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Eco-aquatic engineering of dual-mode bioreactors: Integrated management of organic and nitrogenous loads in koi aquaculture

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ABSTRACT

The intensification of koi fish farming increases the accumulation of organic waste, resulting in decreased water quality and high mortality. Therefore, a more efficient and adaptive ecotechnological approach is required. This study aimed to evaluate the performance of a dual-mode (aerobic–anaerobic) bioreactor based on indigenous microbes in koi fish farming. Three treatments were used in this study: control (without circulation), RAS (recirculation without a bioreactor), and a dual-mode bioreactor. The bioreactor was constructed with four layers of media (cotton fiber, bio-ball, PVA sponge, and zeolite) and operated using continuous flow. The water quality parameters measured included COD, TOM, nitrate, DO, pH, TDS, EC, total bacterial count (TPC), and fish survival rate. The results showed that the bioreactor provided the most significant reduction in COD from 16.63 to 4.30 mg L^{-1} and reduced TOM to 14.00 mg L^{-1}, while the control and RAS experienced accumulation of organic matter. The consistent increase in nitrate in the bioreactor (up to 12.43 mg L^{-1}) was accompanied by a strong negative correlation with TOM (R^2 = 0.9971), indicating the dominance of ammonification and nitrification. DO and pH parameters remained stable in the bioreactor, whereas TDS increased more slowly than in the other two treatments. The TPC was higher in the aerobic column than in the anaerobic column. The improvement in water quality in the bioreactor directly affected koi survival, which reached 93.3%, higher than that in the RAS (75%) and control (25%). This study indicates that the dual-mode bioreactor has the potential to be an ecotechnological solution for waste management in small-to medium-scale koi farming. Further testing is recommended to evaluate the long-term performance and stability of microbial communities under varying waste loads

Keywords: bioreactor, koi aquaculture, nitrification, technology eco-aquatic.

INTRODUCTION

The intensification of aquaculture, particularly the cultivation of high-value koi fish, has resulted in the accumulation of organic and nitrogenous waste within rearing systems (Nuwansi et al., 2021; Rajesh et al., 2024). The escalation in pollutant load comprising dissolved organic matter, ammonia, nitrite, and nitrate deteriorates water quality, disrupts fish physiology, impairs coloration and growth performance, and increases susceptibility to bacterial infections (Menon et al., 2023; Zhang et al., 2025). This scenario necessitates the development of more efficient, low-energy water treatment technologies that can sustainably manage waste loads. Conventional filtration systems and single-mode biofilters are often inadequate in addressing complex waste dynamics, particularly in intensive aquaculture systems operating with limited water volumes (Zhang et al., 2021a).

Recent advancements in eco-aquatic engineering suggest that innovative bioreactor designs are among the most promising strategies for managing aquatic waste. Various studies have demonstrated that multi-zone bioreactors, such as SNAD and AMCIB, can achieve nitrogen removal efficiencies exceeding 75% through a combination of nitrification, denitrification, and anammox processes without the need for external carbon sources (Lu et al., 2020; Wang et al., 2022). Microalgae-based systems, including inverse fluidized-bed bioreactors and microalgae-bacteria photobioreactors, have been shown to remove 84–95% of nitrogen while reducing aeration requirements by utilizing the oxygen produced by the microalgae themselves (Zheng et al., 2025; Yunardi et al., 2019; Leong et al., 2021; Xia et al., 2025). Additionally, hybrid bioreactors incorporating biomaterials, such as wood, moss, or constructed wetlands, exhibit high resistance to environmental variability and low C/N ratios (Kiani et al., 2022; Audet et al., 2021; Von Ahnen et al., 2018; Wang et al., 2024). Further innovations, including FeS_x (Feammox)-based systems and electrochemical integration, have achieved nitrogen removal efficiencies of up to 93%, while also providing anti-fouling functions (Liu et al., 2025; Du et al., 2025; Tian et al., 2024).

Despite the development of various technologies, several research gaps remain. First, most high-performance bioreactors have focused on domestic and industrial wastewater, with limited application in ornamental fish farming, such as koi (Gupta et al., 2024). Second, many advanced systems demonstrate optimal performance under laboratory conditions but have yet to be validated in real-world aquaculture settings, which are characterized by fluctuations in organic load, fish metabolic activity, and environmental variability (Nayoun et al., 2024). Third, the interactions between organic matter decomposition and nitrogen transformation (C–N) in bioreactors based on indigenous microbes are rarely mechanistically described (Ran et al., 2023). Fourth, few studies have quantitatively linked water quality improvements with fish survival rates, despite this parameter being critically important for the economic success of koi farmers (Andrian et al., 2024). Finally, many technologies entail high costs, specialized microbial cultures, or technical materials that are difficult to access, rendering them unsuitable for small- and medium-scale farmers (Chang et al., 2021).

Addressing existing research gaps, this study assessed the efficacy of a dual-mode (aerobic–anaerobic) bioreactor constructed with layered media comprising cotton fiber, BioBall, PVA sponge, and zeolite. The objective of this study was to leverage the capabilities of indigenous microorganisms for the concurrent remediation of organic and nitrogenous waste. Specifically, this investigation focuses on (1) the bioreactor's capacity to diminish the organic load, as measured by COD and TOM, (2) the dynamics of nitrogen transformation, evaluated through nitrate production, (3) the stability of physicochemical parameters, including DO, pH, TDS, and EC, (4) the prevalence of indigenous bacteria within aerobic and anaerobic zones, and (5) the effect of enhanced water quality on the survival rates of koi fish. By integrating biological processes with eco-technological engineering design, this study aims to provide a mechanistic understanding of dual-mode bioreactor performance and offer a scientific foundation for the implementation of environmentally sustainable technology in small- to medium-scale koi aquaculture.

MATERIALS AND METHODS

Experimental design

This study employed three distinct treatments within the koi fish husbandry system. The treatments were as follows: (1) Control (without circulation and bioreactor), (2) Recirculating aquaculture system (RAS) (circulation without bioreactor), and (3) Dual-mode bioreactor (Aerobic-anaerobic). Each treatment was conducted in duplicate (n = 2), utilizing aquariums with dimensions of 90 × 50 × 50 cm, each containing 225 L of water. Koi fish, measuring 6–8 cm, were stocked at a density of 20 individuals per aquarium. A commercial feed with a protein content of 43% was administered at 5% of the biomass weight per day, divided into two daily feedings. The maintenance period spanned three weeks.

Construction of the dual-mode bioreactor

The bioreactor was constructed with a series of anaerobic and aerobic columns, as developed by Perwira et al., (2017). The anaerobic column comprised six chambers with a low flow rate (0.0001 m^3/h), while the aerobic column consisted of three chambers with a high flow rate (0.001 m^3/h) (Figure 1). Each column contained four layers of media: (1) cotton fiber for suspended particle filtration, (2) bioball serving as a biofilm substrate for organic degradation, (3) polyvinyl alcohol (PVA) sponge for the immobilization of indigenous microbes, and (4) zeolite for ammonia and dissolved ion adsorption. Before placement in the bioreactor, PVA sponges were rinsed with distilled water and sterilized by boiling for 15 minutes to eliminate any residual contaminants and ensure a clean substrate for microbial attachment.

This multilayered design aimed to integrate physical filtration, biofilm formation, aerobic nitrification, and ion binding through adsorption mechanisms. The bioreactor was connected to the aquarium via a submersible pump, ensuring a continuous flow.

Water quality monitoring

Water quality assessments were conducted in accordance with the protocols outlined by Indonesian National Standard (SNI). The parameters evaluated included chemical oxygen demand (COD) using the dichromate colorimetry and FAS titration method (SNI 6989.2:2019), total organic matter (TOM) via the permanganate titrimetry method (SNI 06-6989.22-2004), dissolved oxygen (DO) employing the Winkler method (SNI 06-6989.14-2004), and pH, total dissolved solids (TDS), and electrical conductivity (EC) using a digital meter (COM-600).

Total bacteria

The abundance of indigenous bacteria on the polyvinyl alcohol (PVA) sponge was analyzed at the conclusion of the study using the total plate count (TPC) method. PVA sponges were collected from both aerobic and anaerobic columns and serially diluted (10^{-2}). The diluted PVA samples were cultured on plate count agar medium. The TPC procedure adhered to the (SNI 2332.3:2015), which involved weighing 1.75 g of plate count agar medium and placing it into an Erlenmeyer flask. The medium was dissolved in 100 mL of distilled water and homogenized using a hot plate

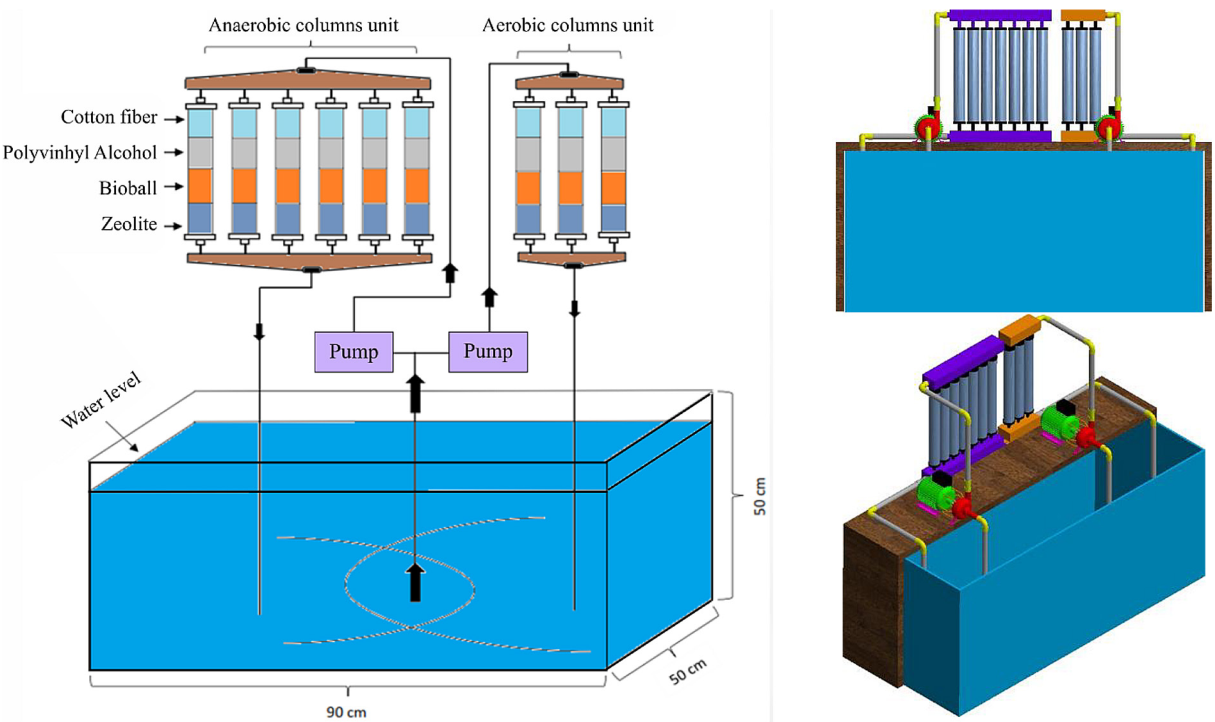

Figure 1. Construction of bioreactor unit

and a stirrer. The flasks were covered with cotton wrapped in aluminum foil. The medium was sterilized by autoclaving at 121 °C for 15 min. The sterilized medium was then cooled to 45–50 °C. The medium was poured into Petri dishes and allowed to solidify before adding the diluted samples using the spread plate technique. The dilution used was 10^2. The media were then incubated for 24 h, and colony counts were performed. The total number of bacteria was subsequently calculated using the following formula (1):

$$Total\ bacteria\ (CFU\ L^{-1}) = \frac{1}{Dilution\ factor} \times colony \quad (1)$$

Survival rate

Koi fish mortality was observed and recorded on a daily basis. The survival rate was calculated at the end of the study using formula (2), where N_0 is the initial number of fish and N_t is the number of live fish at the end of the maintenance period.

$$Survival\ rate\ (\%) = \frac{N_t}{N_0} \times 100 \quad (2)$$

Data analysis

All data are presented as mean ± standard deviation (SD). Statistical analysis was performed using One-way ANOVA with SPSS 16.5, followed by the least significant difference (LSD) test to compare differences between treatments. TOM and nitrate data were analyzed using linear regression to evaluate the relationship between organic matter degradation and nitrogen transformation (R^2).

RESULT AND DISCUSSION

Performance of the dual-mode bioreactor on organic load reduction

The reduction in organic load is a crucial metric for assessing the efficacy of remediation systems in Koi fish aquaculture. The findings indicate that the dual-mode bioreactor exhibited superior performance in reducing organic compound concentrations compared to the RAS and control systems. The COD values in the bioreactor consistently decreased over the three-week maintenance period, starting from 16.63 mg L^{-1} in the initial week, reducing to 11.73 mg L^{-1} in the first week, further declining to 5.13 mg L^{-1}, and ultimately reaching 4.30 mg L^{-1} by the third week. This reduction, approximately 74.1%, highlights the bioreactor's capacity to expedite the decomposition of the dissolved organic compounds. In contrast, the control system exhibited a marked increase in COD levels, peaking at 47.27 mg L^{-1} in the second week, before experiencing a slight decline. Meanwhile, the COD levels in the RAS (Figure 2) continued to rise until the second week, followed by a moderate decrease. A similar trend was observed for the TOM parameter (Figure 3), where the control and RAS systems experienced organic accumulation up to 27.70 mg L^{-1} and 23.70 mg L^{-1}, respectively. Conversely, the bioreactor demonstrated a reduction to 14.00 mg L^{-1} by the end of the maintenance period.

The efficacy of the reduction system in waste treatment processes is significantly influenced by the multilayer design of the bioreactor, which integrates both physical and biological filtration processes. Within this system, the cotton fiber layer serves as a filter to remove coarse organic particulates, whereas the PVA sponge and bioball offer an extensive surface area for the colonization of aerobic and anaerobic microorganisms. This process is crucial because these microorganisms facilitate the ammonification and oxidation of organic compounds, thereby substantially reducing the BOD and COD loads (Leiknes et al., 2009; Kamimoto et al., 2009).

PVA sponges exhibit high porosity and water absorption capacity, which supports microbial growth (Cao et al., 2022; Chen et al., 2024). In biomass treatment, the large pores in the sponge provide space for microbial tissues, which play a role in ammonification, where ammonia is produced from the decomposition of organic compounds by bacteria. Furthermore, PVA offers good stability and tunability, enhancing filtration efficiency and biomass fixation (Phocharoen et al., 2025). When combined with zeolite, the sponge adsorbs dissolved nitrogen and maintains reactor stability (Zhang et al., 2021a). Zeolite is employed in the base layer of this bioreactor because of its ability to adsorb ammonium ions and other nitrogen compounds, which is essential for controlling slow contamination and improving the performance of biochemical processes (Zhang et al., 2021b; Chen et al., 2021).

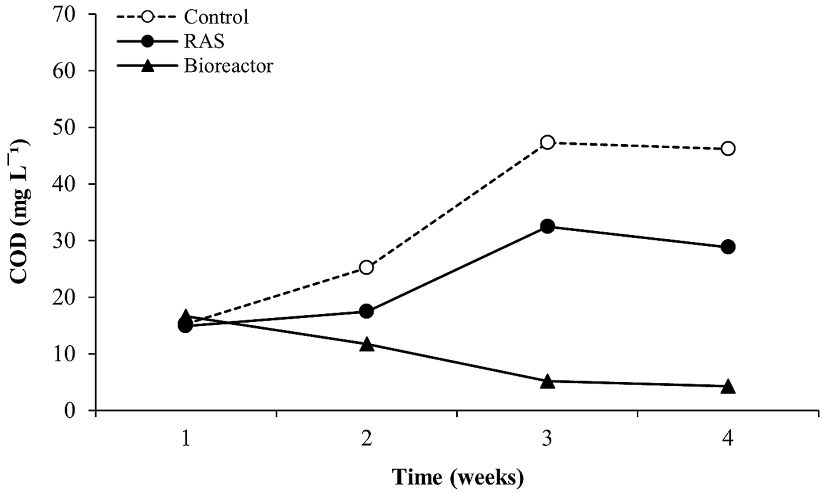

Figure 2. COD fluctuation during maintenance

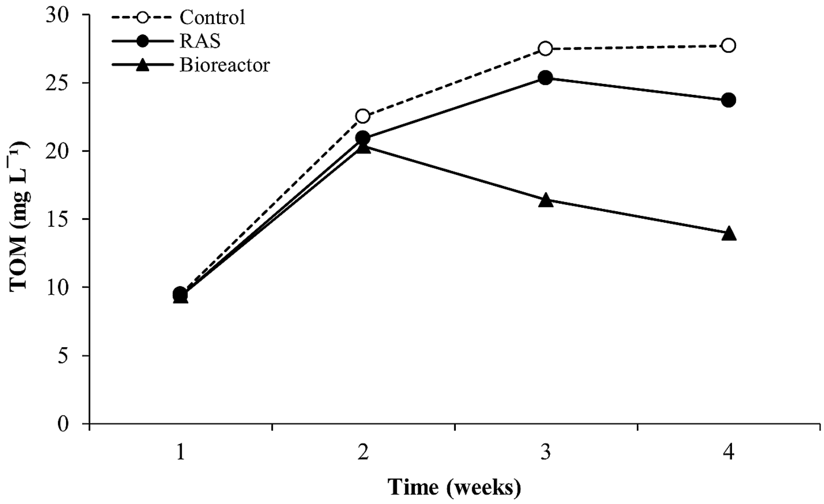

Figure 3. TOM fluctuation during maintenance

The bioreactor system utilizing this multilayer method not only filters particles but also leverages the interactions among different components to expedite the removal of contaminants. Through this interactive mechanism, the use of PVA sponges and zeolites can be optimized to absorb nutrients and harmful ions, thereby maintaining the balance of the microbial ecosystem within the reactor (Phocharoen et al., 2025). In essence, the multilayer bioreactor design that combines physical and biological filtration processes, along with high-performance materials such as PVA and zeolite, presents a progressive approach to managing and mitigating waste impact and achieving greater efficiency in wastewater treatment (Daduon 1998; Phocharoen et al. 2025). These findings demonstrate that the integration of physical and biological components in bioreactors can create an efficient, stable, and adaptive remediation system suited to the high organic loads commonly observed in koi fish farming.

Nitrogen dynamics across culture systems

Variations in nitrate concentration across the three rearing systems revealed distinct nitrogen dynamics and suggested different levels of nitrification process efficacy (Figure 4). In the control system, the nitrate levels gradually increased throughout the rearing period. The initial concentration of 0.73 mg L^{-1} rose to 1.00 mg L^{-1} in the first week, 1.13 mg L^{-1} in the second week, and 1.37 mg L^{-1} in the third week. This slow rate of increase suggests that the environmental conditions in the control system, including the decrease in dissolved oxygen (DO) (Figure 8) and

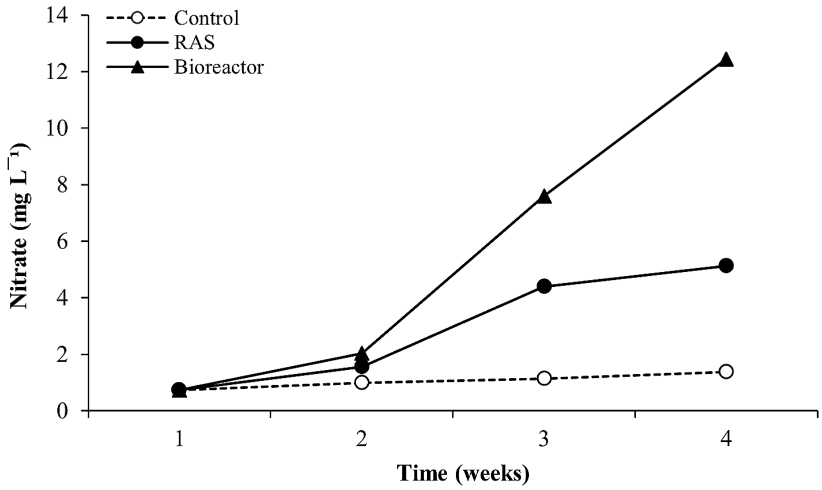

Figure 4. Nitrate fluctuation during rearing

fluctuations in pH (Figure 9), were insufficient to support the activity of nitrifying bacteria (Aisyah and Rofilia, 2023).

Compared to the control, the RAS showed a more pronounced increase in nitrate levels. Specifically, the nitrate concentration increased from 0.73 mg L^{-1} at the beginning of the week to 1.57 mg L^{-1} by the end of the first week, subsequently escalating to 4.40 mg L^{-1} in the second week, and eventually stabilizing at 5.13 mg L^{-1} in the third week. This trend suggests that stable water circulation fosters a favorable environment for nitrification. Nevertheless, the persistent high accumulation of organic matter indicates that the nitrification capacity within the RAS has not yet fully equilibrated with the organic load resulting from farming activities (Jiang et al., 2025).

The bioreactor system exhibited the most significant nitrogen dynamics, characterized by a substantial increase in nitrate from 0.73 mg L^{-1} in the initial week to 2.03 mg L^{-1} in the first week, followed by a sharp rise to 7.60 mg L^{-1} in the second week, and 12.43 mg L^{-1} in the third week. This consistently and significantly higher nitrate increase, compared to the other two systems, indicates that nitrification was highly active in the bioreactor. Factors such as stable and increasing DO support (Jiang et al., 2025), multilayer structures facilitating the retention of nitrifying microbes (Keuter et al., 2011), and a more optimal aerobic environment appear to be the primary drivers of high nitrification efficiency (Sander et al., 2018). The observed nitrogen dynamics pattern suggests that the dual-mode bioreactor possesses an efficient capacity to convert organic nitrogen–ammonia into nitrates. These findings underscore the importance of a combination of stable oxygen supply, bacterial immobilization media, and controlled water flow in determining the effectiveness of nitrogen transformation in koi farming systems.

Coupled carbon–nitrogen transformations

The relationship between organic matter dynamics and nitrogen transformation in koi culture systems was highly consistent and interconnected (Figure 5). Regression analysis between TOM and nitrate demonstrated a very strong negative correlation (R^2 = 0.9971), indicating that a decrease in organic matter levels within the reactor was accompanied by an increase in nitrate concentration. This pattern suggests that the predominant biological processes in the system are ammonification and aerobic nitrification (Hu et al., 2023). TOM continued to increase in the control and recirculating aquaculture system (RAS), reaching 27.70 mg L^{-1} and 23.70 mg L^{-1}, respectively, whereas in the bioreactor, it significantly decreased to 14.00 mg L^{-1}.

In contrast, the nitrate concentration displayed an inverse pattern. In the control treatment, the nitrate increase was gradual, reaching 1.37 mg L^{-1} by the end of the maintenance period. The RAS treatment exhibited a more pronounced increase, reaching 5.13 mg L^{-1}, indicating superior nitrification activity compared with the control. The bioreactor, characterized by more stable aeration and surface structure conditions, recorded the highest increase in nitrate concentration, reaching 12.43 mg L^{-1}. These findings further substantiate that the conversion of organic nitrogen through ammonification, ammonia oxidation to nitrite, and

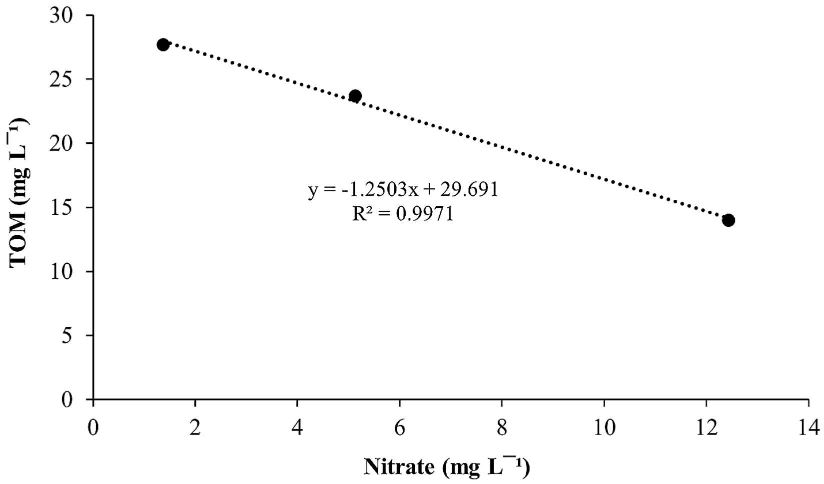

Figure 5. Regression of TOM and nitrate

subsequent oxidation to nitrate is more efficiently facilitated in a bioreactor environment. To validate this inference, it is recommended to measure NH^{4+} and N transformation rates (gross rates), oxygen/redox parameters, and analyze nitrifying/denitrifying microbial communities, as previously suggested (Dubowski et al., 2014; Shi et al., 2023; Kurniawan et al., 2024). The strength of the TOM–nitrate correlation indicates that more effective decomposition of organic matter is correlated with increased nitrification activity. Comparable strong correlations between organic matter decline and nitrate accumulation have been reported in aerobic bioreactor and soil nitrogen studies (Hu et al., 2023; Shi et al., 2023), indicating that systems with stable oxygenation typically exhibit tightly coupled ammonification–nitrification dynamics. Our findings fall within this documented pattern, further confirming that aerobic nitrification is the dominant pathway in the bioreactor. The anaerobic column contributed minimally to denitrification, as evidenced by the significant accumulation of nitrate at the end of the maintenance period in this column. Consequently, the data suggest that the primary remediation mechanism in the bioreactor is robust aerobic nitrification, supported by the structure of the medium and the synergistic function of indigenous microbial activity.

Stability of dissolved solids and ionic strength

TDS and EC are critical indicators for evaluating the accumulation of dissolved ions and stability of the aquatic environment during koi fish cultivation (Figure 6). The findings revealed that each treatment exhibited distinct patterns of TDS and EC variations, reflecting the efficacy of the system in managing ions and dissolved solids. In the control treatment, TDS consistently increased from week 0 to week 3, starting at 334.33 mg L^{-1} and rising to 381.33 mg L^{-1} in the first week, 406.33 mg L^{-1} in the second week, and 433.33 mg L^{-1} by the end of the maintenance period. RAS treatment also demonstrated an upward trend in TDS, albeit at a more moderate rate than the control. TDS increased from 332.33 mg L^{-1} in the initial week to 345.33 mg L^{-1} in the first week, then further to 392.33 mg L^{-1} in the second week, and finally to 416.33 mg L^{-1} in the third week. In contrast, the bioreactor exhibited superior TDS stability during the maintenance period. The initial TDS value of 334.33 mg L^{-1} remained constant in the first week, followed by a slight increase to 342.33 mg L^{-1} in the second week and 351.33 mg L^{-1} in the third.

The continuous rise of TDS in the control system reflects the lack of functional mechanisms to reduce dissolved ions and fine particulates originating from feed residues and metabolic waste. Although the RAS system slowed this accumulation, its filtration capacity remained insufficient to prevent a gradual increase in dissolved solids. In contrast, the dual-mode bioreactor significantly suppressed the rate of accumulation through a combination of adsorption and biological transformation. Other studies have demonstrated the efficacy of zeolite adsorption, the dynamics of nitrifying biofilms on sponges or biocarriers, and the effectiveness of bioremediation units in reducing nutrients and TDS in aquaculture systems (Vaičiukynienė et al., 2020; Sharma et al., 2020; Ismi et al., 2023). Practical recommendations

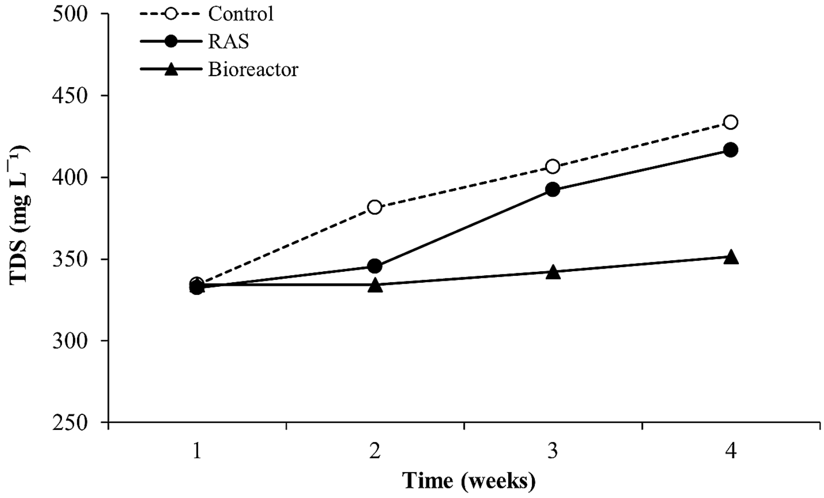

Figure 6. TDS fluctuations during maintenance

include enhancing the combination of mitigation strategies, such as feed management, pre-filtration of solids, adsorptive media, and biofilter optimization, as well as monitoring key parameters to prevent system saturation and further TDS accumulation (Tunçelli and Memiş, 2024; Mulyanto et al., 2023; Lailiyah et al., 2023).

The EC stability, as shown in Figure 7, exhibited a pattern consistent with the changes in TDS, wherein the bioreactor maintained the most stable values and did not exhibit significant fluctuations during maintenance. These findings suggest that the biological processes within the bioreactor did not induce excessive ionic fluctuations, thereby preserving an aquatic environment conducive to fish physiological health (Tunçelli and Memiş, 2024; Mulyanto et al., 2023; Lailiyah et al., 2023). Overall, these results demonstrate that the dual-mode bioreactor offers the most effective regulation of dissolved solids and ions compared to the recirculating aquaculture system (RAS) and the control, rendering it a promising technology for achieving stable water quality in koi culture systems.

Oxygenation and pH stability as key environmental regulators

DO and pH are critical environmental parameters that influence biological processes and the stability of aquatic ecosystems during the cultivation of koi fish (Figure 8). The findings indicated that the three treatment systems exhibited distinct patterns of DO and pH fluctuations, significantly impacting the efficacy of the remediation process and fish physiological conditions. In the control treatment, DO decreased markedly from 6.64 mg L^{-1} in the initial week to 5.80 mg L^{-1} during the first and second weeks, and subsequently declined

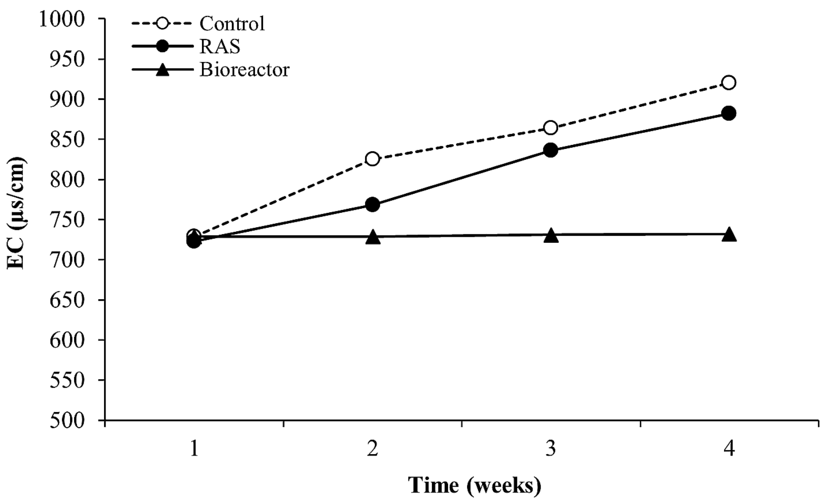

Figure 7. EC fluctuations during maintenance

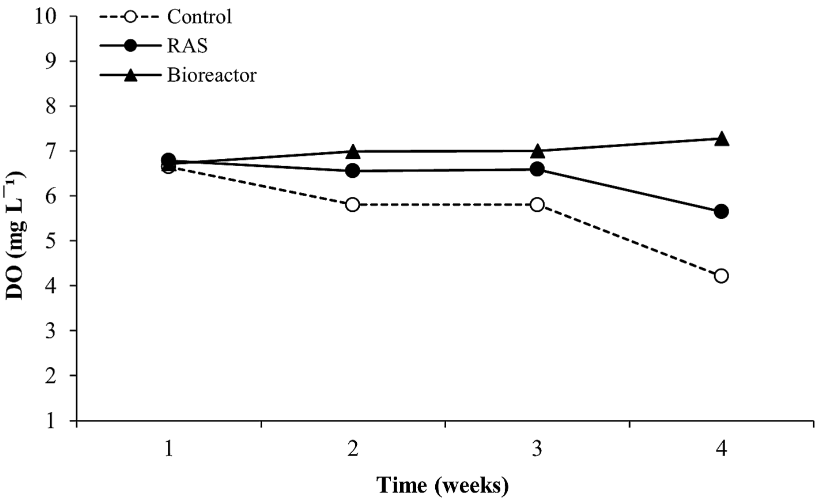

Figure 8. DO Fluctuations during maintenance

further to 4.21 mg L^{-1} in the third week. In contrast, the RAS treatment maintained a more stable DO level, with a decrease from 6.78 mg L^{-1} to 6.55 mg L^{-1} in the first week, remaining within the range of 6.55–6.58 mg L^{-1} until the second week, before dropping to 5.65 mg L^{-1} in the third week. The bioreactor system demonstrated the highest DO stability throughout the maintenance period, with DO increasing from 6.71 mg L^{-1} in the initial week to 6.99 mg L^{-1} in the first week, then slightly rising to 7.00 mg L^{-1} in the second week, and reaching its peak value of 7.28 mg L^{-1} in the third week.

The control system experienced a pronounced decline in DO due to the absence of a technical mechanism to reduce the organic load and TSS, leading to the predominance of heterotrophic organisms in O_2 consumption (Rurangwa and Verdegem, 2014; Li et al. 2023). RAS treatment delayed the decline through circulation and biofiltration; however, inadequate design capacity and pre-solid removal resulted in a DO drop in week 3. Conversely, the dual-mode bioreactor, with its adsorptive configuration and immobilization of indigenous bacteria, along with potentially superior aeration design, created conditions favoring the dominance of autotrophic nitrifiers, thereby maintaining and even increasing the bulk DO. These results are consistent with the relationships between O_2, microbial biofilm communities, and reactor performance (Navada et al., 2020; Noble-Okere et al., 2023). From a construction perspective, the integration of pre-solid filtration, adsorptive media, controlled bacterial immobilization, and adequate aeration capacity are essential steps for sustaining optimal DO levels and long-term remediation performance.

A comparable stability pattern was observed for the pH levels (Figure 9). In the control group, the pH decreased from 8.50 to 7.60 by the end of the maintenance period, suggesting an increased accumulation of organic acids resulting from the decomposition of organic matter. The RAS maintained a more stable pH within the range of 7.70–7.80, whereas the bioreactor exhibited the highest stability, with pH consistently between 8.20 and 8.50. This stability is critical because nitrifying bacteria function optimally at a pH of 7.5–8.5. Consequently, stable pH and DO conditions in the bioreactor directly enhance the efficiency of nitrogen transformation and reduce the organic load. Overall, these findings indicate that the stability of dissolved oxygen and pH are the primary factors differentiating the performance of the bioreactor from that of RAS and the control. Optimal environmental conditions in the bioreactor not only improve the efficiency of biological processes but also support the health and resilience of fish throughout the maintenance period (Lindholm-Lehto et al., 2020).

Indigenous microbial abundance and functional implications

The presence and abundance of indigenous microorganisms within the bioreactor column are crucial to remediation mechanisms, particularly in nitrogen transformation processes and the decomposition of organic matter. TPC analysis revealed a significant disparity between the aerobic and anaerobic columns (Table 1). The aerobic

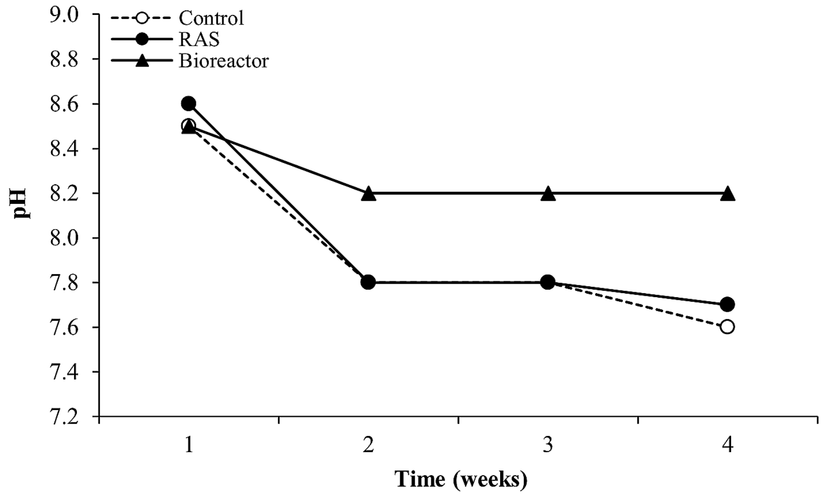

Figure 9. pH Fluctuations during maintenance

Table 1. Total plate count in bioreactor columns

Column	Abundance (× 10^4 CFU mL^{-1})
Aerobic	1.27
Anaerobic	0.6

column exhibited a substantially higher average total bacterial count than the anaerobic column.

Research findings indicate that the aerobic column exhibits a greater abundance of bacteria than the anaerobic zone, suggesting that an ample supply of oxygen facilitates the predominance of ammonification and nitrification processes within the system. Elevated DO conditions promote the intensive oxidation of NH_4^+ to NO_2^- and NO_3^-, whereas the restricted anoxic zone and the presence of dissolved oxygen inhibit the growth and activity of denitrifying bacteria within the biofilm. These results align with those of Adoonsook et al. (2019), who observed that intensive aeration enhances the proliferation of ammonia-oxidizing (AOB) and nitrite-oxidizing (NOB) bacteria while concurrently limiting the development of obligate denitrifiers. Immobilization media, such as polyvinyl alcohol (PVA) sponges, further reinforce this pattern, as their porous structure can retain slow-growing nitrifier biomass and form stable, biofilms. These findings are corroborated by Hou et al. (2015) and Al Zamzami et al. (2025), who demonstrated that porous media create micro-oxygen gradients that remain too oxygen-rich to permit significant denitrification. Furthermore, the low carbon-to-nitrogen (C/N) ratio and high bulk DO in the system constrain the emergence of simultaneous nitrification–denitrification (SND) or heterotrophic nitrification–aerobic denitrification (HNAD) pathways. This condition is consistent with the report by Liu et al. (2020), who stated that both mechanisms develop only under microaerophilic conditions with the availability of easily degradable carbon. Overall, the pattern of increasing nitrate, high abundance of aerobic bacteria, and low levels of anaerobic bacteria in this bioreactor suggests that the reactor design and highly oxygenated operating conditions inherently direct nitrogen transformation along the ammonification–nitrification pathway rather than through denitrification.

Effect of water quality improvement on koi survival performance

The enhancement in water quality achieved by the dual-mode bioreactor system significantly influenced the survival rate of the koi fish during maintenance. Each treatment commenced with an identical number of fish (20 fish per aquarium). Nevertheless, the final outcomes revealed a pronounced disparity in the survival rates between the treatments. In the control system, only five fish survived until the end of the maintenance period, resulting in a survival rate of 25% (SD = 8.7%). This low survival rate aligns with the observed deterioration in water quality, characterized by decreased DO, increased COD, TOM, and a shift in pH towards more acidic conditions. These factors can induce respiratory stress, metabolic disorders, and heightened susceptibility to opportunistic pathogens (Dong et al., 2020; Fu et al., 2021; Ma et al., 2024) (Table 2).

Table 2. Survival rate of koi fish during maintenance

Treatments	Initial amount (ind)	Final amount (ind)	Survival rate (%) ± SD
Control	20.0	5.0	25.0 ± 8.7
RAS	20.0	15.0	75.0 ± 5.0
Bioreactor	20.0	18.7	93.3 ± 2.9

The relationship between water quality and survival rate observed in this study indicates that environmental enhancements achieved through bioreactor technology not only improve the remediation process but also significantly affect fish biological performance. These findings substantiate that eco-aquatic technology utilizing dual-mode bioreactors can serve as a practical and effective strategy for enhancing the success of koi fish cultivation, particularly under intensive conditions that are susceptible to water quality degradation.

CONCLUSIONS

This study demonstrates that a dual-mode bioreactor, operating under both aerobic and anaerobic conditions and utilizing layered media comprising cotton fiber, Bio-Balls, PVA, and zeolite, can enhance water quality in koi fish farming systems by reducing the organic load and increasing the nitrogen transformation efficiency. The bioreactor consistently achieved greater reductions in COD and TOM than the control and recirculating aquaculture system (RAS) and exhibited more robust nitrification dynamics, as evidenced by elevated nitrate levels throughout the maintenance period. The stability of physicochemical parameters, such as DO, pH, and TDS, within the bioreactor system further supports the biological processes occurring therein. Total bacterial analysis revealed a higher abundance of microbes in the aerobic column than in the anaerobic column, indicating that ammonification and nitrification were the predominant processes in the system. The improvements in water quality achieved by the bioreactor system also contributed to a higher survival rate of koi fish compared to that in the other two treatments. Overall, the findings of this study suggest that the dual-mode bioreactor holds promise as an effective ecotechnology approach for waste management in small- to medium-scale koi aquaculture. However, further research is necessary to evaluate the long-term performance, variations in waste load, and more detailed microbial community dynamics. From an application standpoint, the bioreactor can be built using low-cost and locally available materials, making it a practical and scalable technology for small- to medium-scale koi farmers who require stable water quality with minimal operational complexity. Additionally, to enhance the contribution of denitrification within the system, modifications in construction and operation are required to directly establish controlled anoxic conditions in the system.

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