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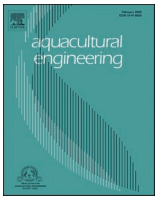

Effect of ozone and hydrogen peroxide on off-flavor compounds and water quality in a recirculating aquaculture system

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ABSTRACT

The recirculating aquaculture system (RAS) is an ever-developing technology for producing fish with a low environmental impact. However, off-flavors can be a major problem in RAS fish production. Off-flavor compounds are of microbial origin and are accumulated in fish flesh. They typically cause a musty and earthy taste and odor, which consumers find unacceptable. Here we hypothesized that oxidizing compounds such as ozone (O_3), hydrogen peroxide (H_2O_2), and their combinations, referred to as advanced oxidation processes (AOP)s, can remove or decrease these compounds in water and prevent their accumulation in fish. In this study, four different oxidative treatments (O_3 low (0.4 mg O_3 L^{-1}), O_3 high (0.8 mg O_3 L^{-1}), H_2O_2 (0.15 µl L^{-1}), AOP (0.4 mg O_3 L^{-1} & H_2O_2 0.10 µl L^{-1}), and controls were applied to 10 experimental RASs for four months. The results showed that the treatments can reduce dissolved organic carbon (DOC) and the off-flavor compounds (geosmin, GSM and 2-methyl isoborneol, MIB) in circulating water, but they were not able to prevent the accumulated off-flavors in fish flesh below the sensory threshold. There was no significant difference in off-flavor removal between the treatments, which indicates that O_3 treatment was ineffective in these conditions. However, H_2O_2 could still reduce the off-flavor concentrations in water.

1. Introduction

As aquaculture becomes a more important method of producing protein in the world (Anon, 2018), there is a growing market for new efficient technologies. The recirculating aquaculture system (RAS) can reduce water consumption and the environmental impact compared with traditional aquaculture methods (Davidson et al., 2016). The RAS technology is relatively new and offers advantages over open systems under specific conditions. In RAS, microbial produced off-flavor-inducing compounds can accumulate to water due to low water exchange rate (Smith et al., 2008). Typically, off-flavor compounds are lipophilic molecules and are quickly transferred to the fish flesh if present in water (Houle et al., 2011).

Geosmin (GSM) and 2-methyl isoborneol (MIB) are the most well-known off-flavor compounds, but there are several other compounds (Podduturi et al., 2017; Lindholm-Lehto, 2022) that can cause unwanted flavors in fish flesh (Cotsaris et al., 1995; Martin et al., 1988). They are formed as metabolic byproducts of microbial metabolism. Although they are not toxic in low concentrations, they are poorly biodegradable (Juttner and Watson, 2007). Different techniques, including oxidizing chemical addition (Lundgren et al., 1988; Swaim et al., 2008; Lindholm-Lehto et al., 2019a, 2019b), bacterial degradation (Azaria et al., 2017), and ultrasound treatment (Nam-Koong et al., 2016) have been studied to remove or reduce these off-flavor compounds from water, but so far without a conclusive result.

Off-flavor compounds can be observed by the human senses at very low concentrations. For example, Robertson et al. (2005) determined human sensory thresholds of 700 ng kg^{-1} for MIB and 900 ng kg^{-1} for GSM in rainbow trout. Off-flavors can lead to financial losses in RAS production, because an unpalatable fish product will not satisfy consumers. Off-flavors can be removed from fish flesh by depurating in clean water from days to few weeks, but this procedure requires a lot of clean water and can be costly (Burr et al., 2012; Lindholm-Lehto et al., 2019a, 2019b).

Ozone (O_3), hydrogen peroxide (H_2O_2), and other oxidizing chemicals are often used in disinfection and in water purification, because

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they react effectively with organic matter and leave no toxic byproducts in the water, at least when freshwater is used (Spiliotopoulou et al., 2018). O_3 has been recorded to reduce fish disease outbreaks (Summerfelt et al., 2009), improve solids removal, and reduce nitrite content and turbidity (Summerfelt et al., 1997). Improved solids removal usually leads to cleaner pipes and tanks, decreasing the required maintenance work (Summerfelt et al., 1997). O_3 has also been shown to enhance the growth rate of fish, possibly because of improved water quality (Davidson et al., 2011, Good et al., 2011, Davidson et al., 2021).

The oxidizing compounds O_3 and H_2O_2 show promise in decomposing off-flavor compounds (Westerhoff et al., 2006). In theory, they react with GSM and MIB (Westerhoff et al., 2006) at reaction rate constants of k_{GSM}= 0.10 $M^{-1}s^{-1}$ and k_{MIB} = 0.35 $M^{-1}s^{-1}$ (Peter and Von Gunten, 2007), which indicates prone reactions. However, the removal results have been insufficient in real systems (Schrader et al., 2010). O_3 forms hydroxyl radicals in advanced oxidation processes (AOP), which combines e.g., H_2O_2, O_3, UV-light, or TiO_2 (Stasinakis, 2008). AOPs have shown promising results in the removal of GSM and MIB in laboratory conditions with RAS water (Park et al., 2006), although Klausen and Gronborg (2010) noted that removal was inhibited in RAS conditions. The hydroxyl radical is known to react at rate constants from 3×10^9 to 10^{10} for GSM and MIB (Peter and Von Gunten, 2007), which can be considered extremely quick. A study by Lindholm-Lehto et al. (2020) showed that the addition of different oxidants can have positive effects even in a commercial-scale RAS, reducing off-flavors and improving water quality. Yet AOPs have not yet been properly tested in full-scale systems.

This experiment was designed to study the effect of O_3, H_2O_2, and their combination (AOP) on the off-flavor compounds in experimental RAS. O_3 experiments have rarely been performed in multiple systems (Lindholm-Lehto et al., 2020). First, the aim was to study the suitability of the added doses for the reared species. O_3 and H_2O_2 have long been underutilized due to their toxic effects on aquatic organisms (Stiller et al., 2020), although their water quality-improving properties are well known. Second, the objective was to study if the addition of O_3 and H_2O_2 could decrease the concentrations of off-flavor-causing compounds (GSM and MIB) in an RAS. Increasing the knowledge and methods for the use of O_3 may become more approachable for commercial RAS fish production in the future.

2. Materials and methods

2.1. Experimental setup

The study was conducted in the spring of 2021 at an experimental recirculating aquaculture research platform using 10 individual RASs, and it lasted for four months. The systems were identical and were maturated for 3 months before the experiment. Each system consisted of a 500 L bottom-drained tank, feed collector, swirl separator, drum filter (60 μm sieve), moving-bed biofilter (80 L, RK-Bioelements, Dania Plast A/S, Denmark), CO_2 removal in a forced-ventilated cascade aeration column, oxygen injection, and pH adjustment. Drum filter activations were monitored and the inlet water was used for rinsing. Adjustment of pH was performed by adding NaOH solution. Water renewal rate was kept at 500 L kg^{-1} d^{-1}, 5.4–6.0 L h^{-1} during the experiment. Inlet water was taken from the oligotrophic Lake Peurunka (62.44886, 25.85201, 694 ha, 59,600 m^3). Mean temperature of water was 13.6 ± 0.4 °C. A full description of the system has previously been reported (Pulkkinen et al., 2018). For this experiment, an additional ozonation loop was attached to each system. Water was circulated from and back into a water reserve tank of 147 L to prevent any overflow in the system (Fig. 1). Each system had individual O_3 and H_2O_2 inlets for the dozing.

There were five duplicated treatments. The treatments were O_3 low (O_3 0.4 mg L^{-1}), O_3 high (O_3 0.8 mg L^{-1}), H_2O_2 (half of the molar amount of O_3 0.4 mg L^{-1} of hydrogen peroxide = 0.15 μl L^{-1}), AOP (O_3 0.4 mg L^{-1} and H_2O_2 0.10 μl L^{-1}), and a control without O_3 and H_2O_2 additions. The O_3 low and O_3 high treatments included 28 g O_3 kg^{-1} feed and 57 g O_3 kg^{-1} feed. Each system was loaded with 23.8 kg of rainbow trout (*Oncorhynchus mykiss*) with an average weight of 403 g per fish at the beginning of the experiment. Feed load was relatively similar for all systems and adjusted for fish biomass, starting with a feeding ratio of 1.2 % of fish biomass per day. This was changed after the first measurement to 0.9 %, and finally to 1.0 %. The feed BioMar Orbit 4.5 mm and 6 mm was administered with automatic feeders, twelve times per day. The feed consisted of crude protein (42–45 % 4.5 mm, 38–41 % 6 mm), crude lipid (26–29 4.5 mm, 29–32 % 6 mm) and a total of 1.0–1.2 % P and 6.3–7.0 % N, as given by the manufacturer. Feed collectors were used to monitor uneaten feed. FCR was calculated from feed load. The O_3 and H_2O_2 injections were unchanged throughout the experiment, although the amount of feed was changed per fish growth to keep O_3 flow stable and to avoid any disturbances.

2.1.1. Ozonation

The ozonation equipment was delivered by BMT Messtechnik GmbH.

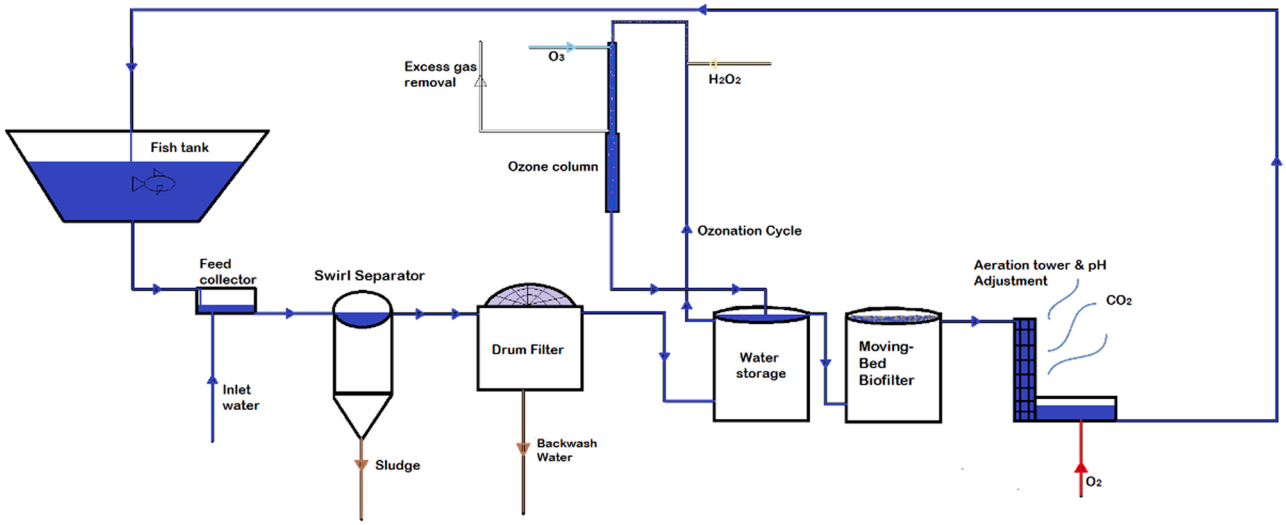

Fig. 1. RAS used in experiment with ozone cycle. Arrows indicate the flow direction.

O_3 was created with a BMT 802 N O_3 generator, and the production was analyzed with a BMT 964 O_3 analyzer (Fig. 2). The generator was fed with oxygen at a constant pressure of 0.9 bar. O_3 was then fed to an analyzer and divided into O_3 loops through gas flow vents. The injected O_3 amount was controlled by leading and adjusting the gas flow through the vents. O_3 was injected directly into the water from the top of the O_3 cycle, producing bubbles that enhanced the ozone solubility. Excess O_3 was fed to an active carbon filter which decomposed O_3 to avoid any residual O_3. Waterflow was maintained at 0.23 L s^{-1} by adjusting the pump power. Water was pumped to the O_3 cycle (Grundfors Magna 3 (25−40) pumps, Denmark)·H_2O_2 was added to the water with the LMI Milton Roy (PD 743–822S2-series) chemical pumps. Each H_2O_2 system had its own H_2O_2 reserve, and 0.2 % solution was pumped, produced from the 50 % solution (H_2O_2, Bang & Bonsomer). The injected H_2O_2 amount was monitored and recorded for each system and based on this, the pumping power was adjusted.

2.2. Sampling

Water samples were collected weekly from the fish tanks below the surface, and any large solid particles were avoided. The samples were taken to be analyzed for dissolved organic carbon (DOC), off-flavors (MIB and GSM), and water quality in general. The samples were collected in 30 mL plastic tubes and filtered through (Sartorius 16555-Q, 0.45 μm, CA) syringe filters directly after sampling, excluding bacterial samples that were collected into 250 mL plastic (HDPE) containers. Samples for DOC and off-flavor analysis were stored in a freezer at − 22 °C for a couple of weeks before the analysis.

Fish were sampled once per month (three times in total + initial sampling) from each system (three individuals per sampling), weighed, humanely euthanized, and gutted. The fish were instantly frozen and stored at − 22 °C for a couple of weeks before the off-flavor analysis.

2.3. O_3 analysis

The production of an O_3 generator was measured by titration with an iodometric standard method (IOA Standardization Committee – Europe, 001/87 (F), 1987) and to ensure the accuracy of the O_3 analyzer. O_3 gas was taken straight from one of the O_3 injection points.

The dissolved O_3 in water was measured with the standard colorimetric indigo-method (IOA Standardization Committee – Europe, 006/89 (F), 1989). Water from the O_3 cycle was introduced to an indigo-trisulfonate solution. The fluorescence of the solution was measured and compared with a control. These measurements were made occasionally to keep track of dissolved O_3 in the system and prevent any harm from O_3 addition to biofilters or fish.

The amount of dissolved O_3 was monitored by following the gas bubbles in the excess gas removal pipe. There were very few bubbles, indicating that most of the produced O_3 was dissolved in the water.

2.4. Water quality analyses

2.4.1. Monitoring of water quality

Water quality was monitored constantly by an online (s::can, Austria) monitoring system, measuring water flowrate, temperature, nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N) and UV-254, turbidity, and suspended solids every six minutes. In addition, the concentrations of dissolved oxygen, (OxyGuard, Farum, Denmark) and carbon dioxide (Franatech, Lüneburg, Germany), and the pH (ProMinent, Heidelberg, Germany) were constantly monitored.

NO_2-N, NO_3-N, and total ammonia-N were analyzed weekly with quick spectrophotometric laboratory tests (Procedure 8038 Nessler, LCK341/342, LCK340, and LCK349 UN3316 9 II, DS 3900, Hach, USA). The alkalinity was measured using a standard titration method (ISO 9963–1:1994, TitraLab AT1000, Hach, Loveland, USA), and the turbidity was measured with a Hach DR 3900 Turbidimeter. The DOC was determined with a Shimdadzu TOC-L analyzer.

2.4.2. Analysis of off-flavors

The off-flavor compounds GSM (geosmin, trans-1,10-dimethyl-trans-9-decalol) and MIB (2-methyl isoborneol, 1-R-exo-1,2,7,7-tetramethyl-bicyclo[2.2.1]heptan-2-ol) were quantified using the method reported in Lindholm-Lehto (2022). In short, the sample extraction was performed by an automated SPME procedure (PAL3 autosampler, CTC Analytics, Switzerland) with an SPME Arrow fiber made of DVB/carbon WR/PDMS (divinylbenzene/carboxene/polydimethyl siloxane). The

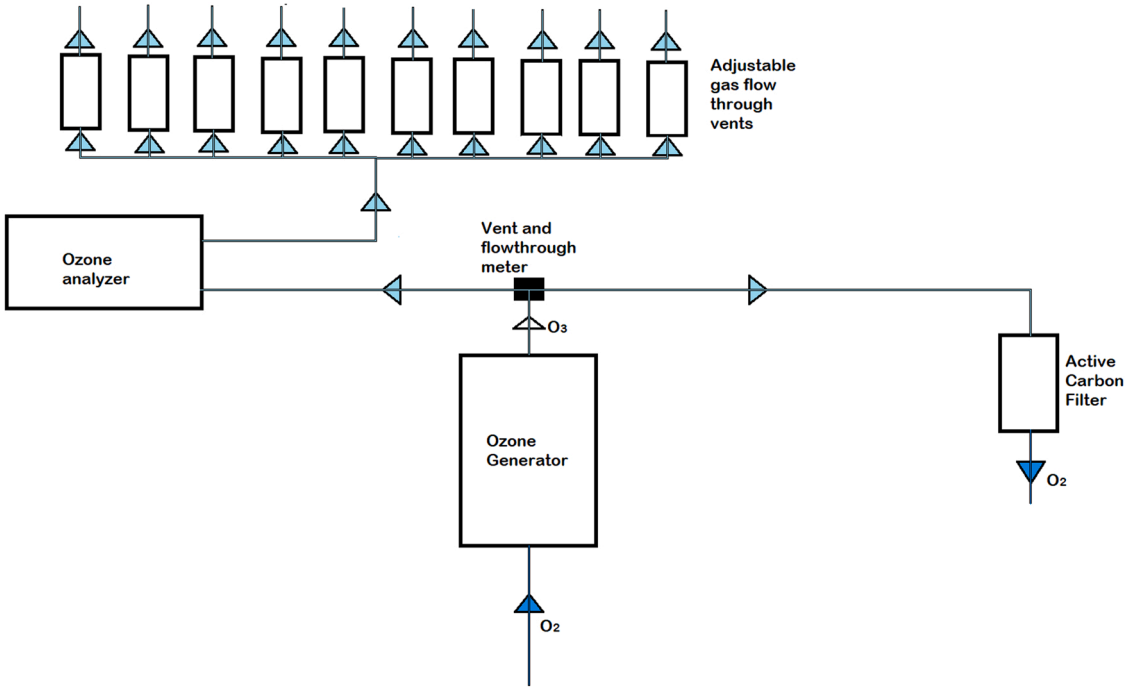

Fig. 2. Ozonation system: O_3 generator, O_3 analyzer and adjustable vents to control the gas flow. An active carbon filter removed any residual O_3.

pretreatment included mixing, heating, adsorption and desorption of analytes, injection into the GC port, and conditioning of the fiber.

The samples were analyzed using a GC-QQQ (7000 Series Triple Quadrupole mass spectrometer, Agilent, Santa Clara, CA, USA). It was operated with a Phenomenex Zebron ZB-5MSi (Torrance, CA, USA) capillary column (30 m × 0.25 mm × 0.25 μm) for the separation and an electron ionization (EI) ion source. The detection was performed in multiple reaction monitoring (MRM) mode. Limits of quantification (LOQ)s were 0.2 ng L^{-1} (GSM) and 0.4 ng L^{-1} (MIB) for aqueous and 65 ng kg^{-1} (GSM) and 107 ng kg^{-1} (MIB) for solid samples. The full method description and validation have been reported in Lindholm--Lehto (2022).

2.4.3. Statistical analyses

In each treatment, there were two replicates in each measurement point. The data were regarded as dependable, as multiple samples were taken from systems. However, n remained below 30, indicating that the preferred test for the data is Friedmann's test, with a significance level of 0.05, allowing to compare multiple treatments at the same time. Tested variables were Redox, DOC, and MIB/GSM concentrations in water and in fish flesh. All the statistical analyses were performed using IBM SPSS statistics version 26.0 (Armonk, NY: IBM Corporation).

3. Results

3.1. Off-flavors GSM and MIB

At the beginning of the experiment and before the oxidant additions, the concentrations of MIB were 13.1 ± 4.5 ng L^{-1} (Fig. 4A) and GSM 2.3 ± 1.4 ng L^{-1} (Fig. 4B). Later, the concentrations increased quickly in the controls but more slowly in the systems with oxidant addition. For MIB, the controls reached 87.3 ± 22.5 ng L^{-1} and later declined to 68.0 ± 12.3 ng L^{-1}. This was followed by a very steep decline in the concentrations of all systems, after which the concentrations ranged between 11.0 and 20.0 ng L^{-1}. At the end of the experiment, MIB increased to 113.4 ± 47.4 ng L^{-1} in the controls, while a very minor increase was observed in the other systems. The slowest increase was observed in systems with a high O_3 dose, reaching a concentration of 17.6 ± 6.1 ng L^{-1} on February 17, 2021, when the others had values between 27.0 and 30.0 ng L^{-1}. In the high O_3 treatment, the concentration then increased rapidly to 59.5 ± 15.9 ng L^{-1}. The concentration of inlet water increased steadily throughout the experiment, from below the LOD to 8.9 ± 0.1 ng L^{-1}. Statistically, treatments had lower MIB concentrations than the control (n = 20, F=4, $p < 0.05$), and between the treatments H_2O_2 was more effective in lowering mentioned MIB concentration than AOP (n = 20, F=4, $p < 0.05$).

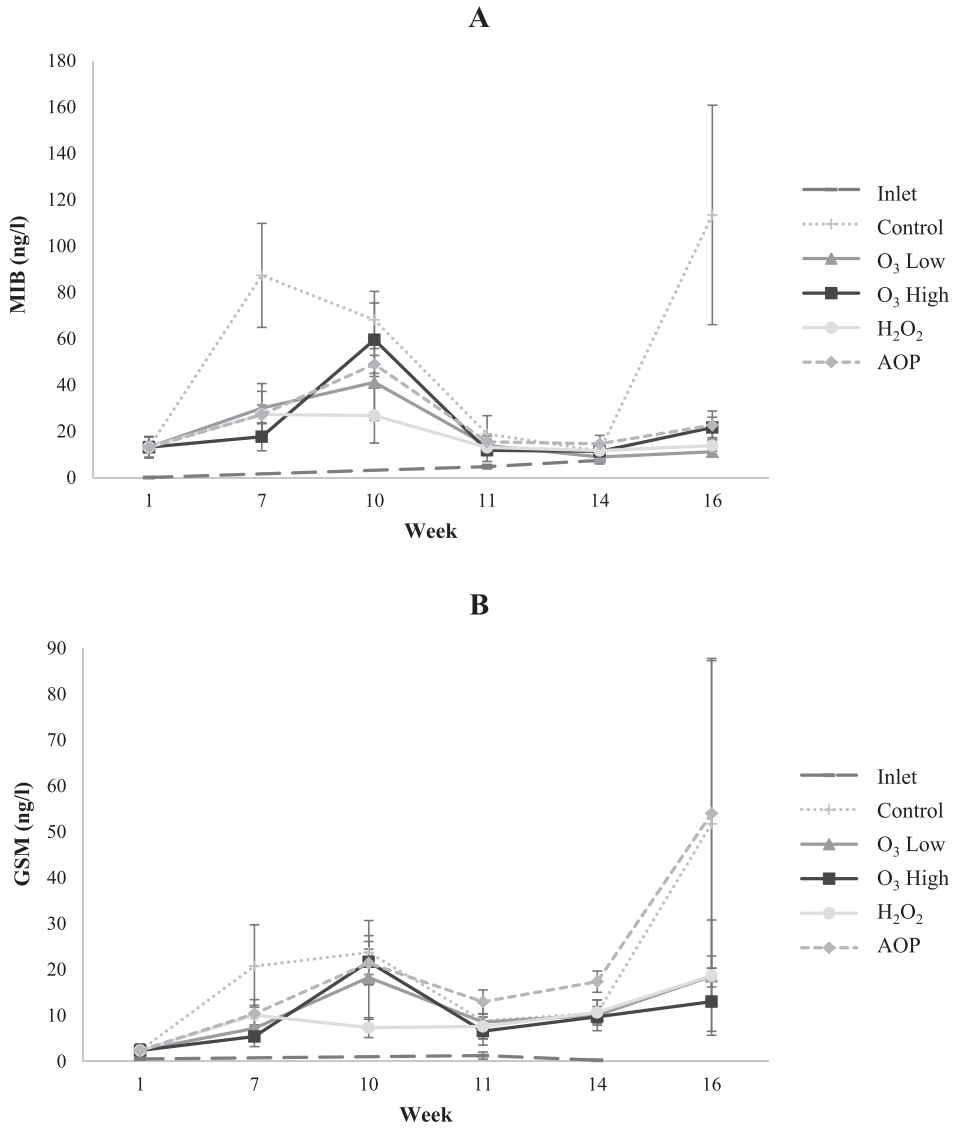

Fig. 3. MIB concentration (A) and GSM concentration (B) in the system water during the experiment (ng L^{-1} ± SD, n = 2).

The concentrations of GSM were at 2.3 ng L^{-1} at the beginning of the experiment and followed the trend of MIB (Fig. 4B). However, the overall concentrations of GSM were lower than those of MIB and decreased after week 11 (Fig. 4B). In the H_2O_2 treatment, the concentrations reached only 7.3 ± 2.2 ng L^{-1}, while the others were close to 20 ng L^{-1}. After the decrease in the middle of experiment, the concentrations again increased. AOP treatment (O_3 +H_2O_2) followed the same trend to 17.3 ± 2.3 ng L^{-1}, the others to 10 ng L^{-1}. As with MIB, an increase in concentrations was observed in the controls and in the AOP systems and reached more than 50 ng L^{-1}. Similar to MIB, the inlet water contained very low concentrations of GSM and increased to 1.2 ± 0.8 ng L^{-1} during the experiment but decreased again below the LOD. Low O_3, high O_3, and H_2O_2 had statistically lower average values than the control (n = 20, F=4, $p > 0.05$), with no statistical difference compared to the control (n = 20, F=4, $p = 0.230$).

Fig. 3.

The concentrations of MIB and GSM in fish flesh are presented in Fig. 5. The concentrations of MIB were initially between 170 and 310 ng kg^{-1}. After approximately one month, the concentrations were at 720 ± 200 ng kg^{-1} in the control and 640 ± 220 ng kg^{-1} in the high O_3. The other treatments increased more slowly in values, and the AOP treatment remained in the range of 300 ± 20 ng kg^{-1} as in the previous week. In the end of the experiment, all the other treatments showed a substantial increase in their values except the high O_3, with the concentration of 730 ± 210 ng kg^{-1}. The control had concentrations of 1190 ± 220 ng kg^{-1} and H_2O_2 1030 ± 270 ng kg^{-1}, which was close to the lower O_3 dose at 1020 ± 230 ng kg^{-1}, and then AOP at 770 ± 170 ng kg^{-1}. Overall, no statistical difference was found between any of the treatments (n = 12, F=4, $p = 0.867$).

In the first measurement with GSM, the high O_3 dose had the concentration of 100 ± 30 ng kg^{-1}. The concentrations in H_2O_2 were at 290 ± 50 ng kg^{-1}, followed by AOP at 240 ± 110 ng kg^{-1}, the control at 180 ± 90 ng kg^{-1}, and the low O_3 dose at 130 ± 40 ng kg^{-1}. In the second measurement, H_2O_2 treatment decreased to 190 ± 110 ng kg^{-1}, while the others increased. AOP showed the concentrations of 380 ± 350 ng kg^{-1}, and the others between 210 and 260 ng kg^{-1}. GSM

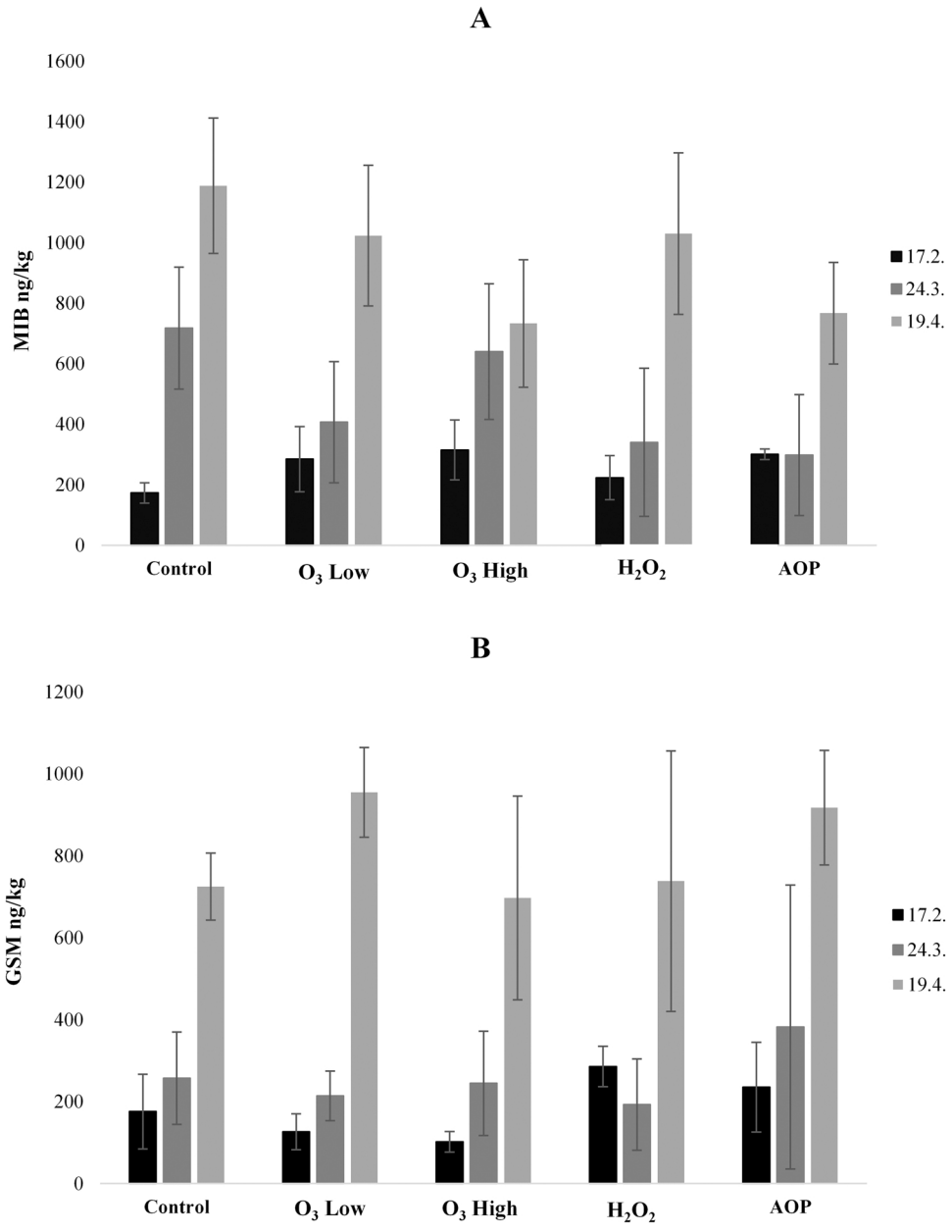

Fig. 4. Concentrations of MIB (A) and GSM (B) in fish flesh (ng kg^{-1}, ± SD, n = 4) in three samplings during the experiment (dates in legend 17.2., 24.3. and 19.4.).

increased substantially in the final measurement, with between 2 and 3 times the values of the previous measurement. Similar to the case for MIB, no statistical difference was found (n = 12, F=4, $p = 0.281$). Fig. 4.

3.2. Water quality

The nitrogen compounds (total ammonia nitrogen, TAN, NO_2-N, NO_3-N) were in the same range in all treatments (Supplementary Table 1): 0.44–1.77 mg L^{-1} for TAN, 0.1–0.58 mg L^{-1} for NO_2-N, and 28–134 mg L^{-1} for NO_3-N.

The pH was at 6.9 ± 0.5 and the alkalinity ranged between 30 and 50 mg L^{-1} (Supplementary Table 1).

The redox values (Supplementary Table 1) fluctuated throughout the experiment, with the lowest values in weeks nine and ten and the highest immediately afterward during weeks ten and eleven. The treatments showed no individual trends, but none of the systems had values greater than 320 mv. The lowest values were 220 mv. No statistical differences were spotted between any of the treatments (n = 24, F=4, $p = 0.090$).

In the controls, DOC first increased to 17 mg L^{-1} and then declined to 12–14 mg L^{-1} (Fig. 5). The DOC was relatively stable at 6 mg L^{-1} in the inlet water from Lake Peurunka. In the high O_3 dose treatment, the DOC concentrations were much lower than in the controls (n = 28, F=4, $p < 0.05$) and only around 2 mg L^{-1} higher than in lake water. The lower O_3 dose performance and AOP treatment were almost similar (n = 28, F=4, $p = 0.800$), but statistically, both differed significantly from the control (n = 28, F=4, $p > 0.05$). The H_2O_2 treatment seemed not to affect the DOC concentrations (n = 28, F=4, $p = 0.866$). In summary, the concentrations of DOC were 2–3 mg L^{-1} lower in the low O_3 and AOP treatments. The *p*-values are listed in Supplementary Table 2.

In this study, in the high O_3 dose treatment values between 0.0800 and 0.150 mg (O_3) mg^{-1} (DOC), an average of 0.106 mg (O_3) mg^{-1} (DOC) was observed, although the values fluctuated (Fig. 5). The lower dose had values between 0.039 and 0.063 mg (O_3) mg^{-1} (DOC), with an average of 0.047 mg (O_3) mg^{-1} (DOC). The AOP treatment had similar values to the lower O_3 treatment, with an average of 0.044 mg (O_3) mg^{-1} (DOC) values, between 0.032 and 0.077 mg (O_3) mg^{-1} (DOC).

3.3. Fish growth

Fish mortality was mortality was very low throughout the experiment (Table 1). The lowest mortalities were recorded in the H_2O_2 treatment and the highest in the high O_3. Overall, there was little variation between treatments (Table 1).

Table 1
Calculated averages (n = 2) and standard deviation (± SD) for specific growth rate (SGR), mortality (MOR), feed conversion ratio (FCR), and fish size (FS, g). Sorted by date and treatment.

	Week	Control	Low O_3	High O_3	AOP	H_2O_2
SGR	7	0.77 ± 0.02	0.73 ± 0.03	0.88 ± 0.14	0.75 ± 0.03	0.84 ± 0.01
	11	0.75 ± 0.03	0.90 ± 0.02	0.86 ± 0.07	0.86 ± 0.11	0.78 ± 0.02
	16	0.69 ± 0.05	0.64 ± 0.03	0.60 ± 0.02	0.60 ± 0.06	0.55 ± 014
MOR	7	4 ± 1	6.5 ± 0.5	6 ± 1	5 ± 3	0.5 ± 0.5
	11	1 ± 1	1.5 ± 0.5	2 ± 1	1.5 ± 0.5	2 ± 2
	16	0.5 ± 0.5	0.5 ± 0.5	2.5 ± 1.5	0.5 ± 0.5	0 ± 0
FCR	7	1.38 ± 0.05	1.36 ± 0.05	1.35 ± 0.08	1.39 ± 0.01	1.43 ± 0.07
	11	1.20 ± 0.02	1.21 ± 0.01	1.27 ± 0.12	1.18 ± 0.02	1.03 ± 0.02
	16	1.19 ± 0.08	1.34 ± 0.01	1.58 ± 0.03	1.38 ± 0.08	1.52 ± 0.18
FS	7	537.0 ± 3.0	528.9 ± 6.1	559.7 ± 29.4	531.4 ± 5	549.5 ± 2
	11	657.0 ± 9.4	675.1 ± 10.6	704.4 ± 23.3	669.7 ± 13.6	678.9 ± 5.5
	16	826.5 ± 25.3	833.2 ± 3.6	858.4 ± 33.2	816.9 ± 33.1	815.7 ± 32.2

Table 1.

The feed conversion ratio (FCR) ranged from 1.03 to 1.58 and the SGR from 0.6 to 0.9 (Table 1). The highest SGR values were observed in the second weighing, and the lowest in the final weighing. The biggest individual fish were observed in the O_3 treatments (around 5 % bigger than in the control), although these treatments had the lowest biomass in total (Table 1).

4. Discussion

4.1. GSM and MIB

Based on the results of this study, O_3, H_2O_2, and AOP treatments had no significant effect on the GSM and MIB concentrations in fish flesh compared to the controls. Additionally, no significant difference between the oxidizing treatments was observed. The lack of effect may be explained by the amount of organic matter present in the water, and that consumed the oxidizing agents before they had an opportunity to react with the off-flavor compounds (Li et al., 2019). Somewhat contradictorily, Liang et al. (2007) found that GSM and MIB were removed more

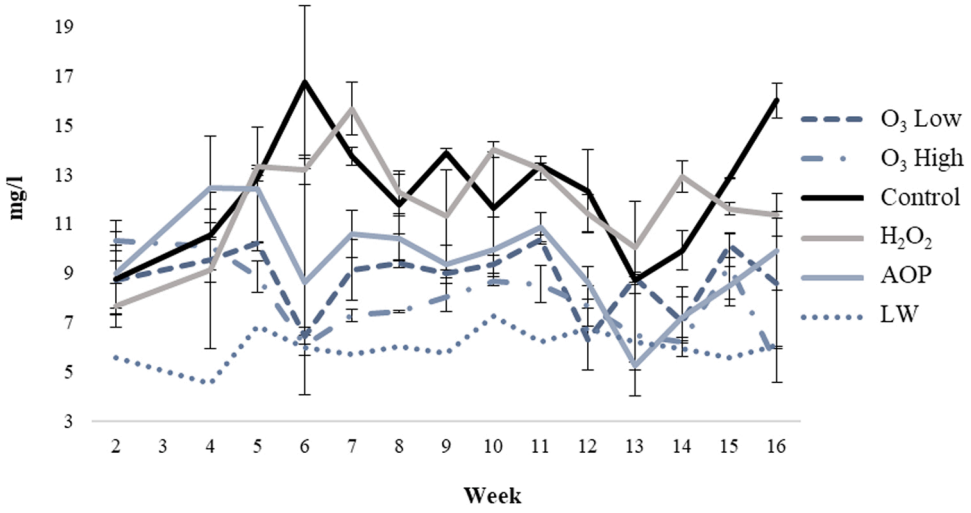

Fig. 5. Averages and standard deviations of DOC (mg L^{-1}) in each treatment, starting from week 2 (± SD, n = 2).

efficiently in water that contained some organic material than in water, which may indicate that hydroxyl radicals can play a greater role in oxidation of these off-flavor compounds than molecular O_3.

A statistical difference was found between the treatments and the controls when the MIB and GSM concentrations in water were studied. However, if the decrease was caused by oxidation, reduction in organic matter and bacterial biomass or their combination, remains unidentified so far. The decrease in organic matter remains H_2O_2 treatment was able to reduce GSM and MIB concentrations, but it did not decrease DOC. However, the decrease in GSM and MIB concentrations in water was not enough to decrease their concentrations in fish flesh. The concentrations of off-flavor compounds in water were much smaller than those of the overall dissolved organic matter, and efficient reactions between O_3 or hydroxyl radicals were not achieved.

The circulating water contains many compounds, for example, organic matter with double bonds, which are oxidized very quickly and more aggressively by O_3 than the terpenoid-based off-flavors GSM and MIB. In the case of H_2O_2, there was a statistically significant difference between H_2O_2 and the controls, and the oxidative chemical concentrations in the H_2O_2 treatment were two times lower than those in the low O_3 treatment. This indicates that H_2O_2 shows higher selectivity toward off-flavor compounds in RAS than O_3. This is accompanied by a trade-off: H_2O_2 offers less to overall water quality but is more effective in decreasing MIB and GSM concentrations, while the opposite for O_3. At least in this study, the water quality improvements were minimal for H_2O_2. On the other hand, the ability of H_2O_2 to improve the water quality has previously been reported (Park et al., 2006; Lindholm-Lehto et al., 2020).

In the AOP, the H_2O_2 was injected into the system before O_3. It is therefore possible that most H_2O_2 was consumed before reaching the point of O_3 injection, possibly explaining the insufficient performance of the AOP treatment. In that case, very few hydroxyl radicals were formed, and advanced oxidation could not occur. This can be improved in the future by moving the place of H_2O_2 addition closer to the O_3 injection point or adding a larger dose of H_2O_2. Interestingly, the addition of H_2O_2 did not seem to improve AOP's performance over a low O_3 dose, even though the amount of oxidizing agent in the water should have been 50 % higher. Another possibility is that the ozonation degraded large organic molecules to a more biodegradable form, the smaller molecules enhance the growth of the microbes that produce the off-flavors. Ozone reduces the overall microbial cell count while enhancing the growth of the microbes that produce GSM has been recorded before (Aalto et al., 2022). This could mean that the treatments remove GSM and MIB from water more efficiently but also increase their production, may subsequently result in poor net removal.

To summarize, it appears that larger amounts of O_3 were incapable of reducing the MIB and GSM concentrations in the RAS water to result in a reduction of their accumulation in the fish flesh. In theory, it is possible to increase the amount of O_3 injected into the system, because the redox values were still in a moderate range. However, the results indicate that even a drastic increase in the O_3 dose may be unable to sufficiently reduce the off-flavor concentrations. This could mean that O_3 addition was insufficient, and H_2O_2 may show more potential.

4.2. Water quality parameters

During the experiment, all the redox values were at a normal (250–280 mv) or low (280–350 mv) level (Stiller et al., 2020). It has been suggested that an O_3 threshold of 350 mV is too limited and presents a minimal risk for raised species (Lazado et al., 2021) so there could still be possibility to increase the treatment size if necessary. However, these referenced studies were carried out for Atlantic salmon in brackish water.

No statistically significant difference was seen in redox-values between the treatments, indicating that the fish did not experience increased amounts of oxidative stress. The lower SGR and higher mortality should not be caused by oxidative stress, based on redox-values. Redox fluctuated during the experiment, but similar fluctuation has also previously been recorded, for example, by Davidson et al. (2021). The control and O_3 treatments redox-values fluctuated similarly throughout the experiment, although O_3 showed higher values. This fluctuation was also apparent in non-oxidative treatments, as seemed evident in the study by Pulkkinen et al. (2021). Such phenomenon should result in more frequent redox measurements in future studies to better understand the fluctuation in RAS systems that lack fixed redox values.

In this experiment, many typical effects of O_3 addition were observed. These include increase in water clarity (Supplementary Table 1), and very similar effects as were observed in Davidson et al. (2021), where the content of DOC and solids decreased (Summerfelt et al., 1997; Spiliotopoulou et al., 2018). This was possibly due to increased drum filter activity (Supplementary Table 3) and the O_3 degraded organic material into smaller fractions. Alkalinity and pH were controlled, and the reduction often caused by O_3 (Powell, 2016), was not observed here.

The systems with the AOP treatment showed high initial TAN concentrations (Supplementary Table 1). During the following weeks, this was transformed to elevated NO_2-N and NO_3-N levels. Ozonation showed no effect on NO_2-N concentrations, although it is easily oxidized by O_3 (Goncalves and Gagnon, 2011). NO_2-N oxidization is proven to be favored more than most organic substances (Schroeder et al., 2011). However, some reports have shown that O_3 may not affect NO_2-N (Krumins et al., 2001) when O_3 is used non-continuously. It is possible that the NO_2-N concentrations were already so low in the circulating water that O_3 had little additional effect. Excluding the elevated nitrogen compound concentrations in the AOP treatment after the first week, NO_2-N concentrations were mostly at the recommended level (Timmons and Ebeling, 2010). NO_2-N was below 1 mg L^{-1}, and NO_3-N was higher than the threshold of 75 mg L^{-1} (Davidson et al., 2014). Later, the concentrations adjusted to a level of 50–60 mg L^{-1}.

4.3. Fish growth

The fish were fed a restricted diet to maintain the same ratio of feeding in treated systems and in the controls, and no major effects on fish performance were expected. Interestingly, the treated systems seemed to have much higher SGR over the period between the first and second weighing and over the last period, SGR was highest for the control treatment. The SGR values were unusually low in all treatments, including the controls. Typical values for the fish, this size can be around 1.5–2 (Colson et al., 2015; Pulkkinen et al., 2019). The FCR was also very high at 0.9–1 (Colson et al., 2015; Pulkkinen et al., 2019) for rainbow trout. Temperature was about three degrees higher in the referenced articles than in this study, which could explain lower measured SGR and higher FCR (Jiang et al. (2021). FCR was also calculated from feed given to fishes and not from ingested feed, which means that uneaten feed can alter the results. Growth of the fishes and reduced feeding later in the experiment can also explain the decrease in SGR.

Low and high O_3 treatments led to slightly larger fish than other treatments, which could possibly be contributed to enhanced water quality. Typically, O_3 treatments enhance the growth and wellbeing of fish due to improved water quality (Summerfelt et al., 1997; Davidson et al., 2011, Good et al., 2011, Davidson et al., 2021), but no substantial differences in growth performances were found in this experiment. Based on this experiment, O_3 treatments had no immediate negative impact on fish growth.

5. Conclusions

The experiment showed that the addition of O_3, H_2O_2 and AOP can decrease concentrations of off-flavor compounds (geosmin, GSM and 2-

methyl isoborneol, MIB) in circulating water. However, they were not effective enough to prevent their accumulation in fish flesh. The treatments did improve water quality O_3 readily attacked the and decreased the DOC content. Due to these issues, even larger doses of O_3 and combination of O_3 and H_2O_2 may be required to fully prevent the accumulation of the off-flavor compounds GSM and MIB in the circulating water and in fish flesh. Possibly, the adjustment of oxidant doses and possibly their addition points may improve their beneficial effects.

Author's contribution

The experiment was planned by Vielma, Pulkkinen, Kiuru, Lindholm-Lehto, and Pettersson. Pettersson and Lindholm-Lehto planned and conducted the sample preparations and the chemical analyses. The manuscript was drafted by Pettersson. Lindholm-Lehto, Vielma, Pulkkinen, and Kiuru critically examined and revised the manuscript.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aquaeng.2022.102277.

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