
Integration of microbial biofiltration and phytoremediation for the development of recirculating aquaculture systems for freshwater fish larvae cultivation

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Abstract Recirculating aquaculture systems (RAS) is optimized by combining microbial biofiltration with phytoremediation to reduce nitrogen and phosphate waste while managing microbial loads. Experiments evaluating filter media and microbial augmentation revealed that pumice stone combined with *Bacillus* (BC) significantly reduced ammonia levels on days 6–9 ($P<0.05$) and nitrite levels on days 9–12 ($P<0.05$) compared with other media. Pumice+BC was selected for integration into phytoremediation systems. Further experiments showed that incorporating *Cabomba caroliniana* into the pumice+BC filter system significantly lowered ($P<0.05$) of ammonia content (day 3) and reduced nitrite levels on day 9 ($P<0.05$) compared to the controls. Significant reduction ($P<0.05$) of nitrate (day 9-12) and orthophosphate concentrations (day 9) were observed in the biological filtration system combined with the phytoremediation unit using *Cabomba caroliniana*. Moreover, in a study on microbial load management, orthophosphate levels were significantly lower ($P<0.05$) in treatments with an ozone generator. Total bacterial counts consistently declined in both UV and ozone treatments, but the ozone treatment showed a significant difference ($P<0.05$) in total bacterial count compared to the control and a UV treatment by day 9. In conclusion, the proposed pumice+BC microorganism biological filter system with *Cabomba caroliniana* for phytoremediation with an ozone generator to manage the microbial load resulted in the increasing of effective for reducing nitrogen and phosphate wastes with low microbial content in water.

Keywords: Water quality, Aquaculture, Microbial augmentation, *Cabomba caroliniana*

Introduction

As global demand for aquaculture products rises, with forecasts projecting that aquaculture will supply 60% of fish for human consumption by 2030 (Rashid *et al.*, 2019), the need for sustainable aquaculture solutions becomes ever more

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urgent. Traditional aquaculture practices, which rely heavily on large water exchanges, confront severe obstacles associated with the quantity, quality, and management of that water, as well as the management of waste (Edwards, 2015). Those challenges threaten ecological status in the environment where aquaculture takes place, as well as the health and viability of fish populations, either in aquaculture or those caught in the wild (Boyd *et al.*, 2020). Freshwater fish hatcheries operate under similarly constrained conditions. They must confront the limitations of their water resources, improve their production efficiency, and maintain the appropriate water quality vital for the healthy development of fish larvae (Uppanunchai *et al.*, 2015). Consequently, the development of more sustainable and efficient aquaculture systems that will need fewer resources while also guaranteeing healthy aquatic environments is necessary. Recirculating Aquaculture Systems (RAS) are one of these alternatives to traditional systems and are a closed-loop system that recycles the water consumed in aquaculture (Ahmed and Turchini, 2021). The RAS filters and cleans the water in the system, which minimizes water exchange, and it also optimizes their sustainability by enhancing nutrients, waste, and microbial load management. The crucial step in RAS is the process of microbial biofiltration, where bacteria develop as biofilms on the media of the filter, and convert harmful compounds such as ammonia and nitrites into less harmful nitrates (Bartelme *et al.*, 2017). Microbial biofiltration also supports healthier fish, reduces water exchange, and mitigates environmental impacts. However, despite these advantages, the accumulation of nitrates over time can still pose a challenge, particularly in long-term aquaculture systems, where aquatic animals are raised for extended periods (Yang *et al.*, 2019). Phytoremediation, the use of aquatic plants to absorb excess nutrients such as nitrogen and phosphates (Handajani *et al.*, 2021) offered a complementary solution to microbial biofiltration. This method helps prevent the eutrophication of water bodies by reducing nutrient pollution and mitigating risks, such as algal blooms, which can lead to oxygen depletion and harm aquatic life (Paerl *et al.*, 2018). Phytoremediation relies on photosynthesis to remove nitrogen and phosphates but requires adequate light for optimal plant growth (Singh *et al.*, 2021). Moreover, regular harvesting of plants is necessary to prevent the decay of accumulated organic material, which can degrade the water quality (Fletcher *et al.*, 2020).

Microbial biofiltration and phytoremediation have their own benefits; they can be complemented by establishing an integrated process by the combination of biofiltration and phytoremediation to obtain a novel waste treatment process in RAS. Thus, an integrated system with microbial biofiltration combined with phytoremediation may offer a very effective method for treating aquaculture

waste. The microbial biofiltration provides a great opportunity for nitrogen waste reduction (Roalkvam *et al.*, 2020), particularly ammonia, and phytoremediation can remove nutrients as nitrate and phosphates (Handajani *et al.*, 2021), thereby mitigating the risk of remaining nutrient in the whole system. This integrative system could be implemented for the direct management of aquaculture systems for fish larvae, which must operate under controlled microbial loads and optimal water quality parameters. Fish larvae are susceptible to high load of microbes in water, as therefore water quality should be ensured for fish growth and survival (Attramadal *et al.*, 2014). Normally, UV treatment and ozone application are employed to reduce microbial loads to allow appropriate water for the fish larval cultivation (Gorito *et al.*, 2021; Goncalves *et al.*, 2023) and they also might be associated with microbial biofiltration and phytoremediation, possibly forming a system able to diminish microbial contamination, generating a balanced environment for suitable larval cultivation. The study aimed to integrate microbial biofiltration and phytoremediation in a recirculating aquaculture system (RAS) to reduce nitrogen, and phosphate wastes and control the microbial load in water.

Materials and methods

Experimental system

The research was conducted at the Faculty of Agriculture, Thaksin University, from March to July 2024. Wastewater treatment systems were developed to treat freshwater fish larvae in a 50-litre rectangle plastic tank, a drainage pipe fitted at the middle of the tank bottom, linked to a plastic pipe that directed wastewater into a 6-liter plastic treatment tank, including a water pump and different types of filtrations medium. The wastewater was further transferred to another 6-liter deposit containing a phytoremediation system consisting of aquatic plants. The water was then channeled into a 6-liter tank containing either a UV system or an ozone generator to reduce number of microorganisms prior to being returned to the fish tank.

Experiment 1: Effect of different filter media on wastewater treatment efficiency

Different filter media were used in this experiment, and 2 types of microorganisms were used in the system: (1) PM. 1, a commercial *Bacillus* mixture made by the Department of Fisheries, Thailand contain 2 isolates of Gram-positive, rod-shaped cell, spore forming bacterial strains. Phylogenetic

analysis on 16S rRNA gene with EzBioCloud database (Yoon *et al.*, 2017) showing both isolates clustered with *Bacillus mexicanus* FSQ1^T (99.88% similarity) and *Bacillus paralicheniformis* KJ-16^T (99.52% similarity); and (2) BC, a lab-scale Bacillus product contain Gram-positive, rod-shaped cell, spore forming bacterial strain clustered with *Bacillus siamensis* KCTC 13613^T (99.60% similarity) which was isolated from a recirculating biofiltration system for freshwater fish farming at the Thaksin University aquatic animal hatchery. The concentration of both microorganisms was 10⁴ CFU/ml when added to the test system. The experiment included 9 treatments, with 3 replicates per treatment, as follows: T1 (NY-), nylon net media without added microorganisms; T2 (NYPM), nylon net media with PM.1; T3 (NYBC), nylon net media with BC; T4 (BB-), Bioball media without added microorganisms; T5 (BBPM), Bioball media with PM.1; T6 (BBBC), Bioball media with BC; T7 (PU-), Pumice rock media without added microorganisms; T8 (PUPM), Pumice rock media with PM.1; and T9 (PUBC), pumice rock media with BC. Synthetic wastewater was introduced into the system, containing 25 ppm of organic matter from fish feed, 0.5, 50, and 5 ppm of ammonia, 0.25 ppm of nitrite, nitrate, and 5 ppm of phosphate. Water flowed into the microbial chamber at a rate of 0.5 liters per minute. Water samples were collected from each treatment at 3 days intervals to analyze the levels of ammonia, nitrite, nitrate, and orthophosphate using the method described by APHA (1998).

Experiment 2: Study on the type and optimal amount of treatment plants for nutrient reduction

The study investigated the effectiveness of different aquatic plants for nitrogen and phosphorus wastes reduction in a phytoremediation system, which connected to the most effective biological filtration system from Experiment 1. The plant species studied were Burhead (*Echinodorus cordifolius*), Canna (*Canna indica*), and Fanwort (*Cabomba caroliniana*). The experiment was divided into 4 treatments, each with 3 replications: T1 (PUBC-), biological filtration system without phytoremediation; T2 (PUBC-CB), biological filtration system+Fanwort (120-150 g/100 L); T3 (PUBC-EH), biological filtration system+Burhead (120-150 g/100 L); T4 (PUBC-CN), biological filtration system+Canna (120-150 g/100 L). Synthetic wastewater was introduced into each experimental unit using the method described for Experiment 1. Water flowed through the biological filtration system, followed by the phytoremediation system at a rate of 0.5 liters per minute. Water samples were collected and analyzed as described in Experiment 1.

Experiment 3: Application of UV and ozone to reduce microorganisms in water

The wastewater treatment system with the highest efficiency, as determined from experiments 1 and 2, was connected to a microorganism reduction system using an ozone generator or UV lamps as equipments for microbial elimination. An ozone generator with a capacity of 120 mg/h was installed and operated for 10 min/hour/100 liters, or a UV lamp was continuously used at a rate of 4 W continuously for 24 h/1,000 liters in the microorganism reduction unit. The experiment included 3 treatments, each with 3 replications: T1 (PUBC CB-), the most efficient water treatment system without microorganism reduction; T2 (PUBC CB UV), the most efficient water treatment system with an ozone generator (Weipro Ozonizer ET-200, China) 120 mg/h for 10 min/h per 100 L; T3 (PUBC CB OZ), the most efficient water treatment system with a UV lamp (SOBO T5-UV9W, China) 4 W/24 h per 1,000 L. Synthetic wastewater, as described in Experiments 1 and 2, was introduced into each system. Water samples were collected and analyzed as described above. Additionally, bacterial counts were performed using the plate count agar method to assess the microbial reduction in each treatment system.

All parameters were statistically analyzed using one-way ANOVA, followed by Duncan's multiple range test to compare means. The effects with a probability of $P < 0.05$ were considered statistically significant.

Results

Experiment 1

The experiment revealed that, on day-3, the ammonia content was significantly higher in the treatment using nylon nets, both without microorganisms and with PM.1, compared to other treatments. In contrast, the experiments using other filter media showed lower and relatively consistent ammonia levels. Notably, from days 6-9, the ammonia content was the lowest in the experiments using pumice as a biological filter. This reduction was particularly significant when pumice was combined with either PM.1 or BC, resulting in a decreasing trend in ammonia levels during day 6-9 of the experiment (Table 1). Between days 3 and 6, nitrite levels ranged from 0.30 to 0.38 mg/L and tended to increase over time. However, in experiments using pumice as a biological medium, nitrite levels were lower during days 9-12 and were significantly different ($P < 0.05$) from the treatment using nylon nets as biological media (Table 2). By the day-6, nitrate levels in the treatment using bioball or pumice with BC supplementation were significantly ($P < 0.05$) lower

than those in the bioball, pumice alone, or pumice with PM.1 (Table 3). Additionally, on days 3 and 12, orthophosphate levels showed an increasing trend in treatments using nylon nets and bioballs as the biofilter media. Notably, on day 12, orthophosphate levels were significantly higher ($P<0.05$) in treatments employing nylon nets or bioballs combined with BC, as well as in treatments using bioballs alone (Table 4). These findings suggest that pumice, when combined with PM.1 or BC, effectively reduced ammonia levels in water between days 6 and 9 of the experiment. Furthermore, using a bioball or pumice with BC tended to decrease nitrate levels by day 6. Therefore, pumice combined with BC was selected for further development of a biofilter system in further experiments.

Table 1. Total ammonia (mg/L) in the RAS with different biological media and bacterial supplements

	0 day	3 days	6 days	9 days	12 days
T1 NY -	0.70±0.09 ^a	1.54±0.23 ^c	0.30±0.10 ^b	0.11±0.05 ^{bc}	0.00±0.00 ^a
T2 NYPM	0.53±0.05 ^a	1.27±0.33 ^{bc}	0.29±0.10 ^b	0.06±0.03 ^{ab}	0.00±0.00 ^a
T3 NYBC	0.65±0.03 ^a	0.85±0.19 ^a	0.60±0.12 ^c	0.07±0.01 ^{ab}	0.03±0.08 ^a
T4 BB -	0.61±0.03 ^a	0.85±0.17 ^a	0.56±0.25 ^c	0.08±0.02 ^{abc}	0.01±0.01 ^a
T5 BBPM	0.66±0.02 ^a	1.03±0.15 ^{ab}	0.89±0.11 ^c	0.07±0.03 ^{ab}	0.02±0.01 ^a
T6 BBBC	0.58±0.03 ^a	0.72±0.13 ^a	0.74±0.13 ^c	0.15±0.06 ^c	0.00±0.00 ^a
T7 PU -	0.62±0.13 ^a	0.73±0.10 ^a	0.11±0.05 ^{ab}	0.06±0.05 ^{ab}	0.01±0.02 ^a
T8 PUPM	0.62±0.11 ^a	0.81±0.08 ^a	0.07±0.01 ^a	0.03±0.05 ^a	0.01±0.02 ^a
T9 PUBC	0.64±0.06 ^a	0.70±0.12 ^a	0.01±0.01 ^a	0.01±0.04 ^a	0.00±0.00 ^a

Means within the same column sharing the same superscripts are not significantly different ($p>0.05$)

Table 2. Nitrite (mg/L) in the RAS with different biological media and bacterial supplements

	0 day	3 days	6 days	9 days	12 days
T1 NY -	0.23±0.00 ^a	0.27±0.00 ^a	0.35±0.03 ^{ab}	0.41±0.00 ^d	0.36±0.01 ^{cd}
T2 NYPM	0.22±0.00 ^a	0.26±0.03 ^a	0.35±0.02 ^{ab}	0.39±0.00 ^{cd}	0.28±0.01 ^c
T3 NYBC	0.23±0.00 ^a	0.33±0.00 ^a	0.38±0.01 ^{ab}	0.48±0.01 ^{bc}	0.44±0.00 ^{cd}
T4 BB -	0.23±0.00 ^a	0.26±0.02 ^a	0.30±0.04 ^{ab}	0.34±0.00 ^{ab}	0.37±0.00 ^d
T5 BBPM	0.22±0.00 ^a	0.26±0.01 ^a	0.33±0.02 ^a	0.38±0.00 ^{bcd}	0.37±0.01 ^{cd}
T6 BBBC	0.22±0.00 ^a	0.26±0.03 ^a	0.33±0.02 ^c	0.31±0.02 ^a	0.33±0.01 ^{cd}
T7 PU -	0.23±0.01 ^a	0.29±0.00 ^a	0.38±0.05 ^b	0.35±0.00 ^{abc}	0.10±0.00 ^a
T8 PUPM	0.22±0.00 ^a	0.29±0.01 ^a	0.37±0.05 ^b	0.32±0.00 ^a	0.05±0.00 ^a
T9 PUBC	0.23±0.00 ^a	0.28±0.02 ^a	0.36±0.07 ^{ab}	0.31±0.01 ^a	0.10±0.00 ^a

Means within the same column sharing the same superscripts are not significantly different ($p>0.05$)

Table 3. Nitrate (mg/L) in the RAS with different biological media and bacterial supplements

	0 day	3 days	6 days	9 days	12 days
T1 NY -	32.11±5.52 ^a	24.47±1.48 ^a	32.30±2.75 ^{abc}	29.73±5.50 ^a	23.21±5.85 ^a
T2 NYPM	38.98±8.58 ^a	31.42±8.42 ^a	31.04±6.62 ^{ab}	25.39±8.40 ^a	23.06±4.95 ^a
T3 NYBC	31.43±1.02 ^a	33.71±2.59 ^a	31.22±2.24 ^{ab}	25.83±5.07 ^a	29.07±5.12 ^a
T4 BB -	43.40±14.05 ^a	27.08±4.01 ^a	34.40±1.39 ^{bc}	27.12±2.48 ^a	28.15±5.98 ^a
T5 BBPM	41.24±12.83 ^a	36.23±8.92 ^a	30.87±5.46 ^{ab}	22.65±4.55 ^a	27.89±4.27 ^a
T6 BBBC	48.11±9.90 ^a	28.55±8.43 ^a	26.53±2.00 ^a	23.24±2.01 ^a	32.59±4.38 ^a
T7 PU -	36.67±2.97 ^a	27.00±1.55 ^a	38.29±2.29 ^c	22.40±6.83 ^a	25.72±10.08 ^a
T8 PUPM	29.37±3.52 ^a	28.90±2.16 ^a	34.79±2.17 ^{bc}	31.41±2.10 ^a	25.83±3.86 ^a
T9 PUBC	29.42±3.14 ^a	27.50±18.64 ^a	26.75±2.84 ^a	26.82±3.39 ^a	26.74±4.89 ^a

Means within the same column sharing the same superscripts are not significantly different ($p>0.05$)

Table 4. Orthophosphate (mg/L) in the RAS with different biological media and bacterial supplements

	0 day	3 days	6 days	9 days	12 days
T1 NY -	4.13±0.36 ^a	5.01±0.15 ^{ab}	5.09±0.07 ^a	8.12±0.46 ^a	9.67±0.22 ^{ab}
T2 NYPM	4.24±0.08 ^a	4.86±0.38 ^{ab}	5.14±0.50 ^a	7.71±0.48 ^a	9.30±1.03 ^a
T3 NYBC	3.07±2.23 ^a	5.11±0.07 ^b	5.59±0.02 ^a	8.20±1.20 ^a	12.11±0.11 ^d
T4 BB -	4.39±0.01 ^a	4.86±0.04 ^{ab}	5.15±0.04 ^a	7.66±0.44 ^a	10.95±0.24 ^c
T5 BBPM	4.30±0.05 ^a	4.79±0.17 ^a	3.72±1.82 ^a	7.80±0.41 ^a	9.47±0.37 ^a
T6 BBBC	4.23±0.12 ^a	4.95±0.06 ^{ab}	5.41±0.04 ^a	8.09±0.15 ^a	10.55±0.17 ^{bc}
T7 PU -	4.68±0.19 ^a	4.90±0.05 ^{ab}	5.08±0.05 ^a	8.26±0.35 ^a	9.89±0.15 ^{ab}
T8 PUPM	4.43±0.02 ^a	4.69±0.06 ^a	4.99±0.04 ^a	8.04±0.69 ^a	9.37±0.85 ^a
T9 PUBC	4.55±0.04 ^a	4.70±0.18 ^a	5.45±0.06 ^a	8.41±0.16 ^a	9.47±0.35 ^a

Means within the same column sharing the same superscripts are not significantly different ($p>0.05$)

Experiment 2

On day-3, the ammonia content was significantly lower ($P<0.05$) in the experiment that employed the biological filter system with CB. From days 6-12, the ammonia levels decreased across all experimental treatments, with no significant differences observed between them ($P>0.05$) (Table 5). By day-9, the nitrite levels in the biological filter system with CB and CN were significantly lower ($P<0.05$) than those in the control (Table 6). Nitrate and orthophosphate levels exhibited a decreasing trend across all treatments over time (Table 7-8), it was found that the biological filter system with CB and EH had lower nitrate concentration on days 9-12, and significantly different to the control group ($P<0.05$). Orthophosphate level reduced in the biological filter system using CB and different to the control or the group using EH and CN ($P<0.05$), however, on day 12 the orthophosphate concentration in all phytoremediation treatment were lower than control ($P<0.05$). These findings demonstrate that the biological filter system with Fanwort, *Cabomba caroliniana* (CB), effectively reduced ammonia content and showed potential for lowering of nitrite, nitrate and orthophosphate

levels. Therefore, the CB-based biological filter system was selected for further study.

Table 5. Total ammonia (mg/L) in the PUBC RAS with different phytoremediation plant species

	0 day	3 days	6 days	9 days	12 days
T1 PUBC-	0.74±0.03 ^a	0.75±0.04 ^d	0.07±0.01 ^a	0.04±0.01 ^a	0.00±0.00 ^a
T2 PUBC-CB	0.67±0.03 ^a	0.25±0.06 ^a	0.04±0.02 ^a	0.05±0.02 ^a	0.00±0.00 ^a
T3 PUBC-EH	0.77±0.06 ^a	0.39±0.07 ^b	0.05±0.05 ^a	0.04±0.03 ^a	0.00±0.00 ^a
T4 PUBC-CN	0.73±0.05 ^a	0.55±0.02 ^c	0.01±0.01 ^a	0.03±0.02 ^a	0.00±0.00 ^a

Means within the same column sharing the same superscripts are not significantly different ($p>0.05$)

Table 6. Nitrite (mg/L) in the PUBC RAS with different phytoremediation plant species

	0 day	3 days	6 days	9 days	12 days
T1 PUBC-	0.24±0.01 ^a	0.23±0.04 ^a	0.31±0.00 ^a	0.22±0.01 ^c	0.06±0.01 ^a
T2 PUBC-CB	0.22±0.00 ^a	0.24±0.04 ^a	0.31±0.01 ^a	0.18±0.01 ^{ab}	0.04±0.01 ^a
T3 PUBC-EH	0.24±0.01 ^a	0.25±0.01 ^a	0.30±0.02 ^a	0.20±0.01 ^{bc}	0.03±0.01 ^a
T4 PUBC-CN	0.22±0.01 ^a	0.24±0.02 ^a	0.32±0.01 ^a	0.17±0.01 ^a	0.05±0.01 ^a

Means within the same column sharing the same superscripts are not significantly different ($p>0.05$)

Table 7. Nitrate (mg/L) in the PUBC RAS with different phytoremediation plant species

	0 day	3 days	6 days	9 days	12 days
T1 PUBC-	39.02±3.59 ^a	29.04±11.64 ^a	26.46±2.08 ^a	27.92±1.70 ^b	29.59±3.49 ^b
T2 PUBC-CB	35.98±4.56 ^a	27.81±11.54 ^a	21.86±1.56 ^a	20.39±1.10 ^a	20.64±2.02 ^a
T3 PUBC-EH	43.33±5.53 ^a	29.53±6.02 ^a	24.22±6.97 ^a	20.21±5.76 ^a	20.77±6.38 ^a
T4 PUBC-CN	37.63±1.73 ^a	30.33±4.43 ^a	21.02±8.41 ^a	26.31±2.62 ^{ab}	27.72±1.48 ^{ab}

Means within the same column sharing the same superscripts are not significantly different ($p>0.05$)

Table 8. Orthophosphate (mg/L) in the PUBC RAS with different phytoremediation plant species

	0 day	3 days	6 days	9 days	12 days
T1 PUBC-	4.65±0.10 ^a	4.04±0.20 ^a	4.42±0.13 ^a	4.43±0.02 ^b	2.85±0.20 ^b
T2 PUBC-CB	4.50±0.27 ^a	4.10±0.38 ^a	4.63±0.20 ^a	4.26±0.13 ^a	2.51±0.06 ^a
T3 PUBC-EH	4.56±0.07 ^a	4.11±0.07 ^a	4.66±0.03 ^a	4.66±0.01 ^c	2.49±0.06 ^a
T4 PUBC-CN	4.54±0.22 ^a	4.11±0.10 ^a	4.45±0.06 ^a	4.48±0.01 ^b	2.51±0.16 ^a

Means within the same column sharing the same superscripts are not significantly different ($p>0.05$)

Experiment 3

Ammonia, nitrite, and nitrate levels decreased consistently from day 3 onwards, with no statistically significant differences observed between the treatments ($P>0.05$) (Table 9-10). From day-3 to the end of the experiment, the orthophosphate levels were significantly reduced ($P<0.05$) in the treatment with ozone generator (Table 11). At the start of the experiment, the total bacterial

count in the water from all the treatments showed no statistical differences ($P>0.05$). However, from day 3-6, the total bacterial count decreased continuously in treatments using UV or ozone generators, with values significantly lower than those of the control ($P<0.05$). By day 9, the ozone generator treatment demonstrated a markedly greater bacterial reduction than the control and UV treatments ($P<0.05$). By the end of the experiment (day 12), the total bacterial counts in the water from both the UV and ozone generator treatments were comparable and significantly lower than those of the control group ($P<0.05$) (Figure 1).

Table 9. Total ammonia (mg/L) in the PUBC-CB RAS with different disinfection system

	0 day	3 days	6 days	9 days	12 days
T1 PUBC CB-	0.49±0.03 ^a	0.20±0.07 ^a	0.01±0.02 ^a	0.01±0.02 ^a	0.00±0.00 ^a
T2 PUBC CB UV	0.42±0.04 ^a	0.14±0.02 ^a	0.00±0.00 ^a	0.01±0.02 ^a	0.00±0.00 ^a
T3 PUBC CB OZ	0.42±0.06 ^a	0.15±0.01 ^a	0.00±0.00 ^a	0.02±0.03 ^a	0.00±0.00 ^a

Means within the same column sharing the same superscripts are not significantly different ($p>0.05$)

Table 10. Nitrite (mg/L) in the PUOM-CB RAS with different disinfection system

	0 day	3 days	6 days	9 days	12 days
T1 PUBC CB-	0.28±0.01 ^a	0.22±0.01 ^a	0.22±0.08 ^a	0.15±0.07 ^a	0.04±0.02 ^a
T2 PUBC CB UV	0.26±0.03 ^a	0.24±0.03 ^a	0.11±0.01 ^a	0.11±0.06 ^a	0.02±0.01 ^a
T3 PUBC CB OZ	0.28±0.01 ^a	0.19±0.04 ^a	0.15±0.00 ^a	0.09±0.02 ^a	0.02±0.00 ^a

Means within the same column sharing the same superscripts are not significantly different ($p>0.05$)

Table 11. Nitrate (mg/L) in the PUBC-CB RAS with different disinfection system

	0 day	3 days	6 days	9 days	12 days
T1 PUBC CB-	35.80±4.58 ^a	23.69±5.51 ^a	24.30±3.30 ^a	28.56±1.15 ^b	21.33±0.62 ^a
T2 PUBC CB UV	34.26±8.95 ^a	22.24±6.36 ^a	24.44±5.05 ^a	22.50±2.16 ^a	21.05±3.83 ^a
T3 PUBC CB OZ	35.01±3.32 ^a	19.73±1.70 ^a	23.03±5.82 ^a	19.36±1.74 ^a	19.58±3.12 ^a

Means within the same column sharing the same superscripts are not significantly different ($p>0.05$)

Table 12. Orthophosphate (mg/L) in the PUBC-CB RAS with different disinfection system

	0 day	3 days	6 days	9 days	12 days
T1 PUBC CB-	4.05±0.06 ^a	3.07±0.05 ^b	2.29±0.10 ^b	2.94±0.04 ^b	2.16±0.07 ^b
T2 PUBC CB UV	3.93±0.06 ^a	3.34±0.05 ^c	2.65±0.03 ^c	3.15±0.02 ^c	2.44±0.03 ^c
T3 PUBC CB OZ	4.00±0.10 ^a	2.78±0.02 ^a	1.55±0.02 ^a	1.97±0.01 ^a	1.52±0.05 ^a

Means within the same column sharing the same superscripts are not significantly different ($p>0.05$)

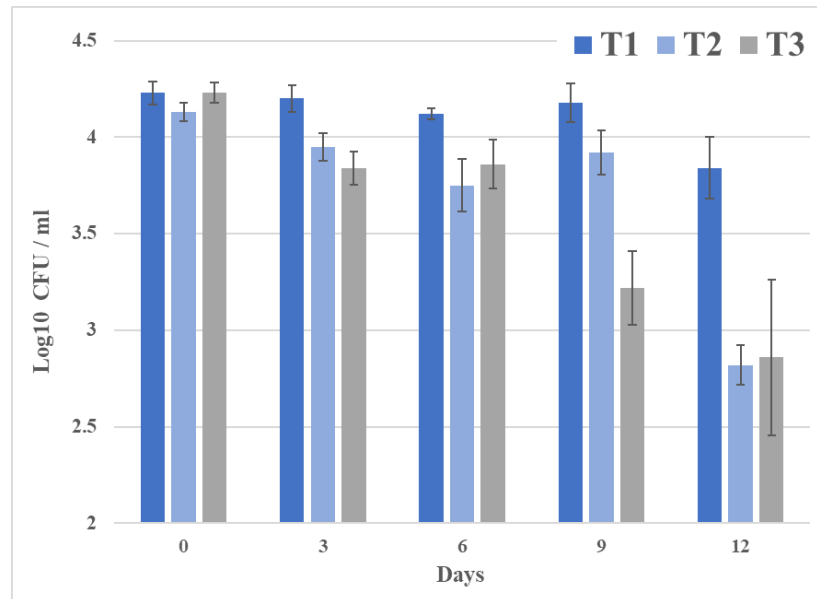


Figure 1. Total bacterial count (Log_{10} CFU/ml) in the water from different disinfection system

Discussion

The most effective nitrogen waste reduction was observed in the RAS using pumice stone as biofiltration media. Our results confirmed the findings of Pungrasmi *et al.* (2016), who reported the capability of pumice stone as an aerobic nitrification biofilter. The reduction of ammonia and nitrite levels to below the fish tolerable range of 1 mg-N/L during long-term RAS operation demonstrated the ability of pumice to provide water quality that complement aquatic organism health. This is consistent with the report on nitrogen removal efficiency by Suantika *et al.* (2016), who documented the significant removal of ammonium (30.95% per day) by a freshwater trickling bio-filtering system with a bacteria-inoculated bioball substrate over a 15-day period. Additionally, Lailiyah *et al.* (2023) also observed the improved ammonia removal efficiency when combining nylon nets with other substrates (Dacron + 25% nets + 75% sand). However, no studies have directly compared the ammonia removal efficiencies of nylon nets, bioballs, and pumice. Our findings indicate that pumice demonstrated higher nitrogen waste removal efficiency compared to the other substrates. This effectiveness can be attributed to high surface area of the pumice (Sepehr *et al.*, 2013), creates an optimal environment for bacterial colonization (Elser *et al.*, 2014) and adsorption capacity, which facilitate the immobilization and activity of nitrifying and denitrifying microorganisms (Xiao

et al., 2019). In addition, pumice has shown a high capacity for ammonium adsorption, and the large surface area of pumice may facilitate the interaction between ammonium ions and the substrate interface (Pratiwi *et al.*, 2019). Our findings also highlight the beneficial effects of microbial bioaugmentation on nitrogen waste removal efficiency in the RAS system. Both PM.1, a commercial *Bacillus* mixture (Department of Fisheries, Thailand), and BC, a lab-scale product of *Bacillus siamensis* (developed by Thaksin University's Laboratory), demonstrated a decreasing trend in ammonia, nitrite and nitrate concentrations in the treatments utilizing bioballs or pumice with microbial product supplement. These results align with Lee *et al.* (2019), who reported that the microbial mixture KBM-1 effectively removed ammonium and nitrate over 8 days, with removal efficiencies of 42% and 50%, respectively. Bioaugmented species were shown to facilitate the growth of indigenous microbial communities, thereby enhancing the degradation of solid wastes into simpler metabolites. Additionally, bioaugmentation in subsurface wastewater infiltration systems has been reported to improve nitrogen removal efficiency by increasing the abundance of nitrogen-removing bacteria and enhancing the activity of nitrogen-removal enzymes (Jia *et al.*, 2019). In our present study, the concentration of orthophosphate increased over the course of the experiment, suggesting that the biological filtration system was ineffective in reducing this substance. This inefficiency may be attributed to the retention of phosphorus within the organic matter of filtration systems rather than its removal from the water (Barak and Rijn, 2000). The retention of phosphorus within these systems limits their capacity to effectively reduce orthophosphate levels. In addition, in integrated multitrophic aquaculture systems, phosphorus can undergo chemical precipitation with calcium and magnesium ions, forming insoluble salts. This precipitation process further reduces the bioavailability of phosphorus, thereby limiting its removal through biological means (Tórz *et al.*, 2022).

Since the aquaculture biological filtration system face to the problem of phosphate and nitrate accumulation, it is necessary to develop other technology for remove those substances, phytoremediation is a promising technology for addressing the accumulation of phosphates and nitrates in aquaculture systems. Nizam *et al.* (2020) reported that various aquatic plants, exhibit significant potential for removing phosphates and nitrates from aquaculture wastewater. In the present study, our findings demonstrated that the combination of a microbial-augmented biological filtration system with *Cabomba caroliniana*-based phytoremediation effectively reduced nitrate and orthophosphate levels in aquaculture wastewater. These results align with (Nesan *et al.*, 2021), who reported that integrating phytoremediation with membrane filtration can enhance the removal of suspended solids, improve overall water quality, and increase

process efficiency. According to Olette *et al.* (2008), *Cabomba* species have demonstrated phytoremediation capabilities, and they are often outperformed by other aquatic plants in terms of efficiency. Nuwansi *et al.* (2018) report that *Cabomba caroliniana* was not the most effective plant in their study, but this plant contributed to the overall reduction of total solids, turbidity, and other water quality parameters, indicating its potential utility in aquaculture system. However, environmental factors can influence the efficiency of nutrient removal efficiency of the plants in phytoremediation such as, metal ions in water (Megateli and Couderchet, 2018) and light intensity (Walsh *et al.*, 2021). Because *Cabomba caroliniana* exhibits high tolerance to low-light conditions (Koleszár *et al.*, 2022), this plant maintains its efficiency in reducing nitrogen and phosphorus levels in recirculating biofiltration systems for fish larvae housed in shaded environments. Consequently, this study found that *Cabomba caroliniana* outperformed other plants in waste treatment within the system.

In addition, ozone generator application in aquaculture recirculation systems has been found to decrease orthophosphate concentrations. This finding is in accord with the findings of Hu *et al.* (2021) found that in a microalgae-based biofiltration system, ozone pretreatment for 0.5 minutes resulted in a total phosphate removal of 91.9%, compared with 77.6% removal efficiency in the control group. This process is based on the breakdown of organic phosphates, exemplified by organophosphate esters, via reactions with hydroxyl radicals generated in ozonation. These highly reactive radicals help to cleave complex organic phosphates and degrade into simpler, less toxic compounds (Pocostales *et al.*, 2010). Total bacterial counts decreased in treatments using either UV generators or ozone generators. However, the ozone generator treatment proved to be far more effective than either the control or the UV treatments in reducing bacterial count. This finding is consistent with the study conducted by Spiliotopoulou *et al.* (2017) also stated that a dosage of 15 g ozone/kg feed in optimal ozone in pilot recirculating aquaculture systems provides for improved water quality whilst reducing the bacterial load and dissolved organic matter. In aquaculture systems, ozone is a potent oxidant that can efficiently inactivate diverse bacterial, viral, fungal, and protozoan pathogens. It acts by destroying the bacterial cell wall, inactivating them (Gonçalves and Gagnon, 2011). In addition, ozone is more effective in eliminating organic matter than ultraviolet (UV) treatment. The use of an ozone generator reduces organic matter concentrations, thus limiting nutrient availability for bacterial growth (Spiliotopoulou *et al.*, 2018). This experimental study shows that pumice stone serving as a biotreatment substrate and microbial augmentation in RAS is efficient in removing ammonia and nitrites from the aquatic environment. *Cabomba caroliniana*-based phytoremediation system was more effective in

removing nitrate and orthophosphate concentrations than the treatment without phytoremediation. In addition, ozone (120 mg/hour, 10 min/hour/100 liters of water supplementation) significantly improved orthophosphate reduction and decreased numbers of total bacterial count. The integrate method showed an improving water quality and low bacterial load, thereby also contributing to sustainable aquaculture.

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Conflicts of interest

The authors declare no conflict of interest.

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