JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2026, 27(7), 1–11
https://doi.org/10.12911/22998993/216359
ISSN 2299–8993, License CC-BY 4.0

Received: 2025.11.18
Accepted: 2026.05.20
Published: 2026.06.01

Biofilter substrates influence microbial dynamics and water quality in the Cherax quadricarinatus recirculating aquaculture system

Juwita Nur Azizah[1], Seto Sugianto Prabowo Rahardjo[1,2*], Yunita Maimunah[1], Muta Harah Zakaria[3,4], Rizky Fadilla Agustin Rangkuti[1], Muhammad Fakhri[1], Rani Yuwanita[1], Arum Pratiwi[5], Tri Budi Prayogo[6]

[1] Study Program of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Brawijaya, Malang 65145, Indonesia
[2] Center for Shrimp Research Commodity, Brawijaya University, East Java, Indonesia
[3] Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia
[4] International Institute of Aquaculture and Aquatic Sciences (I-AQUAS), Universiti Putra Malaysia, Port Dickson 71050, Negeri Sembilan, Malaysia
[5] Sustainable Agricultural Extension Department, Agricultural Development Polytechnic of Malang, Jl. Dr. Cipto Bedali, Lawang 65216, East Java, Indonesia
[6] Department of Water Resources Engineering, Faculty of Engineering, Brawijaya University, Indonesia
* Corresponding author's email: seto.wre@ub.ac.id

ABSTRACT

This study evaluated the effectiveness of four biofilter substrates, Bioball (BB), dead coral (DC), zeolite (Ze), and activated carbon (AC), in supporting microbial colonization and improving water quality in a recirculating aquaculture system (RAS) for freshwater crayfish (*Cherax quadricarinatus*). A substrate-free control (Ct) was used for comparison over a 42-day culture period. Biofilter performance was assessed through bacterial abundance, water quality, and crayfish growth and survival. The substrate type strongly influenced nitrogen dynamics and microbial development. AC and Ze were most effective at reducing total ammonia nitrogen (TAN) and nitrite (NO_2^-), with AC lowering TAN from 0.21 mg/L to 0.11 mg/L and maintaining NO_2^- at 0.01–0.03 mg/L at 42 days of culture. These improvements corresponded to the highest survival rates of about 87% and 80% and to growth of about 1.12 and 1.05 g/day for AC and Ze, respectively. Bacterial counts were highest in AC and Ze, with AC supporting up to 21380 CFU/mL, 17783 CFU/mL, and 14380 CFU/mL of ammonifiers, *Nitrosomonas*, and *Nitrobacter*, respectively. Biochemical profiling revealed minimal diversity in Ct, with only *Streptococcus* sp., whereas BB, DC, and Ze supported complete nitrifying consortia. AC exhibited broad metabolic diversity, though *Nitrosomonas* detection was inconsistent between methods. The superior performance of AC was attributed to its high porosity and adsorption capacity, while Ze combined ion-exchange capability with microbial support. BB and DC performed moderately, whereas Ct was ineffective. Overall, AC and Ze emerged as the most promising substrates, demonstrating that indigenous microbial colonization is sufficient for stable nitrification and sustainable RAS management.

Keywords: recirculating aquaculture system, crayfish, biofilter substrates, nitrifying bacteria, water quality.

INTRODUCTION

Freshwater lobster or crayfish (*Cherax quadricarinatus*) is a high-value aquaculture commodity with growing demand in international markets (Mauro et al., 2022). In some countries, such as Australia, Thailand, Indonesia, and China, crayfish is also favored as an alternative protein source and a leading export commodity (Mauro et al., 2022; Azizah et al., 2022). Nevertheless, crayfish farming still faces several challenges, including low survival rates due to water-quality issues in intensive culture systems. Water quality in intensive aquaculture tends to deteriorate due to the accumulation of organic waste from uneaten feed and lobster excretion. Microorganisms break down this

waste into inorganic compounds such as ammonia (NH_3), nitrite (NO_2^-), and nitrate (NO_3^-), which can become toxic to cultured organisms if not adequately managed (Ahmad et al., 2021). NH_3 and NO_2^-, in particular, are known to have both acute and chronic toxic effects on the respiratory and osmoregulatory systems of aquatic animals (Guo et al., 2025). Therefore, practical strategies for managing nitrogen waste are crucial for maintaining stable water quality (Ramli et al., 2020).

One widely adopted approach in recirculating aquaculture systems (RAS) is the use of biofilters (Liu et al., 2021). Biofilters offer a substrate for the colonization of microorganisms engaged in nitrogen bioconversion through ammonification and nitrification pathways (Han et al., 2016). Ammonification is carried out by heterotrophic bacteria that break down organic nitrogen into NH_3. Subsequently, NH_3 is oxidized into NO_2^- by *Nitrosomonas*, and NO_2^- is further oxidized into NO_3^- by *Nitrobacter*, all of which occur under aerobic conditions (Liu et al., 2020). The effectiveness of this process largely depends on the presence and activity of the microbial community within the biofilter system (Cholet et al., 2025).

The characteristics of the biofilter media play a crucial role in supporting the growth and density of bacteria involved in the nitrogen cycle (Gymnastiar et al., 2025). In this study, four commonly used biofilter media were employed: bioballs (Chang et al., 2019), dead coral (Boshagh et al., 2023), zeolite (Eberle et al., 2022), and activated carbon (Xu et al., 2020). Bioballs have a hollow structure and high surface area that facilitate oxygen circulation (Xin et al., 2024). Dead coral offers a rough surface and natural porosity, making it an ideal substrate for biofilm attachment (Qi et al., 2023). In addition to being porous, zeolite possesses ion-exchange capabilities that support NH_3 absorption. Activated carbon is recognized for its vast surface area and high adsorption capacity for organic compounds, thereby creating a microenvironment conducive to microbial colonization (Lu et al., 2022).

This study did not involve adding external bacteria. Instead, the microorganisms observed developed naturally from the culture environment in response to organic input from feed and the characteristics of the biofilter substrate (Wang et al., 2023). This approach reflects natural conditions commonly encountered in the field, making the findings more practically relevant. Although numerous studies have investigated the effectiveness of biofilters in reducing NH_3 and NO_2^- levels, specific research focusing on the types and densities of naturally occurring nitrifying and ammonifying bacteria across various biofilter media in crayfish RAS remains limited. Therefore, this study aims to evaluate the influence of biofilter substrate type (bioball, dead coral, zeolite, and activated carbon) on the occurrence and density of *Nitrosomonas*, *Nitrobacter*, and ammonifying bacteria in *Cherax quadricarinatus* aquaculture systems using a non-inoculated bacterial approach. The results of this study are expected to provide substantial insights into the effectiveness of biofilter substrates in supporting natural microbial dynamics and improving water quality in closed aquaculture systems.

MATERIAL AND METHODS

Substrates

This study was conducted from October to December 2024 at the Fish Cultivation Laboratory, Faculty of Fisheries and Marine Science, Brawijaya University. The cultivation system used was based on the RAS, as shown in Figure 1. The freshwater lobsters (*Cherax quadricarinatus*) used had an average length of 5 cm and an average weight of 3 gr. The juveniles were obtained from the Sumberpasir Laboratory in Malang Regency, East Java, and were of the red-clawed freshwater lobster strain. The lobsters were acclimatized for 2 days before the treatments began. The stocking density was set at 100 fish/m^2.

The study employed a completely randomized design (CRD) with four biofilter substrate types and three replications. The substrates tested were Bioball (BB) which had a surface area of 200–240 m^2/m^3, porosity >85%, and a diameter of 20–50 mm; dead coral (DC) with a surface area of 20.45 m^2/g, particle size of 5–15 mm, diameter of 5–15 mm, and porosity of 40–60%; zeolite (Ze) with a surface area of 14-30 m^2/g, particle size of 1–3 mm, diameter of 5–10 mm, and porosity of 40–50%; activated carbon (AC) with a surface area of 4320 m^2/g, particle size of 1–5 mm, diameter of 2–5 mm, and porosity of 70–90%. Each treatment was operated in a single laboratory-scale RAS unit, consisting of a rearing tank measuring 50 × 30 × 30 cm with a volume of 45 liters, a biofilter tank measuring 38 × 30 × 12.5 cm, an SP1200 circulation pump, and a HIBLOW HP200 aerator.

Biofilter system and maintenance

Each biofilter system unit used a uniform volume and biological reactor design to maintain consistency across treatments. The rearing tanks had a total volume of 45 L and were filled to 30 L. Each rearing tank was stocked with 15 fish, 15 shelter units, a water heater, and a water circulation pump with a recirculation capacity of 40 L/h and a flow rate of 1 L/min. The biofilter tank, connected to the rearing tank, served as the biological treatment medium, containing 5 L of biofilter substrate. The types and weights of substrates used were 1.1 kg of bioball, 3 kg of dead coral, 4.5 kg of zeolite, and 3 kg of activated carbon, as specified in the respective treatments. Water was continuously circulated from the biofilter tank to the circulation pump and back, forming a closed-loop RAS.

No bacterial inoculum or starter was added at the start of the cultivation; microorganisms were allowed to grow and develop naturally through colonization by leftover feed, lobster excretion, and interactions with the biofilter substrates. Feed was provided twice daily ad libitum using commercial Fengli pellets (MP Adraquatic Co., Indonesia) containing 40% protein, 5% fat, 2% fiber, 13% ash, and 11% moisture. Feeding was conducted at 1.5% of body weight per day, with 30% administered in the morning and 70% in the afternoon.

Microorganism analysis

Biofilm samples were collected from the surface of the biofilter media on day 0 (before culture), day 21 (mid-day of culture), and day 42 (end of culture) of the rearing period. Sampling was conducted by scraping the substrate surface using a sterile spatula. The collected biofilm was then dissolved in 10 mL of sterile phosphate-buffered saline (PBS) at pH 7.2 for microscopic analysis and bacterial culturing. Bacterial isolation was carried out using the streaking method on solid nutrient media to obtain single colonies (Hartmann et al., 2021). The pure isolates obtained were then analyzed by Gram staining and colony morphology. Subsequently, a series of basic biochemical tests was conducted, including the oxidase test, catalase test, indole test, motility, and H_2S production test (using SIM medium), and citrate utilization test on Simmons citrate agar (SCA), as well as functional tests to detect nitrification and ammonification activities (Vetrano et al., 2005). Bacterial quantification was performed using the total plate count (TPC) method with selective media for the three primary bacterial groups (Jennison, 1940). The selective ammonia oxidizer medium for *Nitrosomonas* bacteria typically consists of the following chemicals (all chemicals were purchased from Sigma-Aldrich, USA): $(NH_4)_2SO_4$ (2 g/L), K_2HPO_4 (1 g/L), NaCl (2 g/L), $FeSO_4 \cdot 7H_2O$ (0.4 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), $CaCO_3$ (0.01 g/L), fenol red (0.025 g/L), and bacto agar (20 g/L) (Belser, 1978). The selective nitrite oxidizer medium for *Nitrobacter* bacteria typically consists of the following components KNO_2 (0.06 g/L), K_2HPO_4 (1 g/L), NaCl (0.03 g/L), $MgSO_4 \cdot 7H_2O$ (0.1 g/L), $FeSO_4 \cdot 7H_2O$ (0.03 g/L), $CaCO_3$ (1 g/L), $CaCl_2$ (0,3 g/L), and bacto agar (40 g/L) (Lees, 1957). The selective ammonifying bacteria medium for *amonification* bacteria typically consists of the following components Mn_2HPO_4

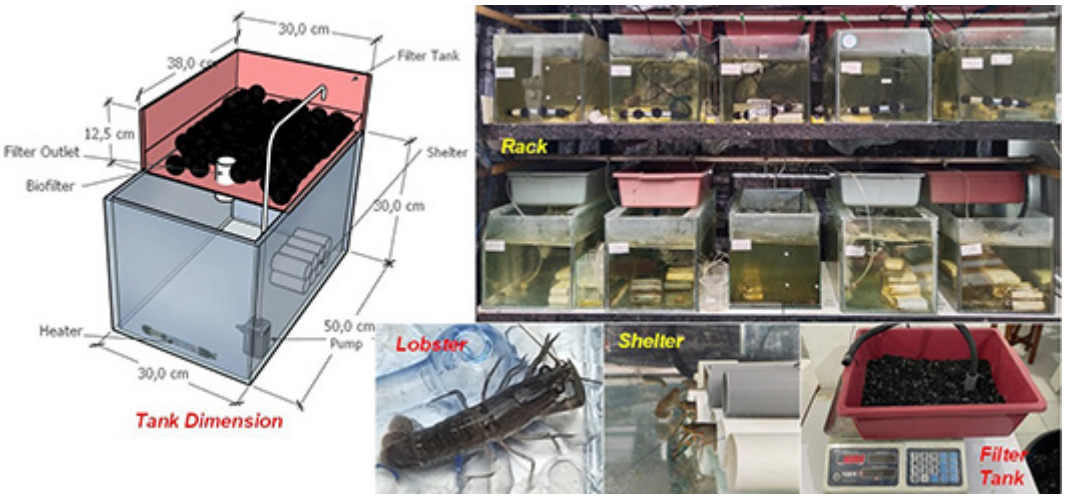

Figure 1. Biofilter design

(0.9 g/L), K_2HPO_4 (0.2 g/L), $MgSO_4 \cdot 7H_2O$ (0.1 g/L), $FeCl_3 \cdot 6H_2O$ (0.005 g/L), $CaCl_2 \cdot 6H_2O$ (18.4 g/L), yeast extract (0.25 g/L), glucose (5 g/L), NaCl (2 g/L), and bacto peptone (5 g/L) (Poirier et al., 2017). Medium for total bacterial abundance counting using TSA medium 40g/L (Sondo et al., 2023). All media were incubated at 28–30 °C for 5–7 days under aerobic conditions. The growing colonies were counted, and the results were expressed in colony-forming units per milliliter (CFU/mL) of the biofilm solution.

Water quality analysis

Water quality parameters, including temperature, pH, and dissolved oxygen (DO), were routinely measured daily using digital instruments. Temperature and DO were measured using a DO meter. (Lutron DO-5510), pH using a water quality checker (Tool Master). Meanwhile, total ammonia nitrogen (TAN), NO_2^-, and NO_3^- were routinely measured on days 0, 21, and 42 using the spectrophotometric method (721G Visible Spectrophotometer) as standard (Thangiah, 2019).

Data analysis and bacterial quantification

The data on bacterial density and water quality parameters were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to evaluate significant differences between treatments at the 5% significance level ($p < 0.05$). All statistical analyses were performed using the latest version of SPSS software (Arroyo et al., 2017). Nitrifying bacteria were quantified using the swab sampling method on a 10 × 10 cm area of the biofilter substrate with a sterile cotton swab, which was then placed into 10 mL of sterile saline solution (0.85% NaCl) (Nie et al., 2022). The initial suspension was serially diluted up to 10^{-2} using the serial dilution method. Then, 0.1 mL of each dilution was inoculated onto sterile, selective media using the spread plate method, and the mixture was evenly spread with a sterile glass rod (Thomas et al., 2015). Petri dishes were tightly sealed with Parafilm and incubated at 28–30 °C for 24–48 hours. The growing colonies were counted and expressed in CFU/mL. Bacterial identification was performed using macroscopic observation (shape, color, edges, and colony surface) and biochemical tests (catalase, oxidase, H_2S production, and indole) as an initial approach to identify nitrifying bacteria (*Nitrosomonas*, *Nitrobacter*) and ammonifying bacteria. For specific validation, molecular analysis is recommended in further studies (Mellbye et al., 2017).

RESULTS

Water quality and production

The water quality parameters remained within suitable ranges for *Cherax quadricarinatus* culture across all treatments, with no significant differences (F-values of 1.32, 0.51, and 1.48 for temperature, DO, and pH, respectively; $p < 0.05$) in temperature, DO, or pH. Water temperature was relatively stable at 27.1–27.6°C, while DO levels (8.86–8.95 mg/L) indicated well-oxygenated conditions across treatments. The pH values ranged from 6.65 to 6.99, remaining within the optimal range for crayfish culture. TDS showed a significant variation (F-values = 4.17; $p < 0.05$), with the highest value recorded in the AC treatment (389.8 ± 17.6 mg/L), which differed significantly from those of Ct, BB, and Ze. The elevated TDS in AC is likely due to the higher adsorption and desorption dynamics of ions associated with its micro- and mesoporous structure.

In contrast to the generally uniform water quality, the production parameters showed statistically significant differences among treatments (F-values of 3.97 and 17.13 for SR and GR, respectively; $p < 0.05$). The survival rate (SR) and growth rate (GR) were both strongly influenced by the substrate type. The AC treatment achieved the highest SR (86%) and GR (84.68 mg/day), which were significantly higher than those of Ct and moderately higher than those of BB, DC, and Ze. Ze showed 80.00% SR and 78.33mg/day growth, while Ct showed the lowest performance (66.67% SR; 47.86 mg/day GR). The superior performance in AC and Ze systems corresponds with their ability to maintain low NH_3 and NO_2^- concentrations, providing a more stable and less toxic environment for crayfish metabolism and growth. Overall, the progression in both SR and GR parameters (Ct<BB/DC<Ze<AC) reflects the increasing efficiency of biofiltration and improved environmental conditions associated with more effective substrates (Table 1).

Figure 2 illustrates the dynamics of inorganic nitrogen (NH_3, NO_2^-, and NO_3^-) concentrations across treatments during the 42-day culture period. Initial NH_3 and NO_2^- levels were low (<0.02

Table 1. The measurement results of water quality and production

Parameters	Ct	BB	DC	Ze	AC
a. Water quality					
Temperature, C	27.6 ± 0.65^{a}	27.12 ± 0.23^{a}	27.55 ± 0.59^{a}	27.25 ± 0.18^{a}	27.6 ± 0.36^{a}
DO, mg/L	8.95 ± 0.09^{a}	8.89 ± 0.03^{a}	8.91 ± 0.06^{a}	8.86 ± 0.04^{a}	8.88 ± 0.01^{a}
pH	6.99 ± 0.21^{a}	6.84 ± 0.12^{a}	6.74 ± 0.14^{a}	6.74 ± 0.05^{a}	6.65 ± 0.06^{a}
TDS, mg/L	326.6 ± 14^{a}	323.9 ± 9.6^{a}	363.7 ± 18.0^{ab}	332.2 ± 4.1^{a}	389.8 ± 17.6^{b}
b. Production					
Survival Rate, %	66.67 ± 6.5^{a}	73.33 ± 6.50^{ab}	73.33 ± 6.51^{ab}	80.00 ± 7.00^{bc}	86.67 ± 6.51^{c}
Growth Rate, mg/day	47.86 ± 3.46^{a}	64.05 ± 6.90^{b}	59.13 ± 0.99^{b}	78.33 ± 2.38^{bc}	84.68 ± 2.84^{c}

Note: $^{a, b, c}$ significant different among treatments at $P < 0.05$.

mg/L), within safe limits for freshwater lobster culture. By day 21, both compounds increased significantly in all treatments, with the highest NH_3 (±0.32 mg/L) and NO_2^- (±0.23 mg/L) observed in the Ct and the lowest in AC (±0.21 mg/L NH_3) and Ze, reflecting more efficient nitrification in these substrates. Although some values exceeded the recommended safe thresholds (NH_3: 0.1–0.2 mg/L), concentrations declined by day 42, with AC and Ze achieving levels near the safe range, confirming their superior performance in removing NH_3 and NO_2^-. NO_3^- levels rose sharply by day 21 (10–15 mg/L), remained far below toxic levels (>90 mg/L), and decreased slightly by day 42, likely due to denitrification or biological uptake, indicating balanced nitrogen cycling within the biofilter systems.

The concentrations of nitrogen compounds and the abundances of key bacterial groups varied significantly among treatments (F-values of 6.58, 4.34, and 11.72 for NH_3, NO_2^-, and NO_3^-, respectively; $p < 0.05$), indicating that substrate type strongly influenced biofiltration efficiency in the crayfish RAS. NH_3 levels were lowest in Ze (0.2418 mg/L) and AC (0.2214 mg/L), which differed significantly from the other treatments (Ct, BB, and DC). Similarly, NO_2^- concentrations were markedly lower in Ze (0.0732 mg/L) and AC (0.0250 mg/L) than in Ct, BB, and DC (0.158–0.175 mg/L). The reduced levels of NH_3 and NO_2^- in Ze and AC treatments reflect more efficient NH_3 oxidation and NO_2^- conversion, consistent with enhanced nitrification activity. NO_3^- concentrations followed an inverse trend, with the lowest values in Ze (10.96 mg/L) and AC (10.69 mg/L), and the highest in Ct (15.01 mg/L). This suggests that improved nitrification efficiency and potential denitrification processes in Ze and AC minimized NO_3^- accumulation.

Bacterial enumeration supported these findings. AC consistently supported the highest densities of nitrifying and ammonifying bacteria:

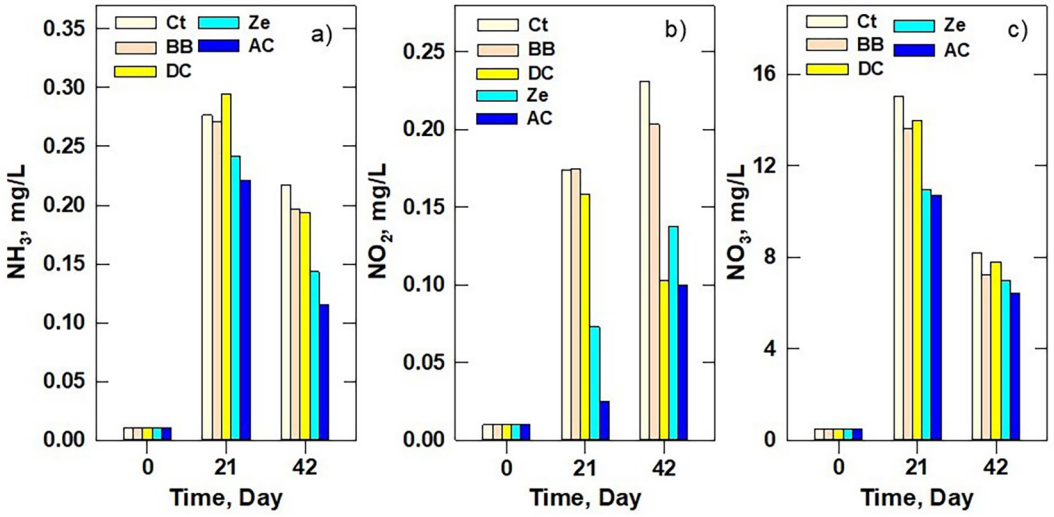

Figure 2. The dynamics of inorganic nitrogen concentrations (a) NH_3, (b) NO_2^-, and (c) NO_3^-

Nitrobacter (33113 CFU/mL), *Nitrosomonas* (17783 CFU/mL), and ammonifiers (21380 CFU/mL), with total bacterial counts reaching 72444 CFU/mL. Ze followed with moderate densities (*Nitrobacter*: 16218 CFU/mL; *Nitrosomonas*: 9120 CFU/mL; total: 36308 CFU/mL), while BB and DC exhibited intermediate levels, and Ct showed the lowest values. The superior performance of AC and Ze corresponds to their physicochemical characteristics: AC's high micro–mesoporosity and adsorption capacity provide stable microhabitats for biofilm formation and NH_3 trapping, while Ze's ion-exchange framework enhances NH_4^+ capture and supports microbial colonization. Conversely, BB and DC supported moderate nitrification due to limited adsorption or ion-exchange capacity, and Ct, lacking a substrate, exhibited the weakest bacterial growth and nitrogen removal efficiency.

Abundance of bacteria in the substrate

At day 0, all treatments started with similar bacterial densities (302 CFU/mL) and balanced functional groups, with ammonifying bacteria (29.5%) and *Nitrosomonas* (26.3%) slightly dominant over *Nitrobacter* (23.4%) and other bacteria (20.7%), as shown in Figure 3. By day 21, substrate effects became apparent. AC supported the highest bacterial density (60256 CFU/mL), followed by Ze (28840 CFU/mL) and BB (26303 CFU/mL). In contrast, DC (13490 CFU/mL) and Ct (11749 CFU/mL) exhibited lower densities. Across all treatments, *Nitrobacter* began to increase, indicating establishment of NO_2^- oxidation, whereas other bacteria declined due to competitive exclusion (Table 2). By day 42, the substrate type had an evident influence on microbial colonization and nitrification efficiency. AC achieved the highest bacterial density (72444 CFU/mL) with *Nitrobacter* dominance (45.7%) and balanced populations of ammonifiers and *Nitrosomonas*, indicating complete nitrification. Ze followed (36308 CFU/mL), exhibiting a similar nitrifier succession, which was supported by its ion-exchange and microbial-carrier properties. BB maintained a moderate density (29512 CFU/mL) with balanced nitrification potential, while DC and Ct remained at the lowest levels (15849 CFU/mL), reflecting limited colonization and weak NH_3 oxidation. Overall, AC and Ze provided optimal conditions for microbial specialization and efficient nitrogen transformation, while BB offered intermediate benefits, DC provided moderate support, and Ct was the least effective.

Biochemical identification of dominant bacteria

The identification of bacteria across different biofilter substrates revealed distinct variations in microbial colonization and nitrification potential. In the Ct treatment, only *Streptococcus* sp. was detected, with no evidence of nitrifying or ammonifying bacteria (John et al., 2020). This suggests that, without a suitable substrate, beneficial microbial communities cannot establish effectively, leading to reduced nitrification capacity. In contrast, the BB substrate supported a more diverse and functional microbial community. The detection of *Bacillus sp.*, *Nitrobacter* sp., and *Nitrosomonas* sp. indicates that both ammonification and the two key steps of nitrification (NH_3 and NO_2^- oxidation) occurred concurrently, showing that BB provides sufficient surface area and favorable conditions for microbial biofilm development (Sepehri et al., 2019).

Table 2. The measurement results of nitrogen and bacteria densities

Parameters	Ct	BB	DC	Ze	AC
a) Nitrogen					
NH_3, mg/L	0.277±0.017^b	0.271±0.012^b	0.294±0.018^b	0.242±0.016^a	0.221±0.019^a
NO_2^-, mg/L	0.174±0.003^b	0.175±0.025^b	0.159±0.008^b	0.073±0.006^a	0.025±0.008^a
NO_3^-, mg/L	15.01±0.295^c	13.62±0.140^b	13.95±0.050^b	10.96±0.037^a	10.69±0.054^a
b) Bacteria					
Nitrobacteria, CFU/mL	6918±2^a	13804±2^{ab}	7079±1^a	16218±2^{bc}	33113±2^c
Nitrosomonas, CFU/mL	3467±2^a	7079±2^{ab}	3890±1^a	9120±2^{ab}	17783±2^b
Ammonification, CFU/mL	4169±2^a	8511±2^b	4677±1^a	10715±2^{bc}	21380±2^c
Total, CFU/mL	15849±2^a	29512±2^{ab}	15849±1^a	36308±2^{ab}	72444±1^b

Note: $^{a, b, c}$ significant different among treatments at $P < 0.05$.

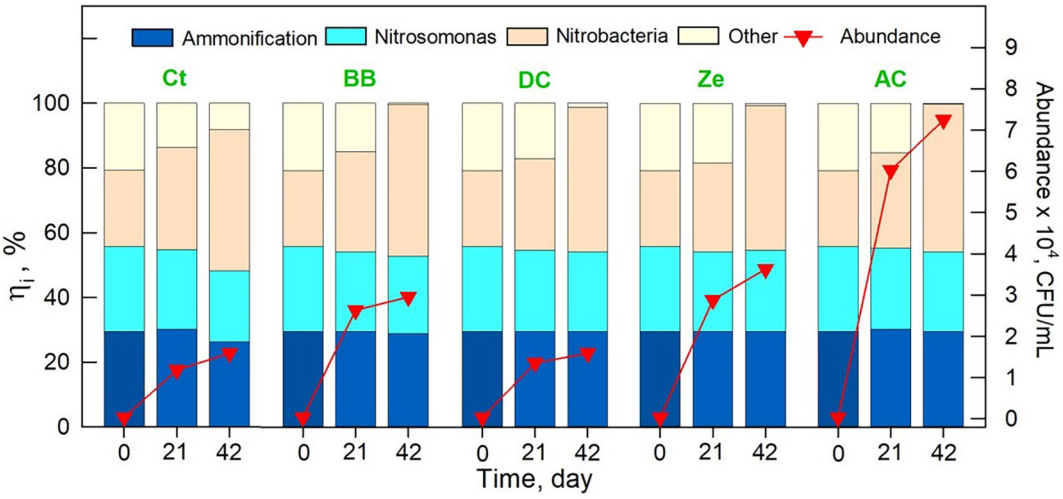

Figure 3. Bacterial density and selectivity

A similar pattern was observed in DC, which harbored *Pseudomonas* sp., *Nitrobacter* sp., and *Nitrosomonas* sp., suggesting a robust potential for complete nitrification. The presence of *Pseudomonas* sp. may enhance heterotrophic activity and denitrification, promoting a balanced nitrogen cycle. Ze exhibited a microbial composition comparable to that of *Bacillus* sp., *Nitrobacter* sp., and *Nitrosomonas* sp. (Xiang et al., 2023). However, Ze's ion-exchange properties provided an additional advantage by directly adsorbing NH_3 while facilitating microbial colonization. This dual function makes Ze highly effective for both biological and physicochemical nitrogen removal (Ye et al., 2019). In contrast, AC supported *Bacillus* sp. and *Nitrobacter* sp. but lacked *Nitrosomonas* sp., suggesting that the initial step of nitrification may be less efficient in AC-based systems (Rent et al., 2020).

Biochemical tests confirmed differences in metabolic profiles across treatments (Bhatt et al., 2024). Ct had only two isolates with limited enzymatic activity (Toyama et al., 2018), while BB, DC, and Ze showed more diverse biochemical characteristics, with catalase-, oxidase-, and SCA-positive isolates supporting nitrification and organic matter degradation. Ze showed the highest diversity and metabolic capacity, followed by AC, which had numerous catalase- and oxidase-positive isolates exhibiting indole, motility, H_2S, and SCA activity (Gevod et al., 2022). Despite its metabolic diversity, AC was less effective in complete nitrification due to the absence of *Nitrosomonas* sp. Overall, Ze and BB emerged as the most effective substrates, supporting complete nitrification and substantial microbial diversity. DC showed moderate performance, AC displayed high metabolic diversity but limited nitrification efficiency, and Ct remained ineffective (Ishaq et al., 2023).

DISCUSSION

Biofilter performance and nitrogen removal

Substrate type significantly influenced biofilter performance in reducing toxic nitrogen compounds. AC and Ze consistently maintained lower NH_3 and NO_2^- concentrations than BB, DC, and Ct. Specifically, AC reduced NH_3 from 0.21 mg/L (DOC 21) to 0.11 mg/L (DOC 42), while maintaining NO_2^- levels at 0.01–0.03 mg/L. This superior performance can be attributed to AC's high microporous surface area, which not only adsorbs organic matter and NH_3 but also provides niches for microbial biofilm development (Bartelme et al., 2017). Ze likewise achieved strong NH_3 suppression due to its ion-exchange capacity, which binds ammonium ions while supporting microbial colonization. In contrast, Ct accumulated the highest levels of NH_3 and NO_2^-, correlating with the lowest survival (67%) and growth (Rahardjo et al., 2026). These results confirm that substrates that combine physicochemical and biological functions, such as AC and Ze, are most effective at maintaining water quality suitable for lobster culture (Tatarri et al., 2017).

Bacterial abundance and community composition

Bacterial density increased substantially in systems with biofilter substrates compared to Ct.

AC consistently supported the highest abundance of ammonifiers, *Nitrosomonas*, and *Nitrobacter* at DOC 42, reaching 21380, 17783, and 14380 CFU/mL, respectively. This reflects AC's dual function: adsorption reduces toxic accumulation, while its porous structure enhances microbial attachment (Neissi et al., 2021). Ze followed closely, benefiting from its negatively charged crystalline framework, which provides stable microhabitats for nitrifiers through the exchange of ammonium cations. BB and DC exhibited moderate densities, consistent with their designs. BB's engineered hollows promote biofilm formation, while DC's natural porosity supports colonization, albeit with weaker chemical interactions (Yuan et al., 2017). The consistently low bacterial numbers in Ct underscore the limited microbial colonization in the absence of substrate support. These trends align with improved nitrogen conversion observed in AC and Ze, confirming a strong link between substrate properties, bacterial abundance, and water quality (Cebron et al., 2003).

Biochemical characteristics of bacterial isolates

Biochemical tests further highlighted functional differences across treatments. Ct yielded only *Streptococcus sp.* with limited traits (catalase and motility positive), reflecting poor nitrification potential. BB and DC supported complete nitrifying communities, including *Bacillus* sp., *Nitrosomonas* sp., and *Nitrobacter* sp.; DC additionally harbored *Pseudomonas* sp., suggesting possible denitrification pathways. Ze hosted diverse bacteria with strong metabolic versatility, supported by its ion-exchange ability that reduces free NH_3 while creating microenvironments favorable for oxidase and indole activity (Su et al., 2025). AC supported the most isolates, all of which were catalase- and mostly oxidase-positive, with additional traits such as indole, H_2S, and SCA (Hink et al., 2024). The adsorption capacity of AC likely allowed heterotrophs and nitrifiers to coexist by minimizing the accumulation of organic residues. Interestingly, *Nitrosomonas* sp. was not detected biochemically despite high plate counts, suggesting methodological limitations rather than an actual absence. Collectively, these profiles confirm that BB, DC, and Ze supported complete nitrification, whereas AC promoted broad metabolic diversity with adsorption-enhanced stability (Lu et al., 2022).

Influence of substrate properties on bacterial colonization

The properties of the substrate directly influence variations in bacterial abundance and function. AC, with its extremely high surface area and micro–mesoporous structure, traps organic matter and NH_3 while reducing oxygen competition between heterotrophs and nitrifiers. This results in stable biofilms and enhanced nitrification (Kim et al., 2006). Ze, with its aluminosilicate framework and high cation-exchange capacity, binds ammonium ions and gradually releases them, supporting Nitrosomonas and Nitrobacter while decreasing free NH_3 toxicity (Peela et al., 2015). BB offers a large engineered surface area with strong water circulation, ensuring oxygen supply, although its inert composition limits adsorption. DC, made of porous calcium carbonate, provides microbial attachment and buffering capacity to stabilize pH, but lacks the physicochemical benefits of AC and Ze (Dang et al., 2015). Conversely, Ct, without any substrate, fails to provide structural or chemical support, resulting in poor microbial colonization and weak nitrification (John et al., 2020).

Adaptation of indigenous microorganisms

This study highlights the resilience and adaptability of indigenous microorganisms in the *Cherax* RAS. Without commercial inocula, naturally occurring microbes effectively colonized all substrates and tolerated fluctuating water quality and elevated NH_3 levels (Rahardjo et al., 2024). Their ability to form biofilms on both engineered (BB, AC, Ze) and natural (DC) substrates demonstrates ecological adaptability. The presence of *Bacillus* sp., *Pseudomonas* sp., and nitrifiers across treatments shows a balanced community capable of ammonification, nitrification, and even partial denitrification. This underlines the ecological advantage of indigenous microbiota in RAS, which ensures long-term stability without requiring costly external supplementation (Oh et al., 2019).

Implications for RAS management

The findings show that AC and Ze are the most effective substrates for biofiltration in freshwater lobster RAS, due to their complementary physicochemical and microbial roles: AC specializes in adsorption and biofilm stabilization, while Ze combines ion exchange with microbial support (Li

et al., 2021). BB and DC offer moderate support by promoting biofilm growth and buffering, but are less effective for nitrogen removal. Ct without substrate was unsuitable for microbial colonization or for maintaining water quality. From a management perspective, combining substrates can optimize performance by utilizing BB or DC upstream to enhance circulation and buffering, followed by Ze or AC downstream to improve adsorption, ion exchange, and microbial retention. Notably, the system developed stable microbial communities without the use of commercial inoculation, highlighting a cost-effective and sustainable approach for aquaculture operations (Kesy et al., 2019).

CONCLUSIONS

Substrate selection significantly influenced biofilter efficiency, microbial colonization, and culture performance in the crayfish RAS. AC and Ze showed the best performance, maintaining the lowest NH_3 and NO_2^- levels, supporting the highest bacterial densities, and achieving superior survival (87%) and growth (1.12 g/day). In contrast, the Ct exhibited poor microbial development, nitrogen accumulation, and the lowest production outcomes. Biochemical analyses revealed that BB, DC, and Ze supported complete nitrifier guilds, whereas AC promoted diverse metabolic activity but inconsistent detection of Nitrosomonas, likely due to methodological factors. The superior functionality of AC and Ze stemmed from their physicochemical properties AC extensive adsorption surface, and Ze ion-exchange capacity. In contrast, BB and DC offered moderate benefits through structural and buffering roles. Indigenous microorganisms naturally colonized all substrates, indicating that external inocula are unnecessary. A staged biofilter design combining BB or DC for circulation and buffering with Ze or AC for adsorption and nitrification offers an efficient, sustainable strategy for freshwater lobster RAS management. Future studies incorporating molecular-based approaches (e.g., NGS) are recommended to resolve the functional dynamics of the microbial community further.

Acknowledgments

We wish to extend our sincere appreciation to Brawijaya University for their invaluable support through the research grant. Furthermore, we extend our heartfelt thanks to all individuals and organizations whose contributions have enriched this research endeavor.

REFERENCES

1. Ahmad, A., Abdullah, S., Hasan, H., Othman, A., Ismail, N. (2021). Aquaculture industry: Supply and demand, best practices, effluent and its current issues and treatment technology. *Journal of Environmental Management, 287*, 112271. https://doi.org/10.1016/j.jenvman.2021.112271
2. Arroyo, M., Ferreira, A., Frota, O., Rigotti, M., de Andrade, D., Brizzotti, N., Peresi, J., Castilho, E., de Almeida, M. (2017). Effectiveness of ATP bioluminescence assay for presumptive identification of microorganisms in hospital water sources. *BMC Infectious Diseases, 17*. https://doi.org/10.1186/s12879-017-2562-y
3. Azizah, U., Hartoyo, S., Wijaya, W. (2022). Competitiveness of Indonesia's fresh lobster market with China. *International Journal of Scientific Research in Science, Engineering and Technology.* https://doi.org/10.32628/ijsrset229119
4. Bartelme, R., McLellan, S., Newton, R. (2017). Freshwater recirculating aquaculture system operations drive biofilter bacterial community shifts around a stable nitrifying consortium of ammonia-oxidizing archaea and comammox *Nitrospira*. *Frontiers in Microbiology, 8*. https://doi.org/10.3389/fmicb.2017.00101
5. Belser, L., Schmidt, E. (1978). Diversity in the ammonia-oxidizing nitrifier population of a soil. *Applied and Environmental Microbiology, 36*, 584–588. https://doi.org/10.1128/aem.36.4.584-588.1978
6. Bhatt, P., Brown, P., Huang, J., Hussain, A., Liu, H., Simsek, H. (2024). Algae and indigenous bacteria consortium in treatment of shrimp wastewater: A study for resource recovery in sustainable aquaculture system. *Environmental Research, 240*, 118447. https://doi.org/10.1016/j.envres.2024.118447
7. Boshagh, F., Rostami, K., Moazami, N. (2023). Dark fermentative hydrogen production in packed-bed bioreactor using the Persian Gulf dead coral, ceramic saddle, and ceramic ball as support matrices. *International Journal of Hydrogen Energy*. https://doi.org/10.1016/j.ijhydene.2023.06.344
8. Cébron, A., Berthe, T., Garnier, J. (2003). Nitrification and nitrifying bacteria in the lower Seine River and estuary (France). *Applied and Environmental Microbiology, 69*, 7091–7100. https://doi.org/10.1128/AEM.69.12.7091-7100.2003
9. Chang, J., Mei, J., Jia, W., Chen, J., Li, X., Ji, B., Wu, H. (2019). Treatment of heavily polluted river water by tidal-operated biofilters with organic/inorganic media: Evaluation of performance and bacterial

community. *Bioresource Technology, 279*, 34–42. https://doi.org/10.1016/j.biortech.2019.01.060

10. Dang, H., Lovell, C. (2015). Microbial surface colonization and biofilm development in marine environments. *Microbiology and Molecular Biology Reviews, 80*, 91–138. https://doi.org/10.1128/MMBR.00037-15
11. Eberle, S., Börnick, H., Stolte, S. (2022). A sequential anammox zeolite-biofilter for the removal of nitrogen compounds from drinking water. *Water*. https://doi.org/10.3390/w14213512
12. Gevod, V., Chernova, A., Zhumadulayeva, A., Dossybayeva, G., Syzdykova, M., Shymelkova, R., Issayeva, A. (2022). Buffer effects in submersed denitrifying biofilter. *Journal of Ecological Engineering*. https://doi.org/10.12911/22998993/146634
13. Guo, H., Li, Y., Ge, H., Sha, H., Luo, X., Zou, G., Liang, H. (2025). Competitive bio-accumulation between ammonia and nitrite results in their antagonistic toxicity to *Hypophthalmichthys molitrix*: Antioxidant and immune responses and metabolic detoxification evidence. *Antioxidants, 14*. https://doi.org/10.3390/antiox14040453
14. Gymnastiar, A. A., Yuniarti, A., Rahardjo, S. S. P., Maimunah, Y., Yuwanita, R., Mahariawan, I. M. D., Widyawati, Y., Sholichin, M., Hariati, A. M. (2025). The role of pure nano oxygen bubbles in the nitrogen cycle of tilapia biofloc systems. *Chemosphere, 384*, 144522. https://doi.org/10.1016/j.chemosphere.2025.144522
15. Han, L., Huang, S., Wei, Z., Chen, P., Zhang, Y. (2016). Performance of a new suspended filler biofilter for removal of nitrogen oxides under thermophilic conditions and microbial community analysis. *Science of the Total Environment, 562*, 533–541. https://doi.org/10.1016/j.scitotenv.2016.04.084
16. Hartmann, R., Jeckel, H., Jelli, E., Singh, P., Vaidya, S., Bayer, M., Rode, D., Vidakovic, L., Díaz-Pascual, F., Fong, J., Dragoš, A., Lamprecht, O., Thöming, J., Netter, N., Häussler, S., Nadell, C., Sourjik, V., Kovács, Á., Yildiz, F., Drescher, K. (2021). Quantitative image analysis of microbial communities with BiofilmQ. *Nature Microbiology, 6*, 151–156. https://doi.org/10.1038/s41564-020-00817-4
17. Ishaq, S., Turner, S., Lee, G., Tudor, M., MacRae, J., Hamlin, H., Bouchard, D. (2023). Water temperature and disease alters bacterial diversity and cultivability from American lobster (*Homarus americanus*) shells. *iScience, 26*. https://doi.org/10.1016/j.isci.2023.106606
18. Jennison, M., Wadsworth, G. (1940). Evaluation of the errors involved in estimating bacterial numbers by the plating method. *Journal of Bacteriology, 39*, 389–397. https://doi.org/10.1128/jb.39.4.389-397.1940
19. John, E., Krishnapriya, K., Sankar, T. (2020). Treatment of ammonia and nitrite in aquaculture wastewater by an assembled bacterial consortium. *Aquaculture, 526*, 735390. https://doi.org/10.1016/j.aquaculture.2020.735390
20. Kim, D., Kim, S. (2006). Effect of nitrite concentration on the distribution and competition of nitrite-oxidizing bacteria in nitratation reactor systems and their kinetic characteristics. *Water Research, 40*(5), 887–894. https://doi.org/10.1016/j.watres.2005.12.023
21. Lees, H., Simpson, J. (1957). The biochemistry of the nitrifying organisms. V. Nitrite oxidation by *Nitrobacter*. *The Biochemical Journal, 65*(2), 297–305. https://doi.org/10.1042/BJ0650297
22. Li, D., Van De Werfhorst, L., Rugh, M., Feraud, M., Hung, W., Jay, J., Cao, Y., Parker, E., Grant, S., Holden, P. (2021). Limited bacterial removal in full-scale stormwater biofilters as evidenced by community sequencing analysis. *Environmental Science & Technology*. https://doi.org/10.1021/acs.est.1c00510
23. Liu, W., Du, X., Tan, H., Xie, J., Luo, G., Sun, D. (2020). Performance of a recirculating aquaculture system using biofloc biofilters with convertible water-treatment efficiencies. *Science of the Total Environment, 754*, 141918. https://doi.org/10.1016/j.scitotenv.2020.141918
24. Liu, W., Xu, B., Tan, H., Zhu, S., Luo, G., Shitu, A., Wan, Y. (2021). Investigating the conversion from nitrifying to denitrifying water-treatment efficiencies of the biofloc biofilter in a recirculating aquaculture system. *Aquaculture, 544*, 737817. https://doi.org/10.1016/j.aquaculture.2021.737817
25. Lu, Z., Jing, Z., Huang, J., Ke, Y., Li, C., Zhao, Z., Ao, X., Sun, W. (2022). Can we shape microbial communities to enhance biological activated carbon filter performance? *Water Research, 212*, 118104. https://doi.org/10.1016/j.watres.2022.118104
26. Mauro, M., Arizza, V., Arculeo, M., Attanzio, A., Pinto, P., Chirco, P., Badalamenti, G., Tesoriere, L., Vazzana, M. (2022). Haemolymphatic parameters in two aquaculture crustacean species *Cherax destructor* (Clark, 1836) and *Cherax quadricarinatus* (Von Martens, 1868). *Animals, 12*. https://doi.org/10.3390/ani12050543
27. Mellbye, B., Giguere, A., Bottomley, P., Sayavedra-Soto, L. (2016). Quorum quenching of *Nitrobacter winogradskyi* suggests that quorum sensing regulates fluxes of nitrogen oxide(s) during nitrification. *mBio, 7*. https://doi.org/10.1128/mBio.01753-16
28. Neissi, A., Rafiee, G., Rahimi, S., Farahmand, H., Pandit, S., Mijakovic, I. (2021). Enriched microbial communities for ammonium and nitrite removal from recirculating aquaculture systems. *Chemosphere*, 133811. https://doi.org/10.21203/rs.3.rs-636504/v1
29. Nie, J., Huang, H., Rao, P., Chen, H., Du, X., Wang, Z., Zhang, W., Liang, H. (2022). Composite functional particle enhanced gravity-driven ceramic membrane bioreactor for simultaneous removal

of nitrogen and phosphorus from groundwater. *Chemical Engineering Journal*, 139134. https://doi.org/10.1016/j.cej.2022.139134

30. Oh, S., Hammes, F., Liu, W. (2018). Metagenomic characterization of biofilter microbial communities in a full-scale drinking water treatment plant. *Water Research, 128*, 278–285. https://doi.org/10.1016/j.watres.2017.10.054
31. Peela, S., Kadiri, D., Gorle, N., Peetala, K. (2015). Isolation and identification of promising ammonifying and nitrifying bacteria from agricultural fields of Visakhapatnam. *International Journal of Pharma and Bio Sciences*.
32. Poirier, S., Madigou, C., Bouchez, T., Chapleur, O. (2017). Improving anaerobic digestion with support media: Mitigation of ammonia inhibition and effect on microbial communities. *Bioresource Technology, 235*, 229–239. https://doi.org/10.1016/j.biortech.2017.03.099
33. Qi, W., Skov, P., De Jesus Gregersen, K., Pedersen, L. (2023). A novel method to estimate biofilm activity based on enzymatic oxygen release from hydrogen peroxide decomposition. *Biofilm, 5*, 100121. https://doi.org/10.1016/j.bioflm.2023.100121
34. Rahardjo, S. S. P., Shih, Y.-J., Fan, C.-S. (2024). Ammonia oxidation by in-situ chloride electrolysis in etching wastewater of semiconductor manufacturing using RuSnOx/Ti electrode: Effect of plating mode and metal ratio. *Journal of Hazardous Materials, 469*, 134042. https://doi.org/10.1016/j.jhazmat.2024.134042
35. Rahardjo, S. S. P., Supriatin, F. E., Septiansyah, A. D., Fadli, M. F., Rahmawati, A., Rangkuti, R. F. A. (2026). Examining blind feeding techniques for growth performance and water quality in the intensive production of Pacific white shrimp (*Penaeus vannamei*). *Aquaculture, 611*, 742958. https://doi.org/10.1016/j.aquaculture.2025.742958
36. Ramli, N., Verreth, J., Yusoff, F., Nurulhuda, K., Nagao, N., Verdegem, M. (2020). Integration of algae to improve nitrogenous waste management in recirculating aquaculture systems: A review. *Frontiers in Bioengineering and Biotechnology, 8*. https://doi.org/10.3389/fbioe.2020.01004
37. Ren, Z., Jia, B., Zhang, G., Fu, X., Wang, Z., Wang, P., Lv, L. (2020). Study on adsorption of ammonia nitrogen by iron-loaded activated carbon from low temperature wastewater. *Chemosphere, 262*, 127895. https://doi.org/10.1016/j.chemosphere.2020.127895
38. Sepehri, A., Sarrafzadeh, M. (2019). Activity enhancement of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria in activated sludge process: Metabolite reduction and CO_2 mitigation intensification process. *Applied Water Science, 9*. https://doi.org/10.1007/s13201-019-1017-6
39. Sondo, M., Wonni, I., Klonowska, A., Koïta, K., Moulin, L. (2023). Quantification of diversity sampling bias resulting from rice root bacterial isolation on popular and nitrogen-free culture media using 16S amplicon barcoding. *PLOS ONE, 18*. https://doi.org/10.1371/journal.pone.0279049
40. Su, Z., Liu, T., Guo, J., Zheng, M. (2025). Kinetic and physiological characterization of acidophilic *Nitrobacter* spp. in a nitrite-oxidizing culture. *Environmental Science & Technology*. https://doi.org/10.1021/acs.est.4c10020
41. Tatari, K., Musovic, S., Gülay, A., Dechesne, A., Albrechtsen, H., Smets, B. (2017). Density and distribution of nitrifying guilds in rapid sand filters for drinking water production: Dominance of *Nitrospira* spp. *Water Research, 127*, 239–248. https://doi.org/10.1016/j.watres.2017.10.023
42. Thangiah, A. S. (2019). Spectrophotometric determination of sulphate and nitrate in drinking water at Asia-Pacific International University Campus, Muak Lek, Thailand. *Rasayan Journal of Chemistry, 12*(3), 1503–1508. https://doi.org/10.31788/RJC.2019.1235201
43. Toyama, T., Kasuya, M., Hanaoka, T., Kobayashi, N., Tanaka, Y., Inoue, D., Sei, K., Morikawa, M., & Mori, K. (2018). Growth promotion of three microalgae—*Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Euglena gracilis*—by in situ indigenous bacteria in wastewater effluent. *Biotechnology for Biofuels, 11*. https://doi.org/10.1186/s13068-018-1174-0
44. Vetrano, A., Heck, D., Mariano, T., Mishin, V., Laskin, D., Laskin, J. (2005). Characterization of the oxidase activity in mammalian catalase. *Journal of Biological Chemistry, 280*, 35372–35381. https://doi.org/10.1074/JBC.M503991200
45. Wang, Y., Lv, Y., Wang, C., Jiang, G., Han, M., Deng, J., Hsi, H. (2023). Microbial community evolution and functional trade-offs of biofilm in odor treatment biofilters. *Water Research, 235*, 119917. https://doi.org/10.1016/j.watres.2023.119917
46. Xiang, Y., Zhou, T., Deng, S., Shao, Z., Liu, Y., He, Q., Chai, H. (2023). Nitrite improved nitrification efficiency and enriched ammonia-oxidizing archaea and bacteria in the simultaneous nitrification and denitrification process. *Water Research X, 21*, 100204. https://doi.org/10.1016/j.wroa.2023.100204
47. Xu, L., Campos, L., Canales, M., Ciric, L. (2020). Drinking water biofiltration: Behaviour of antibiotic resistance genes and the association with bacterial community. *Water Research, 182*, 115954. https://doi.org/10.1016/j.watres.2020.115954
48. Ye, J., Liu, S., Liu, W., Meng, Z., Luo, L., Chen, F., Zhou, J. (2019). Photocatalytic simultaneous removal of nitrite and ammonia via a zinc ferrite/activated carbon hybrid catalyst under UV–visible irradiation. *ACS Omega, 4*, 6411–6420. https://doi.org/10.1021/ACSOMEGA.8B00677