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Screening and Immobilizing the Denitrifying Microbes in Sediment for Bioremediation

Yixin Yan, Dafang Fu * and Jiayuan Shi

School of Civil Engineering, Southeast University, Nanjing 210096, China; 18252772836@163.com (Y.Y.); sjy_012@163.com (J.S.)

* Correspondence: fdf@seu.edu.cn

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Abstract: In this study, immobilized microbial beads were proposed as a solution for excessive nitrogen concentration of the river sediment. The predominant denitrifying microbes were screened from the river sediment. The optimized production of immobilized microbial beads and long-term nitrogen removal efficiency were investigated. 16S rRNA gene sequencing analysis showed that denitrifying bacteria such as *Pseudomonas*, *Alcaligenes*, *Proteiniclasticum*, *Achromobacter* and *Methylobacillus* were dominant microflora in the enriched microbial agent, which accounted for 94.43% of the total microbes. *Pseudomonas* belongs to Gammaproteo bacteria, accounting for 49.22% and functioned as the most predominant denitrifying bacteria. The material concentration of 8% polyvinyl alcohol, 0.5% sodium alginate and 12.5% microbial biomass were found to be the optimal immobilizing conditions. The NH_4^+-N and total nitrogen (TN) removal rates in sediment with dosing immobilized microbial beads were estimated as 68.1% and 67.8%, respectively, when compared to the dosing liquid microbial agent were 50.5% and 49.3%. Meanwhile, the NH_4^+-N and TN removal rates in overlying water went up from 53.14% to 59.69% and from 68.03% to 78.13%, respectively, by using immobilized microbial beads.

Keywords: immobilized microbial beads; sediment; bioremediation; denitrifying bacteria

1. Introduction

Excessive nitrogen in the river ecosystem is constantly deposited on the surface of sediment, making the sediment of a river and lake a significant pollution source [1]. Sediment pollution is a significant problem in China and worldwide [2], especially in the Taihu basin of Southeast China [3], where even if exogenous pollution has been cleaned up, water quality cannot be improved. Due to the frequent exchange between surface sediment and overlying water, contaminated sediment will become a potential threat to the internal source pollution of rivers and lakes [4]. Additionally, a series of very complex biochemical reactions constitute a dynamic transformation system [5,6]. It has been reported that coupled nitrification–denitrification occurring in overlying water with suspended sediments (SPS) where aerobic and anoxic/low oxygen zones may coexist is ignored for N-loss in rivers [7]. Denitrification technology in surface sediment deserves further study as it is the main site for biological nitrogen fixation and denitrification [8–10].

Bioremediation technology has become a research focus in the treatment of river sediment pollution. Moreover, economically viable, good effect, low-energy consumption and the absence of secondary pollution is also significant aspect of research [11]. Traditional microbial remediation is characterized by the use of dominant liquid bacteria, which is low concentration of effective degradation bacteria, easy loss of bacteria under the intense water conservancy, and weak competition with indigenous bacteria [12,13]. In order to solve the respective problems, immobilization technology is gradually introduced into the microbial remediation process. The immobilization of microbial strains

can keep the activity of biological enzymes [14], improve the microbial concentration [15], effectively avoid microbial loss [16], and enable them to exert their bioremediation ability in a stable and efficient manner under complex environmental and intense hydraulic conditions [17].

There has been in-depth research on using immobilized microbial technology for wastewater treatment and ammonia oxidation starts. Albert Magríet et al. used polyvinyl alcohol (PVA) cryogels to encapsulate slow-growing anammox bacteria for deammonification treatment of wastewater, achieving 93% of nitrogen removal efficiency [18]. Ali et al. successfully start up the anammox process by immobilized PVA-sodium alginate (SA) gel beads with less quantity of biomass [19]. In a previous study, heterotrophic nitrification and aerobic denitrification were identified in secondary effluent nitrogen removal process via microbial entrapment with poly (vinyl alcohol) sodium alginate gel modified with alumina nanoparticles [20]. Immobilized cells had a higher ammonium removal rate (21.84%, 43.59% and 41.46%) than free living cells (14.35%, 38.57% and 40.59%) under autotrophic, heterotrophic, and micro-aerobic conditions [21]. Liu et al. developed a PVA cell immobilization technology in degrading diesel in seawater with a degradation rate of 88% [22]. In the Hyokwan Baes study, poly (vinyl alcohol)/sodium alginate gel beads were utilized as an immobilization system to entrap activated sludge and successfully started up the anaerobic ammonium oxidation (ANAMMOX) reactor with a total nitrogen removal efficiency of 88.9% [23]. However, research on its application in the river sediment ecosystem has not been undertaken comprehensively.

Commonly used embedded carrier materials include polyethylene glycol (PEG) [24], SA [25], carboxy methyl cellulose (CMC) [26], PVA [18], and waterborne polyurethane (WPU) [27]. PVA is widely used in the fields of enzyme and cell immobilization. PVA has the advantages of good chemical stability, strong anti-microbial decomposition ability, non-toxic to microorganisms and low cost. However, previous studies also concluded that PVA is a highly viscous substance that can easily expand and agglomerate in water [28]. Moreover, it exhibits strong mass transfer resistance, reducing the degradation efficiency of microorganisms [29]. Studies have reported that the addition of SA and $CaCl_2$ during PVA-H_3BO_3 cross-linking can solve the agglomeration problem of PVA particles, enhance the beads forming ability of PVA gel, and alleviate the toxic effect of boric acid on microorganisms [30,31].

Therefore, the main aim of the present study was to remediate nitrogen pollution from contaminated sediment by using microbial immobilization technology. Compared with previous research, the innovations were: (i) screen and culture predominant denitrifying bacteria from river sediment and make mixed bacteria agent for immobilization; (ii) determine the optimal immobilizing condition including PVA concentration, sodium alginate concentration, and microbial biomass to avoid adhesion and trailing; (iii) evaluate the performance of immobilized microbial activated beads on the nitrogen pollution remediation of sediment.

2. Material and Methods

2.1. Detection and Analysis of Sediment

Sediment (grab type sediment sampler) samples were collected from various sampling sites of Shedu River (Taihu lake basin). Piston columnar sediment samplers (catalog no. #G60316, Beijing Anode Instrument Co. Ltd., Beijing, China) were used for sediment sampling. The samples were stored in an ice box and delivered to the lab for nutrient analysis. Sediment samples were air dried and coarse concretions, stones, pieces of roots, leaves, stones, plastic and other decomposed organic residue were removed.

Ammonia nitrogen (NH_4^+-N) and total nitrogen (TN) were estimated by the following standard methods of analysis [32]. Finally the microbial communities of the sediments were isolated and characterized by high-throughput sequencing (Zhongyi Jinda Analysis and Detection Co., LTD, Nanjing, China).

Screening and culture of dominant denitrifying bacteria.

The screening of dominant denitrifying bacteria has 2 steps [33]. The optimal culture conditions were obtained by preliminary experiments, which are 5%, carbon source of calcium carbonate + citric acid sodium, carbon source concentration of 3 g/L, temperature of 35 °C, pH of 8 and trace elements.

Step 1: Initial screening. 10 g sediment taken from Shedu River was inoculated in triplicate in sterile denitrifier enrichment medium and cultivated in static incubator for 4 days. The content of NH_4^+-N and NO_3-N in the culture medium was detected.

Step 2: Enrichment of optimization culture. The initial screened bacteria solution was inserted into the sterile denitrifier enrichment medium, and maintained at 35 °C biochemical incubator, for 7 days as a culture cycle.

Microelement solution: $ZnSO_4 \cdot 7H_2O$ 3.93 g/L; $CaCl_2$ 5.5 g/L; $MnSO_4 \cdot H_2O$ 4.32 g/L; $FeSO_4 \cdot 7H_2O$ 5.0 g/L; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 1.1 g/L; $CuSO_4 \cdot 5H_2O$ 1.57 g/L; $CoCl_2 \cdot 6H_2O$ 1.61 g/L, respectively.

2.2. Immobilization of Active Breads

The PVA and sodium alginate were selected as the carrier to prepare the beadsto optimize the preparation scheme of the immobilized beads and evaluate the quality of the beads according to the disadvantages of the large water solubility and expansibility of the polyvinyl alcohol beads in the preparation process. Production process of the immobilized microbial beads is presented in Figure 1.

Step 1: Preparation of gels. A known amount of PVA and sodium alginate was infiltrated in 100 mL water and dissolved in a water bath for 24 h at 85 °C. Then a known amount of additives (3.5% SiO_2, 0.4% $CaCO_3$, 0.5% attapulgite powder) were added and the mixture was maintained at room temperature for 4 h (previous experiments showed that it was difficult to form beads immediately after cooling, while the effect of embedding after 4–5 h was better), the inoculation was added into the liquid. The bacterial count (most probable number) in the optimized inoculation was about 4.2×10^7/mL~1.9×10^8/mL.

Step 2: Preparation of crosslinking agent. The mixture of saturated boric acid and 2% $CaCl_2$ was crosslinked for 24 h. pH was adjusted to 7.5.

Step 3: Immobilization. The mixed solution of PVA and sodium alginate was heated and dissolved, and was placed at room temperature for more than 4 h. Then it was added to the crosslinking agent with a peristaltic pump. Meanwhile, the drip-acceleration of the peristaltic pump was controlled to form of white beads of about 2 mm in diameter. The prepared immobilized beads were cross-linked in the crosslinking agent in 4 °C for 36 h to ensure its strength and were washed with normal saline (0.9% of NaCl solution) before use [34].

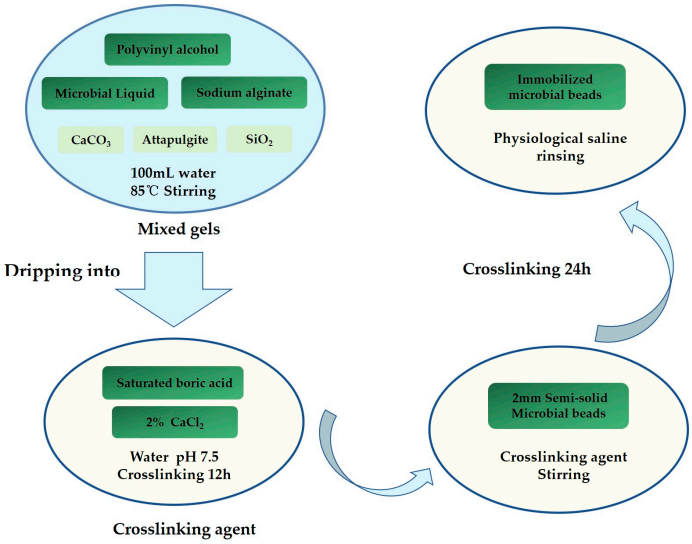


Figure 1. Production process of the immobilized microbial beads.

The preliminary experiment found that immobilized microbial beads are easy to adhesion and trailing. The effect of PVA, sodium alginate concentrations and microbial biomass on spherification of colloids was studied. The concentration range of sodium alginate (w/v) was 0.25%, 0.5%, 1%, 2% and 3%. The concentration of PVA (w/v) was 7%, 8%, 9%, 10% and 11%. The microbial biomass (w/v) was 5%, 6%, 7% and 8%.

The mass transfer rates were also used as criteria to evaluate the immobilization process. The same amount of diluted blue ink and the immobilized beads in the same size were added to the beakers and soaked for 18 h. Absorbance of the solution in the beaker was determined with distilled water as the reference at 406 nm. High absorbance indicates that the immobilized pellets absorb less pigment and have poor mass transfer performance.

2.3. Simulation Experiment of Sediment Remediation by Activated Beads

The plexiglass container was used as the reaction device with a size of 35 × 35 × 60 cm. The contaminated sediment was spread up to 10 cm depth and the overlying water was 30 cm. The peristaltic pump was used to enter and exit water with constant and equal water velocity. The actual water flow state was simulated and the water level in the device was kept at constant. Device 1# was the control group, devices 2~4# were corresponding devices for the addition of 1000 mL bacteria liquid, 200 g immobilized beads without microbe, 200 g immobilized microbial beads. Water temperature was around 25 °C, dissolved oxygen 2–3 mg/L and pH 7–8. The simulation experiment lasted for 30 days, and every 5 days, samples were taken to measure the indexes of NH_4^+-N, TN in sediment and TN, NH_4^+-N in overlying water bodies for comparison and evaluation of the remediation effect.

3. Results and Discussion

3.1. Diversity Analysis of Microflora in Sediment

A total of 47,333 original sequences were obtained by high-throughput sequencing of Shedu River sediment samples, including 26 phyla and 291 genera, with an average length of 300 bp. At the phylum level, 4050 high-quality sequences were analyzed, accounting for 84.61% of the total sequence number (Figure 2). Further classification analysis showed that only 10,627 high-quality sequences could be classified into 291 known genera, accounting for 22.45% of the total sequence number, indicating the presence of unauthenticated microbial colonies found in the sediments.

Out of the 26 phyla detected, Proteobacterium, Bacteroidetes, Acid-bacillus, Actinomycetes, Firmicutes, Chlorobacterium, Verruca microflora, and Cyanobacteria were the dominant microorganisms in the sediment, accounting for 83.76% of the total microbial population. The Proteobacteria, the largest phylum was accounted for up to 61.61%, follow by the Bacteroidetes which is the second largest phylum. Steroidobacter belongs to the Gammaproteo bacteria, which is the main class of bacteria in the Shedu River sediment, accounting for 2.64% of the total sequenced DNA. There is only one species of Steroibacdoter denitrificans that has been effectively identified, which can degrade sterols and reduce nitrate to NO by separating from activated sediment enriched in waste water treatment plant.

The deformation detected in the collected samples of Shedu River sediment includes 5 classes, named Epsiprotelonobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, and Alphaproteobacteria. Among them, Deltaproteobacteria (19.91%), Betaproteobacteria (17.46%) and Gammaproteobacteria (12.33%) are the main Proteobacteria in the sediment samples of Shedu River, accounting for 80.67% of the total amount of Proteobacteria. Proteobacteria is usually the main prokaryotes in the water system, which are distributed in both aerobic and anaerobic environments [34]. Betaproteobacteria is the main phytoplankton group, accounting for a large proportion of the collected sediment samples. Deltaproteobacteria is the main bacteria in lake sediments, and its proportion in lake sediments is much higher than that in water bodies. Meanwhile, Deltaproteobacteria play an important role in the anaerobic sediment layer of lakes. An earlier study concluded that the

Deltaproteobacteria used as sulfate reduction [35]. Bacteroidete, Acidobacteria and Actinobacteria accounted for about 17.06% of the total microbial biomass in the collected sediments of Shedu River. An earlier study also reported that the predominant bacteria in the fresh water lake [36]. Chloroflexi is another abundant bacterial group in the sedimentary samples. Zhao et al. found that organic pollutants in lake sediments are conducive to the growth of Chloroflexi bacteria, and it play a crucial role in degrading oil substances. Chloroflexi exist in sediments as a major bacterial group, indicating that sediments contain a high proportion of total organic carbon (TOC) [37].

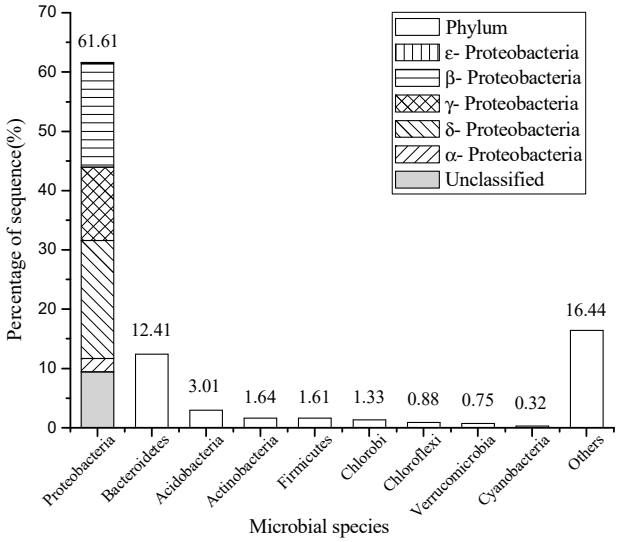


Figure 2. Microbial community of sediment at phylum level.

Cyanobacteria, another major group of bacteria were also found in the sediment samples from the Shedu River. In nutrient-rich environment, especially in lakes and rivers with rich nitrogen and phosphorus, it would multiply in large numbers to form bloom (algal bloom). Cyanobacteria were also detected in the sediments of Shedu River, indicating that water in the region has already shown signs of eutrophication [38].

There are 291 genera of microorganisms were detected and the top 20 genera of the total sequences are listed in Table 1 [39]. At the main genus level, anaerobic bacteria account for a large proportion, and a large number of sulfate reductants and methanol-oxidizing bacteria are found in sediments. The largest proportion of *Steroidobacter* belongs to Proteobacteria, which can degrade organic substrates and simultaneously remove the nitric nitrogen in anaerobic environment [40]. In addition, *Vogesella* generally grows and breeds with water bloom, and the content of 0.18% in sediments is detected, which also indicates that the water body of Shedu River is prone to eutrophication.

Table 1. Microbial abundance and function at the level of genus.

Genera	Proportion %	Function
Steroidobacter	2.64	Anaerobic denitrification
Dechloromonas	0.65	Anaerobic denitrification
Methylotenera	0.61	Anaerobic denitrification
Thiobacillus	0.60	Denitrification
Pseudomonas	0.38	Aerobic denitrification, heterotrophic nitrification
Acinetobacter	0.21	Denitrification, heterotrophic nitrification
Paracoccus	0.18	Aerobic denitrification
Thauera	0.08	Heterotrophic nitrification, aerobic denitrification
Hyphomicrobium	0.06	Aerobic denitrification
Nitrospira	0.05	Nitrifying bacteria

Table 1. *Cont.*

Genera	Proportion %	Function
Halomonas	0.02	Low temperature high salt denitrification
Bacillus	0.01	Aerobic denitrification, heterotrophic nitrification
Propionibacterium	0.01	Aerobic denitrification
Rhizobium	0.01	Aerobic denitrification
Corynebacterium	0.01	Heterotrophic nitrification
Rhodococcus	0.01	Aerobic denitrification, heterotrophic nitrification

3.2. Screening and Culture of Dominant Denitrifying Bacteria

The NH_4^+-N and TN removal rate of the enrichment and optimization culture reactor in one experimental period are 84.4% and 84.8%, respectively. The concentration of bacteria in the optimized culture mixture was about 4.2×10^7 /mL~1.9×10^8 /mL. A total of 19 major phyla were detected in the optimized cultured microbial agent, among which Proteobacteria, Firmicutes, Chlorobi, Actinomycetes and Bacteroidetes are dominant in the mixture, accounting for about 99% of the total microbial population. Table 2 shows that Proteobacteria, Firmicutes and Chlorobi have been optimized to become the dominant denitrifying bacteria. Among them, Proteobacteria has obvious advantages, and it is believed that aerobic denitrification and heterotrophic nitrifying bacteria have proliferated in large numbers.

Table 2. Predominant strains at the level of phylum.

Category / Sample	*Proteobacteria* %	*Firmicutes* %	*Chlorobi* %	*Actinobacteria* %	*Bacteroidetes* %
Shedu sediment	15.31	0.65	0.53	2.72	1.04
5% inoculation	89.12	8.98	4.84	3.55	2.55
10% inoculation	94.33	3.98	3.98	3.62	2.66

A total of 204 strains of *Pseudomonas*, *Alcaligenes*, *Proteiniclasticum*, *Achromobacter* and *Methylobacillus* were detected at the genus level, accounting for 82.73% and 94.43% of the total microbial population (Table 3). Among them, *Pseudomonas* belonging to Gammaproteo bacteria accounted for about 50%, which was the most dominant denitrifying strain [41]. *Alcaligenes*, *Achromobacter* and *Methylobacillus* are also strains of Proteobacteria with high efficiency of denitrification [42]. *Proteiniclasticum* is the most important type of denitrification in Firmicutes. It can be concluded that *Pseudomonas* and *Alcaligenes*, which were screened out by the optimized culture scheme, showed great growth advantages and could effectively remove the concentration of nitrogen in the medium.

Table 3. Predominant strains at the level of genus.

Category / Sample	*Pseudomonas* %	*Alcaligenes* %	*Proteiniclasticum* %	*Achromobacter* %	*Methylobacillus* %
Shedu sediment	0.38	Nil	0.01	Nil	0.08
5 % inoculation	49.87	19.98	8.77	3.89	0.22
10% inoculation	49.22	40.87	3.82	0.26	0.26

3.3. Preparation of Microbial Activated Beads

The beads were too thin to form into beads with a PVA concentration (w/v) of 7%. When PVA concentration was 8%, particles were most likely to form into beads with a little bit of adhesion. When the concentration of PVA is 9–11%, it was easy to drag the tail, and the viscosity increases accordingly. At the same time, a higher concentration of PVA will affect the diffusion of pollutants, microbial metabolites and oxygen transfer in water, thus affecting cell activity. Therefore, 8% is optimal concentration for further experiments (Table 4). Figure 3 reveal the **m**icrobial beads at different PVA

concentrations. Huang et al. reported that the appropriate concentration of PVA in immobilized microbial technology is 7.5–10% [43]. Other studies reported higher PVA concentrate. Bae et al. used 15% PVA to immobilized ammonia-oxidizing bacteria (AOB) and ANAMMOX bacteria and gain an 80.4% nitrogen removal rate of wastewater [44]. Albert Magrí et al. made immobilized beads with a PVA concentrate of 20% [18].

Table 4. Effect of polyvinyl alcohol (PVA) concentration on spherification of colloids.

No	PVA Concentration (%)	Adhesion	Stability	Mass Transfer Rate A_{406}
a	8	General	Good	0.182
b	9	Easy	Good	0.191
c	10	Easy	Good	0.205
d	11	Easy	Good	0.198

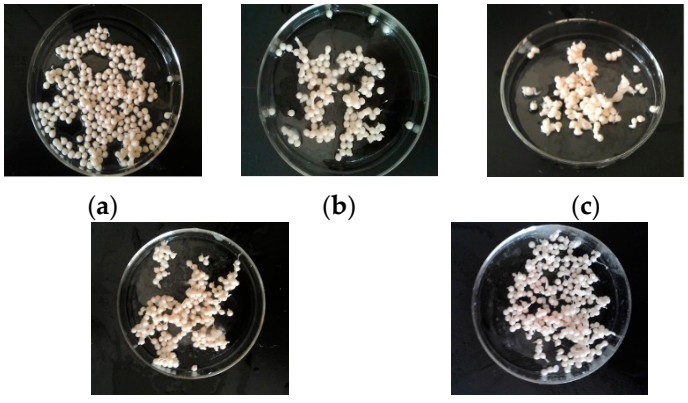
(**a**) (**b**) (**c**) (**d**)

Figure 3. Microbial beads at different PVA concentrations. (**a**) 8% PVA; (**b**) 9% PVA; (**c**) 10% PVA; (**d**) 11%PVA.

As shown in Table 5 and Figure 4, if the concentration of sodium alginate (w/v) is low, the immobilized particles are easy to form pellets. If the concentration of sodium alginate is too high, it is easy to drag tail and adhesion. Therefore, the concentration of sodium alginate was 0.5% for follow-up study. Other studies report that dosage of sodium alginate in microbial beads immobilization were 0.8% [45], 0.9% [30] and 2% [44].

Table 5. Effect of sodium alginate concentration on spherification of colloids.

No	Sodium Alginate Concentration (%)	Adhesion	Stability	Mass Transfer Rate A_{406}
a	0.25	General	General	0.193
b	0.5	General	Good	0.182
c	1	Easy	Good	0.179
d	2	Easy	Bad	0.181
e	3	Easy	Bad	0.154



(**a**) (**b**) (**c**)

(**d**) (**e**)

Figure 4. Microbial beads at different concentrations of sodium alginate (SA). (**a**) 0.25% SA; (**b**) 0.5% SA; (**c**) 1% SA; (**d**) 2% SA; (**e**) 3% SA.

It can be seen from Table 6 and Figure 5 that the amount of microbial agent significantly affected the spherification of colloids. Immobilized microbial beads with 12.5% of microbial biomass in the experiment have the best bead-forming effect, which can overcome the problem of adhesion in the immobilization process.

Table 6. Effect of microbial biomass on spherification of colloids.

No	Microbial Biomass (%)	Adhesion	Stability	Mass Transfer Rate A_{406}
a	5	Easy	General	0.190
b	7.5	General	Good	0.182
c	10	General	Good	0.179
d	12.5	Difficult	Good	0.171
e	15	Easy	General	0.169

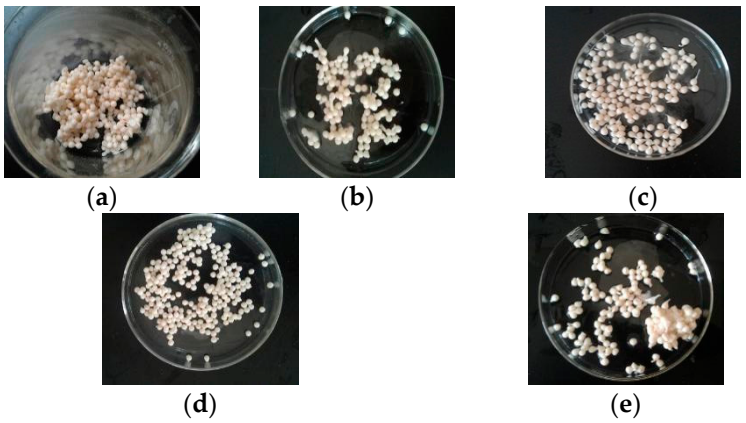


Figure 5. Microbial beads at different microbial biomass. (**a**) 5% biomass; (**b**) 7.5% biomass; (**c**) 10% biomass; (**d**) 12.5% biomass; (**e**) 15% biomass.

3.4. Nitrogen Removal Efficiency of Immobilized Microbial Beads

The changes in concentration of NH_4^+-N and TN in the overlying water of 4 sets of devices over time are presented in Figure 6. Concentration of NH_4^+-N and TN in devices 2# and devices 4# first went up sharply, then continued to decline, and finally stabilized within a range of decrease. At the beginning of the experiment, the microbial degraded organic nitrogen in the sediment, and the NH_4^+-N produced by the ammoniation reaction entered the overlying water body, resulting in the increase of NH_4^+-N and TN concentration in the overlying water body. The peak concentrations of NH_4^+-N and TN in device 2# were the highest, while this was relatively low in device 4# as immobilized particles had certain adsorption effect to inhibit sediment release. The degradation rate of nitrogen in the early stage of device 2# was significantly better than device 4#. This is because the floating microbe is soluble in water and directly in contact with the pollutant while immobilized microbe is in the embedding medium with slow transfer speed. At the later stage of experiment, the NH_4^+-N and TN degradation rate of the floating microbe in overlying water was lower than that of the immobilized microbial beads.

At the beginning of the 5th day of the experiment, the volume of the microsphere expanded slightly and the hardness of the microsphere was decreased. White foam appeared in the water sample, mainly due to the precipitation of PVA monomer in the particles. At the same time, the sodium alginate and dead microbial residues used in the formula will have a certain degree of dissolution in the initial stage, which makes the chemical oxygen demand (COD) of water significantly increase in the initial stage. After several days of experiments, PVA was no longer dissolved, no white foam was generated, the volume of microspheres remained unchanged, and the physical properties of particles basically stabilized. In the process of immobilization, both surface and internal structure of the immobilized

particles are changing, from dense to loose. As the activation process continued, the granules gradually changed from the initial white to gray-brown and integrated with the sediment.

The immobilized microbial beads successively forms anaerobic zone, anoxic zone and aerobic zone from inner to outer side, which can simultaneously form an anaerobic and aerobic environment and carry out synchronous nitrification and denitrification reaction. The rate and efficiency of denitrification are higher than the traditional nitrification and denitrification process. The concentrations of NH_4^+-N and TN generally showed a trend of continuous decrease and eventually tended to be stable. The final NH_4^+-N and TN removal rates of immobilized microbial beads in overlying water were 78.3% and 68.1%, respectively. The NH_4^+-N and TN removal rates of immobilized beads without microbe in device 3# were 10.3% and 15.3%, which may be due to the adsorption effect of PVA particles. The NH_4^+-N and TN removal rate of the microbial agents were 59.69% and 53.1%, respectively. Zhou et al. capped sediment with thin-layer biozeolite and gained a 90% TN removal rate while removal of nitrogen from sediment was not obvious [46].

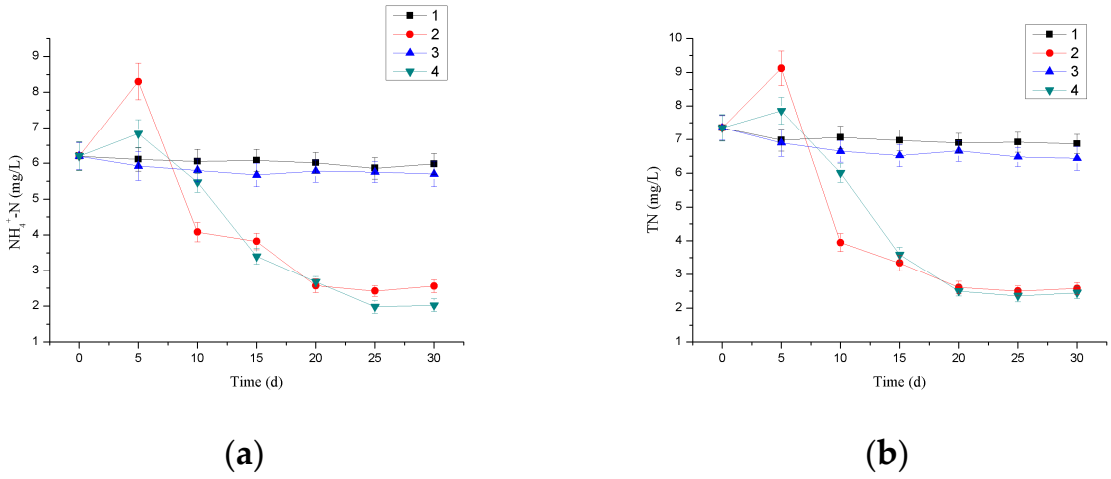
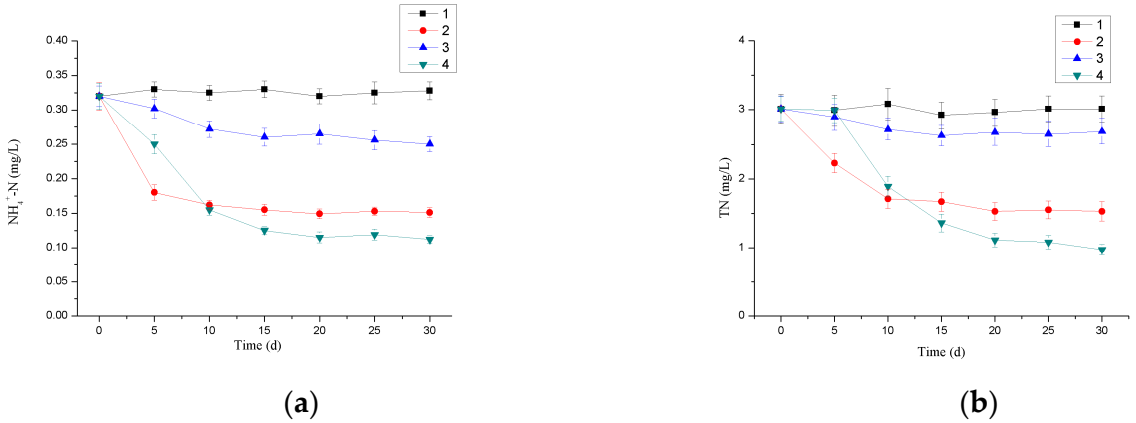


Figure 6. Change in concentration of (**a**) ammonia nitrogen and (**b**) total nitrogen in overlying.

The NH_4^+-N and TN concentration in the sediment of 4 sets of devices over time is shown in Figure 7. It can be seen that the NH_4^+-N and TN removal rate of devices 2, 3 and 4 was mainly concentrated in the early stage of the experiment and finally stabilized. The removal rate of the NH_4^+-N and TN by immobilized beads without microbe reached 14.6% and 12.1%, respectively. The special reticular pore structure inside the immobilized microbial beads increases the specific surface area, enabling it to absorb a certain amount of pollutants. The immobilized beads capture and enrich nitrogen pollutants at the junction of surface sediment and overlying water where microbial denitrification is most intense. After 20 days of the experiment, the concentration of nitrogen pollutant echoes in the phase when the beads are completely dissolved. Some previous studies reported that the microbial immobilization with inorganic nanoparticles can increase the functional specific surface area and loading rate of cells. Owing to their high specific surface area and adsorption capacity, alumina nanoparticles have been widely used for reducing various contaminants in water and material modification [28,31]. Moreover, PVA immobilized beads have a good adsorption effect on metal ions and phosphorus pollutants in water [47].

In the first 10 days of the experiment, the microbial agent had a faster degradation rate of sediment pollution than the immobilized microbial beads. The removal rate of NH_4^+-N by bacteria solution and immobilized microbial beads reached 88.5% and 75% of the overall removal efficiency. During the 10–25th days of the experiment, the degradation rate continued to rise in device 4 by using immobilized microbial beads while it remained stable in the microbial agent device 2. The immobilized microbial beads had a longer-lasted degradation of NH_4^+-N and TN in sediment. The removal rates of NH_4^+-N and TN by immobilized microbial beads were 67.8% and 68.1%. Xu et al. used the coupling sediment

microbial fuel cells (SMFCs) with submerged aquatic plants to remove nitrogen in sediment and gain a TN removal rate of up to 25.3% [44]. Aerobic denitrifiers coupled with a denitrifying agent were applied in the sediment of an urban river and the TN removal rate was 14.7% in sediment [48]. Compared to previous studies, the immobilized microbial beads have a higher nitrogen removal of sediment.



(**a**) (**b**)

Figure 7. Change in concentration of (**a**) ammonia nitrogen and (**b**) total nitrogen in sediment.

3.5. Practical Applications and Future Research Aspects of Current Study

The immobilized microbial activated bead shows the potential effectiveness for in-situ remediation sediment bioremediation. Compared with liquid biological agents, immobilized microbial beads are in solid form, easy to store and transport. Current work provides an alternative and effective method for microbial immobilizing, which can effectively reduce trailing and adhesion. According to our knowledge, this is the first attempt where immobilized microbial activated beads were applied to a highly contaminated river in the Taihu lake area, China. Further research on the production, storage and implication of microbial activated beads with indigenous microbial community is highly recommended. Moreover, the implication of microbial beads in different climatic regions and role of different microbial communities in sediment bioremediation required further detail studies.

4. Conclusions

High-throughput sequencing analysis showed an abundance of denitrifying bacteria in the sediment. After the enrichment and optimal cultivation, denitrifying bacteria such as *Pseudomonas*, *Alcaligenes*, *Proteiniclasticum*, *Achromobacter*, *Methylobacillus* became dominant microflora in the enriched microbial agent, which accounted for 94.43% of the total microbial species. *Pseudomonas* belonging to Gammaproteo accounted for 49.22% and functioned as the most predominant denitrifying bacteria. The optimal immobilization condition of microbial broth is 8% PVA, 0.5% Na-alginate and 12.5% microbial biomass. The NH_4^+-N and TN removal rates of sediment with dosing liquid microbial agent were 50.5% and 49.3%, respectively, while the removal rates with dosing immobilized microbial beads were 68.1% and 67.8%. Meanwhile, the NH_4^+-N and TN removal rates in overlying water went up from 53.14% to 59.69% and from 68.03% to 78.13% respectively by using immobilized microbial beads. Moreover, experimental results showed that an immobilized microbial bead has a longer-lasting denitrification performance than a liquid microbial agent. Microbial immobilization technology has a broad application prospect in the in-situ remediation of river sediment.

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