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# Off-Flavour Removal With Advanced Oxidation Process and Hydrogen Peroxide Treatments in Recirculating Aquaculture Systems

Samu Pettersson[1] | Petra C. Lindholm-Lehto[2] | Jani T. Pulkkinen[2] | Tuula Tuhkanen[1]

[1]Department of Biological and Environmental Sciences, University of Jyväskylä, Jyväskylä, Finland | [2]Natural Resources Institute Finland (Luke), Aquatic Production Systems, Jyväskylä, Finland

**Correspondence**: Petra C. Lindholm-Lehto (petra.lindholm-lehto@luke.fi)

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## ABSTRACT

Aquaculture is becoming increasingly important for the world's food production. Recirculating aquaculture system (RAS) has a reduced water requirement and better possibilities for waste handling. Unfortunately, off-flavours can be formed in RAS and concentrate on fish flesh. Off-flavour compounds cause earthy, musty or other unwanted flavours to fish flesh that consumers find objectionable. Typically, off-flavours are removed by depurating the fish in clean water, but it often takes from days to weeks to fully remove these unwanted flavours that causes additional costs to fish producers. Therefore, reliable methods to reduce the need for depuration are needed. In this study, two methods were investigated for the removal of off-flavours in RAS rearing rainbow trout *Oncorhynchus mykiss*: an advanced oxidation process (AOP) using a combination of ozone ($O_3$) and hydrogen peroxide ($H_2O_2$), and a treatment with $H_2O_2$ alone. Two treatments (AOP and $H_2O_2$) and a control without oxidants were applied across nine identical experimental RASs for 8 h day$^{-1}$ over 10 days, and selected off-flavour compounds in water and fish were analysed. In fish, the concentrations of GSM and MIB were on average 776 and 962 ng kg$^{-1}$ (AOP) and 688 and 919 ng kg$^{-1}$ ($H_2O_2$) compared to 1071 and 1205 ng kg$^{-1}$ in the controls. The results showed that intensive oxidant treatments reduced the off-flavour concentrations in the recirculating water and in fish, which can potentially lead to reduced depuration time and production costs. Further optimization of the treatment is needed to improve off-flavour removal efficiencies.

## 1 | Introduction

Recirculating aquaculture system (RAS) was developed as a more environmentally friendly method to produce food (FAO 2022). Its main appeal is to reduce the water requirement (Davidson et al. 2016) and a more flexible placement of facilities and easier treatment of wastewater, although good quality raw water is still a priority. Water recirculation includes multiple water treatment steps, before leading water back to rearing tanks (Badiola, Mediola, and Bostock 2012).

One of the problems in RAS is off-flavour compounds that cause muddy or other unwanted taste and odour to fish flesh (Smith, Boyer, and Zimba 2008). These compounds are not toxic at low concentrations (Jüttner and Watson 2007), but unpleasant taste and smell in fish products will not appeal to customers. Currently the only way to efficiently remove off-flavours from fish is to depurate them in clean water before sale, which often causes additional costs to fish farmers. This leads to many additional costs: requirement of large volumes of clean water, additional tanks and production space, pumping and

fish often lose weight (Burr et al. 2012, Lindholm-Lehto et al. 2019).

Off-flavours are produced as by-products of microbial metabolism (Houle et al. 2011). Many of the off-flavour compounds are lipophilic, such as terpene-based compounds (Podduturi et al. 2017), which makes them easily accumulate in fish flesh (Howgate 2004). RAS environment is also very concentrated with organic matter, even though solids are removed constantly from the circulating water (Fossmark et al. 2020). The unremoved organic matter can then act as a growth medium for microbes that support the production of off-flavour compounds (Lindholm-Lehto and Vielma 2018). Fortunately, oxidation treatments, such as ozone ($O_3$), hydrogen peroxide ($H_2O_2$) and advanced oxidation processes (AOP) have shown potential in removing off-flavours from water (Powell and Scolding 2018; Spiliotopoulou et al. 2018; Pettersson et al. 2022).

In an AOP treatment, very reactive hydroxyl radicals (OH) are formed (Powell and Scolding 2018). There are a lot of different AOPs combinations, such as $O_3$/UV, $H_2O_2$/UV and $O_3$/ $H_2O_2$/UV (Dewil et al. 2017), but in this study, a combination of $O_3$ and $H_2O_2$ was used to form the very reactive hydroxyl radical.

Use of $O_3$ and $H_2O_2$ in RAS is beneficial to overall water quality (Pedersen and Pedersen 2012; Powell and Scolding 2018) by reducing water turbidity and pathogens. The oxidants degrade large organic molecules when reacting with dissolved organic matter (DOM). Both $O_3$ and $H_2O_2$ are decomposed to oxygen and water in the process (Arvin and Pedersen 2015; Powell and Scolding 2018) without forming harmful by-products in freshwater systems (Pedersen and Pedersen 2012; Spiliotopoulou et al. 2018). However, treatment must be carefully planned, because O3 is harmful to the raised species and its entry to rearing tanks must be avoided (Leynen et al. 1998; Gaikowski et al. 1999). This can be done by adjusting the treatment properly and all $O_3$ consumed or removed, for example, with UV-light or activated carbon filters (Goncalves and Gagnon 2011).

In previous studies, $O_3$ treatment in RAS has been inefficient in fully removing off-flavours at low concentrations (Schrader et al. 2010, Pettersson et al. 2022). This is probably due to large amounts of organic matter because $O_3$ reacts unselectively with all organic matter, not only with the off-flavour compounds (Li et al. 2019). $H_2O_2$ and hydroxyl radicals formed in AOP have been more efficient in off-flavour removal even in large-scale applications (Lindholm-Lehto, Kiuru, and Hannelin 2020), but addition of suitable and sufficient oxidant dose is of high importance (Pettersson et al. 2022).

In this study, it was investigated if off-flavour compounds can be removed with intense oxidation treatment. We hypothesized that the intensive treatment with suitable oxidants could reduce the off-flavour concentrations without compromising the fish health. Oxidant treatments have potential to remove or decrease the time required for the depuration of off-flavours that can potentially reduce the depuration and, ultimately the production costs.

## 2 | Materials and Methods

### 2.1 | Experimental Setup

The experiment was conducted in the RAS-platform of Natural Resources Institute Finland (Luke). The experimental setup has been previously explained in Pettersson et al. (2022). In short, nine small individual and identical experimental RASs (total volume 1440 L) were used, all using the same inlet/replacement water. Each treatment (control, $H_2O_2$, AOP) was performed with three replicates and treated identically, excluding the oxidant additions. Oxygen saturation was kept above 90% in the fish tanks, and the $CO_2$ content below 15 mg $L^{-1}$. The water temperature was 13.5 ± 0.7°C and the lights were kept on 24 h $day^{-1}$.

The freshwater RASs consisted of a rearing tank, solids removal setup (swirl separator, drum filter), water reserve tank, fixed bed bioreactor (80-L Saddle-Chips, KSK Aqua, Skive, Denmark), aeration device, pH control unit (20% sodium hydroxide solution) and an oxygen diffuser. Replacement water was added before the swirl separator and came from an oligotrophic Lake Peurunka (62.44886, 25.85201, 694 ha, 59,600 $m^3$). The relative water renewal rate was 500 L $kg^{-1}$ feed.

The rearing tanks were stocked with 23.5 kg of rainbow trout (*Oncorhynchus mykiss*), on average 334 g per individual. The fish were fed by an automated feeding system (T Drum 2000, Arvo-Tec, Huutokoski, Finland) with a commercial fish feed of 0.21 kg $d^{-1}$ (BioMar Orbit 4.5 mm), containing crude protein 42%–45%, crude lipid 26%–29%, 1.0%–1.2% phosphorous and 6.3%–7.0% nitrogen, as given by the manufacturer.

Due to the short experimental period, there was no need to remove supernumerary fish. The fish were visually inspected on a daily basis. The study followed the protocols approved by the Luke Animal Care Committee, Helsinki, Finland, and EU Directive 2010/63/EU (Council Directive 2010/63/EU) for animal experiments.

The treatments were $H_2O_2$, AOP and the control without oxidative treatments. $H_2O_2$ treatment had 1.2 μL $L^{-1}$ of pure $H_2O_2$ (1% solution), 53 g $H_2O_2$ $kg^{-1}$ feed added to water during the loop. AOP had 1.2 mg $L^{-1}$ of $O_3$ (37.9 g $O_3$ $kg^{-1}$ feed) added to water with 0.6 μL $L^{-1}$ of $H_2O_2$ (26.5 g $H_2O_2$ $kg^{-1}$ feed). $H_2O_2$ and AOP had a side loop where the oxidants were added via a pump in a continuous mode. The water was led from a water reserve tank and returned to the same tank. The flow in the loop was 0.23 L $s^{-1}$. The treatments were active for 8 h $day^{-1}$. In total, the experiment lasted from 31 January 2022 to 11 February 2022. The treatments were paused for the weekend due to safety reasons.

The $O_3$ generator was fed with oxygen at a constant pressure of 0.9 bar and injected directly into the water. Full description of the $O_3$ production can be found in Pettersson et al. 2022). The produced $O_3$ was monitored with $O_3$-analyser (BMT 964 $O_3$ analyser, BMT Messtechnik GmbH, Stahnsdorf, Germany) to ensure the correct concentrations injected to water. New batches of injected $H_2O_2$ solutions were made every two days to refill the

pump canisters at selected concentration (1%) by diluting 50% $H_2O_2$ solution (Bang & Bonsomer, Helsinki, Finland).

## 2.2 | Sampling

Aqueous samples were taken from the culture tanks in the morning (at 7:00 AM) before the start of the ozonation and in the afternoon (at 3:00 PM) after the treatment. Water was taken from the rearing tank. Samples were then filtered through syringe filters (0.45 μm, prewashed cellulose acetate, Sartorius, 16555-Q) to 50-mL sample tubes (polypropylene (PP) and high-density polyethylene (HDPE), VWR). Samples were stored in a freezer (at −22°C) until the analysis.

Fish were sampled four times: in the beginning, before the first weekend, after the first weekend and in the end of the experiment. Three individuals were randomly taken from each tank and immediately euthanized with a sharp blow on the head. A skin-free part of flesh (10 g) was taken from the lateral part of fish flesh as reported by Hathurusingha and Davey (2016). The samples were immediately frozen and stored at −22°C until the analysis.

## 2.3 | Water Quality Analyses

Water quality was monitored constantly by an online monitoring system (S::can, Vienna, Austria) that measured water flow rate, temperature, nitrite nitrogen ($NO_2$-N), nitrate nitrogen ($NO_3$-N), TOC, turbidity, total suspended solids (TSS) and UV-254 every 6 min. Additionally, the concentrations of dissolved oxygen (Oxy-Guard, Farum, Denmark), carbon dioxide (Franatech, Lüneburg, Germany) and the pH (ProMinent, Heidelberg, Germany) were constantly monitored.

Additionally, $NO_2$-N, $NO_3$-N and total ammonia-N were analysed weekly with quick spectrophotometric laboratory tests (Procedure 8038 Nessler, LCK341/342, LCK340 and LCK349 UN3316 9 II, DS 3900, Hach, Loveland, Colorado, USA). Redox-values (three replicates from each tank) were measured from culture tanks two times a day with pH/ORP/conductivity meter D-74 (LAQUAact, Horiba, Irvine California, USA).

### 2.3.1 | Off-Flavour Analyses

The off-flavours were analysed by using a method based on automated head space solid phase microextraction (HS-SPME) with a PAL3 autosampler (CTC Analytics, Switzerland) coupled with gas chromatography and tandem mass spectrometry (GC-MS/MS, 7000 Series Triple Quadrupole mass spectrometer, Agilent, Santa Clara, CA, USA). The method allowed detection and quantification of 14 selected off-flavour compounds in circulating water and in fish flesh. The validated method, levels of detection (LOD) and quantification (LOQ) have been previously presented in Lindholm-Lehto (2022).

### 2.3.2 | Statistical Analyses

The normality of the data was tested with a Shapiro-Wilk test. The data were not normally distributed, and Kruskal–Wallis (at a significance level 0.05) test was chosen to test the difference of off-flavour concentrations between treatments in water and in fish flesh. Wilcoxon's test was used to compare the difference between morning and evening samples (at a significance level 0.05).

**TABLE 1** | FCR, SGR and mortality (%) of control, AOP and $H_2O_2$ treatments (± std, $n = 3$).

| | FCR | SGR | Mortality (%) |
|---|---|---|---|
| Control | 1.05 ±0.03 | 0.79 ± 0.02 | 0.0 ± 0.01 |
| AOP | 1.04 ± 0.07 | 0.78 ± 0.07 | 0.03 ± 0.03 |
| H2O2 | 0.99 ± 0.05 | 0.93 ± 0.16 | 0.08 ± 0.08 |

## 3 | Results

## 3.1 | Fish Growth and Water Quality

The fish growth parameters are presented in Table 1. Feed conversion ratio (FCR) was close to 1 in all treatments. Specific growth rate (SGR), however, was higher in the $H_2O_2$ treatments compared to control and AOP, but it also demonstrated higher mortality. None of the treatments showed harmful effects (Table 1) and statistically significant difference was not found between the treatments (Table S1).

Dissolved organic carbon (DOC) concentrations were high in all systems (18–20 mg $L^{-1}$) and gradually declined throughout the experiment (17–18 mg $L^{-1}$ in the end, Figure S1). Statistical difference was not found in DOC between the treatments or between morning and evening samplings (Table S2).

Total ammonia nitrogen (TAN) concentrations were within 0.8–1.8 mg $L^{-1}$, although $H_2O_2$ treatment peaked at 4.2 mg $L^{-1}$ in the end of the experiment (Figure S2). Otherwise, concentrations were in similar range in all treatments. Nitrite-N concentrations, however, were higher in $H_2O_2$ and AOP treatments than in the control (Table S2), ranging from 0.50 to 1.43 mg $L^{-1}$ (Figure S2). For Nitrate-N, the concentrations were higher in $H_2O_2$ and AOP treatments than in the controls, but the difference was not statistically significant (Table S2). Nitrate concentrations ranged within 38–68 mg $L^{-1}$ (Figure S2).

Significant difference was not found between any of the treatments for Redox values (Table S2). Redox fluctuated during the experiment from 100 to 280 mv, but the fluctuation was observed in all treatments (Figure S3).

## 3.2 | Off-Flavours

### 3.2.1 | Off-Flavours in Water

The AOP and $H_2O_2$ treatments had a significant ($p < 0.05$) effect on most off-flavour concentrations in water. In general treatments, off-flavour concentrations decreased, but with some exceptions. For example, 3-isobutyl-2-methoxypyrazine (IBMP) values were statistically higher in $H_2O_2$ treatments than in the controls (Table 2, Table S3). The AOP treatment had statistically

**TABLE 2** | Off-flavour concentrations in water of control, AOP and $H_2O_2$ treatments (ng $L^{-1}$, ± std, $n = 60$, except for LW, $n = 20$, from 31 January 2022 to 11 February 2022).

| | Control | std (±) | AOP | std (±) | $H_2O_2$ | std (±) | LW | std (±) |
|---|---|---|---|---|---|---|---|---|
| Hexanal | 22.2 | 12.7 | 19.0 | 12.4 | 18.4 | 11.5 | 52.9 | 9.2 |
| Methional | 12.5 | 7.2 | 7.3 | 0.6 | 8.2 | 2.6 | 5.9 | 2.9 |
| Octanal | 8.3 | 6.0 | 6.2 | 1.6 | 9.4 | 5.1 | 2.3 | 5.9 |
| Hexenoic acid | 0.5 | 0.5 | 0.2 | 0.2 | 0.4 | 0.3 | 0.4 | 0.7 |
| PhenA | 18.9 | 7.3 | 6.6 | 0.9 | 30.0 | 14.5 | 3.0 | 1.6 |
| IPMP | 53.3 | 13.9 | 29.5 | 7.7 | 38.1 | 18.3 | 6.0 | 1.2 |
| Acetoin | 3.1 | 2.1 | 1.8 | 0.2 | 2.1 | 0.7 | 1.2 | 3.5 |
| Octanoic acid | 52.6 | 14.1 | 35.7 | 18.1 | 41.9 | 12.5 | 26.1 | 13.8 |
| IBMP | 5.7 | 5.7 | 22.0 | 7.9 | 13.9 | 4.9 | 19.5 | 3.2 |
| MIB | 38.9 | 12.6 | 13.6 | 3.5 | 42.6 | 4.2 | 9.3 | 6.7 |
| Terpineol | 2.7 | 1.7 | 1.1 | 0.5 | 1.5 | 0.7 | 1.4 | 1.8 |
| TCA | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.1 |
| Vanillin | 18.6 | 9.2 | 9.8 | 3.3 | 12.0 | 6.7 | 7.5 | 2.7 |
| GSM | 11.6 | 5.4 | 6.9 | 2.4 | 11.1 | 7.8 | 1.6 | 0.8 |

lower concentrations in majority of the measured off-flavours than in the controls, except for hexanal, octanal, octanoic acid, 2,4,6-trichloroanisole (TCA) and vanillin (Table 2, Table S3). $H_2O_2$ treatment had lower effect on off-flavour compounds than the AOP treatment (Table S3).

From the measured off-flavours, only GSM and MIB were relatively constant and abundant throughout the experiment. Other compounds showed more fluctuation (an example in Figure S4).

No statistical difference was observed between morning and evening in $H_2O_2$ treatments and in the controls. On the other hand, in the AOP treatment, on average 4 ± 0.5 ng $L^{-1}$ higher concentrations of off-flavours were observed in the morning (Table S4).

There was a significant correlation between the off-flavours in the inlet water and in the circulating water (Table S5). Methional, hexenoic acid, acetoin, α-terpineol, TCA and vanillin correlated with all treatments. On the other hand, 2-isopropyl-3-methoxypyrazine (IPMP), IBMP, 2-methylisoborneol (MIB) and geosmin (GSM) did not correlate with the inlet water (Table S5).

### 3.2.2 | Off-Flavours in Fish

AOP and $H_2O_2$ treatments reduced the off-flavours concentrations in fish compared to the controls (Figure 1). Among the measured off-flavours, only MIB, α-terpineol and GSM were reduced, while others did not show any statistical difference (Table S4). In the AOP treatment, there were on average 242 ± 72 ng $kg^{-1}$ lower MIB, 8 ± 4 ng $kg^{-1}$ lower α-terpineol and 295 ± 203 ng $kg^{-1}$ lower GSM concentrations. In the $H_2O_2$ treatment, fish contained on average 285 ± 92 ng $kg^{-1}$ lower MIB and 383 ± 281 ng $kg^{-1}$ lower GSM concentrations in fish flesh than in the controls. However, no statistically significant difference was

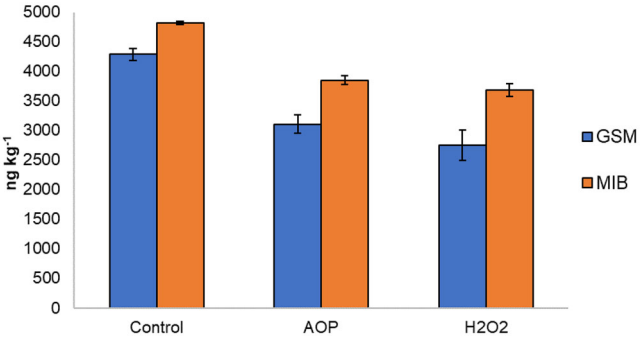


**FIGURE 1** | Average GSM and MIB concentrations in fish (rainbow trout *Oncorhynchus mykiss*) flesh (ng $kg^{-1}$, ± std, $n = 20$) in total (from 31 January 2022 to 11 February 2022).

observed in α-terpineol concentrations between $H_2O_2$ and the controls (Table S6). $H_2O_2$ showed slightly better results compared to AOP treatment, but not with a statistical significance (Figure 1, Table S6).

The total amounts of off-flavours (Figure 2) in the AOP and $H_2O_2$ treatments were lower than in the controls. Despite of some fluctuation in concentrations, fish had systematically lower concentrations after the AOP and $H_2O_2$ treatments (Table S7).

### 3.2.3 | Off-Flavour Composition in Water and in Fish

Overall, the inlet water had different composition of off-flavours compared with the RAS water. For example, the RAS water contained four times more IPMP, four times less hexanal and four times more GSM than in the inlet water. Similar amounts of octanoic acid were present both in the inlet water and in RAS water (Figure 3).

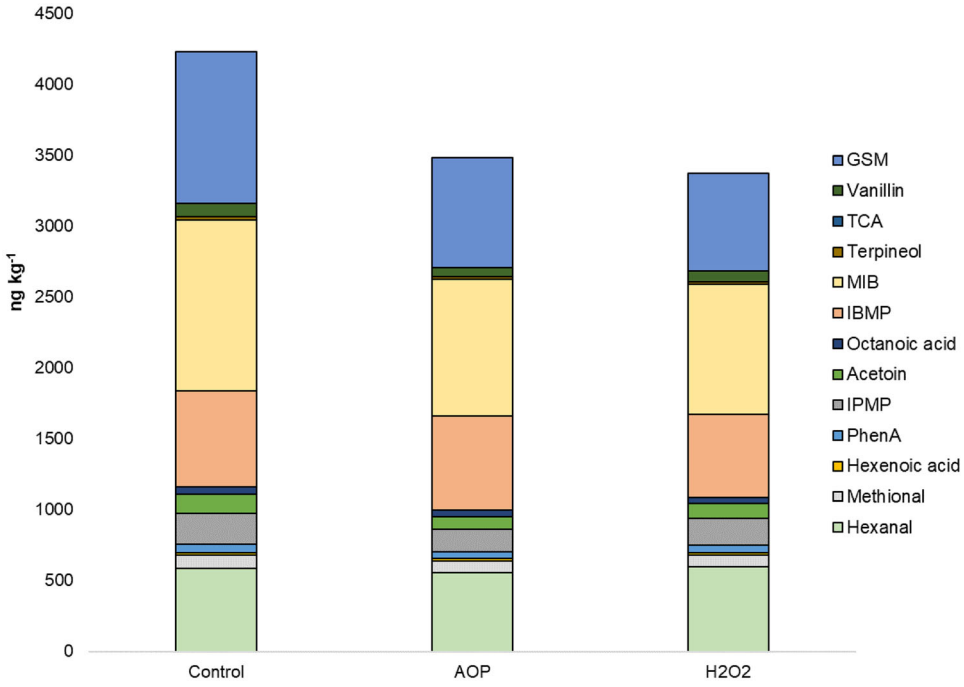


**FIGURE 2** | Average off-flavour concentrations of control, AOP and $H_2O_2$ treatments in fish (rainbow trout *Oncorhynchus mykiss*) flesh (ng $kg^{-1}$, $n = 20$), in total (31 January 2022 to 11 February 2022).

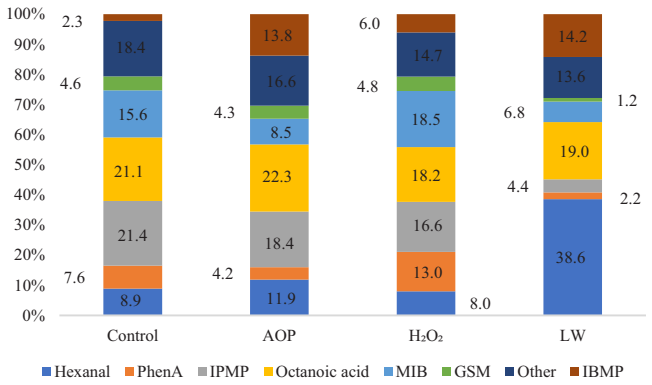


**FIGURE 3** | Off-flavour profiles (%) in water of control, AOP and $H_2O_2$ treatments, and inlet water (LW) (ng $L^{-1}$, $n = 20$, from 31 January 2022 to 11 February 2022).

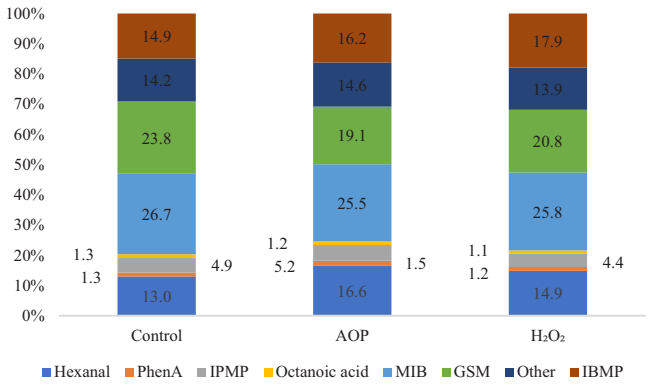


**FIGURE 4** | Off-flavour profiles (%) in fish flesh (rainbow trout *Oncorhynchus mykiss*) of control, AOP and $H_2O_2$ treatments (ng $kg^{-1}$, $n = 20$, from 31 January 2022 to 11 February 2022).

In water, the off-flavour profiles were quite similar in all RASs, but some differences were present. The concentration of phenyl acetaldehyde (PhenA) was two times higher in $H_2O_2$ than in the controls, while in the AOP, there was approximately two times lower content of PhenA than in the controls. The MIB concentration was also about half in AOPs than in other RASs. Additionally, IPMP, octanoic acid and MIB were responsible for about 50% of measured off-flavours. In the inlet water, the most abundant components were octanoic acid and hexanal (Figure 3).

In fish, the off-flavour profiles in fish flesh were very similar in all RASs. The majority of off-flavours (approximately 50%) consisted of MIB and GSM. The proportion of GSM was less than 5% of off-flavours in water, while over 20% in fish flesh. Other major components were IBMP and hexanal, both having a slightly higher proportion in flesh than in water. Octanoic acid, IPMP and PhenA were much more abundant in water than in fish flesh. Furthermore, the proportion of IPMP was 5%, while those of octanoic acid and PhenA were less than 2% in the fish flesh (Figure 4).

## 4 | Discussion

Present results clearly demonstrates that some of the measured off-flavours strongly correlated with the compounds detected in the inlet water (methional, hexenoic acid, acetoin, α-terpineol, TCA and vanillin). This suggests that those off-flavours can be derived from the inlet water rather than produced in the RAS. Furthermore, treatment of the inlet water, for example

with oxidizers, could lead to the removal of these compounds. Oxidization of water with reduced organic matter (and DOM) content, AOP treatment has shown very good effects against off-flavours (Kropp et al. 2022). In this study, however, the off-flavours found in the inlet water, were in minor role among those found in the circulating water and in fish flesh.

The off-flavours that did not correlate with the inlet water (IPMP, IBMP, MIB, GSM), consisted of over 75% of the detected off-flavours in fish flesh. Many of these off-flavours can originate from the RAS via microbial actions, inhabiting the biofilter or other system parts (Moretto et al. 2022), or derived from feed (Mahmoud and Buettner 2017). This emphasizes the requirement of depuration period with an inlet water of good quality.

In this study, off-flavours concentrations fluctuated in a similar way in every treatment. Some of the compounds showed increased values in the inlet and in circulating water (with a delay though). In all RASs, the concentrations of PhenA were mostly below 10 ng $L^{-1}$. For octanoic acid, the concentrations were on average at 10 ng $L^{-1}$ but peaked occasionally up to 120 ng $L^{-1}$. Davidson et al. (2022) also displayed considerable deviation and peaked off-flavour concentrations in their experiment, although their experiment was performed in marine water.

Off-flavour concentrations of similar range have been detected in previous studies (Lindholm-Lehto, Kiuru, and Hannelin 2020; Lindholm-Lehto 2022). A study performed in a commercial RAS suggested that $H_2O_2$ addition can reduce off-flavours in RAS water (Lindholm-Lehto, Kiuru, and Hannelin 2020). Later, a study by Pettersson et al. (2022) showed fluctuations of off-flavours. These results should provoke future experiments to study off-flavour dynamics in RAS water with very frequent sampling and longer time periods as it would be valuable to understand how these fluctuations occur.

Hexanal (control and AOP systems correlated) and octanoic acid (correlated with AOP) were extremely abundant in lake water, so it is possible that they were mainly led to the RASs via the inlet water. Origin of octanal (correlated with control) and PhenA (correlated with AOP) remained more uncertain. Origins of different compounds would be a valuable information to understand the function, and definitely a subject for future studies.

MIB and GSM consisted of almost 50% of off-flavours in fish flesh. In the circulating water, their proportion was much smaller. Interestingly, MIB and GSM were relatively well removed from water compared to, for example, octanoic acid that was hardly removed by the treatments. Similarly, IPMP was only partly removed by the AOP treatment. Both octanoic acid and IPMP compounds are easily oxidized and aggressively attacked by $O_3$ and $H_2O_2$ (Spiliotopoulou et al. 2018), but this was not observed by the results of this study. The results may be explained by the unselectivity of the hydroxyl radical as it is a strong oxidizer but may have reacted with other organic matter first before these compounds at low concentrations. On the other hand, IPMP and octanoic acid may be locally decomposed in the ozonation, but then regenerated quickly in the other parts of the system, leading to reduced differences between the morning and the evening measurements.

In general, the oxidative treatments were able to reduce the off-flavour concentrations in water and in fish flesh. The AOP had a slight statistical reduction in total off-flavours during evenings. Although the results showed reduced concentrations in water, similar behaviour was not observed in fish flesh. For example, the octanol/water partition coefficients ($K_{ow}$) of GSM and MIB are 3.57 and 2.58 (Céondo 2024) or 3.31 (Howgate 2004) that were highly concentrated in the lipids of fish flesh. On the other hand, the octanol/water partition coefficient of PhenA is 1.43 suggesting lower lipophilicity and decreased accumulation compared to GSM and MIB. Similarly, IPMP with $K_{ow}$ of 1.61 was only moderately concentrated in fish flesh. PhenA, octanoic acid and IPMP demonstrated poor accumulation to fish flesh as their part in water was much bigger than in fish. Hexanal ($K_{ow}$ 1.77) and IBMP ($K_{ow}$ 1.68) did the opposite with higher concentrations in fish than in water. This is likely explained by the low $K_{ow}$ values compared to GSM and MIB with high tendency for accumulation. The increase in their concentrations was not as extensive as in case of MIB and GSM.

The off-flavour profiles did not change with the treatments in fish flesh. In water, they remained very similar, AOP with less MIB than the control or the $H_2O_2$ treatment. The inlet water contained hexanal as it is by far the most dominant ingredient (about 40%), but it made only about 10% of the RAS water. Part of that can be accumulated to fish, but it is also possible that microbes in the system use this fatty acid as their energy source, decreasing its concentration. Another notable observation was the 14.2% of IBMP in inlet water which is one of the off-flavours that are thought to be derived from fish feed (Mahmoud and Buettner 2017; Lindholm-Lehto et al. 2023). IBMP is also found in plants, mainly bell peppers and grapes for example (Zamolo and Wüst 2023), so it is being derived from the plants or algae in the water. This can vary depending on the inlet water quality.

The treatments were able to significantly reduce MIB, GSM and $\alpha$-terpineol concentrations in fish flesh; the first two compounds were responsible for most of the off-flavours. Concentrations possibly below the sensory threshold were achieved for GSM being slightly below 310–900 ng $kg^{-1}$ (threshold 250–900 ng $kg^{-1}$, Robertson et al. 2005; Lindholm-Lehto and Vielma 2018) in oxidized systems. Sensory thresholds for MIB were recorded to be 700 ng $kg^{-1}$ in fish flesh and oxidative treatments were able to reduce concentrations very close to it, ranging from 770 to 1000 ng $kg^{-1}$. It is very possible to have these concentrations to be reduced below those sensory thresholds with optimization of this method in future. Fish from the controls already had higher GMS and MIB concentrations in the initial sampling. However, the concentrations in the controls either remained constant or increased during the experiment compared to other RASs with declining concentrations.

The $\alpha$-terpineol was reduced in fish flesh due to oxidative treatment. This off-flavour was found at low concentrations (11–29 ng $kg^{-1}$ in AOP and $H_2O_2$); thus, its effect was most likely negligible in terms of sensory profile (Mahmoud and Buettner 2017; Podduturi et al. 2017).

The aim of depuration is to decrease and remove off-flavours from water before they concentrate in fish. However, IBMP can possibly enter the fish through feed. Another problem with

IBMP is that although its sensory threshold in fish is yet to be determined, in water it is very low, only 2 ng $L^{-1}$ (Li et al. 2016). In this study, 360–800 ng $kg^{-1}$ of IBMP was found in fish flesh in the AOP and $H_2O_2$ treatments. The sensory threshold is at similar range for IPMP too (Li et al. 2016), but its concentrations in fish flesh were much lower ranging from 82 to 274 ng $kg^{-1}$.

Dupre et al. (2023) showed that GSM can also concentrate in fish through feed but concluded that it should not be an issue with high-quality feed (Schrader 2023). However, it was found that starvation and change in gut microbiota reduced off-flavours in fish flesh during depuration period (Zou et al. 2023). As our treatments had an effect on off-flavour concentrations in fish flesh, at least some GSM and MIB accumulate in fish flesh via water. IBMP and IPMP were not affected by our treatments, which supports the theory of them being feed derived. One of the main off-flavour components in fish flesh was hexanal. Its sensory threshold in fish has not been determined. However, in a study by Lindholm-Lehto et al. (2023), hexanal was the least found off-flavour in fish flesh, while in this study, it was the fourth most abundant.

According to present results, the fish were not affected by the treatments, although the measuring period for growth data included only 10 days. However, nitrite concentrations were increased in the treated systems that could indicate effect on nitrification biofilm. Especially nitrite concentrations were higher in $H_2O_2$ treatments, sometimes exceeding the recommended threshold of 1 mg $L^{-1}$. TAN concentrations were similar among the treatments. Nitrate concentrations were slightly higher for the treatments, but still below known toxicity (Dauda and Akinwole 2015). Rurangwa and Verdegem (2015) suggested that intense AOP treatment in RAS would kill the microbial populations in RAS. It seems that the treatments had some effect on the nitrifying microbes.

Redox values were pretty identical in every treatment, which suggests that oxidative stress was under control. Interestingly, the usually reported decrease in DOC was not seen here. There was also no difference between morning and evening samples, although it was speculated that before the treatment of the day, DOC and off-flavours would be higher, than in the evening when treatment was stopped. The DOC was very high in the beginning of the experiment already (about 20 mg $L^{-1}$) and decreased gradually in every system as the experiment lasted. It could be that systems had for some reason accumulated more organic matter than normally and DOC started to level off during the experiment. This hid any changes to DOC made by treatments.

All in all, as suggested in our original hypothesis, the oxidant treatments were able to reduce off-flavour concentrations in water and in fish. There was a slight reduction in the total off-flavour content in AOP in the evening. Furthermore, the treatments may have disinfected water and reduced microbial load reducing the production of off-flavour, although nitrification bacteria may have also been affected. Most likely, both processes occurred simultaneously. Previous studies suggest that pre-treatment for water would be required to $O_3$ have substantial effect on off-flavours (Klausen and Grønborg 2010; Rurangwa and Verdegem 2015). This study showed that a sufficient oxidizing treatment can decrease off-flavours in RAS and in fish flesh. Both AOP and $H_2O_2$ treatments were successful, but AOP proved to be better than $H_2O_2$. In the future, more attention should be focused on research on the off-flavour dynamics and optimization of these treatments.

## 5 | Conclusions

The part-time treatments were found to be able to reduce some of the studied off-flavour compound below or very close to the sensory thresholds in water and fish flesh. However, treatments did not have any statistical effect on IPMP, IBMP or hexanal concentrations that also appeared in fish. AOP was able to affect most of the measured off-flavours in water, but due to the unpredictable nature of the off-flavour dynamics, the magnitude of this effect was not clearly observed. Four of the off-flavours were identified to be produced in the system rather than be derived from inlet water while six of the off-flavours were clearly derived from inlet water. None of them had big impact on the off-flavour composition in fish flesh. Nonetheless, treatment of the inlet water could be worth of investigating in the future. Surprisingly, the treatments did not reduce the DOC in water. To conclude, following studies are required regarding the off-flavour dynamics in RAS and optimization of the oxidation treatment to benefit from its full potential.

### Author Contributions

**Samu Pettersson**: Data curation, investigation, writing–original draft, conceptualization. **Petra C. Lindholm-Lehto**: Writing–original draft, validation, writing–review and editing, project administration, conceptualization, methodology, funding acquisition. **Jani T. Pulkkinen**: Conceptualization, methodology, writing–review and editing. **Tuula Tuhkanen**: Conceptualization, investigation, funding acquisition, writing–review and editing, supervision.


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### Consent

The authors have nothing to report.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

All data used in this manuscript can be obtained by emailing the corresponding author.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.