., Guest, R. K., Finch, G. R., & Haas, C. N. (2000). Correlating Cryptosporidium removal using dissolved air flotation in water treatment. *Water Research*, *34*, 4116–4119. https://doi.org/10.1016/S0043-1354(00)00164-0

Huyben, D., Bevan, D., Stevenson, R., Zhou, H., & Moccia, R. (2018). Evaluation of membrane filtration and UV irradiation to control bacterial loads in recirculation aquaculture systems. *Aquaculture International*, *26*, 1531–1540. https://doi.org/10.1007/s10499-018-0301-z

Jørgensen, T. R., Larsen, T. B., & Buchmann, K. (2009). Parasite infections in recirculated rainbow trout (Oncorhynchus mykiss) farms. *Aquaculture*, *289*, 91–94. https://doi.org/10.1016/j.aquaculture.2008.12.030

Lahnsteiner, F., & Weismann, T. (2007). Treatment of ichthyophthiriasis in rainbow trout and common carp with common and alternative therapeutics. *Journal of Aquatic Animal Health*, *19*, 186–194. https://doi.org/10.1577/H07-002.1

Landau, M., Richard, C., & Erstfeld, K. (2002). The Effect of Suspended Clay on Protein Removal during Foam Fractionation. *North American Journal of Aquaculture*, *64*, 217–219. https://doi.org/10.1577/1548-8454(2002)064<0217:teosco>2.0.co;2

Lekang, O. I. (2020). *Aquaculture Engineering*, 3rd ed. (p. 524).

Leonard, N., Blancheton, J. P., & Guiraud, J. P. (2000). Populations of heterotrophic bacteria in an experimental recirculating aquaculture system. *Aquaculture Engineering*, *22*, 109–120. https://doi.org/10.1016/S0144-8609(00)00035-2

Linley, E., Denyer, S. P., Mcdonnell, G., Simons, C., & Maillard, J. (2012). Use of hydrogen peroxide as a biocide : new consideration of its mechanisms of biocidal action. *The Journal of Antimicrobial Chemotherapy*, *67*, 1589–1596. https://doi.org/10.1093/jac/dks129

Martins, C. I. M., Eding, E. H., Verdegem, M. C. J., Heinsbroek, L. T. N., Schneider, O., Blancheton, J. P., Roque d'Orbcastel, E., & Verreth, J. A. J. (2010). New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquaculture Engineering*, *43*, 83–93. https://doi.org/10.1016/j.aquaeng.2010.09.002

Park, J., Kim, P. K., Park, S., & Daniels, H. V. (2015). Effects of two different ozone doses on total residual oxidants, nitrogen compounds and nitrification rate in seawater recirculating systems for black seabream Acanthopagrus schlegelii (Bleeker). *Aquaculture Engineering*, *67*, 1–7. https://doi.org/10.1016/j.aquaeng.2015.05.003

Patterson, R. N., Watts, K. C., & Timmons, M. B. (1999). The power law in particle size analysis for aquacultural facilities. *Aquaculture Engineering*, *19*, 259–273. https://doi.org/10.1016/S0144-8609(98)00054-5

Pedersen, L. F., Oosterveld, R., & Pedersen, P. B. (2015). Nitrification performance and robustness of fixed and moving bed biofilters having identical carrier elements. *Aquaculture Engineering*, *65*, 37–45. https://doi.org/10.1016/j.aquaeng.2014.10.005

Pedersen, L. F., & Pedersen, P. B. (2012). Hydrogen peroxide application to a commercial recirculating aquaculture system. *Aquaculture Engineering*, *46*, 40–46. https://doi.org/10.1016/j.aquaeng.2011.11.001

Pedersen, L. F., Rojas-Tirado, P., Arvin, E., & Pedersen, P. B. (2019). Assessment of microbial activity in water based on hydrogen peroxide decomposition rates. *Aquaculture Engineering*, *85*, 9–14. https://doi.org/10.1016/j.aquaeng.2019.01.001

Pedersen, P. B., von Ahnen, M., Fernandes, P., Naas, C., Pedersen, L. F., & Dalsgaard, J. (2017). Particle surface area and bacterial activity in recirculating aquaculture systems. *Aquaculture Engineering*, *78*, 18–23. https://doi.org/10.1016/j.aquaeng.2017.04.005

Peng, L., & Jo, J.-Y. (2003). Performance of a foam fractionator in a lab scale seawater recirculating aquaculture system. *Journal of Fisheries Science and Technology*, *6*, 187–193. https://doi.org/10.5657/fas.2003.6.4.187

Peng, L., Oh, S.-Y., & Jo, J.-Y. (2003). Protein removal by a foam fractionator in simulated seawater aquaculture system. *Ocean and Polar Research*, *25*, 269–275. https://doi.org/10.4217/OPR.2003.25.3.269

Piedrahita, R. H. (2003). Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture*, *226*(1-4), 35–44.

Rach, J. J., Gaikowski, M. P., Howe, G. E., & Schreier, T. M. (1998). Evaluation of the toxicity and efficacy of hydrogen peroxide treatments on eggs of warm- and coolwater fishes. *Aquaculture*, *165*, 11–25. https://doi.org/10.1016/S0044-8486(98)00248-8

Rahman, M. M., Kadowaki, S., Linn, S. M., & Yamada, Y. (2012). Effects of protein skimming on water quality, bacterial abundance and abalone growth in land based recirculating aquaculture systems. *Journal of Fisheries and Aquatic Science*, *7*, 150–161. https://doi.org/10.3923/jfas.2012.150.161

Rojas-Tirado, P. A., Pedersen, P. B., Vadstein, O., & Pedersen, L. F. (2018). Changes in microbial water quality in RAS following altered feed loading. *Aquaculture Engineering*, *81*, 80–88. https://doi.org/10.1016/j.aquaeng.2018.03.002

Saez, J. A., & Bowser, P. R. (2001). Hydrogen Peroxide Concentrations in Hatchery Culture Units and Effluent during and after Treatment. *North American Journal of Aquaculture*, *63*(1), 74–78. https://doi.org/10.1577/1548-8454(2001)063<0074:hpcihc>2.0.co;2

Schmidt, L. J., Gaikowski, M. P., & Gingerich, W. H. (2006). *Environmental Assessment for the Use of Hydrogen Peroxide in Aquaculture for Treating External Fungal and Bacterial Diseases of Cultured Fish and Fish Eggs*. 54603(608).

Schumann, M., & Brinker, A. (2020). Understanding and managing suspended solids in intensive salmonid aquaculture: a review. *Reviews in Aquaculture*, *1–31*, https://doi.org/10.1111/raq.12425

Summerfelt, S. T. (1999). Waste Handling Systems. CIGR Handbook of Agriculture Engineering. *Animal Production and Aquaculture Engineering*, *2*, 309–350.

Timmons, M. B., & Losordo, T. M. (1994). *Use of foam fractionators in aquaculture*. Elsevier Science, New York, EEUU. NL. 1994. 247-279.

Wilson, W. W., Wade, M. M., Holman, S. C., & Champlin, F. R. (2001). Status of methods for assessing bacterial cell surface charge

properties based on zeta potential measurements. *Journal of Microbiological Methods*, *43*, 153–164. https://doi.org/10.1016/S0167-7012(00)00224-4

Wold, P. A., Holan, A. B., Øie, G., Attramadal, K., Bakke, I., Vadstein, O., & Leiknes, T. O. (2014). Effects of membrane filtration on bacterial number and microbial diversity in marine recirculating aquaculture system (RAS) for Atlantic cod (Gadus morhua L.) production. *Aquaculture*, *422–423*, 69–77. https://doi.org/10.1016/j.aquaculture.2013.11.019

How to cite this article: Jafari, L., de Jesus Gregersen, K. J., Vadstein, O., & Pedersen, L.-F. (2022). Removal of microparticles and bacterial inactivation in freshwater RAS by use of foam fractionation, H_2O_2 and NaCl. *Aquaculture Research*, 53, 3274–3282. https://doi.org/10.1111/are.15804