



## Performance of microporous carrier for removing nitrogen from aquaculture wastewater

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### ABSTRACT

It is critical when dealing with the huge emissions of low-strength wastewater (LSW) to improve the total nitrogen (TN)-removal performance of the treatment strategy. In this study, a kind of microporous carrier (polyvinyl alcohol gel beads) was studied for its N-removal performance in treating low-strength wastewater (LSW) in a biofilm reactor. The results showed that the best N-removal performance within three reactors with different carriers was achieved under the condition of chemical oxygen demand/TN = 8 and aeration strategy S2. In these conditions, the effluent TN of the polyvinyl alcohol (PVA)-bead group was stably maintained below 2 mg/L, the microbial richness of the biofilm on PVA-gel beads was significantly greater than that of the control (suspended polypropylene plastic sphere); furthermore, the percentage of microorganisms related to N-removal on the PVA-gel carriers was 39.04%, approximately 9.5 times higher than the control (4.13%). Therefore, compared with the traditional polypropylene plastic sphere, microporous PVA-gel carriers performed better for the advanced treatment of LSW.

**Keywords:** Polyvinyl alcohol-gel carriers; Low-strength wastewater (LSW); Aeration strategies; Chemical oxygen demand/total nitrogen

### 1. Introduction

Low-strength wastewater (LSW), which is characterized by a low chemical oxygen demand (COD), such as aquaculture wastewater and tailwater from municipal sewage treatment plants, has recently drawn extensive attention because of its poor total nitrogen (TN)-removal performance. In comparison, biological nitrogen removal

(BNR) is a more effective, sustainable, cost-effective, and easily implemented system for wastewater treatment. BNR employs nitrifying and denitrifying bacteria to convert various forms of nitrogen (N) in water into N gas (N<sub>2</sub>) through a series of enzymatic reactions. However, anoxic denitrification removal systems need carbon sources as electronic donors, and a lack of carbon sources in the wastewater affect N-nitrogen removal performance considerably.

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It was reported that COD lower than 200 mg/L in the LSW seriously restricted denitrification [1,2]. In addition, the traditional N-removal theory says that, to achieve complete denitrification, the ratio of carbon to nitrogen sources (C/N) should be about 2.86 at the lowest. In fact, due to the growth of microorganisms, the actual required ratio is usually more than 4.0 [3]. In view of the typical mass of LSW emissions, it is critical that we improve BNR performance under low carbon source conditions.

Biofilm technology is a recent wastewater biological treatment technology used to support bacterial colonization. Nitrifying and denitrifying bacteria can also be maintained in biofilms at the same time, and biofilm technology has been applied to improve the efficiency of BNR in many biofilm reactors [4]. Carrier material and shapes play core roles in biofilm reactors, as they greatly influence biofilm formation and development and the microbial community structure. Hence, it is vital to select an appropriate carrier for application in a biofilm reactor. Plastic is widely utilized in carriers because of its low mass, low cost, toughness, and anti-corrosion properties, and polypropylene plastic (PP) is the most commonly used plastic material in modern systems. However, PP is lighter than water and usually floats on the surface, which is conducive to the attachment of aerobic rather than anaerobic microorganisms, subsequently restricting denitrification. Recently, another material, polyvinyl alcohol (PVA)-gel, has attracted more attention for use in biofilm reactors, as it is non-toxic and easily implemented in carriers. Moreover, PVA-gel beads have adequate elasticity and a comparatively high mechanical strength that can withstand high shear forces [5]. Most of all, the larger surface area potential of this type of bead is conducive to the aggregation and colonization of various microorganisms (aerobic and anaerobic), which can form stable and extensive biofilm consortia. In view of these distinguishing features, PVA-gel beads are promising carriers in biofilm reactors.

Biofilm reactor is not only easy to operate but, like other immobilized cell technologies, it also has a low cost [6]. Recently, several research groups have explored the use of PVA-gel beads as biomass carriers for N-removal in biofilm reactors. Shi et al. [7] treated high-strength ammonium ( $\text{NH}_4^+\text{-N}$ ) wastewater with PVA-gel beads as carriers in batch reactors. The results showed that, at low  $\text{NH}_4^+\text{-N}$  concentrations (50 and 100 mg/L), the removal rate reached 100%; subsequently, when  $\text{NH}_4^+\text{-N}$  rose to 400 mg/L, the removal rate still reached 48% after 58 reaction cycles (about 20 d). Further studies by the group showed that the heterotrophic nitrifying and aerobic denitrifying bacteria on the carriers provided additional pathways for BNR in the reactor. Bae et al. [8] used core-shell-structured PVA-gel beads for single-stage autotrophic N-removal (SANR) in a continuous bioreactor with synthetic ammonia wastewater. The reactor showed a maximum N-removal efficiency of  $80.4 \pm 1.20\%$ , with a total N-loading rate of  $590 \pm 12.1$  g-N/ $\text{m}^3\text{d}$ . These results also suggested that using core-shell structured PVA-gel beads in a start-up strategy in SANR could minimize the adaptation period without sacrificing anaerobic ammonium bacteria activity. Ge et al. [9] treated synthetic N wastewater using PVA-gel beads in an aerobic reactor, and the results showed that, when the

TN in the influent was 460 mg/L, the removal rate reached 90%, representing a high removal efficiency. These studies all focused on the removal of high-concentration N from wastewater, and there are few reports on the effectiveness of biofilm reactors with PVA-gel beads for N-removal in low-strength wastewater.

In this study, a lab-scale biofilm reactor (SBBR) containing PVA-gel beads as carriers was fabricated to study the removal of N from low-strength wastewater. The aims of this study were (1) to explore the optimum conditions (COD/TN ratio and dissolved oxygen [DO] content) for running reactors, (2) to investigate the N-transformation characteristics of reactors with different carriers, and (3) to assess the diversity of biofilm microbes and their functional roles in the reactors.

## 2. Materials and methods

### 2.1. Synthetic wastewater

Synthetic low-strength wastewater was used in the experiments. The initial  $\text{NH}_4^+\text{-N}$  concentration, initial  $\text{NO}_3^-\text{-N}$  concentration, initial TN concentration, and pH were  $5 \pm 0.3$  mg/L,  $3 \pm 0.5$  mg/L,  $10 \pm 2$  mg/L, and 7.0–7.5, respectively; the COD concentration ranged from 50 to 110 mg/L.

### 2.2. Carriers

Two kinds of carriers with different materials were used in this study: (A) white porous PVA-gel beads of  $\Phi$  (3–5 mm),  $\rho$  (1.025 g/ $\text{cm}^3$ ), and pore apertures of approximately 20  $\mu\text{m}$ ; and (B) white PP suspension spheres of  $\Phi$  (25 mm) and  $\rho$  (0.90–0.98 g/ $\text{cm}^3$ ).

### 2.3. Setup and operation of reactors

Three groups of reactors were set up in this study, namely, a PVA-gel bead group (GPVA, with PVA-gel beads as carriers); a PP suspension sphere group (GPPS, with PP suspension spheres as carriers); and a control group (GC, without carriers). Each reactor was composed of two units (R1 and R2), and each unit had a capacity of 4 L (Fig. 1). The carriers were fed into the reactors (GPVA and GPPS) at a volume ratio of 12%.

There was an aeration device at the bottom of the R1 unit for aeration or carrier suspension. Reactors were placed in a room at a constant temperature of  $28^\circ\text{C} \pm 3^\circ\text{C}$ . The reactors run for 75 d in total, and different operating parameters were applied at different stages as follows (the settling stages are not included in the following experimental timetable):

#### 2.3.1. Start-up stage

Activated sludge from Suzhou Chengdong Sewage Treatment Plant was inoculated into three reactors filled with 4 L synthetic wastewater, which were subsequently aerated and drained 24 h later. The effluents were sampled at the draining times and analyzed. This cycle was repeated until the water quality was stable (results not shown). Stable concentrations of COD and TN in the effluent samples meant mature biofilm formation.

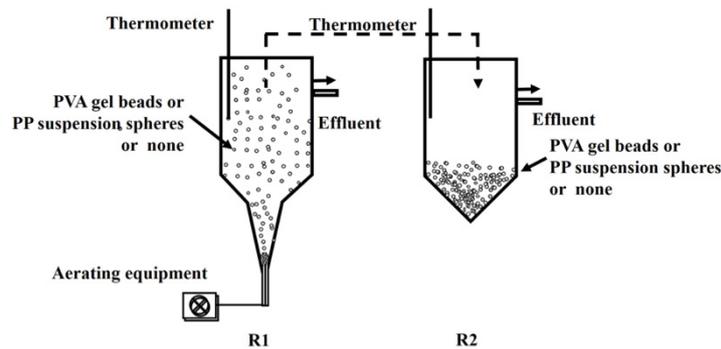


Fig. 1. Biofilm reactor diagram (R1 as aeration unit; R2 as anaerobic unit).

### 2.3.2. Aeration optimization stage

At first, only the R1 unit of the GC was applied to explore changes in DO in the wastewater during operation with an S1 aeration strategy (aeration for 2 h after water exchange). The reactor was fed with 4 L synthetic wastewater and replaced with 2 L fresh synthetic wastewater every 24 h. DO content was monitored every other hour.

After examining the results (Fig. 2a), S2 and S3 aeration modes were set up for the next studies (Table 1), which were performed using three reactors to determine the optimal running conditions. The effluent concentrations of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and TN in the three reactors were measured at the draining times. The reactors ran for 10 d in total with two different aeration modes. The more effective aeration mode was chosen for the next stage.

### 2.3.3. Nitrogen removal study stage

In this stage, R1 and R2 were combined into one reactor and used as aerobic and anaerobic areas, respectively (Fig. 1). The R1 units of the three reactors were in the appropriate aeration mode according to the aeration optimization experiment, and the effluent from R1 entered the anaerobic zone (R2) for advanced N-removal. The hydraulic retention time of the combined reactors was 4 d, and each cycle lasted 48 h. The synthetic wastewater was prepared to achieve various COD/TN ratios with different proportions of soluble starch and glucose (carbon sources) vs. potassium nitrate and ammonium chloride (N sources). During operation, changes in  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and TN were detected over time.

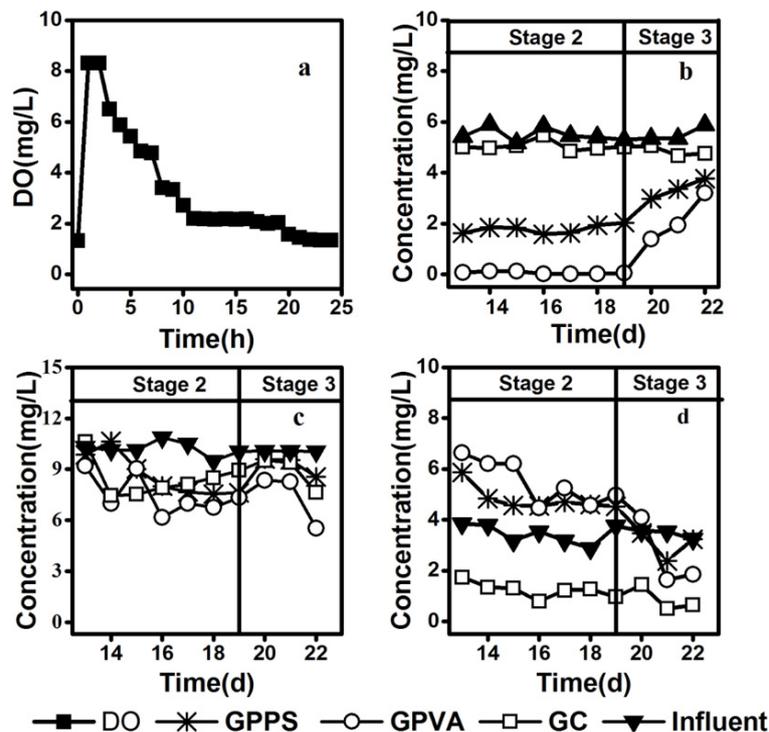


Fig. 2. The DO changes during running cycles (a) and removal performance of  $\text{NH}_4^+\text{-N}$  (b), TN (c), and  $\text{NO}_3^-\text{-N}$  (d) with different aeration modes (GPVA and GPPS respectively represent sequencing biofilm batch reactors filled with PVA-gel beads and PP suspension spheres, and GC represents control reactor without carriers).

Table 1  
Reactor operating conditions

Modes	Aeration period every day	Aeration duration (h)	HRT* (d)	COD/TN
S1	0–2 h	2	2	5
S2	15–17 h	2	2	5
S3	15–15.5 h	0.5	2	5

\*HRT: hydraulic retention time

#### 2.3.4. Microbial community analysis stage

The reactors ran for 1 week under optimized conditions, and  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and TN were detected every 3 h. After 24 h, biofilm samples on the carriers from the R1 units of GPVA and GPPS were collected and refrigerated for later analysis.

#### 2.4. Analysis of effluent samples

During the operation, effluent samples collected at pre-defined time intervals from the top of the reactors were filtered through a 0.45  $\mu\text{m}$  membrane before analysis.

$\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  concentrations were measured using a fully automated flow-injection system (AA3, SEAL, Germany). TN and COD were measured using the alkaline potassium persulfate digestion-UV spectrophotometric method and the potassium dichromate titration method, respectively. Water temperature, pH, and DO were immediately measured in situ using a portable multi-parameter water quality meter (SG68-SevenGo, Mettler, Toledo, Switzerland).

#### 2.5. Analysis of the microbial communities of biofilms

Biofilm DNA was extracted using the Power Soil DNA Isolation Kit (MO BIO, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Triplicate DNA extracts from each reactor were equally mixed for downstream manipulation. The extracted DNA was stored at  $-80^\circ\text{C}$  for later use. The V3–V4 regions of the bacterial 16S ribosomal RNA genes were amplified by PCR ( $95^\circ\text{C}$  for 2 min; followed by 25 cycles at  $95^\circ\text{C}$  for 30 s,  $57^\circ\text{C}$  for 30 s, and  $72^\circ\text{C}$  for 30 s; and a final extension at  $72^\circ\text{C}$  for 5 min) using primers F341 (5'-ACTCCTACGGGSGCAGCAG-3') and R806 (5'-GGACTACVGGGTATCTAATC-3'). PCR reactions were performed in triplicate from a 20  $\mu\text{L}$  mixture containing 4  $\mu\text{L}$  of 5  $\times$  Fast Pfu Buffer, 2  $\mu\text{L}$  of 2.5 mM dNTPs, 0.8  $\mu\text{L}$  of each primer (5  $\mu\text{M}$ ), 0.4  $\mu\text{L}$  of Fast Pfu Polymerase, and 10 ng of template DNA. PCR products were separated on 2% agarose gels, and the amplicons were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions, and quantified using QuantiFluor<sup>TM</sup>-ST (Promega, Madison, WI, USA). Purified amplicons were pooled in equimolar solutions and paired-end sequenced (2  $\times$  250) on an Illumina MiSeq platform based on standard protocols. High-throughput sequencing was carried out on an Illumina MiSeq platform, and the obtained sequences were processed by Suzhou Genewiz Biotechnology Co., Ltd., (Suzhou, China).

### 3. Results and discussion

#### 3.1. Aeration optimization in R1 units

It is known that  $\text{DO} > 2 \text{ mg/L}$  is usually an essential condition for maintaining nitrification in reactors [10]. The changes in DO content during each cycle (24 h) are shown in Fig. 2a, and DO gradually decreased to 2 mg/L after aeration (during 16–17 h). Therefore, two other aeration modes, S2 and S3, were designed to aerate at 15 h (as shown in Table 1) to ensure enough DO for these reactors, and aeration was maintained for 2 h (S2) or 0.5 h (S3). The N-removal performances of these two modes were compared (Fig. 2b–d). As can be seen in Fig. 2b, effluent  $\text{NH}_4^+\text{-N}$  decreased in the three reactors, especially in GPVA and GPPS. However, TN did not significantly decrease (Fig. 2c), which might be attributed to the new  $\text{NO}_3^-\text{-N}$  species generated by nitrification that were not converted by denitrification within the experimental time (Fig. 2d). Compared with the S3 aeration mode (Fig. 2), higher  $\text{NH}_4^+\text{-N}$  and TN removal rates were achieved in the S2 mode. Therefore, we concluded that S2 was the optimal aeration mode for subsequent experiments.

#### 3.2. Nitrogen removal performance with different COD/TN rates

Carbon source is a key factor in BNR progresses, especially for denitrifiers, which are facultative heterotrophic bacteria that use organic matter as electron donors. Consequently, an appropriate carbon source needs to be added to LSW to ensure sufficient denitrification reactions for effective N-removal. However, too much organic matter promotes the rapid proliferation of heterotrophic bacteria, preventing nitrifiers (autotrophic bacteria) from dominating in the reactor, which would weaken the nitrification reaction. Therefore, to ensure effective TN removal, the optimal amount of carbon source should be determined to achieve a balance between nitrification and denitrification. In this study, a series of COD/TN ratios were set up to study the N-removal performance of three different reactors, GPPS, GPVA, and GC. To ensure complete N-removal, we assembled an anaerobic R2 unit behind the R1 unit. The R1 units of all three reactors operated under S2 aeration conditions.

The results (Fig. 3) show that, as the COD/TN ratio increased from 5 to 11, the removal of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and TN was clearly different among three reactors.

In the GPPS reactor, the removal rate of  $\text{NH}_4^+\text{-N}$  was higher at  $\text{COD/TN} = 8$  than that at  $\text{COD/TN} = 5$ , and it decreased slightly at  $\text{COD/TN} = 11$ . The effluent  $\text{NH}_4^+\text{-N}$  in the GC reactor continuously increased to 5.8 mg/L after

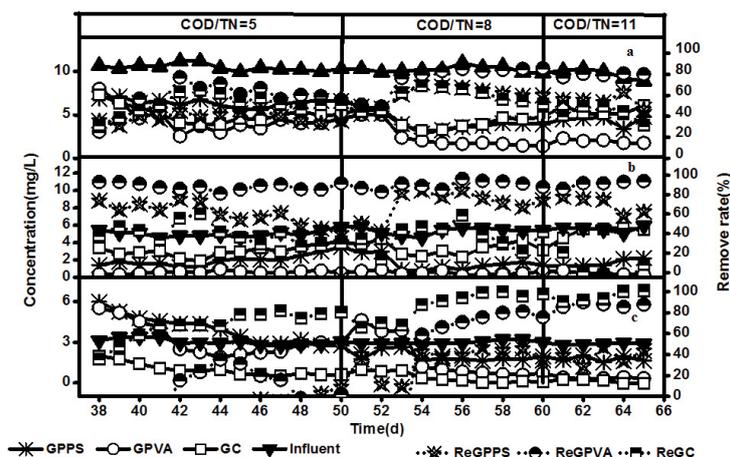


Fig. 3. Removal performance of TN (a),  $\text{NH}_4^+\text{-N}$  (b), and  $\text{NO}_3^-\text{-N}$  (c) with different COD/TN (GPVA and GPPS respectively represent sequencing biofilm batch reactors filled with PVA-gel beads and PP suspension spheres, and GC represents control reactor without carriers).

reaching the lowest value on the 54th day, indicating that the ability of GC to remove  $\text{NH}_4^+\text{-N}$  gradually decreased at higher COD/TN rates. In contrast to GPPS and GC,  $\text{NH}_4^+\text{-N}$  removal in GPVA was relatively stable and was maintained at about 93% throughout.

The effluent  $\text{NO}_3^-\text{-N}$  decreased significantly in all three reactors after treatment, and the average  $\text{NO}_3^-\text{-N}$  removal rates were 40% (GPPS), 77% (GPVA), and 98% (GC) when COD/TN = 8, which were higher than at COD/TN = 5. When COD/TN increased to 11, the average removal rates of GPPS and GC decreased to 31% and 91%, respectively, while the rate in the GPVA reactor increased slightly to 86%. Interestingly, the  $\text{NO}_3^-\text{-N}$  removal rate in the GC reactor was higher than that in the other two reactors, and it was speculated that the nitrate load in the GC reactor was relatively low and was within the treatment capacity of the reactor.

In terms of TN removal, as shown in Fig. 3c, the average effluent TN of GPPS and GC at COD/TN = 8 was the lowest among the three stages, and the effluent TN of GPVA was relatively stable, remaining below 2 mg/L, as COD/TN increased from 8 to 11.

Overall, it can be concluded that when COD/TN was 8, N-removal achieved the best performance in the three reactors. The effluent TN concentration of GPVA met the requirements of class V water of the environmental quality standards for surface water (GB 3838-2002), while the effluent TN concentration of GPPS and GC were about twice as high as the standards for class V. It was speculated that when COD/TN increased to 11, COD loading promoted the growth of heterotrophic bacteria in the reactor, inhibiting the reproduction of nitrifiers and weakening the nitrification, which subsequently affected denitrification. Therefore, COD/TN = 8 was the optimal ratio for this series of biological N-removal systems.

### 3.3. Nitrogen removal performance of different reactors

After examining the above experimental results, the N-removal capacities of the different reactors were studied at COD/TN = 8 under S2 aeration mode. The contents of

different forms of N in one cycle (48 h) in the three reactors were detected every 3 h, and the results are shown in Fig. 4.

TN concentration at different times in the GPPS and GC reactors did not change significantly, while the TN in GPVA gradually decreased with time. After one cycle (48 h), TN in GPPS, GPVA, and GC decreased from 7.5, 7.4, and 6.8 to 5.3, 1.9, and 6.3 mg/L, respectively. Therefore, the GPVA reactor showed the best performance over one cycle.

N-removal in the aerobic zone (0–24 h) and anaerobic zone (24–48 h) was further analyzed (Fig. 4). In the earliest stage (0–6 h), there was little difference in the N-removal capacity of the different reactors. After 2 h of aeration (15–17 h),  $\text{NH}_4^+\text{-N}$  decreased from 3.9 to 2.0 mg/L and  $\text{NO}_3^-\text{-N}$  increased from 0.0 to 2.4 mg/L in GPVA, while the contents of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  did not noticeably change in the other two reactor groups. In the anaerobic zone, TN concentrations showed no significant changes in three reactors, while  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  showed obvious changes, especially in GPVA, that is,  $\text{NH}_4^+\text{-N}$  increased and  $\text{NO}_3^-\text{-N}$  decreased. TN fluctuated slightly and reached the lowest value at the 27th hour.  $\text{NO}_3^-\text{-N}$ ,  $\text{NH}_4^+\text{-N}$ , and TN were 0.6 mg/L, 1.1 mg/L, and 1.9 mg/L respectively in GPVA on the 48th hour. According to the above results, GPVA showed the most effective N-removal, which was mainly completed within the R1 unit.

### 3.4. Microbial community analysis of biofilm species and function

It is well-known that biofilms on carriers play an important role in pollutant degradation. The performance of a reactor directly relates to the biofilm community structure. After achieving the above results, the microbial communities of the aerobic units of GPVA and GPPS were characterized via high-throughput sequencing. As shown in Table 2, the read lengths were 120,796 bp and 88,750 bp for GPVA and GPPS, respectively. After 97% similarity classification, there were 114.4 (GPVA) and 106.1 (GPPS) OTUs. The dilution curves of these two communities are shown in Fig. 5, and both curves tended to be stable, which indicates that the sequencing was reasonably complete. Coverage index

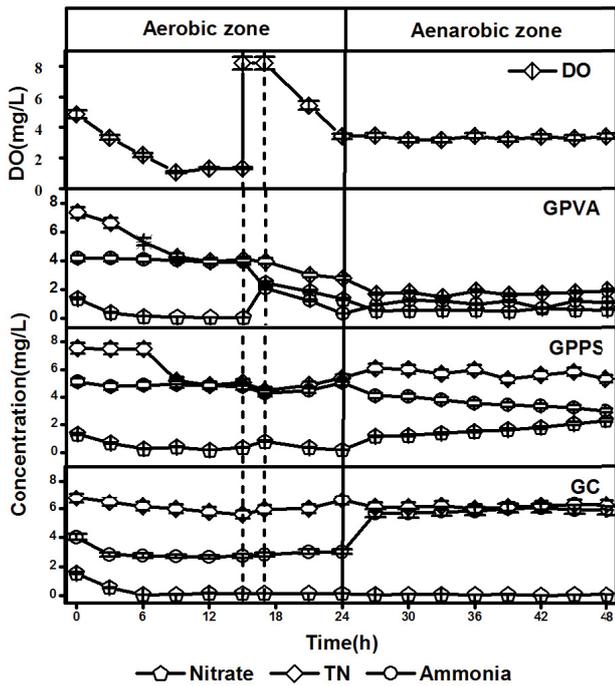


Fig. 4. Typical changes in total nitrogen (TN), ammonia nitrogen ( $\text{NH}_4^+\text{-N}$ ), and nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ) during reaction cycles (GPVA and GPPS respectively represent sequencing biofilm batch reactors filled with PVA-gel beads and PP suspension spheres, and GC represents control reactor without carriers).

theoretically represents the proportion of the microbial species contained in the clone library compared with all the microorganisms in the sample. In this study, the coverage for each sample was >99%, which meant the results reflected the differences in the microbial community of these two BNR systems.

The abundance and diversity indices of the microbial community in GPVA were greater than those of GPPS, suggesting that the PVA-gel beads were more conducive to microbial attachment and colonization (Table 2). The results of the relative abundance (to phylum level) analysis of the PPS and PVA biofilms are presented in Fig. 6. The predominant species in the PVA biofilm included Proteobacteria (81.7%), Bacteroidetes (9.52%), *Nitrospirae* (2.17%), and Actinobacteria (1.18%). Whereas the predominant species in the PPS biofilm included Proteobacteria (35.36%), *Nitrospirae* (27.03%), Actinobacteria (12.11%), and Bacteroidetes (11.79%). The total abundance of Proteobacteria and Bacteroidetes was high, which is consistent with the community composition of activated sludge reported by other studies [11,12]. What is more interesting is that the relative abundances of *Nitrospirae* in the two biofilms were significantly different. *Nitrospirae* is an important microorganism for the promotion of the nitrification reaction, which can oxidize  $\text{NO}_2^-\text{-N}$  to  $\text{NO}_3^-\text{-N}$  and reduce the accumulation of nitrite [13,14]. This reaction is conducive to denitrification and improves the N-removal efficiency of the system.

This study mainly addressed the N-removal effect of biofilms enriched by different carriers in BNRs, and the

Table 2  
Alpha diversity indices of microbial communities

Sample ID	Ace	Chao	Reading values
GPPS	117.487	116.167	88750bp
GPVA	126.726	131.167	120796bp

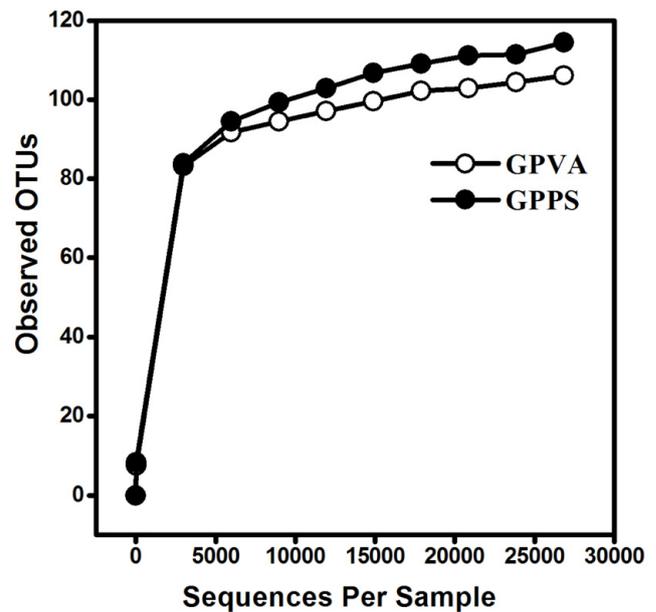


Fig. 5. Rarefaction curves based on Miseq of biofilm microbial communities (GPVA and GPPS respectively represent sequencing biofilm batch reactors filled with PVA-gel beads and PP suspension spheres).

various bacteria at the genus level, are also shown in Fig. 7 and Table 3.

According to Fig. 7, two genera, *Nitrosomonas* and *Nitrospira*, were involved in the nitrification process in both GPVA and GPPS, but the richness of these two genera in GPVA was greater than that in GPPS (Table 3). It was interesting that *Nitrosomonas* was the only ammonia-oxidizing bacteria (AOB) identified in this experiment, and these play an important role in transforming  $\text{NH}_4^+\text{-N}$  to  $\text{NO}_2^-\text{-N}$ . Compared with PPS, the biofilms attached to PVA contained more *Nitrosomonas* (1.22%), which may be due to the larger number of hydrophilic groups on PVA, making it suitable as a carrier for ammonia-oxidizing bacteria (AOB) colonization and preventing loss. Meanwhile, AOB were easily lost on the surface of the PP spheres in GPPS because of agitation by the water current, which was confirmed by the *Nitrosomonas* proportion (0.03%) in biofilms attached on PPS and the lower N-removal rate (Fig. 4) in GPPS. *Nitrospira* belongs to the NOB (nitrite-oxidizing bacteria) and can thus transform  $\text{NO}_2^-\text{-N}$  to  $\text{NO}_3^-\text{-N}$  and reduce the accumulation of nitrite [15], which is conducive to improving the N-removal efficiency of the system.

In this study, seven denitrifying bacteria were detected: *Denitratisoma*, *Hyphomicrobium*, *Methyloversatilis*, *Dechloromonas*, *Pseudoxanthomonas*, *Acidovorax*, and *Zoogloea*.

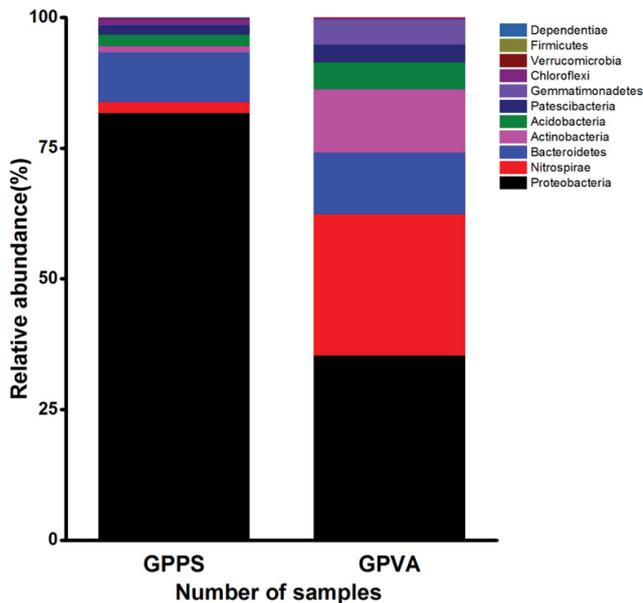


Fig. 6. Microbial community structure of carriers at the phylum level (GPVA and GPPS respectively represent sequencing biofilm batch reactors filled with PVA-gel beads and PP suspension spheres).

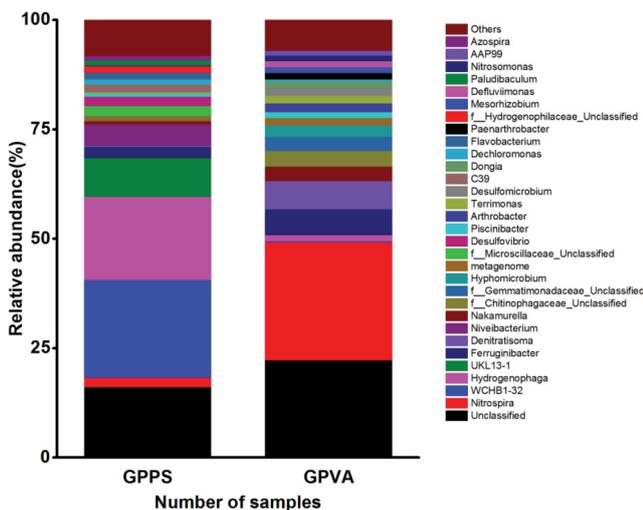


Fig. 7. Microbial community structure of carriers at the genus level (GPVA and GPPS respectively represent sequencing biofilm batch reactors filled with PVA-gel beads and PP suspension spheres).

Most of these are denitrifying bacteria, and were commonly used in wastewater treatment. Based on the results in Table 3, the proportion of denitrifying bacteria in GPVA (10.78%) was five times that in GPPS (1.93%). We speculated that the attachment abilities of the biofilms on the two carriers differed, which led to the different proportions of microorganisms. Among these, *Hyphomicrobium* belongs to aerobic denitrifying bacteria, which can directly transform  $\text{NO}_3^-$  to  $\text{N}_2$  under aerobic conditions [16,17]. Therefore, simultaneous nitrification and denitrification

Table 3

Relative abundance of nitrifiers and denitrifiers at the genus level

Genus		Relative abundance (%)	
		GPPS	GPVA
Denitrifiers	<i>Denitratisoma</i>	0.2	6.41
	<i>Hyphomicrobium</i>	0.01	2.58
	<i>Methyloversatilis</i>	0.01	1.04
	<i>Dechloromonas</i>	1.14	0.42
	<i>Pseudoxanthomonas</i>	0	0.3
	<i>Acidovorax</i>	0.42	0.02
	<i>Zoogloea</i>	0.15	0.01
Total		1.93	10.78
Nitrobacterium	<i>Nitrospira</i>	2.17	27.03
	<i>Nitrosomonas</i>	0.03	1.22
Total		2.2	28.25

can be achieved with this genus. The proportion of this genus in the GPVA (2.58%) reactors was about 258 times higher than that in GPPS (0.01%). *Pseudoxanthomonas*, which only existed on GPVA, has been reported to have complex denitrification abilities that can not only catalyze nitrite but also nitrate [18,19], and it can reduce the accumulation of nitrite and accelerate the denitrification process. More interestingly, the strictly anaerobic *Denitratisoma* appeared in GPVA, which indicated that PVA-gel beads allowed a stable anoxic zone to form because of their high density ( $1.025 \text{ g/cm}^3$ ) and porosity. This phenomenon is consistent with the findings of Madhawa et al. [20].

Overall, we provided evidence that more nitrifying and denitrifying bacteria can exist simultaneously on PVA beads in GPVA than other common materials and complete simultaneous nitrification and denitrification reactions, resulting in better N-removal performance.

#### 4. Conclusion

N-removal from LSW is an urgent problem in the world today. It is of great significance to find effective removal methods. Shortcut nitrification–denitrification is a hot spot in biological nitrogen removal process, which is worth exploring and promoting with different carriers in reactors.

This study, using biofilm reactors with PVA-gel beads carriers, showed that after one cycle under optimum conditions, the TN in GPVA quickly decreased from 7.4 to 1.9 mg/L and showed the best performance of all the groups. The abundance and diversity indices of the microbial community in GPVA were greater than those in GPPS, especially of the nitrifying bacteria *Nitrosomonas* and *Nitrospira*, and the denitrifying bacteria *Hyphomicrobium* and *Pseudoxanthomonas* etc. Overall, these results demonstrated PVA-gel beads can be used as promising carriers for N-removal biofilms in LSW treatment.

#### Declarations

-Ethical Approval  
Not applicable

-Consent to Participate

**Not applicable**

-Consent to Publish

**Not applicable**

### Authors contributions

W. Zhou conducted the reactor experiments. J.L. Yuan analyzed the microbial communities in the biofilms. T.F. Ni analyzed and interpreted the reactor data. Y.H. Yang interpreted data for the microbial communities. M.Y. Li helped to solve new problems in the research. Z.Q. Cheng was a major contributor in interpreting and writing the manuscript. All authors read and approved the final manuscript.

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### Competing interests

The authors declare that they have no competing interests.

### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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