

Removing Earthy and Musty Odors in Secondary Effluent Using Ozone and Zeolite for Culturing *Plecoglossus altivelis*

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1. Introduction

The potential for treated municipal wastewater reuse in freshwater aquaculture represents a significant opportunity for resource utilization, particularly in developed urban areas with advanced wastewater treatment infrastructure. However, earthy and musty odors in effluent from wastewater treatment plant (WWTP), primarily attributed to volatile organic compounds (VOCs) such as 2,4,6-Trichloroanisole (TCA), Geosmin (GSM), and 2-Methylisoborneol (MIB)¹⁾, pose a significant challenge for water reuse applications, including aquaculture. Since 2021, our group has focused on developing a land-based freshwater aquaculture system utilizing secondary effluent (SE) from a Tokyo WWTP. Our study has demonstrated the feasibility of culturing Ayu sweetfish (*P. altivelis*) under controlled SE conditions; however, the bioaccumulation of the persistent VOCs poses a potential threat to fish quality²⁾. In 2022, we developed a novel ozone-zeolite treatment system for VOC removal from SE, achieving average removal efficiencies of 61% for TCA, 66% for GSM, and 33% for MIB³⁾. While analysis of VOC accumulation in fish flesh suggested that odorant removal from SE could mitigate bioaccumulation, a comprehensive sensory evaluation to validate the effectiveness of this novel treatment system was lacking.

This study aimed to enhance the VOC removal efficiency of the ozone-zeolite system by incorporating a separate ozone-SE water reaction column and by investigating the impact of increased zeolite loading (238g vs. employed 199g in the corrugated honeycomb structure) on VOC removal. Furthermore, the study evaluated the influence of dietary supplementation with algae, cultivated concurrently in the SE, on VOC bioaccumulation in Ayu and the subsequent sensory attributes (odor and taste) of the fish.

2. Methods

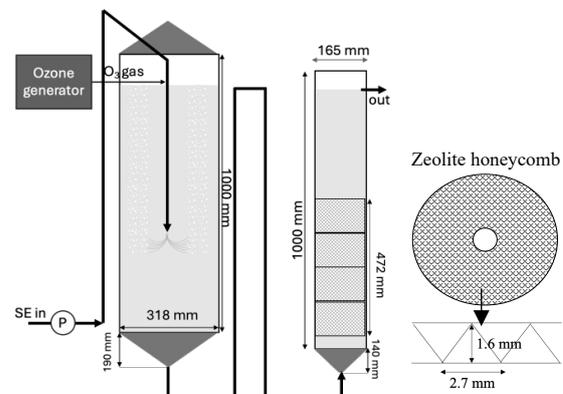
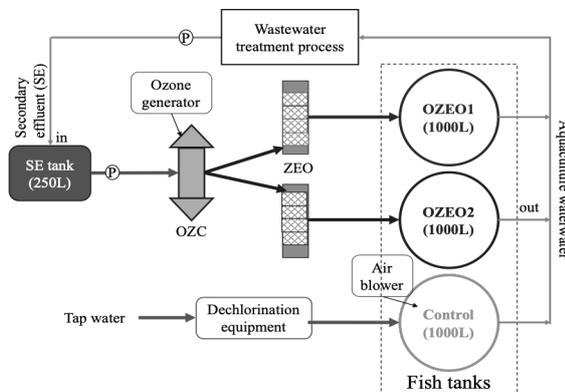


Figure 1 Layout of aquacultural system and treatments.

OZC: ozone injection column; ZEO: zeolite column

Figure 2 Illustration of ozone and zeolite columns

Culture system: Ayu were cultured using a land-based aquaculture system located in a Tokyo WWTP from April to July 2024. Layout of the system is depicted in Figure 1. Detailed descriptions of the system design and operational parameters can be found in our previously published works²⁻³⁾. A key modification to the system was the incorporation of an ozone reaction column (approximately 68.5 L), designed to enhance the contact time between ozone and the SE (Figure 2).

Experimental treatments and condition: The aquaculture experiment comprised three treatment groups: two experimental treatments employing the ozone-zeolite treatment system and one control group utilizing dechlorinated municipal tap water (Figure 1). In the ozone-zeolite system, SE was pumped from the secondary sedimentation tank of the WWTP to a receiving reservoir (SE tank) and subsequently introduced into the ozone injection column. Two distinct zeolite columns were employed, each containing corrugated honeycombs packed with Y-type zeolite (Si/Al = 95) at different masses: 199 g (OZEO1) and 238 g (OZEO2). The effluent from the ozone reaction column was then directed to the respective zeolite columns, following a configuration similar to that described by Ogata et al. (2024). The flow rate within the ozone reaction column was maintained at 3–4 L/min, resulting in a corresponding flow rate of 1.5–2 L/min into each fish rearing tank. Consequently, the ozone injection rate was controlled within the range of 16–22 mg O₃/L. This ozone dosage was maintained in accordance with Japanese guidelines for ozone application in wastewater treatment (>10

mg O₃/L).

Sampling and analysis: Water samples were collected at a minimum frequency of once per week throughout the experimental period. Fish tissue samples were collected at the initiation and termination of the experiment. VOC analysis of both water and fish samples was performed using headspace gas chromatography-mass spectrometry (GC-MS), following methodologies consistent with our previously published studies²⁻³.

Sensory blinding test: Harvested fish samples were prepared for sensory evaluation by grilling using a gas cooker without the addition of salt to avoid confounding taste factors. The odor and taste of the grilled fish samples were assessed by a panel of 30 participants collaborating in this study. During the assessment, the control fish group was explicitly identified to the participants, while the other groups was not to minimize potential bias. A 7-point scale, ranging from -3 to +3, was employed. A score of 0 indicated no preference (equal to the control fish group), scores from -1 to -3 indicated decreasing preference, and scores from +1 to +3 indicated increasing preference. Ethical approval for the sensory evaluation was obtained from the Research Ethics Committee of the University of Tokyo (Approval No. KE24-10).

3. Results and Discussion

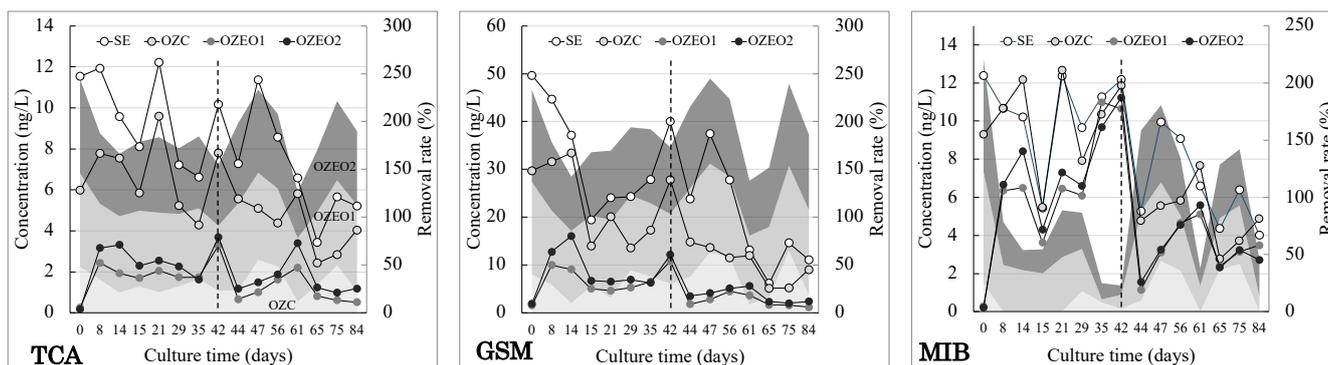


Figure 3 Concentrations (lines) and removal rates (areas) of VOCs in water samples (unit: ng/L). Dotted line shows time of changing a new zeolite honeycomb; OZC: water samples from ozone injection column

Removal of VOCs in SE by ozone and zeolite treatment. Figure 3 presents the temporal variation of VOC concentrations in SE and treated SE water samples, alongside corresponding removal rates over the culture period. Removal rate (%) was calculated as $[(\text{VOC concentration in SE} - \text{VOC concentration in treated SE}) / \text{VOC concentration in SE}] * 100$. Notably, VOC concentration in water samples from OZEO1 and OZEO2 treatments were significantly lower ($p < 0.05$) compared to those in the SE and the outflow from ozone injection column (OZC). Furthermore, the removal rates of TCA and GSM in both test treatments ranged 49–99% and 57–97%, respectively, indicating a substantial reduction in the VOCs. Conversely, with the exception of initial measurements at 0 and 44 days post-honeycomb injection, MIB removal rates ranged 2.5–50%. This lower MIB removal is potentially attributable to several factors influencing adsorption onto the employed zeolite: (i) less favorable interactions or hindered intraparticle diffusion of MIB due to its molecular dimensions or configuration (ii) a lower degree of hydrophobicity in MIB compared to GSM and TCA, resulting in weaker interactions with hydrophobic sites within the zeolite pores⁴⁻⁵. Additionally, the rate constants for the reaction of ozone with MIB are likely lower than those for GSM and TCA⁶, contributing to its less effective removal. Furthermore, the initial concentrations of TCA and GSM in SE frequently exceeded their respective odor threshold values (2 and 10 ng/L) while MIB concentrations rarely surpassed its threshold (10 ng/L), suggesting that TCA and GSM as the primary contributors to earthy and musty odor in the WWTP effluent. These findings suggests the potential efficacy of the odor treatment system, employing ozone in conjunction with zeolite as an adsorbent, in removing earthy and musty odor the effluent. Notably, no clear difference was observed between the removal efficiencies of the OZEO1 and OZEO2 treatments, indicating that the increased zeolite loading in the honeycomb structure of OZEO2 did not substantially enhance the removal of the investigated musty odor compounds under the tested conditions.

Odor bioaccumulation in fish. Figure 4 presents the concentrations of VOCs detected in the flesh tissue of Ayu harvested from the experiment. Statistical analysis revealed no significant difference ($p > 0.05$) in the concentrations of TCA and GSM between the test treatments (OZEO1 and OZEO2) and the control. This observation indicates that the effective removal of TCA and GSM from the SE using ozone and zeolite significantly contributed to reducing their bioaccumulation in the fish tissue. This finding aligns with the results of a prior study conducted in 2022³). Furthermore, the concentration of MIB in fish from the control was higher than that in the test systems. This suggests that, under the conditions of this experiment, MIB may have exerted a less pronounced influence on the overall odor and taste profile of the fish compared to TCA and GSM. Notably, fish from the OZEO1 and OZEO2 treatments that were additionally fed SE-cultivated algae for one week exhibited significantly lower ($p < 0.05$) concentrations of TCA and GSM in their flesh compared to fish that were not fed algae. This finding suggests that dietary supplementation with algae cultivated in the treated SE has a discernible effect on the bioaccumulation dynamics of these specific VOCs in the fish.

Sensory evaluation of fish. Figure 5 presents sensory evaluation of grilled SE-cultured Ayu. For smell, 63–90 % of

participants rated the odor of the experimental fish groups as equivalent to or superior to that of the control. However, the olfactory evaluation did not reveal a clear difference in perceived odor between the test fish groups with and without algal feed. This suggests that residual earthy/musty odor compounds in the fish flesh had a limited impact on the overall sensory perception of the grilled fish smell. In contrast, the gustatory evaluation indicated a less favorable perception of the SE-cultured fish. Only 30% to 47% of participants rated the initial taste of the test fish groups as equivalent to or better than the control fish, with a similar range of 30% to 57% for the aftertaste. With the exception of the OZEO2_algae group, more than half of the participants perceived the taste of the test fish groups as inferior to that of the control. This highlights the non-negligible influence of VOC odorants from the SE water, which bioaccumulated in the fish flesh, on taste perception. Notably, a discernible increase was observed in the proportion of participants who rated the taste (and aftertaste) of the algae-supplemented fish groups as better than the control, compared to the non-algae-supplemented fish groups. These results suggest that reducing the bioaccumulation of odor compounds in fish flesh through algal dietary supplementation may exert a significantly positive impact on the perceived taste of the fish.

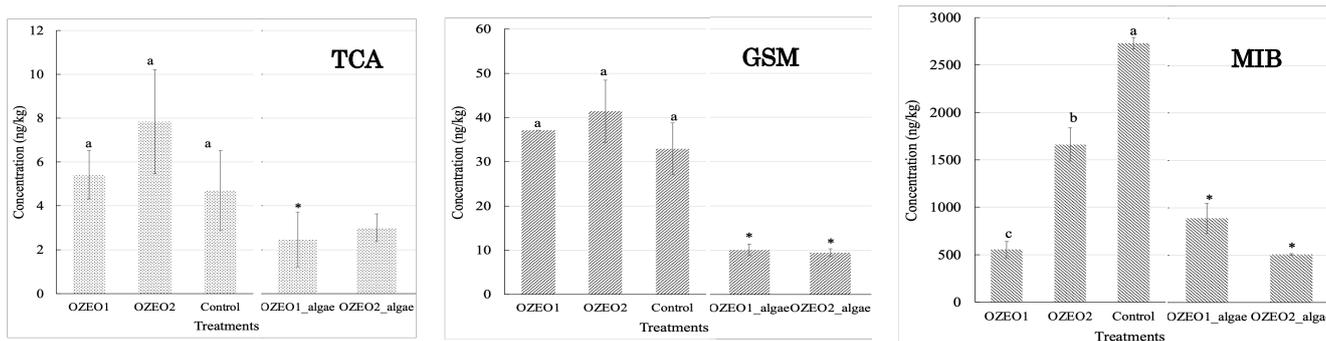


Figure 4 Concentrations of VOCs in fish flesh (unit: ng/kg). Different letters indicate significant differences among treatments (ANOVA). Asterisk indicates significant difference between treatments with and without feed SE-cultivated algae (t-test).

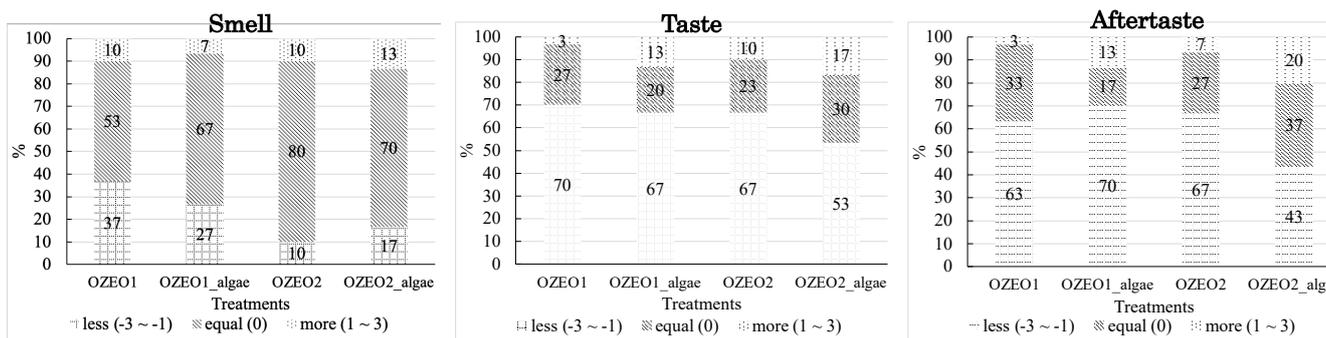


Figure 5 Sensory evaluation (smell, taste and aftertaste) of grilled fish. The results are the number of participants who assessed the odor and taste of SE-cultured fish out of the total number of participants in the sensory experiment.

4. Conclusions

The ozone-zeolite treatment system effectively reduced the concentrations of the primary earthy and musty odorants, TCA and GSM, in the secondary effluent, thereby limiting their bioaccumulation in fish flesh to levels comparable to those in tapwater-cultured fish. Sensory evaluations indicated that the treated SE resulted in fish with acceptable odor, but the bioaccumulation of residual VOCs can be negatively impacted taste. Notably, dietary supplementation with algae cultivated in the treated SE demonstrated a significant reduction in TCA and GSM bioaccumulation in fish and a corresponding improvement in their perceived taste, highlighting a potential strategy to further mitigate off-flavors in aquaculture using treated municipal wastewater. Increased zeolite loading in the treatment system did not significantly enhance VOC removal.

References

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