Evaluating protein skimmer performance in a commercial seawater recirculating aquaculture system (RAS)

Kovács, Bence Dániel; de Jesus Gregersen, Kim João; Rüppel, Florian; von Danwitz, Arndt; Pedersen, Lars-Flemming

Published in:
Aquacultural Engineering

Link to article, DOI:
10.1016/j.aquaeng.2023.102369

Publication date:
2023

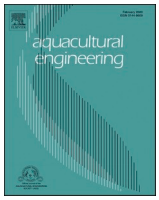
Document Version
Publisher's PDF, also known as Version of record

Aquacultural Engineering 103 (2023) 102369

Contents lists available at ScienceDirect

Aquacultural Engineering

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



Evaluating protein skimmer performance in a commercial seawater recirculating aquaculture system (RAS)

Bence Dániel Kovács [a,*], Kim João de Jesus Gregersen [a], Florian Rüppel [b], Arndt von Danwitz [b], Lars-Flemming Pedersen [a]

[a] Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, 9850 Hirtshals, Denmark
[b] Danish Salmon A/S, 9850 Hirtshals, Denmark

ARTICLE INFO

Keywords:
Ozone
Protein skimming
Water quality
Commercial seawater RAS
Atlantic salmon

ABSTRACT

Combining protein skimming with ozone (O_3) is a common method for removing microparticles in recirculating aquaculture systems (RAS). Nevertheless, there is a limited number of studies that have validated protein skimming's performance at a commercial scale. Additionally, variations in protein skimmer designs and operational variables may yield different performance outcomes. In the present study, the performance of two types of full-scale protein skimmer (S1 and S2) were compared and evaluated under two levels of hydraulic retention time (HRT) (1.8 and 2.2 min) and three levels of O_3 doses (0, 7, and 14 g O_3/kg feed) in a commercial seawater RAS facility. Samples from the inlet and outlet of the protein skimmers were collected at each combination of operational variables. They were analysed for several relevant water quality parameters to quantify the treatment efficiency. O_3 dose significantly improved water quality and reduced the numbers of microparticles and bacterial activity in a single pass. Besides that, doses as high as 14 g O_3/kg feed significantly increased total residual oxidant (TRO) concentration. Additionally, an increase in HRT exerted a moderate effect on removing microparticles and a strong effect on redox potential (ORP) and TRO. Finally, the type of protein skimmer only affected the ORP, causing no significant changes to other water quality metrics. The correlations between the investigated water quality parameters defined a clear pattern of the ongoing processes and particle characteristics. Overall, the results demonstrated that protein skimming combined with carefully selected O_3 doses can improve general water quality and control critical factors such as bacterial activity and microparticles under commercial operations.

1. Introduction

Recirculating aquaculture systems (RAS) are closed fish production systems in which the culture water is reused and continuously treated before being discharged into the environment (Piedrahita, 2003; Lekang, 2019). As the technology allows large volumes of fish production, under controlled conditions, it is considered a more production-efficient and environmentally sustainable option than conventional sea cages or flow-through systems (Martins et al., 2010; Ahmed and Turchini, 2021). However, because of increasing feeding rates and limited water exchange, maintaining ideal water quality conditions in a RAS is a challenge.

One of the major issues of intensive RAS is the accumulation of waste products including dissolved and solid wastes resulting from fish metabolism and feed spills (Badiola et al., 2012). While commercial RAS applies highly effective water treatment processes to eliminate large solids by drum filters, fine solids or microparticles (1–100 μm) tend to accumulate in the system (Chen et al., 1993; Fernandes et al., 2014). Aside from the anticipated but uncertain negative effects on fish welfare and gill health (Becke et al., 2018; Lu et al., 2018), the accumulation of microparticles have several water quality implications. The eutrophication and prolonged retention time promote bacterial proliferation (Pedersen et al., 2017; de Jesus Gregersen et al., 2019), leading to the accumulation of organic matter, off-flavor formation, elevated biochemical oxygen demand (BOD), reduced oxygen concentration, and increased carbon dioxide production. This built-up of microbial biomass may facilitate ammonia leaching, unwanted biofilm growth which can potentially limit nitrification and promote the formation of toxic hydrogen sulfide (Ling and Chen, 2005; Kvåle et al., 2006; Pedersen et al., 2017; Letelier-Gordo et al., 2020).

* Corresponding author.
E-mail address: kovacsbenced@gmail.com (B.D. Kovács).

https://doi.org/10.1016/j.aquaeng.2023.102369
Received 6 June 2023; Received in revised form 20 September 2023; Accepted 23 September 2023
Available online 26 September 2023

Several approaches have been developed and evaluated for the removal of microparticles, such as reduced drum filter mesh sizes, membrane filtration, low pore cartridge filtration or electro coagulation (Dolan et al., 2013; Holan et al., 2014; de Jesus Gregersen et al., 2020; Xu et al., 2021). While these strategies can be effective, their large-scale application, raises some economic and feasibility concerns. Currently, a common and viable practice includes protein skimming (Barrut et al., 2012; de Jesus Gregersen et al., 2021) and ozone (O_3) treatment, which are often combined for maximum efficiency (Lekang, 2019).

Protein skimming is a technology used to remove microparticles and dissolved organic matter. The method relies on a fundamental process known as adsorptive bubble separation (Lekang, 2019), and the device used is called a protein skimmer. Studies investigating protein skimmer performance have revealed significant total suspended solids and protein removal, decreased microbial activity and positive contribution to dissolved oxygen concentration and carbon dioxide degassing (Peng and Jo, 2003; Brambilla et al., 2008; Barrut et al., 2012; Orellana and Wecker, 2013). Protein skimming exhibits greater effectiveness in seawater due to its higher surface tension compared to freshwater (Lekang, 2019; Jafari et al., 2022).

O_3 is a highly reactive oxidant that can break down organic matter, flocculate microparticles, oxidize nitrite, improve water clarity and potentially reduce off-flavour issues (Summerfelt, 2003; Li et al., 2009; Davidson et al., 2011). Additionally, O_3 can destroy cell membranes and nucleic acids, making it a strong disinfectant (Summerfelt, 2003; Sharrer and Summerfelt, 2007; Guilherme et al., 2020). When O_3 is introduced into the protein skimmer, these advantages are complemented by improved skimming efficiency. This synergy occurs as O_3 rearranges molecular charges during the break down or flocculation of organic matter, promoting particle adhesion to the rising bubbles by altering surface tension and charge (Lekang, 2019).

The positive effects of O_3 combined with protein skimming have been reported in several studies. Park et al. (2011) found that adding O_3 to a pilot-scale seawater protein skimmer improved the removal of dissolved organic carbon, suspended and volatile solids. Furthermore, in a freshwater study by de Jesus Gregersen et al. (2021), the combination of O_3 and protein skimming resulted in a significant reduction in particle counts, bacterial activity, bioavailable organic matter and turbidity, as well as a considerable increase in ultraviolet transmittance (UVT) when compared to each treatment alone. These treatments directly and indirectly impact RAS performance by enhancing fish health, improving biofilter efficiency, potentially reducing costs, conserving water, and environmental sustainability. Although combining protein skimming with O_3 is highly effective, both approaches pose some challenges. Firstly, protein skimmer performances are influenced by several interrelated chemical and physical factors. Among these factors some of the most important ones are protein skimmer design, gas transfer and hydraulic retention time (HRT) (Wheaton et al., 1979; Weeks et al., 1992; Peng et al., 2003; Buckley et al., 2021).

Secondly, under suboptimal O_3 dosing, the action of excessive molecular O_3 and hydroxyl radicals formed during decomposition can result in harmful ozonation by-products, particularly in seawater, which contains higher concentration of bromide ions (Legube, 2003; Stiller et al., 2020).

So far, no studies have reported the performance of industrial protein skimmers under commercial conditions or quantified central operational conditions, such as HRT and O_3 dosing. The aim of the study was to evaluate the performance of two different protein skimmers during full-scale operation in a seawater RAS growing Atlantic salmon (*Salmo salar*). The study included replicated tests of two different HRTs (1.8 and 2.2 min) and three levels of O_3 doses (0, 7, and 14 g O_3/ kg feed) using several relevant water quality parameters to quantify the treatment efficiency.

2. Materials and methods

2.1. Experimental setup

The experimental setup consisted of a multi-factorial (2 × 2 × 3) design with two types of commercial scale protein skimmers: S1 and S2 (see detailed description in Section 2.1.2); two levels of HRT: 1.8 min (Low) and 2.2 min (High); and three levels of O_3 doses: 0 (T0), 7 (T7) and 14 (T14) g O_3/kg feed. The design included four sets of trials, where each trial involved operating one protein skimmer at a particular HRT and performing three separate runs with the different O_3 doses (Fig. 1). Each trial was replicated three times (n = 3) and one trial replication was completed daily, resulting in a total experimental period of twelve days. The selection of O_3 doses and HRT levels was grounded in a synthesis of previous studies and recommendations from both skimmer manufacturers and experienced operators in commercial scale RAS operational contexts.

2.1.1. Recirculation system

The experiment was carried out at Danish Salmon A/S, Hirtshals, Denmark.

The two types of protein skimmer were connected to the water treatment system of a 7000 m^3 full-strength saline grow-out facility with 0.1–4 kg Atlantic salmon. The yearly fish production volume of the particular grow-out facility was 500 tons.

The water treatment system in the grow-out facility consisted of four drum filters (Hydrotech HDF 2007–2 S, Hydrotech AB, Sweden) with 80 μm mesh size, two rows of 90 m^3 degassers/trickling filters, two rows of three aerated 95 m^3 serial fixed bed biofilter followed by a 110 m^3 unaerated fixed bed biofilter considered as a microparticle-filter, UV filters (MR8 320 PP, Ultra Aqua A/S, Denmark), oxygen cones, pH compensation unit using sodium hydroxide (NaOH), an O_3 generator and the two protein skimmers (Fig. 2). Throughout the trials, the grow-out facility maintained average standing biomass of 234 ± 25 tons with a daily feeding rate of 1.0 ± 0.4% of body weight and a daily water exchange rate of 11.5 ± 2.1% of the total system water volume. The intensity of recirculation and feed loading was 3 ± 0.8 kg feed/m^3 of make-up-water. The feed (4.5–7 mm EFICO Enviro 940, Biomar A/S, Denmark), which had 38–44% protein content, was fed continuously by automatic feeders from 8:00 a.m. to 1:00 a.m. Throughout the experiment, the water treatment system maintained a pH of 7.2 ± 0.1, a temperature of 12.4 ± 0.3 °C, and a salinity of 34 ± 1 ppt, while total ammonia nitrogen averaged at 0.7 ± 0.2 mg/L, nitrite nitrogen at 0.7 ± 0.4 mg/L and nitrate nitrogen at 114 ± 28 mg/L.

2.1.2. Protein skimmers

The two types of protein skimmer (Fig. 3), S1 (Ratz 2500 Hi, CM Aqua Technologies Aps, Denmark) and S2 (Helgoland 2500 ×4500 LE-315, Erwin Sander Elektroapparatebau GmbH, Germany), were both venturi types and had a counter-current flow pattern. S1 was equipped with seven bubble injection units, with five of them being powered by five 0.7 kW pumps (Badu 21–40/53 AK, SPECK Pumpen Verkaufsgesellschaft GmbH, Germany), and the remaining two by a single 2.2 kW pump (DM 15 PP, DEBEM srl, Italy). In contrast, S2 had two injection units that were powered by two 3 kW pumps (Badu 21–60/45 AK, SPECK Pumpen Verkaufsgesellschaft GmbH, Germany). Furthermore, in S1, five out of the seven injection units had the suction tube connected to the foam chamber, allowing a fraction of the degassed air and O_3 to be recycled. Meanwhile, S2 received only freshly supplied gases. The total capacity of the reaction chamber was approx. 17.5 m^3 in S1 and 17 m^3 in S2.

2.1.3. Ozone generator

O_3 was generated with the corona discharge method (Primozone GM48 2.0, Primozone Production AB, Sweden). Pure oxygen blended with 2% nitrogen was used as the feed gas to supply O_3 at a

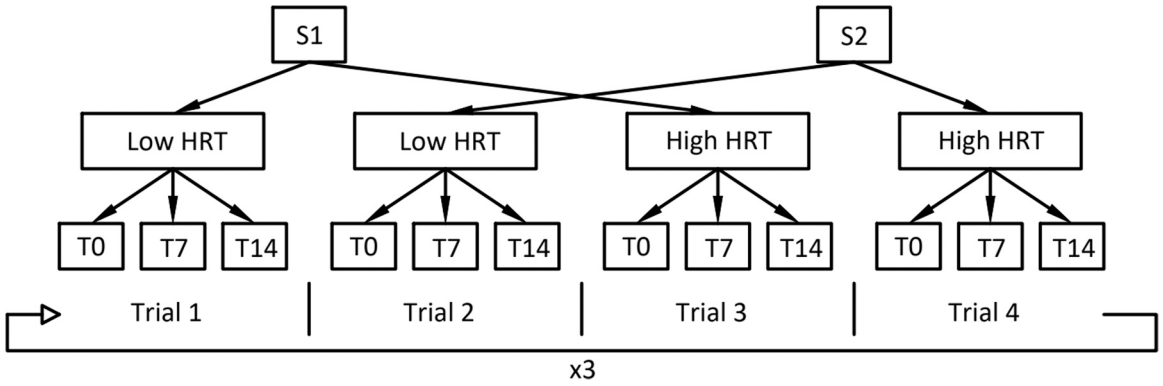


Fig. 1. Schematic diagram illustrating the combination of experimental factors, levels and workflow. The treatment combinations included two types of protein skimmers (S1 and S2), two levels of hydraulic retention times (HRT) (1.8 and 2.2 min as Low and High) and three O_3 doses (0, 7 and 14 g O_3/kg feed as T0, T7 and T14).

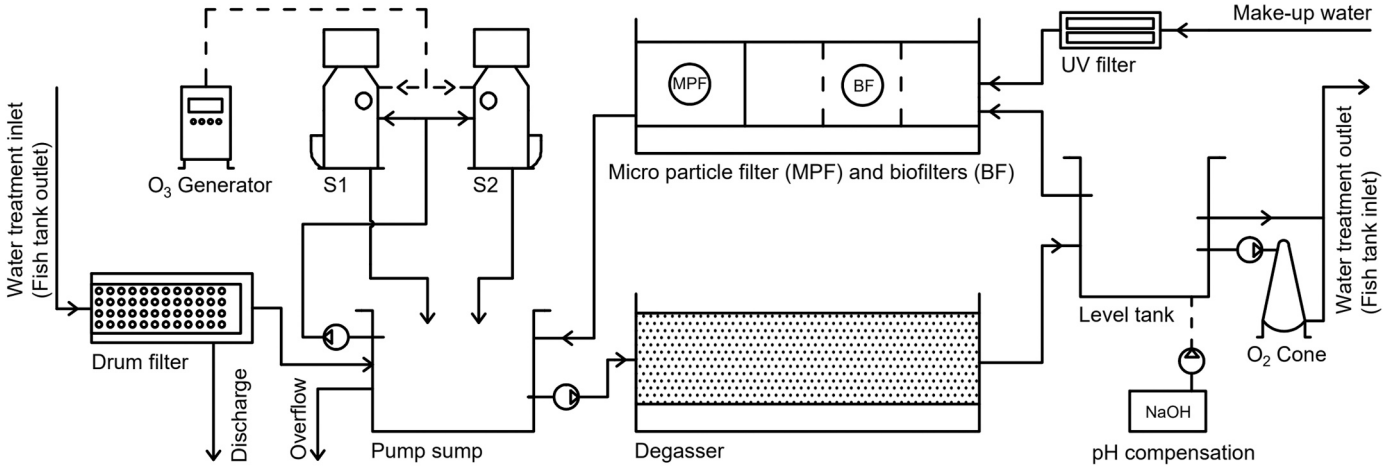


Fig. 2. Process flow diagram and main components of the water treatment system.

concentration of 200 g/Nm^3 and a pressure of 2.5 bar. The gas was delivered to the protein skimmer through ozone-resistant polytetrafluoroethylene hoses. The incoming O_3 concentration on the protein skimmer side was measured (Ozone Analyzer BMT 964 BT, BMT Messtechnik Gmbh, Germany) at the beginning of the experiment to account for potential decomposition.

2.2. Operational procedures for the experiments

Each trial followed the same standard protocol. The protein skimmer not subjected to analysis was bypassed/turned off prior to each test, allowing all the water and O_3 to reach the tested protein skimmer. Then the overflow height was set to the centre of the cone of the reaction chamber based on the manufacturer's recommendations and the operator's observations of optimal foam production. The water flow required to achieve the desired HRT was then set by adjusting the frequency of the supply pump. A water flow meter (TF-100 P Ultrasonic Flow Meter, Tofting, Denmark) clamped to the inlet pipe was used for continuous flow rate monitoring. Following the water flow adjustments, the airflow rate was set using a 1:1.3 water volume (m^3) to airflow (m^3/h) ratio, based on S1, as this protein skimmer had no built-in airflow meter. Instead, airflow in S1 was measured at the beginning of the experiment with an airflow meter (TSI 4000 Mass Flow Meter, TSI Inc, USA). After adjusting the physical parameters, O_3 doses were calculated based on the previous day's feed amount and were adjusted directly on the generator. The primary operational parameters of both protein skimmers are listed in Table 1.

During each trial, three consecutive, separate runs with a different O_3 dose, followed the sequence of 1) a 30 min stabilization time; 2) a 20 min sampling and analysis; and 3) a 10 min readjustment of the O_3 dose for the next run. The experiments were performed at the same time of the day, with sampling times scheduled at 08:30, 10:00 and 11:30 for the first, second and third run. Days when farming operations deviated from normal, i.e., harvest of fish or biofilter cleaning, were excluded to avoid potential uncontrolled changes in water quality.

2.3. Water sampling and analysis

Within the sampling period of a trial run, two sets of water samples were collected; a 0.3 L grab water sample and a 2.5 L pooled water sample (see below) both from the inlet and the outlet of the protein skimmer tested.

The 0.3 L grab samples were promptly examined for bacterial activity based on the BactiQuant assay (Mycometer A/S, Denmark), and the results were expressed as standardized BactiQuant value (BQV). Additionally, the samples were tested for total residual oxidant (TRO) concentration using the N, N-diethyl-p-phenylenediamine (DPD) colorimetric method (DPD Total Chlorine Reagent Powder Pillow, Hach Lange, USA). This method detects O_3 and potential harmful ozonation by-products (chlorine, bromine, iodine species) considered O_3 produced oxidants (OPO), as described by Schroeder et al. (2011). The method has a detection range of 0.02–2.00 mg/L Cl_2).

The 2.5 L pooled samples were stored on ice until transported to DTU Aqua's laboratory at the end of each trial. First, the samples were

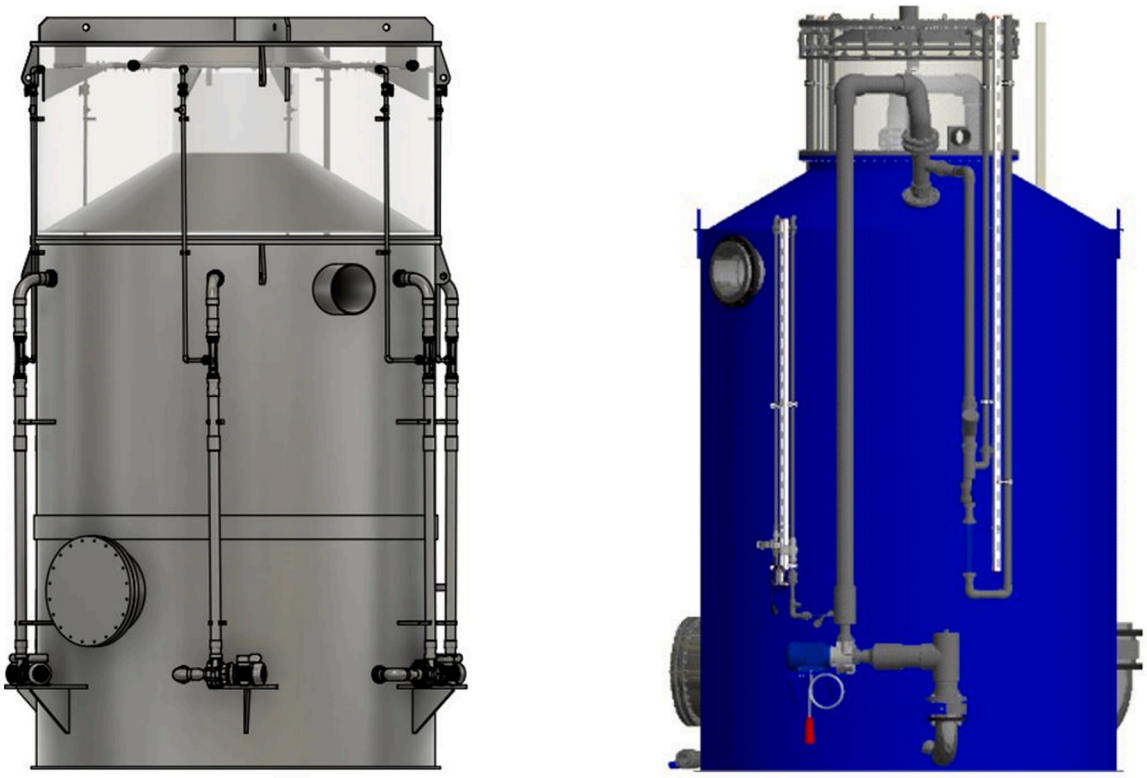


Fig. 3. The two types of protein skimmer models tested: S1 (Ratz 2500 Hi, CM Aqua Technologies Aps, Denmark) on the left and S2 (Helgoland 2500 x 4500 LE-315, Erwin Sander Elektroapparatebau GmbH, Germany) on the right.

Table 1
Specifications and primary operational parameters of the two types of protein skimmer (S1 = Ratz 2500 Hi, CM Aqua Technologies Aps, Denmark; S2 = Helgoland 2500 ×4500 LE-315, Erwin Sander Elektroapparatebau GmbH, Germany) during the experiments.

Parameters	***S1***	***S2***
Water volume (m^3)	17.2	16.7
Water flow (m^3/h)		
Low HRT (1.8 min)	573 ± 5	556 ± 5
High HRT (2.2 min)	469 ± 5	455 ± 5
O_3 dose (g/h) (doses varied based on feeding)		
T0	0	0
T7	390–520	390–520
T14	780–1040	780–1040
Air flow rate (m^3/h)	23 ± 2	22 ± 2

analysed for water clarity by UVT (UV Spectrophotometer DU-520, Bechman Coulter Inc, USA) and turbidity (Hach 2100Q Portable Turbidimeter, Hach Lange, USA). After that, particle size distribution (PSD) analysis was conducted using a particle counter (Multisizer 4e Coulter Counter, Bechman Coulter Inc, USA). The device detected particle numbers (#/mL), volumes (mm^3/mL) and surface areas (mm^2/mL) in the 1–12 μm particle size range.

During the trials redox potential (ORP) was continuously monitored at the inlet and outlet using ORP handy probes (OxyGuard Portable pH/Redox Meter, OxyGuard International A/S, Denmark).

2.4. Data analysis

2.4.1. Calculations

The differences between the inlet and the outlet of the investigated water quality parameters were evaluated based on relative efficiencies (%). For the decreasing turbidity, PSD and bacterial activity, one-pass removal efficiencies (RE) were calculated using Eq. (1). For the increasing UVT, ORP and TRO, one-pass enhancement efficiencies (EE) were calculated using Eq. (2).

$$\%\ \text{Removal } \textit{efficiency}(\text{RE}) = \left(\frac{\text{Vi} - \text{Vo}}{\text{Vi}}\right) \times 100 \quad (1)$$

$$\%\ \text{Enhancement } \textit{efficiency}(\text{EE}) = \left(\frac{\text{Vo}}{\text{Vi}}\right) \times 100\text{–}100 \quad (2)$$

Where RE: removal efficiency (%); V_i: inlet value (FNU, #/mL, μm^3/mL, μm^2/mL, BQV, mV, mg/L, %); V_o: outlet value (FNU, #/mL, μm^3/mL, μm^2/mL, BQV, mV, mg/L, %); EE: enhancement efficiency (%).

2.4.2. Statistical analysis

The data were tested for homogeneity of variances and normality by the Levene's and the Shapiro-Wilk tests. Three-way ANOVA was used to compare the main and interaction effects of the protein skimmer, HRT and O_3 dose on the relative efficiencies. Following that, a secondary normality test on the residuals evaluated the model. When the ANOVA revealed statistical significance ($p < 0.05$), Tukey's test was conducted for multiple comparisons. To examine the size-based removal efficiencies, the data were merged based on HRT, as HRT had no significant effect on particle counts. Outliers were removed according to the interquartile ranges. As the merged data did not pass the homogeneity test, the non-parametric Kruskal-Wallis test was used to explore the effect of particle size on the relative efficiencies. Finally, the relationship between relative efficiencies of water quality parameters was assessed by Pearson's correlation. All statistical analysis was carried out using R v.4.1.2 and R Studio (RStudio Inc, USA).

3. Results

3.1. Effect of operational variables

The main effects and significant interactions of operational variables

extracted from the three-way ANOVA are shown in Table 2. Average inlet and outlet values of turbidity, UVT, PSD, bacterial activity, ORP and TRO are given in Supplementary Table A.1 and Table A.2.

3.1.1. Water clarity

The type of protein skimmers and HRT did not significantly affect turbidity or UVT in a single pass measurement ($p > 0.05$) (Table 2). High dose of O_3, on the other hand, caused significant improvements in turbidity compared to the absence of O_3 ($p = 0.01$). Average turbidity decreased by 1.2 ± 6.1, 2.7 ± 5.8 and 13.7 ± 3.1%, in T0, T7 and T14, respectively (Fig. 4a). UVT in the outlet improved compared to the inlet across all treatments by an average of 0.7 ± 0.5% (Fig. 4b).

3.1.2. Particle size distribution

The results obtained from the PSD measurement showed an exponentially decreasing curve with a peak at the lowest 1–2 μm size range, representing > 95% of the total particle number (Supplementary Fig. A.1).

The removal of particle numbers, particle volumes and particle surface area increased significantly with increasing O_3 doses ($p < 0.001$) (Table 2). Simultaneously, significant removal of particle volumes was observed with increasing HRT ($p = 0.03$). The types of protein skimmer had no significant effect ($p > 0.05$). The highest reduction was observed in particle numbers by an average of 2.6 ± 3.4, 37.7 ± 3.6 and 51.8 ± 4.5% in T0, T7 and T14, respectively (Fig. 5a). A lower but identical pattern was demonstrated by surface area, which reduced by 3.4 ± 3.8, 28.2 ± 4.1 and 41.8 ± 5.6% in T0, T7 and T14, respectively (Fig. 5b). Finally, the lowest removal in particle volumes yielded 4.2 ± 3.9, 18.2 ± 4.7 and 33.6 ± 7% on low HRT, and 4.5 ± 7.2, 27.3 ± 6.5 and 38.5 ± 4.9% on high HRT, in T0, T7 and T14, respectively (Fig. 5c).

The size of the particles had a significant impact ($p = 0.02$) on the removal of particle numbers using both S1 (Fig. 6a) and S2 (Fig. 6b). Without O_3, the numbers of particles in the 1–6 μm particle fractions reduced by 3.2 ± 1.3% with S1 and 4.0 ± 5.9% with S2. The removal increased for larger particles to 5.1 ± 19.2% for S1 and 14.7 ± 8.9% for S2 in the 6–9 μm size range, and 10.8 ± 20.5% for S1 and 8.7 ± 30.8% for S2 in the 9–12 μm size range. With O_3, the removal increased in each size range. The highest decrease was observed in the most abundant 1–2 μm particle ranges, yielding 44.1 ± 4.3 and 52.5 ± 6.3% with S1 and 41.7 ± 1.7 and 59.2 ± 2.5% with S2, in T7 and T14, respectively.

3.1.3. Bacterial activity

The bacterial activity was not affected by the type of protein skimmer, or the HRT tested ($p > 0.05$) (Table 2). However, O_3 caused significant bacteria inactivation with increasing O_3 doses ($p < 0.001$). Bacterial inactivation (expressed as removal efficiencies in %) during a single pass were 4.1 ± 9.3, 28.9 ± 7.6 and 60.3 ± 8.2%, in T0, T7 and T14, respectively (Fig. 7).

3.1.4. Redox potential

O_3 significantly increased ORP which directly related to the doses ($p < 0.001$) (Table 2). Following that, there was a significant increase with increasing HRT ($p = 0.01$). Finally, the effect of the protein skimmer demonstrated significantly higher performance with S1 than S2 ($p = 0.01$). Aside from the main factors, an interaction effect between HRT and protein skimmer indicated that HRT had significantly less impact on S1 than S2 ($p = 0.02$) (Fig. 8a). The average ORP increase with S1 was 84.3 ± 12.7, 155 ± 11 and 219 ± 7% at low HRT and 83.7 ± 9.6, 156 ± 6 and 230 ± 12% at high HRT, in T0, T7 and T14, respectively (Fig. 8b). Simultaneously, ORP in S2 increased by 77 ± 4.6, 110 ± 18 and 183 ± 30% at low HRT and 95.3 ± 17, 140 ± 28 and 226 ± 18% at high HRT, in T0, T7 and T14, respectively.

Table 2
Three-way ANOVA summary table based on the effect of operational variables on the relative efficiencies of the investigated water quality parameters. The treatment combinations included two types of protein skimmer (PS) = S1 and S2; two levels of hydraulic retention times (HRT) = 1.8 and 2.2 min as Low and High; and three O_3 doses = 0, 7 and 14 g O_3/kg feed as T0, T7 and T14.

Parameters (RE, EE (%))	*Variables*	*SS*	*F*	*p*	*Tukey HSD*
Turbidity	PS	57.5	0.45	0.51	
	HRT	14.3	0.11	0.74	
	O_3 dose	1433	5.58	0.01 *	T0 < T14
Ultraviolet transmittance (UVT)	PS	0.0	0.11	0.75	
	HRT	0.2	1.21	0.28	
	O_3 dose	0.4	1.21	0.32	
P. number	PS	2.6	0.13	0.72	
	HRT	4.1	0.22	0.65	
	O_3 dose	1.5E+ 04	386	< 0.001 *	T0 < T7 < T14
P. volume	PS	7.8	0.19	0.67	
	HRT	204	5.06	0.03 *	Low < High
	O_3 dose	6097	75.6	< 0.001 *	C < T7 < T14
P. surf. area	PS	0.1	0	0.94	
	HRT	55.1	2.59	0.12	
	O_3 dose	9090	214	< 0.001 *	T0 < T7 < T14
Bac. activity	PS	0.8	0.01	0.92	
	HRT	108	1.42	0.25	
	O_3 dose	1.9E+ 04	125	< 0.001 *	T0 < T7 < T14
Redox potential (ORP)	PS	2336	8.66	0.01 *	S1 > S2
	HRT	2601	9.65	0.01 *	Low < High
	O_3 dose	1.0E+ 05	187	< 0.001 *	T0 < T7 < T14
	PS × HRT	1573	5.83	0.02 *	
Total residual oxidants (TRO)	PS	3750	0.05	0.83	
	HRT	4.0e+ 05	5.48	0.03 *	Low < High
	O_3 dose	4.3e+ 06	59.28	< 0.001 *	T0 < T7 < T14

*Statistical significance at $p < 0.05$.

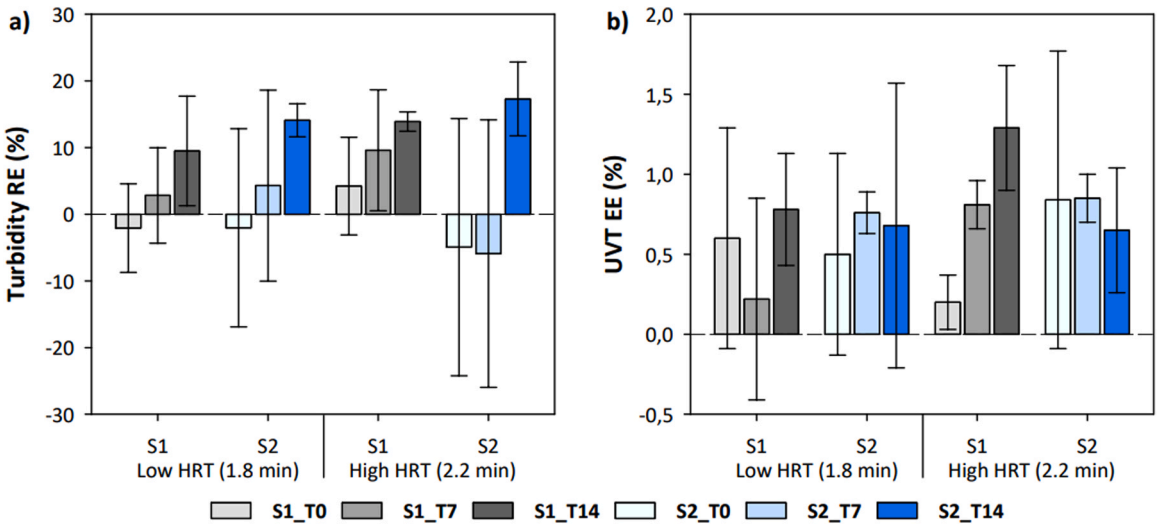


Fig. 4. Mean ± sd (n = 3) one-pass **a)** turbidity removal efficiencies (RE) and **b)** ultraviolet transmittance (UVT) enhancement efficiencies (EE). The treatment combinations included two types of protein skimmers (S1 and S2), two levels of hydraulic retention times (HRT) (1.8 and 2.2 min as Low and High) and three O_3 doses (0, 7 and 14 g O_3/kg feed as T0, T7 and T14).

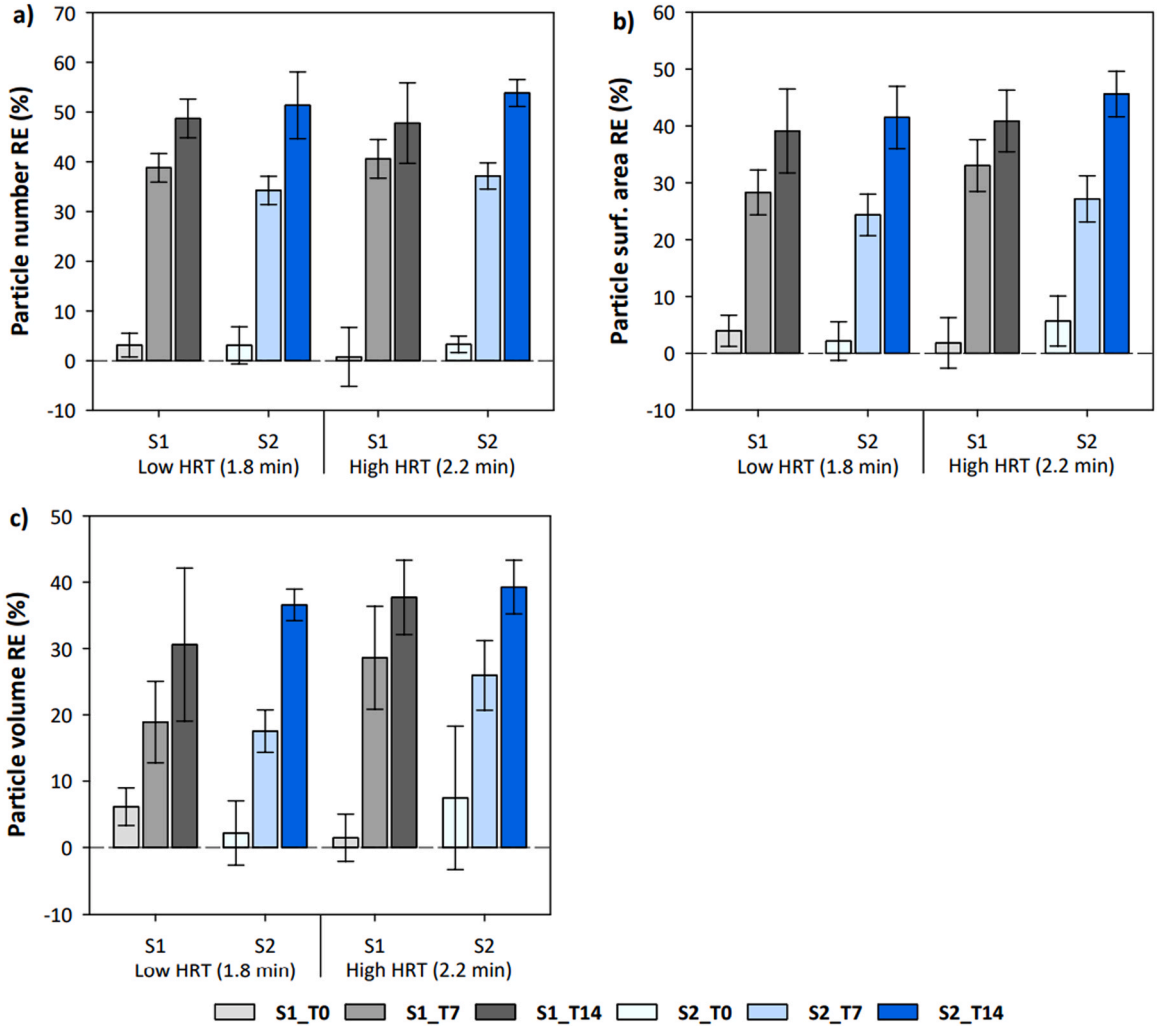


Fig. 5. Mean ± sd (n = 3) one-pass removal efficiencies (RE) of total **a)** particle number, **b)** surface area and **c)** volume. The treatment combinations included two types of protein skimmers (S1 and S2), two levels of hydraulic retention times (HRT) (1.8 and 2.2 min as Low and High) and three O_3 doses (0, 7 and 14 g O_3/kg feed as T0, T7 and T14).

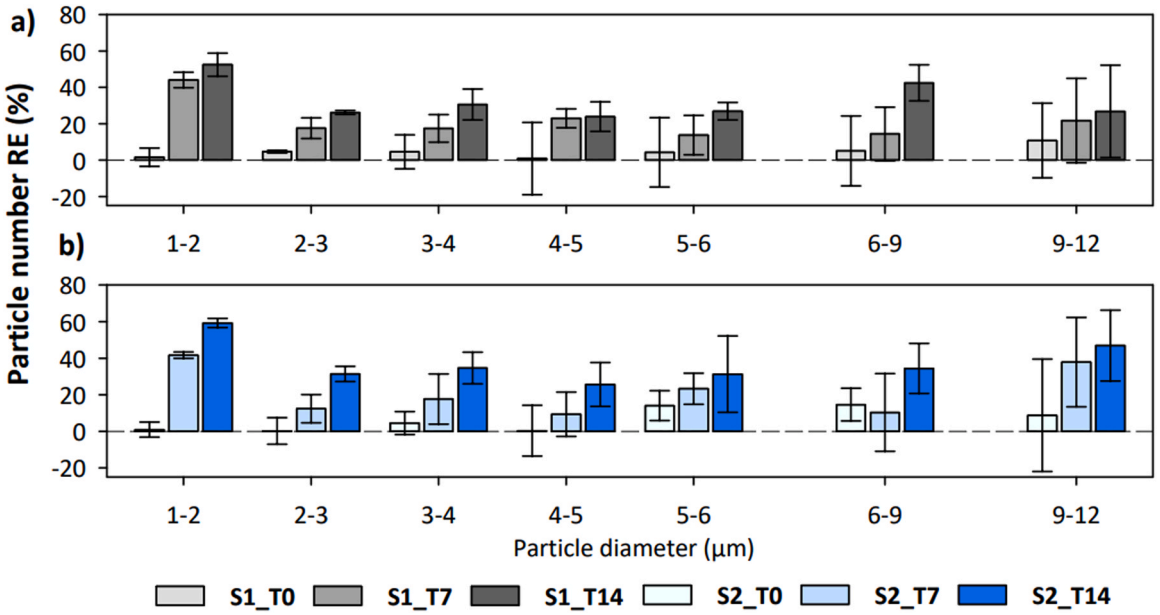


Fig. 6. Mean ± sd (n = 3) size-based removal efficiencies (RE) through one-pass with two types of protein skimmers **a)** S1 and **b)** S2, based on merged data. The treatment combinations included three O_3 doses (0, 7 and 14 g O_3/kg feed as T0, T7 and T14).

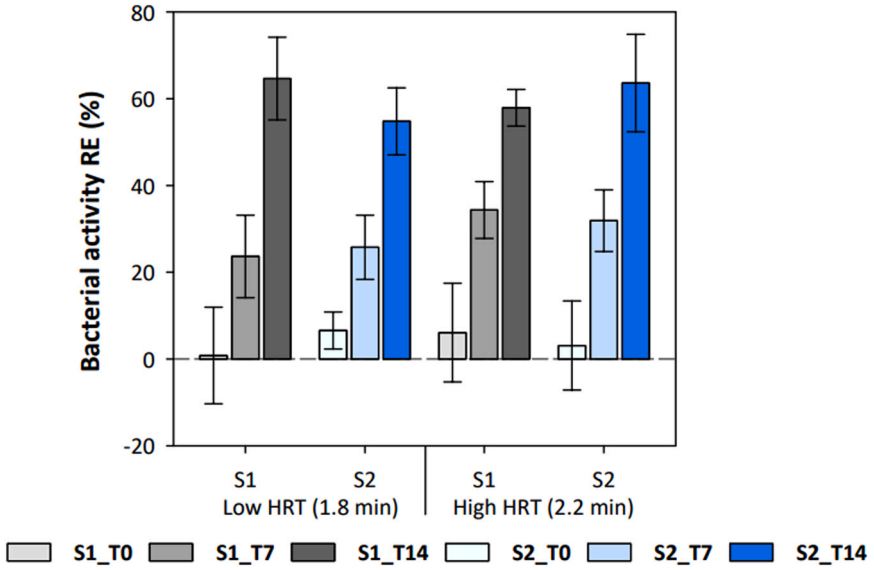


Fig. 7. Mean ± sd (n = 3) of bacterial activity removal efficiencies (RE) based on the BactiQuant assay. The treatment combinations included two types of protein skimmers (S1 and S2), two levels of hydraulic retention times (HRT) (1.8 and 2.2 min as Low and High) and three O_3 doses (0, 7 and 14 g O_3/kg feed as T0, T7 and T14).

3.1.5. Total residual oxidants

Without O_3 the outlet TRO measurements were similar to the inlet in both protein skimmer. However, the addition of O_3 led to a significant TRO increase ($p < 0.001$) (Table 2). Furthermore, HRT was responsible for a minor but significant efficiency increase with increasing HRT ($p = 0.03$). The type of protein skimmer had no significant effect ($p > 0.05$). TRO increased by 267 ± 39 and 1017 ± 234% on low HRT, and 425 ± 133 and 1375 ± 432% on high HRT, in T7 and T14, respectively (Fig. 9).

3.2. Water quality relations

Pearson's correlation matrix was employed to examine the relations between the relative efficiencies of water quality parameters; the results indicated strong association between most metrics. The specific relations of the relative efficiencies of water quality parameters are listed in Table 3.

4. Discussion

The study showed that O_3 had the highest impact on enhancing water quality in commercial seawater RAS through protein skimming. The types of protein skimmer design and the HRT tested had only minor effect. As the study was conducted on full-scale, it offers valuable insights into the real-world implications of protein skimming in the aquaculture industry, without the scaling effects often present in laboratory experiments (i.e., Guilherme et al., 2020; Jafari et al., 2022).

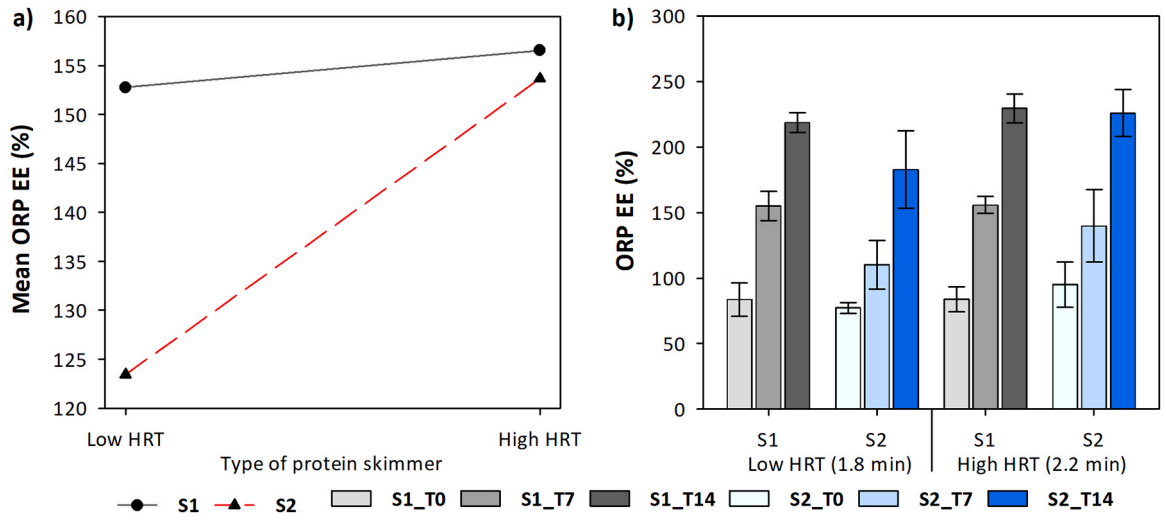


Fig. 8. a) Interaction effect of the protein skimmer type and hydraulic retention time (HRT) on the mean redox potential (ORP) enhancement efficiency (EE) and **b)** mean ± sd (n = 3) of ORP EEs. The treatment combinations included two types of protein skimmers (S1 and S2), two levels of HRT (1.8 and 2.2 min as Low and High) and three O_3 doses (0, 7 and 14 g O_3/kg feed as T0, T7 and T14).

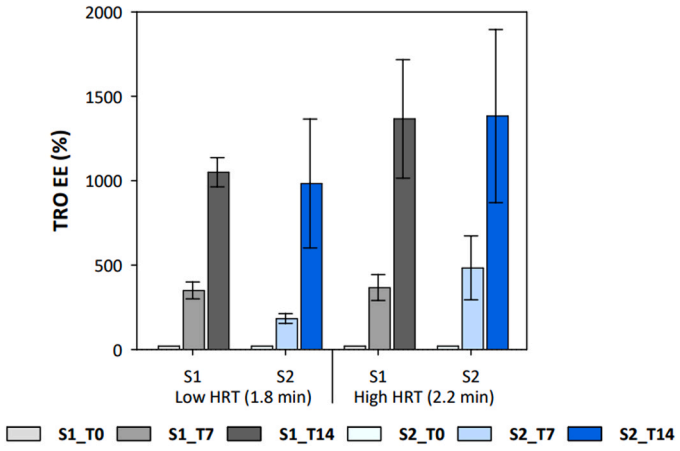


Fig. 9. Mean ± sd (n = 3) of total residual oxidants (TRO) enhancement efficiencies (EE). The treatment combinations included two types of protein skimmers (S1 and S2), two levels of hydraulic retention times (HRT) (1.8 and 2.2 min as Low and High) and three O_3 doses (0, 7 and 14 g O_3/kg feed as T0, T7 and T14).

4.1. *Effect of protein skimmer design*

The two types of protein skimmer were found to have almost identical performance across multiple evaluated water quality parameters, likely attributed to similar configuration, including flow pattern and dimensions. ORP was the only parameter affected by protein skimmer design, which was higher in S1 compared to S2. ORP is a measure that reflects the "oxidation power" of the water (Gonçalves and Gagnon, 2011); hence an increased ORP indicates a higher concentration of O_3 and O_3 produced oxidative radicals inside the protein skimmer. The reason why S1 led to higher ORPs than S2 is due to its gas recycling system, which turns a portion of the degassed air and O_3 from the foam chamber back to the reaction chamber. In contrast, S2 was only supplied with new gases. Potential consequences of gas recycling such as the reintroduction of stripped gasses like carbon dioxide, nitrogen, and ammonia, were not investigated in the current study.

While O_3 utilisation is an important attribute, particularly from an economic perspective, the higher ORP with S1 did not relate to significant improvements in other water quality metrics. Therefore, the protein skimmer performance under the given conditions can be considered equally effective in both S1 and S2.

Ensuring optimal protein skimmer performance in RAS under fluctuating water conditions requires a combination of adjusting skimmer settings, monitoring and automation, and adopting appropriate feeding and RAS management strategies. The specific approach will depend on the aquaculture system's goals, the species being cultured, and the available resources and technology.

4.2. *Effect of hydraulic retention time*

HRT is an important operational factor when it comes to protein skimming. Longer HRT increases the contact time between gas and water, boosting surfactant extraction (Wheaton et al., 1979; Buckley et al., 2021). However, some studies have reported no discernible or even negative long-term effect of greater HRTs on solid removal (Weeks et al., 1992; Peng et al., 2003).

4.2.1. *Effects of hydraulic retention time on particle size distribution*

By increasing HRT from 1.8 to 2.2 min, a moderate improvement

Table 3
Pearson's correlation matrix with correlation coefficients between relative efficiencies of the investigated water quality parameters. Stars represent statistically significant differences at p = 0–0.001 '* ** ', p = 0.001–0.01 '* *' and p = 0.01–0.05 '* '.

Turbidity							
0.32	**UVT**						
0.48 * *	0.27	**P. num.**					
0.47 * *	0.34 *	0.91 * **	**P. vol.**				
0.53 * *	0.31	0.98 * **	0.96 * **	**P. s.a.**			
0.54 * *	0.25	0.87 * **	0.86 * **	0.89 * **	**Bac. act.**		
0.49 * *	0.26	0.87 * **	0.86 * **	0.90 * **	0.89 * **	**ORP**	
0.48 * *	0.21	0.83 * **	0.79 * **	0.83 * **	0.90 * **	0.91 * **	**TRO**

was observed in the reduction of total particle volume. Given that most particles were small (1–2 μm) and had low volume, the increase in volumetric removal, but not in numeric suggests that higher HRT assisted in the removal of larger particles. This could be because a longer contact time at the liquid-gas interface enhances the possibility of the particles being caught in the bubbles, (Wheaton et al., 1979) or enhances the possibility of oxidation and disinfection (Summerfelt, 2003). However, due to the scarcity of particles larger than 2 μm, the moderate effect might be attributed to the occasional presence of a few large particles that randomly entered the samples. Considering the uncertain effects, running on high HRT is potentially offset by the higher daily water turnover when running on low HRT.

4.2.2. Effects of hydraulic retention time on redox potential and total residual oxidants

A higher HRT significantly increased the ORP in S2 while the TRO levels increased in both protein skimmers. This is because prolonging the contact time exposes the same water to a larger volume of O_3, increasing the oxidation potential and allowing for more by-product formation (Summerfelt, 2003). Our study did not investigate the potential formation of THM or halogenated bromates, which is a potential risk when ozone is overdosed in saltwater (Legube, 2003). In addition, a strong interaction effect between protein skimmer and HRT revealed that changes in ORP were greater with S2 than S1, which already levelled on low HRT. This suggests that, in the current system, the actual dissolved O_3 concentration approached a near saturation point at the highest average ORP level of 500 mV (Summerfelt and Hochheimer, 1997).

4.3. Effect of ozone dose

Ozone is a strong oxidizing agent, which by coupling with protein skimming, can improve the decomposition of organic compounds and the breakdown of complex molecules into fragments that are more likely to be removed with the foam (Lekang, 2019).

4.3.1. Effects of ozone on water clarity

While O_3 improved the turbidity and increased the UVT, the results were inconsistent. Part of this inconsistency is due to the accuracy of measurements and the minute changes during a single passage. Additionally, the RAS water was relative clean due to permanent ozonation and protein skimming prior to and during the sampling. Different studies reported similar observations about turbidity when using O_3 alone, and the explanations have been related to micro flocculation and the resulting change in particle concentration and size (Tango and Gagnon, 2003; Park et al., 2013). Due to the limited size range of current PSD analysis, the assumption of flocculation could not be confirmed. Still, the inconsistency of both metrics was most likely related to the initially high UVT and low turbidity paired with the limited accuracy of the methods, making it difficult to obtain a clear pattern through a single passage. Even though the relative efficiencies of turbidity and UVT have not been documented in a comparative context, the increased water clarity when protein skimming is combined with O_3 is consistent with observations of de Jesus Gregersen et al. (2021) during long-term operation.

4.3.2. Effects of ozone on microparticles

The PSD analysis showed limited effectiveness of the protein skimmer in the absence of O_3 in reducing the particle number, volume, and surface area. The highest removal was demonstrated by particle volumes, indicating that protein skimming alone was more efficient at removing larger (6–12 μm) particles than smaller ones. This was supported by the size-based investigation, which revealed a gradual increase in removal efficiencies from 1 to 12 μm. Simultaneously, with O_3, the total removal increased significantly in all parameters, and the highest removal shifted from the large to the small (1–2 μm) size ranges. Based on this, it is hypothesised that, while small particles were difficult to remove by protein skimming alone, the exposure to O_3 caused their direct decomposition or transformation into a surface-active state that was readily caught by the bubbles (Lekang, 2019). Removal efficiencies of PSD metrics were significantly higher in T14 than T0 and T7; however, doubling the dose approximated only 1.3-, 1.5- and 1.6-fold increase in the removal of total particle number, surface area and volume, respectively. This suggests that the effect of doubling the dose could not be maximised in the 1–12 μm range, perhaps due to the low inlet concentrations and the low O_3 demand of the particles (Spiliotopoulou et al., 2018). This trend was somewhat consistent with the findings of Park et al. (2011), who found that increasing the O_3 dose from 20 to 40 g O_3/kg feed in a protein skimmer, increased the removal of suspended solids, volatile suspended solids, and dissolved organic carbon by only 1.1-, 1.2-, and 1.1-fold, respectively.

4.3.3. Effects of ozone on bacterial inactivation

Bacterial activity was not consistently reduced during a single passage through the protein skimmers without O_3. However, significant bacterial inactivation was observed with O_3, and the removal almost doubled with doubling the O_3 dose. Research from the wastewater industry has shown that most free-living bacteria are hydrophilic with low adhesion properties at the liquid-gas interface (Zita and Hermansson, 1997), making them difficult to remove with protein skimming. However, the addition of O_3 can destroy cell membranes and the cell's nucleic acids (Summerfelt, 2003; Sharrer and Summerfelt, 2007). The observations substantiate those of Guilherme et al. (2020), who discovered limited microalgae removal with protein skimmer alone, but found complete elimination with O_3 and enhanced removal efficiencies with increasing doses. Furthermore, a previous study by de Jesus Gregersen et al. (2020) demonstrated that a significant portion of the particulate organic matter and microparticles in RAS consists of living microorganisms which can be removed through disinfection, as observed in this study.

4.3.4. Effects of ozone on redox potential and total residual oxidants

ORP and TRO concentrations were significantly correlated to the O_3 doses, and the TRO concentration nearly tripled with increasing doses (Supplementary Table A.1, A.2), reflecting a relative overdose. One of the main risks of O_3 overdosing in seawater is the formation of bromines as the main by-products and its high toxicity to aquatic organisms (Gonçalves and Gagnon, 2011). The by-product formation is influenced by the O_3 demand of organic matter in the system, O_3 dose, water matrix, and contact time (Legube, 2003; Spiliotopoulou et al., 2018). Previous studies have suggested O_3 doses between 3 and 24 g O_3/kg feed or an ORP of less than 300 mV in freshwater and 400 mV in seawater (Powell and Scolding, 2018). The toxicity of O_3 in aquaculture can vary between species, life stage, exposure length, and water matrix. Out of the few studies investigating the topic, Stiller et al. (2020) specified a safety limit for post-smolt Atlantic salmon in brackish water at an ORP of 350 mV, corresponding to a TRO concentration of 0.01 mg Cl_2/L. In the present study, the direction and magnitude of change in TRO and ORP levels were highly correlated. Outlet ORP levels of 361.3 mV and 477.3 mV equalled a TRO concentration of 0.09 and 0.27 mg Cl_2/L in T7 and T14, respectively (Supplementary Table A.1, A.2). The different magnitude of the ORP-TRO relation between this study and Stiller et al. (2020) indicated that the current system's O_3 demand was modest, and once reaching the limit, the extra O_3 reacted so rapidly to form by-products that the TRO concentration could not be maintained (Tango and Gagnon, 2003). Fortunately, the overall system TRO never surpassed 0.02 mg Cl_2/L (lowest detectable limit of the DPD kit) even while operated continuously at 400–430 mV. The explanation for the low system TRO is that the outlet water from the protein skimmers was mixed with the water in the pump sump and went through subsequent treatment units before entering the culture tanks (Fig. 2). Indeed, biofilters can play an important role in the degradation of various oxidants (Pedersen et al., 2006, 2015; Schroeder et al., 2015). Nevertheless, the

risk of prolonged exposure of the system to doses as high as T14 may outweigh the few water quality benefits, both in terms of fish welfare, the disruption of nitrifying bacteria and costs related to O_3 generation (Spiliotopoulou et al., 2018).

4.4. Water quality relations

The turbidity correlated moderately with each metric except UVT. Turbidity describes the cloudiness of water caused by suspended solids, including microorganisms (Schumann and Brinker, 2020). Therefore, when particles and bacteria are removed, turbidity improves similarly.

All PSD metrics correlated significantly to the increase in ORP and TRO. On the one hand, this reveals the apparent effect of the O_3 transferred into the water. On the other hand, it reflects the O_3 demand of the system. When O_3 is introduced into the water, it reacts promptly with readily biodegradable compounds, causing O_3 decomposition (Spiliotopoulou et al., 2018). However, as the demand decreases, residual O_3 reacts with other compounds to form by-products (e.g., bromines in seawater), increasing the TRO concentration (Legube, 2003). The O_3 demand varies from system to system and is affected by feed loading, feed intensity, water exchange rate, water matrix and treatment units (Summerfelt et al., 2009). In the current RAS, the moderate increase in particle removal from T7 to T14 accompanied by a steep increase in TRO concentration indicated a low O_3 demand (Summerfelt et al., 2009; Davidson et al., 2011; Spiliotopoulou et al., 2018). The explanation for the low O_3 demand is that the current RAS was operating on full-scale for years with effective water treatment, keeping steady and low levels of particulate organic matter. Furthermore, the strong correlation between ORP and TRO suggests that besides established baselines, variations in ORP can serve as a safety measure for TRO concentration (Spiliotopoulou et al., 2018).

Bacterial activity was significantly correlated to the PSD metrics, suggesting that most of the removed particles in the 1–12 μm size range were bacteria. This also provides an explanation for the previously discussed low O_3 demand. Bacteria can exist in RAS as free-living or associated with microparticles or embedded in biofilm or bioflocs (Pedersen et al., 2017; Rojas-Tirado et al., 2019; de Jesus Gregersen et al., 2019). Given that the PSD analysis revealed a high abundance of particles between 1 and 2 μm, it is presumed that most particles were primarily free-living bacteria. Therefore, the PSD tendencies reflect a significant bacterial drive where low bacterial inactivation in the absence of O_3, due to limited surface activity (Zita and Hermansson, 1997), resulted in low particle removal. However, with the addition of O_3, bacterial removal increased promptly due to cell destruction (Summerfelt, 2003; Sharrer and Summerfelt, 2007; Guilherme et al., 2020), leading to a subsequent increase in particle removal. Although the magnitude of change between particles and bacterial activity regarding O_3 was different. While bacterial inactivation doubled from T7 to T14, particle removal only slightly increased. This demonstrates the presence of a significant amount of particle or biofilm bounded bacteria, potentially exceeding the 1–12 μm size range, that was only removed with the dose of T14. According to de Jesus Gregersen et al. (2019), bacterial activity in Danish Model Trout Farms is strongly correlated to particle surface area above 10 μm, suggesting that particles can serve as a substrate for bacteria to grow. Investigating particles in a more extensive size range (e.g., 1–80 μm) would be essential for further conclusions on the PSD and bacterial activity relations. Nevertheless, it is assumed that in an intensive commercial scale RAS microparticle concentrations are primarily bacteria-driven.

Inhibition of bacterial activity was found to significantly correlate with the TRO concentration. While molecular O_3 is quite selective in what it reacts with, ozonation by-products can react with a wide range of molecules and are considered strong oxidisers (Summerfelt and Hochheimer, 1997). Since many of the detected microparticles in the 1–12 μm range were bacteria, the system had a relatively low O_3 demand causing high by-product formation and consequent increase in TRO concentration. While molecular O_3 can inactivate bacteria, the disinfection credit depends on the TRO concentration and contact time. Hence, there was a direct linear correlation between the increase in TRO concentration and the bacterial inactivation. The high TRO, in turn, facilitated the bacterial inactivation. The effect of TRO on disinfection was described by Sugita et al. (1992), who reported 99% elimination of specific pathogens in sterile seawater when exposed to a TRO of 0.06–0.1 mg Cl_2/L for 1 min HRT. In the present study, using real RAS water, similar TRO concentration in T7 resulted in 28.9 ± 7.6% bacterial inactivation in a single passage. This reduction rate was much lower than the above mentioned; however, the current system was running effectively for years, throughout which the microbial community potentially stabilised with species that are more tolerant to O_3 (Schroeder et al., 2015; Aalto et al., 2022).

5. Conclusion

The current study evaluated and compared the performance of protein skimming in a commercial scale seawater RAS under multiple operational conditions. The O_3 dose was the primary factor that significantly affected several water quality metrics, but higher doses near the system's O_3 demand promoted TRO formation. Therefore, a dose of 7 g O_3/kg feed in the current system appeared to deliver enhanced performance without compromising production. Increasing HRT only affected the removal of particle volumes and O_3 related measurements. This indicates that the marginal benefits of running on the higher 2.2 min HRT may be offset by the increased daily water turnover achieved by the lower 1.8 min HRT. Lastly, protein skimmer design only affected the ORP levels, with no significant improvements in other water quality metrics. Consequently, both S1 and S2 can be deemed equally efficient.

Correlations between the investigated water quality parameters revealed a clear pattern of the ongoing processes and particle characteristics. Overall, the results suggest that protein skimming combined with moderate O_3 doses improves general water quality and controls critical factors such as bacterial activity and microparticles in commercial operations. Further investigations involving mass balances based on foamate will provide additional knowledge of the protein skimmer removal processes and its dependence on O_3.

CRediT authorship contribution statement

Bence Dániel Kovács - Formal analysis, Investigation, Methodology, Validation, Writing - original draft. **Kim João de Jesus Gregersen** - Conceptualization, Methodology, Supervision, Writing - review & editing. **Florian Rüppel** – Resources, Supervision, Writing - review & editing. **Arndt von Danwitz** – Resources, Supervision, Writing - review & editing. **Lars-Flemming Pedersen** - Conceptualization, Supervision, Writing – review and editing, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Lars-Flemming Pedersen reports financial support was provided by European Maritime and Fisheries Fund (EMFF). Lars-Flemming Pedersen reports financial support was provided by Danish Ministry of Food, Agriculture and Fisheries.

Data availability

No data was used for the research described in the article.

Acknowledgement

The study was funded by the European Maritime and Fisheries and

Aquaculture Fund (EMFAF) and the Danish Ministry of Food, Agriculture and Fisheries in the "ProBiOzon" project (J.no. 33111-I-21-076). We would like to extend our sincere gratitude to Danish Salmon A/S and its management team for their valuable assistance and cooperation, and for allowing us to conduct this study. We would also like to express our thanks to Philip Haargaard Gyldenkærne form CM Aqua Technologies Aps and Dr. Jaime Orellana from Erwin Sander Elektroapparatebau GmbH for their technical support an comments to the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aquaeng.2023.102369.

References

Aalto, S.L., Syropoulou, E., de Jesus Gregersen, K.J., Tiirola, M., Pedersen, P.B., Pedersen, L.F., 2022. Microbiome response to foam fractionation and ozonation in RAS. Aquaculture 550, 737846.
Ahmed, N., Turchini, G.M., 2021. Recirculating aquaculture systems (RAS): Environmental solution and climate change adaptation. J. Clean. Prod. 297, 126604.
Badiola, M., Mendiola, D., Bostock, J., 2012. Recirculating Aquaculture Systems (RAS) analysis: Main issues on management and future challenges. Aquac. Eng. 51, 26–35.
Barrut, B., Blancheton, J.P., Champagne, J.Y., Grasmick, A., 2012. Mass transfer efficiency of a vacuum airlift—Application to water recycling in aquaculture systems. Aquac. Eng. 46, 18–26.
Becke, C., Schumann, M., Steinhagen, D., Geist, J., Brinker, A., 2018. Physiological consequences of chronic exposure of rainbow trout (Oncorhynchus mykiss) to suspended solid load in recirculating aquaculture systems. Aquaculture 484, 228–241.
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. Aquac. Eng. 39 (1), 37–42.
Buckley, T., Xu, X., Rudolph, V., Firouzi, M., Shukla, P., 2021. Review of foam fractionation as a water treatment technology. Sep. Sci. Technol. 57, 929–958.
Chen, S., Timmons, M.B., Aneshansley, D.J., Bisogni Jr, J.J., 1993. Suspended solids characteristics from recirculating aquacultural systems and design implications. Aquaculture 112 (2–3), 143–155.
Davidson, J., Good, C., Welsh, C., Summerfelt, S., 2011. The effects of ozone and water exchange rates on water quality and rainbow trout Oncorhynchus mykiss performance in replicated water recirculating systems. Aquac. Eng. 44 (3), 80–96.
Dolan, E., Murphy, N., O'Hehir, M., 2013. Factors influencing optimal micro-screen drum filter selection for recirculating aquaculture systems. Aquac. Eng. 56, 42–50.
Fernandes, P.M., Pedersen, L.F., Pedersen, P.B., 2014. Daily micro particle distribution of an experimental recirculating aquaculture system—A case study. Aquac. Eng. 60, 28–34.
Gonçalves, A.A., Gagnon, G.A., 2011. Ozone application in recirculating aquaculture system: an overview. Ozone.: Sci. Eng. 33 (5), 345–367.
Guilherme, M.F., de Jesus Gregersen, K.J., Pedersen, L.F., 2020. Effects of foam fractionation and chemical disinfection on the removal of different microalgae cultures. Aquac. Res. 51 (8), 3274–3283.
Holan, A.B., Wold, P.A., Leiknes, T., 2014. Intensive rearing of cod larvae (Gadus morhua) in recirculating aquaculture systems (RAS) implementing a membrane bioreactor (MBR) for enhanced colloidal particle and fine suspended solids removal. Aquac. Eng. 58, 52–58.
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, H2O2 and NaCl. Aquac. Res. 53 (9), 3274–3282.
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. Aquac. Eng. 84, 60–66.
de Jesus Gregersen, K.J., Pedersen, P.B., Pedersen, L.F., Liu, D., Dalsgaard, J., 2020. UV irradiation and micro filtration effects on micro particle development and microbial water quality in recirculation aquaculture systems. Aquaculture 518, 734785.
de Jesus Gregersen, K.J., Pedersen, L.F., Pedersen, P.B., Syropoulou, E., Dalsgaard, J., 2021. Foam fractionation and ozonation in freshwater recirculation aquaculture systems. Aquac. Eng. 95, 102195.
Kvåle, A., Yúfera, M., Nygård, E., Aursland, K., Harboe, T., Hamre, K., 2006. Leaching properties of three different micropaticulate diets and preference of the diets in cod (Gadus morhua L.) larvae. Aquaculture 251 (2–4), 402–415.
Legube, B., 2003. Ozonation by-products. Haloforms and Related Compounds in Drinking Water. Springer,, Berlin, Heidelberg, pp. 95–116.
Lekang, O.I., 2019. Aquaculture Engineering, 3rd ed.,. Blackwell Pub,.
Letelier-Gordo, C.O., Aalto, S.L., Suurnäkki, S., Pedersen, P.B., 2020. Increased sulfate availability in saline water promotes hydrogen sulfide production in fish organic waste. Aquac. Eng. 89, 102062.
Li, T., Yan, X., Wang, D., Wang, F., 2009. Impact of preozonation on the performance of coagulated flocs. Chemosphere 75 (2), 187–192.
Ling, J., Chen, S., 2005. Impact of organic carbon on nitrification performance of different biofilters. Aquac. Eng. 33 (2), 150–162.
Lu, C., Kania, P.W., Buchmann, K., 2018. Particle effects on fish gills: An immunogenetic approach for rainbow trout and zebrafish. Aquaculture 484, 98–104.
Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P., d'Orbcastel, E.R., Verreth, J.A.J., 2010. New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. Aquac. Eng. 43 (3), 83–93.
Orellana, J., Wecker, B., 2013. Degassing to reduce carbon dioxide during live transport of adult salmon in wellboats: A model approach. J. Appl. Ichthyol. 30 (2), 411–414.
Park, J., Kim, Y., Kim, P.K., Daniels, H.V., 2011. Effects of two different ozone doses on seawater recirculating systems for black sea bream Acanthopagrus schlegeli (Bleeker): removal of solids and bacteria by foam fractionation. Aquac. Eng. 44 (1), 19–24.
Park, J., Kim, P.K., Lim, T., Daniels, H.V., 2013. Ozonation in seawater recirculating systems for black seabream Acanthopagrus schlegelii (Bleeker): effects on solids, bacteria, water clarity, and color. Aquac. Eng. 55, 1–8.
Pedersen, L.F., Pedersen, P.B., Sortkjær, O., 2006. Dose-dependent decomposition rate constants of hydrogen peroxide in small-scale bio filters. Aquac. Eng. 34 (1), 8–15.
Pedersen, L.-F., Oosterveld, R., Pedersen, P.B., 2015. Nitrification performance and robustness of fixed and moving bed biofilters having identical carrier elements. Aquac. Eng. 65, 37–45.
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. Aquac. Eng. 78, 18–23.
Peng, L., Jo, J.Y., 2003. Performance of a foam fractionator in a lab-scale seawater recirculating aquaculture system. Fish. Aquat. Sci. 6 (4), 187–193.
Peng, L., Oh, S.Y., Jo, J.Y., 2003. Protein removal by a foam fractionator in simulated seawater aquaculture system. Ocean Polar Res. 25 (3), 269–275.
Piedrahita, R.H., 2003. Reducing the potential environmental impact of tank aquaculture effluents through intensification and Recirculation. Aquaculture 226 (1–4), 35–44.
Powell, A., Scolding, J.W., 2018. Direct application of ozone in aquaculture systems. Rev. Aquac. 10 (2), 424–438.
Rojas-Tirado, P., Pedersen, P.B., Vadstein, O., Pedersen, L.-F., 2019. Microbial Dynamics in RAS water: Effects of adding acetate as a biodegradable carbon-source. Aquac. Eng. 84, 106–116.
Schroeder, J.P., Croot, P.L., Von Dewitz, B., Waller, U., Hanel, R., 2011. Potential and limitations of ozone for the removal of ammonia, nitrite, and yellow substances in marine recirculating aquaculture systems. Aquac. Eng. 45 (1), 35–41.
Schroeder, J.P., Klatt, S.F., Schlachter, M., Zablotski, Y., Keuter, S., Spieck, E., Schulz, C., 2015. Impact of ozonation and residual ozone-produced oxidants on the nitrification performance of moving-bed biofilters from marine recirculating aquaculture systems. Aquac. Eng. 65, 27–36.
Schumann, M., Brinker, A., 2020. Understanding and managing suspended solids in intensive salmonid aquaculture: a review. Rev. Aquac. 12 (4), 2109–2139.
Sharrer, M.J., Summerfelt, S.T., 2007. Ozonation followed by ultraviolet irradiation provides effective bacteria inactivation in a freshwater recirculating system. Aquac. Eng. 37 (2), 180–191.
Spiliotopoulou, A., Rojas-Tirado, P., Chhetri, R.K., Kaarsholm, K.M., Martin, R., Pedersen, P.B., Andersen, H.R., 2018. Ozonation control and effects of ozone on water quality in recirculating aquaculture systems. Water Res. 133, 289–298.
Stiller, K.T., Kolarevic, J., Lazado, C.C., Gerwins, J., Good, C., Summerfelt, S.T., Espmark, Å.M., 2020. The effects of ozone on Atlantic salmon post-smolt in brackish water—Establishing welfare indicators and thresholds. Int. J. Mol. Sci. 21 (14), 5109.
Sugita, H., Asai, T., Hayashi, K., Mitsuya, T., Amanuma, K., Maruyama, C., Deguchi, Y., 1992. Application of ozone disinfection to remove Enterococcus seriolicida, Pasteurella piscicida, and Vibrio anguillarum from seawater. Appl. Environ. Microbiol. 58 (12), 4072–4075.
Summerfelt, S.T., 2003. Ozonation and UV irradiation—an introduction and examples of current applications. Aquac. Eng. 28 (1–2), 21–36.
Summerfelt, S.T., Hochheimer, J.N., 1997. Review of ozone processes and applications as an oxidizing agent in aquaculture. Progress. Fish Culturist 59 (2), 94–105.
Summerfelt, S.T., Sharrer, M.J., Tsukuda, S.M., Gearheart, M., 2009. Process requirements for achieving full-flow disinfection of recirculating water using ozonation and UV irradiation. Aquac. Eng. 40 (1), 17–27.
Tango, M.S., Gagnon, G.A., 2003. Impact of ozonation on water quality in marine recirculation systems. Aquac. Eng. 29 (3–4), 125–137.
Weeks, N.C., Timmons, M.B., Chen, S., 1992. Feasibility of using foam fractionation for the removal of dissolved and suspended solids from fish culture water. Aquac. Eng. 11 (4), 251–265.
Wheaton, F.W., Lawson, T.B., Lomax, K.M., 1979. Foam fractionation applied to aquacultural systems. Proc. World Maric. Soc. 10 (1–4), 795–808.
Xu, J., Qiu, T., Chen, F., Zhou, L., Sun, J., Du, Y., 2021. Construction and application of an electrocoagulation and filtration linkage control system in a recirculating aquaculture system. J. Water Process Eng. 44, 102379.
Zita, A., Hermansson, M., 1997. Determination of bacterial cell surface hydrophobicity of single cells in cultures and in wastewater in situ. FEMS Microbiol. Lett. 152 (2), 299–306.