Effects of potassium monopersulfate on nitrification activity and bacterial community structure of sponge biocarrier biofilm in Litopenaeus vannamei aquaculture system

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Abstract

Effects of potassium monopersulfate (KMPS) on nitrification activity, growth performance of *Litopenaeus vannamei* and bacterial community structure of sponge biocarrier with pre-cultured biofilm (SBBF) were analyzed through shaking flask experiments and *L. vannamei* aquaculture experiment. The change of ammonia oxidation rate (AOR) and nitrite oxidation rate (NOR) of SBBF under six KMPS concentration treatments (0 mg/L, 1 mg/L, 2 mg/L, 3 mg/L, 4 mg/L and 5 mg/L) was studied, the results showed that the AOR and NOR of SBBF treated with high concentrations of KMPS (3 mg/L, 4 mg/L and 5 mg/L) were significantly lower than those of the control group (CK) ($p < 0.05$). However, compared with the first dosing of NH_4Cl and $NaNO_2$, the inhibition of KMPS on AOR and NOR was weakened after the second and third dosing times. The *L. vannamei* aquaculture experiment was set to four concentrations of KMPS (0 mg/L, 2 mg/L, 4 mg/L, 8 mg/L), the results showed that with the increase of KMPS dosage, the average and peak concentrations of NH_4^+-N and NO_2^--N in each system significantly increased ($P < 0.05$). The final body weight of shrimps significantly decreased ($P < 0.05$), high dose (8.0 mg/L) of KMPS reduced the survival rate by 9.33% than CK. High-throughput sequencing analysis of the biofilm structure showed that the relative abundance of Nitrospirota, *Nitrosomonas* and *Nitrococcus*, which is related to nitrogen cycling, and beneficial bacteria including Firmicutes and *Bacilli* decreased with the addition of KMPS ($p < 0.05$).

Introduction

Over the last number of years, the world-wide production of *Litopenaeus vannamei* has increased due to the increasing demand for human consumption (Bauer et al. 2018). The negative impacts of traditional aquaculture are widespread, including environmental hazards from the discharge of farm effluent, waste of water due to the absence of a water recycling system, nutrient pollution as well as frequent disease outbreaks in aquaculture animals. A recirculating aquaculture system (RAS) are designed for aquaculture production with restricted water exchange. The RAS system is characterized by relying on biological filtration to reduce ammonia toxicity and nutrients, ensure water quality safety, and provide a suitable living space for shrimps (Zhu et al. 2016). RAS has been gradually accepted by people for its advantages of environmental protection, energy savings and controllable farming conditions and is considered an inevitable trend for aquaculture development in the future. The biofilm method refers to a community of microorganisms attached to a substrate involved in the transfer of organic matter and the biochemical cycling of aquatic ecosystems to purify water. The stable and viable nitrifying biofilm is the key component of the intensive RAS. Ammonia and nitrite are the primary environmental factors in the aquatic culturing system that pose a great threat to the survival of shrimp. Nitrifying microorganisms use nitrite as an intermediate to oxidize ammonium ions to nitrate. However, nitrifying microorganisms are vulnerable to competition for survival in aquaculture waters, and their activity and composition are susceptible to environmental factors that can lead to reduced or inactive activity, thus necessitating the application of biofilms in aquaculture systems to accelerate the process of establishing nitrification systems. Several studies have shown that the application of biofilm technology in aquaculture can not

only control water quality but also improve shrimp production as an additional food source (Kim et al. 2019; Cuzon et al. 2004; Liu et al. 2019).

In closed environments, RAS exacerbates the risk of pathogen transmission caused by high-density farming. (Lu et al. 2022). For example, *Vibrio*, *Edwardsiella* and *Pseudomonas* can be toxic to *L. vannamei* and often lead to severe disease outbreaks, seriously affecting yield (Arunkumar et al. 2020; Qiu et al. 2016). Current disinfection methods for aquaculture water include the use of antibiotics, chemical disinfectants, ozone and UV irradiation treatments to reduce the bacterial load in the water and avoid the proliferation of potentially disease-causing microorganisms (Hess-Erga et al.2018). These treatments are effective but have some drawbacks. For example, the chlorination process can lead to by-products that are toxic to the environment (David et al. 2018; Moreno-Andres et al. 2019); cultured organisms can only absorb 20–30% of the antibiotic, the rest is dissolved in the water, leading to a gradual accumulation in the water environment or even making aquatic animals resistant to the drugs (Mo et al. 2017). UV radiation affects the growth and repair mechanism of microorganisms, causing growth cell death or regenerative cell death (Hess-Erga et al. 2018; Grob et al. 2016). In addition, the formation of ozone disinfection byproducts and the toxicity of residual cyclohexanone in shrimp have limited ozone wide application in RAS (Liu et al. 2018).

A possible alternative disinfectant is potassium monopersulfate, usually applied in the form of potassium monopersulfate triple salt ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) (KMPS), whose active ingredient and oxidation potential energy source is $KHSO_5$ (PMS), which has been shown to be effective in water treatment (Ike et al. 2018; Wacławek et al. 2017; Hess-Erga et al. 2019). On the one hand, KMPS was more likely to be activated to produce sulfate radical anions due to its asymmetrical molecular structures (Oh et al. 2016). Compared to oxidative lipid peroxidation induced by hydroxyl radicals (Wacławek et al. 2017), sulfate radicals are more selective and have a more stable half-life, altering cell membrane permeability and inhibiting cellular metabolic processes to inactivate cells (Rodríguez-Chueca et al. 2018). On the other hand, KMPS is traded in the form of powder, which is convenient to store, handle and transport. Furthermore, it is considered "green" because it can be broken down into nontoxic, environmentally friendly by-products that are cheap and easily soluble in water (Ghanbari et al. 2017; Moreno-Andres et al. 2019). It has been reported that KMPS has been widely used in epidemic prevention, medical environment elimination, foreign pathogen defence (COVID-19) and other fields (Rodriguez-Chueca et al. 2017; Sonthipet et al. 2018; Hashizume et al. 2019). A prerequisite for using KMPS in aquaculture, however, is that KMPS neither poses sublethal effects to *L. vannamei* nor impairs the nitrification process in the aquaculture system at the dosages applied. However, to our knowledge, the effects of KMPS on water quality, nitrification and changes in microbial communities under aquaculture conditions have not been reported.

Sponge biocarriers (SB) with low cost, high porosity and suitability for stable attachment and growth of microorganisms have received increasing attention from researchers in recent years (Zhu et al. 2016 ; Chu et al. 2014; Wang et al. 2021). SB fillers can effectively enrich native microorganisms in water and remove some eutrophic substances, such as nitrogen and phosphorus, to purify water and are thus

widely used in major industrial wastewater treatment and occasionally used in aquaculture (Wang et al. 2022).

Consequently, this study aimed to (i) determine the effects of KMPS on the ammonia oxidation rate (AOR) and nitrite oxidation rate (NOR) of sponge biocarriers with precultured biofilm (SBBF) at different concentrations; (ii) analyse the effects of different doses of KMPS on the water quality and growth of *L. vannamei* in aquaculture systems; and (iii) compare the structural composition and diversity of SBBF biofilm bacterial communities during culture by high-throughput sequencing methods.

Materials And Methods

Systems and experimental design

Culture method of SBBF

SB (Banhor, specification: 2 cm×2 cm×2 cm, porosity: 98%, density: 0.011 g/cm3, specific surface area: 4000 m^2/m^3) was utilized as the artificial substrate. The culture method of SBBF was described by Wang et al. (2022).

Effect of KMPS on nitrification activity of SBBF

To find out the stabilisation of ammonia oxidation activity and nitrite oxidation activity under the regulation of KMPS. The experiments were carried out in six beakers, each with 1000 mL of experimental water and 10% (V/V) SBBF with initial KMPS (Shanghai Macklin Biochemical Co., Ltd, CAS:70693-62-8) concentrations of 0 mg/L (CK), 1.0 mg/L (Group A), 2.0 mg/L (Group B), 3.0 mg/L (Group C), 4.0 mg/L (Group D) and 5.0 mg/L (Group E). Once KMPS was added, the solutions were treated with a six-joint electric stirrer for 2 h.

Five pieces (10% V/V) of KMPS-treated SBBF were randomly removed from each of the above six beakers and added to six 1000 ml flasks containing 400 ml artificial seawater and 0.1% (V/V) trace element solution. Ammonium chloride was added to make the initial concentration of NH_4^+-N 5 mg/L. Another six 1000 ml flasks were taken with other elements unchanged, and the ammonium chloride was changed to sodium nitrate to make the initial concentration of NO_2^--N 50 mg/L. The flasks were incubated at 150 rpm and 25°C with shaking. Ammonia and nitrite nitrogen were measured every 12 h. When NH_4^+-N and NO_2^--N concentrations were undetectable, a second and third addition was performed. NH_4^+-N and NO_2^--N were measured every 12 hours, and the ammonia oxidation rate (AOR) and nitrite oxidation rate (NOR) were calculated according to the following formulas.

AOR or NOR = K•V/W, where K is the slope of a scatter plot of NH_4^+-N or NO_2^--N concentration (mg/L) against time, V is the volume of solution (L), W is the total dry weight (g) of SBBF.

Effect of KMPS on water quality, L. vannamei **growth performance and SBBF bacterial community structure in aquaculture systems**

The experiment was carried out in a 0.3×0.3×0.3 m tank with 18 L of artificial seawater (salinity 15‰). The concentration of KMPS was set as 0.0 mg/L (T0), 2.0 mg/L (T1), 4.0 mg/L (T2) and 8.0 mg/L (T3). During the experimental period, there was no water renewal, and KMPS was added every five days. SBBF in 2mm polyester mesh was immersed in the shrimp culture pond at a rate of 5% (V/V) and the SBBF is cleaned every 15 days to restore adsorption capacity.

Each culture system was fed with 16 shrimp larvae (density: 800 shrimp/m^3). The temperature was controlled at 24–26°C, and DO was maintained between 7.0 and 8.5 mg/L. Shrimp were obtained from Haida shrimp hatchery (Guangzhou), with an average body length of 1.50 ± 0.10 cm and average weight of 0.1 ± 0.004 g. Feeding 4 times a day with bait (Shenzhen Aohua Group Co., LTD) at approximately 4.0% of the total shrimp weight.

Analytical methods

Physicochemical analysis of water quality

The water quality of each culture system was monitored as follows: temperature, dissolved oxygen (DO), pH (HQ30D water quality analyser, Harker, Loveland CO, USA) and turbidity (2100Q turbidity analyser, Hach company, USA) in each tank were measured daily during the test. NH_4^+-N and NO_2^--N were measured daily by Nessler's reagent spectrophotometry and N-(1-naphthyl)-ethylenediamine spectrophotometry; NO_3^--N was determined by the naphthylethylenediamine hydrochloride spectrophotometry method every 3 days.

Growth performance of L. vannamei

At the end of the experiment, body weight (g), survival rate (%) and feed conversion rate (FCR) were determined. After that, the following variables were calculated:

Survival rate (percent) = (survival shrimp volume/initial shrimp volume) × 100

FCR = [total dry feed intake (grams)/weight gain (grams)]

DNA extraction and highthroughput sequencing method

Biofilm samples from each container were collected on Days 15 and 30 of the experiment. The samples were placed into a beaker, distilled water was added, and the samples were shaken in an ultrasonic instrument bath for 15 minutes. The supernatant was removed after centrifugation, and the biofilm sample was obtained and denoted T0_15, T1_15, T2_15, T3_15, T0_30, T1_30, T2_30, and T3_30. The initial biofilm sample of SBBF was denoted P0. DNA extraction using Soil DNA Extraction Kit (OMEGA Bio-Tek, Norcross, GA, USA), its purity and concentration was checked by ultramicro spectrophotometer P360 (Implen GmbH, München, Germany).

The DNA samples obtained were completed by Shanghai Major Biotechnology Co., Ltd., based on Illumina MiSeq sequencing platform for high-throughput sequencing, and BLAST analysis of all phyla and the first 30 genera.

Statistical analysis

Origin and SPSS were used for data processing, and the data were expressed as mean and standard error (means ± SE), error bars indicate standard error. ANOVA was used to determine significant differences, and $P < 0.05$ was considered significant.

Results

Variation in NH_4^+-N and NO_2^--N at different KMPS concentrations

To determine the influence of different KMPS concentrations on the ammonia oxidation activity and nitrite oxidation activity of SBBF, the changes in ammonia nitrogen and nitrite nitrogen concentrations in different experimental groups after three doses of ammonium chloride (sodium nitrite) were analysed (Fig. 1, Fig. 2). After the first addition of ammonium chloride, NH_4^+-N conversion in Groups B, C, D and E within 48 h (0.35%-4.04%) was significantly lower than that in groups CK and A (33.50% and 32.15%) ($P < 0.05$). The time required for NH_4^+-N decline to be undetectable in Groups D and E was twice as long as that in the CK group, which meant that NH_4^+-N conversion was reduced by 50%. After adding ammonium chloride for the second and third time, the total transformation time of NH_4^+-N in each group was reduced by 14.3%, 25.0%, 33.3%, 45.5%, 53.8%, and 53.8%, respectively, compared with the first time ($P < 0.05$). After the first addition of sodium nitrite, the conversion rate of CK group (4.67 $mg/L \cdot h^{-1}$) was significantly higher than that of the other groups (4.27 $mg/L \cdot h^{-1}$, 3.99 $mg/L \cdot h^{-1}$, 3.32 $mg/L \cdot h^{-1}$, 1.59 $mg/L \cdot h^{-1}$, 1.35 $mg/L \cdot h^{-1}$; $P < 0.05$), and the complete transformation time of NO_2^--N in Group E was three times longer than that in CK. Similarly, after the second and third additions of sodium nitrite, the NO_2^--N conversion rates of different concentrations of KMPS were 5.42–5.83 $mg/L \cdot h^{-1}$ and 5.78–6.16 $mg/L \cdot h^{-1}$, respectively. There was no significant difference among the groups ($P > 0.05$), but it was significantly higher than that of the first treatment ($P < 0.05$) (Fig. 2).

Effect of KMPS on AOR and NOR of SBBF

The AOR in each group increased gradually with increasing ammonium chloride addition (Fig. 3). The AOR of each group decreased to different degrees after three additions. AOR in the CK was the highest, while in Groups D and E, it was the lowest. The change trend of NOR was similar to that of AOR and increased gradually with increasing sodium nitrite addition time. After adding sodium nitrite for the first time, the NOR decreased with increasing KMPS concentration to different degrees. The NOR of the CK

was the highest, and those of the D and E groups the lowest. After adding sodium nitrite for the second and third time, the NOR in each system was higher than that of CK.

Effects of KMPS on water quality in L. vannamei aquaculture systems

Table 1 shows the changes in water quality during the aquaculture of *L. vannamei*. Without significant differences between systems in mean temperature, pH or DO. The turbidity of each system showed an upwards trend, but at a low level, it did not differ significantly among systems. NH_4^+-N and NO_2^--N were maintained at low levels in the T0 systems (NH_4^+-N < 0.05 mg/L and NO_2^--N < 1.0 mg/L) (Fig. 4). The concentrations of NH_4^+-N and NO_2^--N fluctuated with KMPS addition, and the fluctuation degree was T3 > T2 > T1 > T0. The NH_4^+-N content was higher overall in the experimental groups than in T0. NH_4^+-N in T1, T2 and T3 increased sharply after the addition of KMPS and then declined after 3 days, with average decreases of 27.27%, 14.28% and 5.77% in 6 cycles, respectively. The peak value of NH_4^+-N in the experimental groups was higher than that in T0 ($P < 0.05$), specifically, T3 > T2 > T1 > T0. The concentration of NO_2^--N varied greatly; the NO_2^--N mean concentrations in the T1, T2 and T3 systems were 1.09 mg/L, 2.14 mg/L and 3.46 mg/L, respectively, showing a 2.11-fold, 5.11-fold and 8.89-fold increase compared with T0. Similarly, NO_2^--N accumulated immediately after the addition of KMPS and then declined after 4 days, and the fluctuations in T3 were significantly lower than those in T1 and T2 ($P < 0.05$). The NO_3^--N concentrations in all groups tended to rise throughout the experiment, and the accumulation was T0 > T1 > T2 > T3, with significant differences among the different systems ($P < 0.05$).

Effect of KMPS on the growth performance of L. vannamei

The final body weight of shrimp was significantly differences among different systems ($P < 0.05$) (Table 2). T0 had the highest average weight gain at harvest, which was significantly better than T1, T2 and T3. With the increase in KMPS dosage, the survival rate of shrimp decreased. T0, T1 and T2 were 100%, which was significantly higher than that of T3 ($P < 0.05$). For FCR, significant differences ($p < 0.05$) were observed between T3 and the others, but no significant difference among T0, T1 and T2 ($p > 0.05$).

Table 1
Physical and chemical parameters of water in four treatments in the *L. vannamei* culture system.

Parameter	T0	T1	T2	T3
Temperature (℃)	25.3 ± 0.7	25.2 ± 1.2	25.3 ± 0.8	25.1 ± 0.9
DO (mg/L)	7.98 ± 0.25[a] (7.82–8.23)	7.69 ± 0.21 [a] (7.49–7.90)	7.70 ± 0.18 [a] (7.50–7.88)	7.69 ± 0.17 [a] (7.56–7.86)
pH	8.31 ± 0.06 [a] (8.23–8.37)	8.23 ± 0.06 [a] (8.16–8.29)	8.33 ± 0.06 [a] (8.23–8.39)	8.32 ± 0.07 [a] (8.23–8.39)
Turbidity (NTU)	1.12 ± 0.37 [a] (0.53–2.01)	1.21 ± 0.43 [a] (0.57–2.10)	1.37 ± 0.23 [a] (0.67–2.02)	1.49 ± 0.44 [a] (0.65–1.98)
Total phosphorus	0.23 ± 0.04 [a] (0.00-1.30)	0.18 ± 0.04 [a] (0.00-0.84)	0.17 ± 0.04 [a] (0.00-0.80)	0.17 ± 0.04 [a] (0.00-0.80)
Different superscripts in the same row indicate significant differences ($p < 0.05$)				

Table 2
Growth performance of *L. vannamei* cultivated in different experimental treatments during culture (30 days)

Parameter	T0	T1	T2	T3
Initial weight (g)	0.1 ± 0.004	0.1 ± 0.004	0.1 ± 0.004	0.1 ± 0.004
Final weight (g)	3.34 ± 0.28[a]	2.82 ± 0.42[b]	2.54 ± 0.16[c]	2.08 ± 0.37[d]
Average weight gain ($g \cdot week^{-1}$)	0.81 ± 0.05[a]	0.68 ± 0.07[b]	0.61 ± 0.03[c]	0.52 ± 0.06[d]
FCR	1.59 ± 0.07[b]	1.66 ± 0.04[b]	1.72 ± 0.07[b]	1.89 ± 0.06[a]
Survival (%)	100[a]	100[a]	100[a]	91.67 ± 1.91[b]
Values are means of 3 replicates ± standard deviation. Different superscripts in the same row indicate significant differences ($p < 0.05$)				

Effect of KMPS on the bacterial community structure of the SBBF

Numbers of operating units (OTUs) and alpha diversity indices of bacterial communities in SBBF biofilm samples from different systems are shown in Table 3. The coverage rate of each sample was greater than 99%, indicating that the detected sequence results could truly reflect the diversity of microbial communities. A total of 1214 OTUs were observed in nine samples, and a low average similarity and low number of shared OTUs among the samples were observed, representing 10.71% of the total reads (Fig. 4). The P0_0 OTU numbers were the lowest, followed by 15-d and 30-d OTU numbers. The Chao index and Ace index of P0_0 were the lowest. When the samples of the same experimental group on the 30th day were compared with those on the 15th day, the Shannon index increased, while the Simpson index decreased.

The hierarchical clustering of the nine samples is shown in Fig. 5. The structural composition of the bacterial community was more different in P0_0 than in the other eight groups of samples. The microbial communities differed significantly under different KMPS concentration conditions; biofilm samples from different time periods of T0 and T1 clustered together, while T2 and T3 were dispersed. This indicates that the similarity of bacterial community composition in the T2 and T3 systems was lower than that in the T0 and T1 systems, and the difference in bacterial community composition in the T2 and T3 culture systems was greater than that in T0 and T1 systems.

Figure 6 shows the composition of microbial community structure at phylum to genus level. Thirty-four bacterial phyla and 544 bacterial genera were detected. The diversity of bacteria in the P0 sample was lower than that in the other samples. The dominant phyla among the nine groups were Proteobacteria (38.55–71.60%), Bacteroidetes (7.29–12.01%), Actinobacteriota (0.35–16.01%), Planctomycetes (2.31–22.46%) and Chloroflexi (2.31–13.25%). In addition, there are bacteria such as Nitrospirota, Firmicutes and others (Fig. 6 up). After the addition of KMPS, the relative abundance of Proteobacteria, Actinobacteriota, Nitrospirota and Chloroflexi decreased, while the relative abundance of Bacteroidetes increased.

The top 30 dominant genera in terms of total abundance are presented in the form of heatmap plots (Fig. 6 down). The relative abundance of dominant genera overall in biofilms were norank_f_Rhodobacteraceae (2.46–13.50%), *Nitrococcus* (1.38–9.16%) and *Bacillus* (0.27–8.04%). Consistent with the results of the phylum level analysis, the genus diversity in P0 was lower than that in the other samples, but the abundance of bacteria related to nitrification, such as *Nitrococcus*, *Nitrosomonas* and *Nitratireductor*, was more plentiful than that in the other biofilm samples. The abundance of norank_f_Rhodobacteraceae and *Flavobacterium* increased with increasing KMPS concentrations, whereas the abundance of *Bacillus* decreased with an increase in KMPS concentration.

Table 3
OTU numbers and alpha diversity index of the SBBF biofilm

Sample ID	Coverage	Number of OUTs	Alpha diversity			
			Shannon	Simpson	Ace	Chao
P0_0	0.999	382	3.81	0.04	463.32	458.56
T0_15	0.998	700	4.46	0.03	811.00	813.97
T1_15	0.998	594	4.17	0.05	683.80	690.76
T2_15	0.997	643	4.31	0.04	793.65	818.47
T3_15	0.998	627	4.31	0.04	744.16	739.99
T0_30	0.998	729	4.68	0.03	838.67	827.21
T1_30	0.998	754	4.74	0.03	873.15	873.47
T2_30	0.998	762	4.97	0.01	861.00	851.01
T3_30	0.997	653	4.85	0.02	762.36	790.60

Discussion

The results showed that KMPS inhibited both ammonia oxidation activity and nitrite oxidation activity of SBBF, and the AOR and NOR of CK were higher than those of the experimental group. When KMPS concentrations were above 1.0 mg/L, ammonia oxidation activity was inhibited, while KMPS concentrations above 2.0 mg/L inhibited nitrite oxidation activity. Hagopian et al. (1998) found that H_2O_2 could inhibit nitrification processes, which was similar to the results of this study. KMPS is characterized by strong oxidizing properties, is extremely water soluble and corrosive, and is capable of providing nonchlorine oxidizing potential and reactive oxygen derivatives; the higher the concentration is, the stronger the inhibitory effect on microbial activity (Anipsitakis et al. 2008). Peroxide addition to the biofilter significantly reduced ammonium removal efficiency. Pedersen et al. (2012) added 2.0 mg/L peracetoacetic acid (PAA) to a biofilter and found that the concentration of NO_2^--N increased significantly over a long time. AOR was more affected by KMPS in the low concentration groups (1 mg/L, 2 mg/L, 3 mg/L), while nitrite oxidation activity was more easily inhibited by KMPS in the high concentration groups (4 mg/L, 5 mg/L). With increasing dosing times of ammonium chloride and sodium nitrite, AOR and NOR increased in each group, which shows that the inhibition of KMPS on the SBBF nitrification process is temporary and recoverable, similar to the results obtained by Chen et al. (2021).

Water quality is considered to be a major limiting factor for shrimp survival, especially pH, DO, NH_4^+-N and NO_2^--N concentrations (Santacruz-Reyes et al. 2012). Water temperature and DO were maintained within the range suitable for shrimp growth. The turbidity of each system showed an increasing trend but was maintained at a low level, with no significant differences between systems ($p < 0.05$). Inorganic

nitrogen is an important environmental factor in shrimp culture and has an important effect on the growth, survival and physiological functions of shrimp. In this experiment, the NH_4^+-N and NO_2^--N concentrations in T0 were significantly lower than those in the other experimental groups. The average concentrations of NH_4^+-N in the T1, T2 and T3 systems were 9.52%, 76.19% and 119.05% higher than those in T0, respectively. The average concentration of NO_2^--N was 23.11, 6.11 and 9.91 times higher than that of T0 (0.35 mg/L), which was due to the continuous inhibition of nitrification microorganisms by KMPS, resulting in NH_4^+-N and NO_2^--N accumulation. NO_3^--N in the system was mainly converted from the nitrification process. With the increase in KMPS dosing, ammonia oxidation activity and nitrite oxidation activity were inhibited, and the result of nitrate concentration in each experimental group was T0 > T1 > T2 > T3.

The biomass of shrimps is affected by inorganic nitrogen, especially nitrite and nitrate, which negatively affects the growth and survival of the organisms (Lin & Chen 2003; Kim et al. 2019). With the increase in KMPS dosage, the concentrations of NH_4^+-N and NO_2^--N increased, and the final mean body weight of shrimp decreased. A low dose (2.0 mg/L, 4.0 mg/L) of KMPS had no significant effect on the survival rate of *L. vannamei*, while a high dose (8.0 mg/L) of KMPS reduced the survival rate by 9.33% compared with T0. Peppler et al. (2020) found lower body weights in PAA-treated *L. vannamei* than in the control group, which is consistent with the results of this study. Meanwhile, the FCR of T3 was relatively high, indicating that the addition of high-dose KMPS weakened the natural productivity of the culture system, thus reducing the feed conversion efficiency and slowing down the growth rate of shrimp (Cuzon et al. 2004).

Microbial diversity is a key component in maintaining stable ecological functions, and in aquaculture systems microbial diversity is closely related to the shrimp diseases (Xiong et al. 2015). The results indicated that the diversity of the bacterial community in the T0 system was higher than that in other culture systems, and the addition of KMPS reduced the abundance and diversity of bacterial communities in the culture system (Liu et al. 2019). There were significant differences in unique OTUs between the near zero levels (0 mg/L and 2 mg/L groups) and other concentration levels. Similar to other studies on the dominant phyla of shrimp, Proteobacteria, Bacteroidetes, Actinobacteriota and Chloroflexi were the dominant phyla (Fan et al. 2019; Yang et al. 2018). As the KMPS level increased from near zero to 8 mg/L, the relative abundance of some bacteria decreased, including Proteobacteria, Planctomycetes, Actinobacteria and Firmicutes.

Proteobacteria is the predominant gastrointestinal bacterial phylum present in the gut of healthy shrimps (*L. vannamei)* (Niu et al. 2022), a Gram-negative bacterium with extremely diverse shapes, mainly participating in complexes and nitrogen degradation (Cottrell & Kirchman 2000; Klase et al. 2019). Firmicutes breaks down carbohydrates such as starch and fibre that are difficult to digest in food, which helps to make more effective utilization of energy in the diet and reduces FCR and feed costs. Butyrate-producing bacteria in Firmicutes produce substances beneficial to the health of the host gut. Li et al. (2013) found that the relative abundance of Firmicutes was one of the factors promoting rapid growth in

transgenic common carp (Fan et al. 2019; Canani et al. 2011). Furthermore, Firmicutes also played key roles in NH_4^+-N, NO_2^--N and NO_3^--N consumption (Shu et al. 2015). The reduction of ammonia nitrogen in aquatic systems is also closely related to the abundance of Planctomycetes. The Planctomycetes contain a large number of anaerobic ammonia-oxidising bacteria that play an important role in the nitrogen cycle by reducing inorganic nitrogen to produce nitrogen (N_2). Planctomycetes are widespread in natural and artificial systems and has been shown to play an important role in denitrification systems for agricultural and domestic wastewater. (Van et al. 2010; He et al. 2022). The reduced abundance of Proteobacteria, Firmicutes and Planctomycetes caused by a high dose of KMPS may further interfere with both the digestive and immune capacity of *L. vannamei*, leading to a decrease in the shrimp survival rate and an increase in FCR. Actinobacteria are an important part of the degradation of organic matter, mainly macromolecules such as starch and protein, and also play a significant position in the natural nitrogen cycle (Zothanpuia et al. 2018; Duan et al. 2020). Although the relative abundance of Actinobacteria varies with shrimp habitat and feed composition, studies have shown that Actinobacteria are always one of the dominant phylum in the gastrointestinal tract of the *L. vannamei* gut. (Li et al. 2018; Niu et al. 2022). Nitrospirota are closely related to the biological nitrification process and their relative abundance directly influences the ammonia/nitrate/nitrite cycling system, which in turn affects water quality and shrimp health (Wang et al. 2022; Harms et al. 2003). Gao et al. (2020) believed that nitrification performance was significantly correlated with the number of nitrifying bacteria. In this experiment, when the level of KMPS addition was 4 mg/L and 8 mg/L, the abundance of Nitrospirota in the T2_30 and T3_30 samples decreased by 72.99% and 66.67%, respectively, compared with T0, which is also the reason why T0 can maintain good water quality in the cultivation process. Bacteroidetes are considered to be one of the richer heterotrophic bacteria in the aquatic environment, with the ability to use a wide range of carbohydrates and break down organic matter to provide nutrients and energy for the host (Diez-Vives et al. 2012; Williams et al. 2013). With the increase in KMPS concentration, Bacteroidetes increased from 7.75–17.67%, and Flavobacteriaceae, pathogenic bacteria in Bacteroidetes, also peaked in T3_30 (Zhao et al. 2018).

Nitrosomonas is a typical ammonia-oxidizing bacterium (AOB) responsible for the nitrification of NH_4^+-N and NO_2^--N (He et al. 2020). Nitrate is the main form of biologically active nitrogen, which is produced through the nitrite oxidation process by nitrite oxidizing bacteria (NOB) such as *Nitrococcus* and *Nitrobacter* (Hubot et al. 2020). *Nitratireductor* are aerobic gram-negative bacteria with the ability to reduce nitrate to nitrite under anoxic conditions and also participate in the degradation of organic pollutants (Nguyen et al. 2019; Manickam et al. 2012); the relative abundance of these bacteria was higher in aquaculture systems with a low dose of KMPS. The abundance of *Nitrosomonas* and *Nitrococcus* in T3 decreased by 34.02% and 45.22%, respectively, which further confirmed that the deterioration of water quality in the T3 system was due to the inhibition of AOB and NOB by KMPS and the reduction of AOR and NOR. The existence of *Rhodobacteraceae* can effectively remove phosphorus (Zilles et al. 2002; Zaman et al. 2021). The relative abundance of *norank_f__Rhodobacteraceae* in T3 was highest, followed by T2 and T1. From the experimental results, the average concentration of total phosphorus in T3 was also the lowest. Bacilli are common probiotics whose abundance corresponds to

the health of *L. vannamei*. The relatively high abundance of these bacteria in systems with low doses of KMPS indicates that KMPS can directly influence the presence of beneficial bacteria in the culture system.

In this study, KMPS showed inhibitory effects on ammonia oxidation activity and nitrite oxidation activity of SBBF. The inhibition of KMPS on AOR and NOR was weakened after the second and third dosing times of ammonium chloride and Sodium nitrite. With the increase of KMPS dosage, the concentrations of NH_4^+-N and NO_2^--N in each system significantly increased. High doses of KMPS (greater than or equal to 4mg/L) can affect the survival of *L.vannamei*, reduce the final body weight of *L.vannamei* and increase FCR. High-throughput sequencing analysis of the biofilm structure showed that the relative abundance of Nitrospirota, *Nitrosomonas* and *Nitrococcus* decreased with the addition of KMPS

Declarations

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Author contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Yazhi Luan, Yang Wang, Ailing Xu and Zhiwen Song. The frst draft of the manuscript was written by Yazhi Luan, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

References

1. Anipsitakis GP et al (2008) Chemical and microbial decontamination of pool water using activated potassium peroxymonosulfate. Water Res 42(12):2899–2910. https://doi.org/10.1016/j.watres.2008.03.002
2. Arunkumar M, LewisOscar F et al (2020) In vitro and in vivo biofilm forming *Vibrio spp*: A significant threat in aquaculture. Process Biochem 94:213–223. https://doi.org/10.1016/j.procbio.2020.04.029
3. Bauer J, Teitge F, Neffe L, Adamek M et al (2018) Recommendations for identifying pathogenic Vibrio spp. as part of disease surveillance programmes in recirculating aquaculture systems for Pacific

white shrimps (*Litopenaeus vannamei*). J Fish Dis 41(12):1877–1897. https://doi.org/10.1111/jfd.12897

4. Canani RB, Costanzo MD et al (2011) Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. World J Gastroenterol 17(12):1519–1528. https://dx.doi.org/10.3748/wjg.v17.i2.1519
5. Chen J, Liu X, Pavlostathis SG (2021) Long-term evaluation of the effect of peracetic acid on a mixed aerobic culture: organic matter degradation, nitrification, and microbial community structure. Water Res 190:116694. https://doi.org/10.1016/j.watres.2020.116694
6. Chu L, Wang J, Quan F et al (2014) Modification of polyurethane foam carriers and application in a moving bed biofilm reactor. Process Biochem 49(11):197–1982. https://doi.org/10.1016/j.procbio.2014.07.018
7. Cottrell Matthew T, Kirchman David L (2000) Appl Environ Microbiol 66(4):16921697. http://dx.doi.org/10.1128/AEM.66.4. 1692-1697.2000. Natural Assemblages of Marine Proteobacteria and Members of the Cytophaga-Flavobacter Cluster Consuming Low- and High-Molecular-Weight Dissolved Organic Matter
8. Cuzon G, Lawrence A, Gaxiola G et al (2004) Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. Aquaculture 235(1):513–551. https://doi.org/10.1016/j.aquaculture.2003.12.022
9. David M, Linders J, Gollasch S, David J (2018) Is the aquatic environment sufficiently protected from chemicals discharged with treated ballast water from vessels worldwide - a decadal environmental perspective and risk assessment. Chemosphere 207:590–600. https://doi.org/10.1016/j.chemosphere.2018.05.136
10. Diez-Vives C, Gasol JM, Acinas SG (2012) Evaluation of Marine Bacteroidetes-Specific Primers for Microbial Diversity and Dynamics Studies. Microb Ecol 64(4):1047–1055. http://dx.doi.org/10.1007/s00248-012-0087-x
11. Duan Y, Tang Y, Huang J, Zhang J, Lin H, Jiang S, Wang R, Wang G (2020) Changes in the microbial community of *Litopenaeus vannamei* larvae and rearing water during different growth stages after disinfection treatment of hatchery water. J Microbiol 58(9):741–749. http://dx.doi.org/10.1007/s12275-020-0053-0
12. Fan L, Wang Z, Chen M, Qu Y, Li J, Zhou A, Xie S, Zeng F, Zou J (2019) Microbiota comparison of Pacific white shrimp intestine and sediment at freshwater and marine cultured environment. Sci Total Environ 657:11941204. https://doi.org/10.1016/j.scitotenv.2018.12.069
13. Gao Y, Wang X, Li J et al (2020) Effect of aquaculture salinity on nitrification and microbial community in moving bed bioreactors with immobilized microbial granules. Bioresour Technol 297:122427. https://doi.org/10.1016/j.biortech.2019.122427
14. Ghanbari F, Moradi M (2017) Application of peroxymonosulfate and its activation methods for degradation of environmental organic pollutants. Rev Chem Eng J 310:41–62. https://doi.org/10.1016/j.cej.2016.10.064

15. Grob C, Pollet BG(2016) Regrowth in ship's ballast water tanks: think again!Marine Pollution Bulletin109 (1)–48. https://doi.org/10.1016/j.marpolbul.2016.04.061
16. Hagopian DS, Riley JG (1998) A closer look at the bacteriology of nitrification. Aquacult Eng 18(4):223–244. https://doi.org/10.1016/S0144-8609(98)00032-6
17. Hashizume M, Aoki K, Ohno S, Kitaichi N, Yawata N, Gonzalez G, Nonaka H et al (2019) Disinfectant potential in inactivation of epidemic keratoconjunctivitis-related adenoviruses by potassium peroxymonosulfate. Eur J Ophthalmol 31(2):379–384. http://dx.doi.org/10.1177/1120672119891408
18. Harms G, Layton AC, Dionisi HM, Gregory IR et al (2003) Real-Time PCR Quantification of Nitrifying Bacteria in a Municipal Wastewater Treatment Plant. Environ Sci Technol 37(2):343–351. http://dx.doi.org/10.1021/es0257164
19. Hess-Erga OK, Moreno-Andres J, Enger Ø, Vadstein O (2019) Microorganisms in ballast water: disinfection, community dynamics, and implications for management. Sci Total Environ 657:704–716. https://doi.org/10.1016/j.scitotenv.2018.12.004
20. He Q, Song J, Zhang W, Gao S, Wang H, Yu J. Enhanced simultaneous nitrification, denitrification and phosphorus removal through mixed carbon source by aerobic granular sludge.Journal of Hazardous Materials 2020, 382,121043. https://doi.org/10.1016/j.jhazmat.2019.121043
21. He Y, Zhang N, Wang A, Wang S, Che Y et al (2022) Positive effects of replacing commercial feeds by fresh black soldier fly (Hermetia illucens) larvae in the diets of Pacific white shrimp (*Litopenaeus vannamei*): Immune enzyme, water quality, and intestinal microbiota. 9. http://dx.doi.org/10.3389/fmars.2022.987363
22. Hubot ND, Giering SLC, Füssel J, Robidart J, Birchill A, Stinchcombe M, Dumousseaud C, Lucas CH (2021) Evidence of nitrification associated with globally distributed pelagic jellyfish. Limnol Oceanogr 66(6):2159–2173. https://doi.org/10.1002/lno.11736
23. Ike IA, Linden KG et al (2018) Critical review of the science and sustainability of persulphate advanced oxidation processes. Chem Eng J 338:651–669. https://doi.org/10.1016/j.cej.2018.01.034
24. Kim JH, Kang YJ et al (2019) Toxic effects of nitrogenous compounds (ammonia, nitrite, and nitrate) on acute toxicity and antioxidant responses of juvenile olive flounder, *Paralichthys olivaceus*. Environ Toxicol Pharmacol 67:73–78. https://doi.org/10.1016/j.etap.2019.02.001
25. Klase G, Lee S, Liang S, Kim J, Zo YG, Lee J (2019) The microbiome and antibiotic resistance in integrated fishfarm water: Implications of environmental public health. Sci Total Environ 649:1491–1501. https://doi.org/10.1016/j.scitotenv.2018.08.288
26. Li E, Xu C, Wang X, Wang S, Zhao Q, Zhang M, Qin JG, Chen L (2018) Gut Microbiota and its Modulation for Healthy Farming of Pacific White Shrimp *Litopenaeus vannamei*. Reviews in Fisheries Science & Aquaculture 26(3):381–399. http://dx.doi.org/10.1080/23308249.2018.1440530
27. Li XM, Hu W, Hu Z et al (2013) Gut Microbiota Contributes to the Growth of Fast-Growing Transgenic Common Carp (*Cyprinus carpio* L.). PLoS ONE 8(5):e64577. https://doi.org/10.1371/journal.pone.0064577

28. Lin YC, Chen JC (2003) Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone) juveniles at different salinity levels. Aquaculture 224(1):193–201. https://doi.org/10.1016/S0044-8486(03)00220-5
29. Liu D, Li C, Guo H, Kong X, Lan L, Xu H, Zhu S, Ye Z (2019) Start-up evaluations and biocarriers transfer from a trickling filter to a moving bed bioreactor for synthetic mariculture wastewater treatment. Chemosphere 218:696–704. https://doi.org/10.1016/j.chemosphere.2018.11.166
30. Liu D, Straus DL, Pedersen LF, Meinelt T (2018) Periodic bacterial control with peracetic acid in a recirculating aquaculture system and its long-term beneficial effect on fish health. Aquaculture 485:154–159. https://doi.org/10.1016/j.aquaculture.2017.11.050
31. Lu GL, Jiang B, Li ZC, Li AX (2022) Ultraviolet light and ozone controls *Cryptocaryon irritans* infection in factory aquaculture. Aquaculture 548:737598. https://doi.org/10.1016/j.aquaculture.2021.737598
32. Manickam N, Pareek S, Kaur I, Singh NK, Mayilraj S (2012) *Nitratireductor lucknowense sp*. nov., a novel bacterium isolated from a pesticide contaminated soil. Antonie Van Leeuwenhoek 101(1):125–131. http://dx.doi.org/10.1007/s10482-011-9623-9
33. Mo WY, Chen Z, Leung HM, Leung AOW (2017) Application of veterinary antibiotics in China's aquaculture industry and their potential human health risks. Environ Sci Pollut Res 24(10):8978–8989. http://dx.doi.org/10.1007/s11356-015-5607-z
34. Moreno-Andres J, Farinango G et al (2019) Application of persulfate salts for enhancing UV disinfection in marine waters. Water Res 163:114866. https://doi.org/10.1016/j.watres.2019.114866
35. Moreno-Andres J et al (2019) Operational and environmental factors affecting disinfection byproducts formation in ballast water treatment systems. Chemosphere 232. https://doi.org/10.1016/j.chemosphere.2019.05.152
36. Nguyen LN, Nghiem LD, Pramanik BK, Oh S (2019) Cometabolic biotransformation and impacts of the anti-inflammatory drug diclofenac on activated sludge microbial communities. Sci Total Environ 657:739–745. https://doi.org/10.1016/j.scitotenv.2018.12.094
37. Niu GJ, Yan M, Li C, Lu PY, Yu Z, Wang JX (2022) Infection with white spot syndrome virus affects the microbiota in the stomachs and intestines of kuruma shrimp. Sci Total Environ 839:156233. https://doi.org/10.1016/j.scitotenv.2022.156233
38. Oh WD, Dong Z, Lim TT (2016) Generation of sulfate radical through heterogeneous catalysis for organic contaminants removal: current development, challenges and prospects. Appl Catal B 194:169–201. https://doi.org/10.1016/j.apcatb.2016.04.003
39. Pedersen LF, Pedersen PB (2012) Hydrogen peroxide application to a commercial recirculating aquaculture system. Aquacult Eng 46:40–46. https://doi.org/10.1016/j.aquaeng.2011.11.001
40. Qiu T, Liu L, Gao M, Zhang L, Tursun H, Wang X (2016) Effects of solid-phase denitrification on the nitrate removal and bacterial community structure in recirculating aquaculture system. Biodegradation 27(2):165–178. http://dx.doi.org/10.1007/s10532-016-9764-7
41. Rodríguez-Chueca J, Silva T, Fernandes JR, Lucas MS, Puma GL, Peres JA, Sampaio A (2017) Inactivation of pathogenic microorganisms in freshwater using HSO_5^-/UV-A LED and

HSO_5^-/Mn+/UV-A LED oxidation processes. Water Res 123:113–123. https://doi.org/10.1016/j.watres.2017.06.021

42. Rodríguez-Chueca J, García-Cañibano C, Lepistö RJ, Encinas Á et al (2019) Intensification of UV–C tertiary treatment: Disinfection and removal of micropollutants by sulfate radical based Advanced Oxidation Processes. J Hazard Mater 372:94–102. https://doi.org/10.1016/j.jhazmat.2018.04.044
43. Santacruz-Reyes RA, Chien YH (2012) The potential of *Yucca schidigera* extract to reduce the ammonia pollution from shrimp farming. Bioresour Technol 113:311–314. https://doi.org/10.1016/j.biortech.2012.02.132
44. Shu D, He Y, Yue H, Wang Q (2015) Microbial structures and community functions of anaerobic sludge in six full-scale wastewater treatment plants as revealed by 454 high-throughput pyrosequencing. Bioresour Technol 186:163–172. https://doi.org/10.1016/j.biortech.2015.03.072
45. Sonthipet S, Ruenphet S, Takehara K (2018) Bactericidal and virucidal efficacies of potassium monopersulfate and its application for inactivating avian influenza virus on virus-spiked clothes. J Vet Med Sci 80(4):568–573. http://dx.doi.org/10.1292/jvms.17-0599
46. Teitge F, Peppler C, Steinhagen D et al (2020) Effect of disinfection with peracetic acid on the microbial community of a seawater aquaculture recirculation system for Pacific white shrimp (*Litopenaeus vannamei*). J Fish Dis 43(9):991–1017. https://doi.org/10.1111/jfd.13207
47. Van Kessel MAHJ, Harhangi HR, Van de Pas-Schoonen K, Van de Vossenberg J et al (2010) Biodiversity of N-cycle bacteria in nitrogen removing moving bed biofilters for freshwater recirculating aquaculture systems. Aquaculture 306(1):177–184. https://doi.org/10.1016/j.aquaculture.2010.05.019
48. Wacławek S, Lutze HV, Grübel K et al (2017) Chemistry of persulfates in water and wastewater treatment: A review. Chem Eng J 330:44–62. https://doi.org/10.1016/j.cej.2017.07.132
49. Wang R, Xu Q, Chen C, Li X, Zhang C, Zhang D (2021) Microbial nitrogen removal in synthetic aquaculture wastewater by Fixed-bed baffled reactors packed with different biofilm carrier materials. Bioresour Technol 331:125045. https://doi.org/10.1016/j.biortech.2021.125045
50. Wang Y, Sun MZ, Tang YZ, Xu AL, Tang JC, Song ZW (2022) Effects of *Haematococcus pluvialis* on the water quality and performance of *Litopenaeus vannamei* using artificial substrates and water exchange systems. Aquacult Int 30(4):1779–1797. http://dx.doi.org/10.1007/s10499-022-00872-0
51. Williams TJ, Wilkins D, Long E, Evan F, DeMaere MZ et al (2013) The role of planktonic Flavobacteria in processing algal organic matter in coastal East Antarctica revealed using metagenomics and metaproteomics. Environ Microbiol 15(5):1302–1317. https://doi.org/10.1111/1462-2920.12017
52. Xiong J, Wang K, Wu J, Yang K, Qian Y, Zhang D (2015) Changes in intestinal bacterial communities are closely associated with shrimp disease severity. Appl Microbiol Biotechnol 99(16):6911–6919. http://dx.doi.org/10.1007/s00253-015-6632-z
53. Yang W, Qiu H, Lu K et al (2018) Succession of bacterioplankton community in intensive shrimp (*Litopenaeus vannamei*) aquaculture systems. Aquaculture 497:200–213. https://doi.org/10.1016/j.aquaculture.2018.07.053

54. Zaman M, Kim M, Nakhla G (2021) Simultaneous partial nitrification and denitrifying phosphorus removal (PNDPR) in a sequencing batch reactor process operated at low DO and high SRT for carbon and energy reduction. Chem Eng J 425:131881. https://doi.org/10.1016/j.cej.2021.131881
55. Zhao Z, Zhang Z, Liu C, Wang J, Zhang Y, Shi H (2018) Contrasting microbial community composition and function perspective of the biofilms in shrimps (*Macrobrachium nipponense*) cultured systems. J Environ Manage 222:268–274. https://doi.org/10.1016/j.jenvman.2018.05.063
56. Zhu S, Shen J, Ruan Y, Guo X, Ye Z, Deng Y, Shi M (2016) The effects of different seeding ratios on nitrification performance and biofilm formation in marine recirculating aquaculture system biofilter. Environ Sci Pollut Res 23(14):14540–14548. http://dx.doi.org/10.1007/s11356-016-6609-1
57. Zothanpuia, Passari AK, Leo VV, Chandra P, Kumar B, Nayak C, Hashem A et al (2018) Bioprospection of actinobacteria derived from freshwater sediments for their potential to produce antimicrobial compounds. Microb Cell Fact 17. http://dx.doi.org/10.1186/s12934-018-0912-0
58. Zilles JL, Hung CH, Noguera DR (2002) Presence of *Rhodocyclus* in a full-scale wastewater treatment plant and their participation in enhanced biological phosphorus removal. Water Sci Technol 46(1–2):123–128. http://dx.doi.org/10.2166/wst.2002.0467

Figures

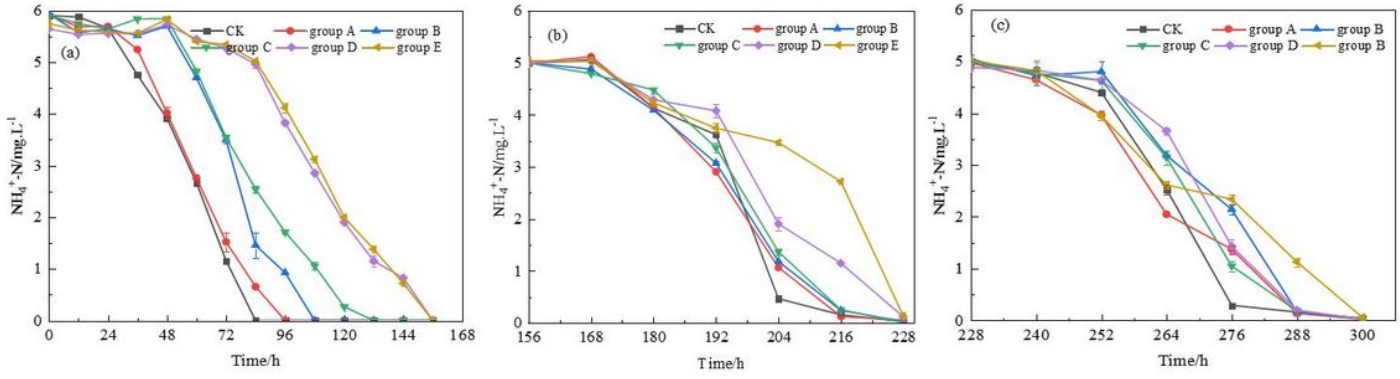

Figure 1

Removal process of NH_4^+-N in each experimental group after treatment with different concentrations of KMPS. (a) First dosing of NH_4Cl; (b) Second dosing of NH_4Cl; (c) Third dosing of NH_4Cl.

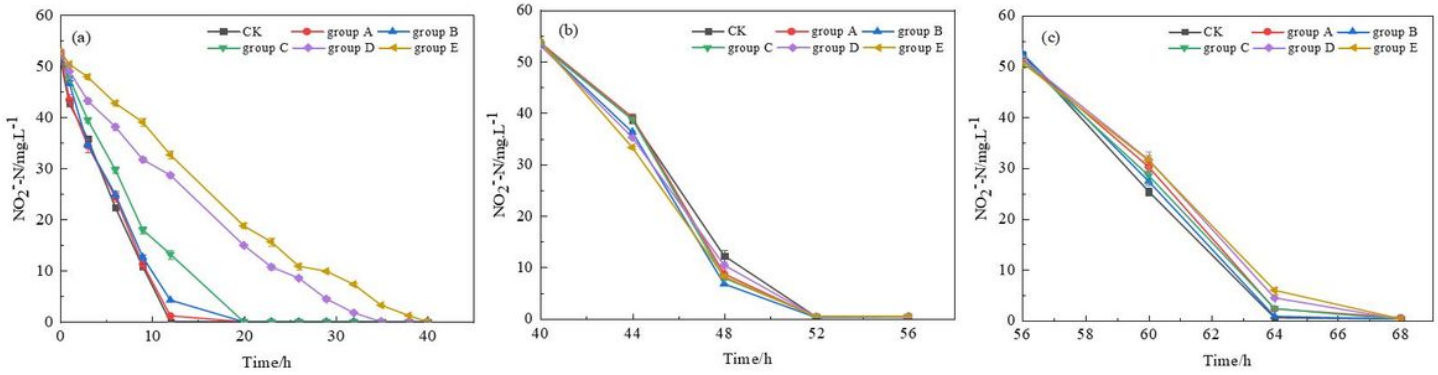

Figure 2

Removal process of NO_2^--N in each experimental group after treatment with different concentrations of KMPS. (a) First dosing of $NaNO_2$; (b) second dosing of $NaNO_2$; (c) third dosing of $NaNO_2$.

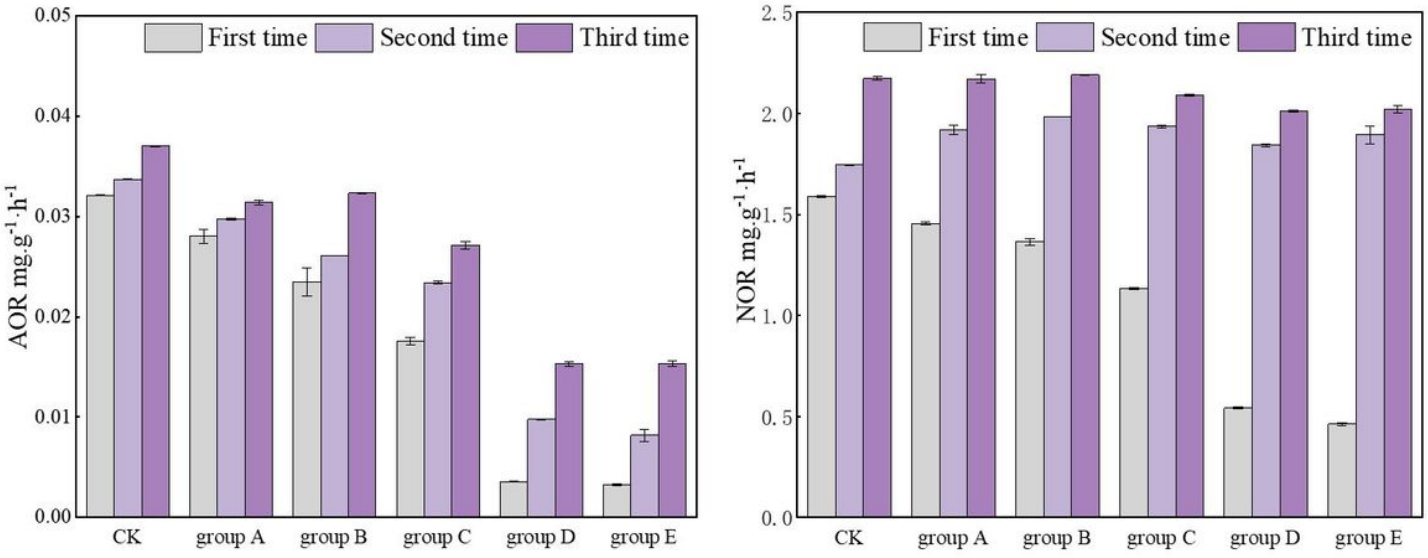

Figure 3

Variation in AOR and NOR in each experimental group after treatment with different concentrations of KMPS.

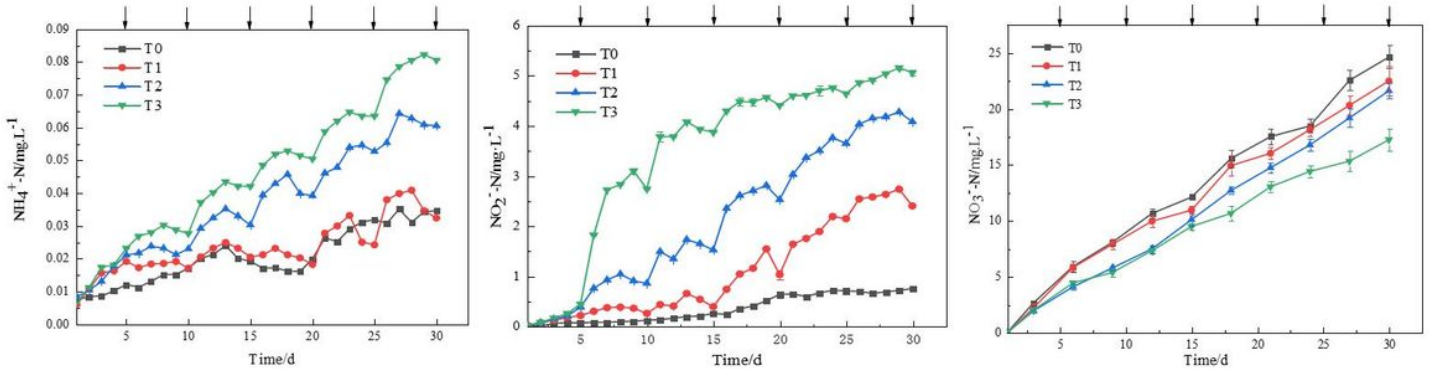

Figure 4

The concentration variation of NH_4^+-N, NO_2^--N and NO_3^--N in *L. vannamei* aquaculture systems (period 30 d, ↓ indicates the addition of KMPS).

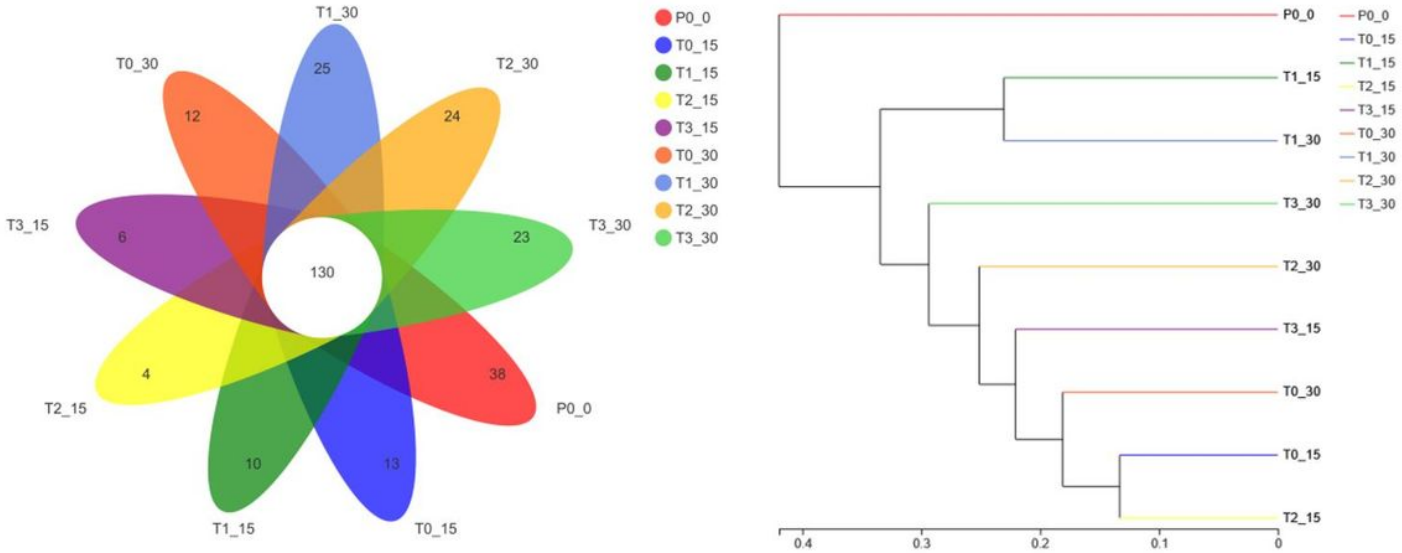

Figure 5

Comparison of OTUs (left) and hierarchical clustering (right) of the SBBF biofilm samples.

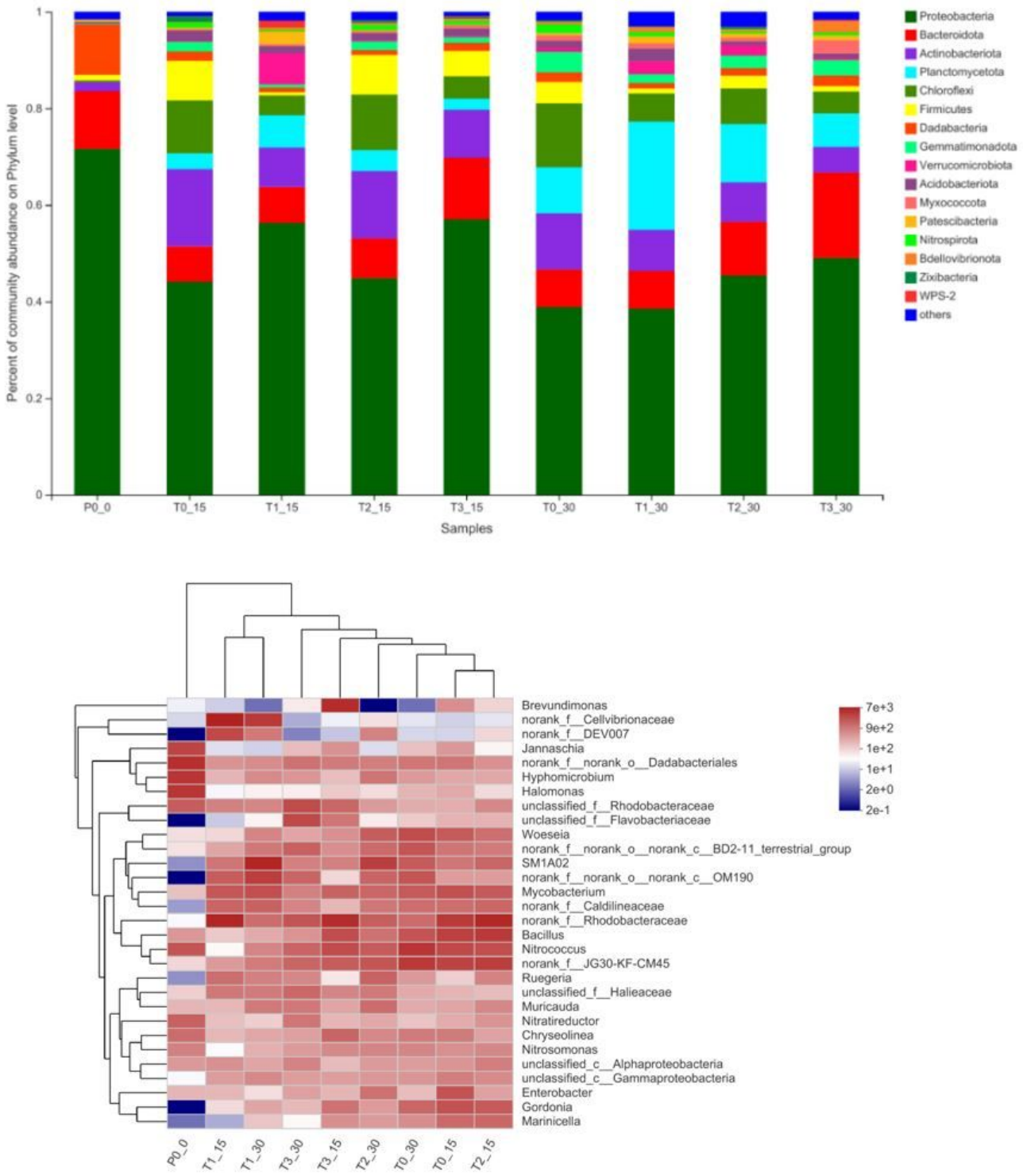

Figure 6

The bacterial community in all samples at the phylum (top) and genus (bottom) levels.