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Performance Enhancement Mechanisms and Community Ecology of an Immobilised Carrier System for Low-Temperature Partial Nitrification-Denitrification

Yahang Chen, Hong Yang*

Key Laboratory of Beijing for Water Quality Science and Water Environment Recovery Engineering, College of Architectural and Civil Engineering, Beijing University of Technology, Beijing, China
Email: *yhong@bjut.edu.cn

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Abstract

Low temperature has long been recognized as a critical constraint limiting the engineering application of mainstream partial nitrification-denitrification processes in cold regions. Compared with conventional activated sludge systems, immobilised carrier technology exhibits outstanding advantages in biomass retention, microenvironmental stability, and anti-shock capacity, making it a promising alternative for low-temperature wastewater treatment. However, its long-term operational performance and microbial adaptation mechanisms under continuous and gradually decreasing low-temperature stress (down to 6˚C) remain insufficiently understood. In this study, an A/O-type immobilised carrier partial nitrification-denitrification system was developed, and its performance was evaluated through a 175-day stepwise cooling experiment (from 22˚C to 6˚C). The results indicated that the stable operating temperature range of the system was extended to 8˚C. When the temperature was above 8˚C, the Total Nitrogen Removal Efficiency (TNRE) could be restored to nearly 60% (the theoretical maximum) by adjusting Hydraulic Retention Time (HRT) and Dissolved Oxygen (DO), and the system recovered rapidly from temperature shocks within 1 - 2 days. Batch tests and microbial community analysis confirmed that the unique microenvironment inside the carriers effectively maintained stable partial nitrification (nitrite accumulation rate, NAR > 90%) at temperatures ≥ 8˚C by continuously inhibiting the dominant nitrite-oxidising bacteria Nitrospira. Although denitrification was the rate-limiting step, the immobilised carriers acted as microbial refuges, driving community succession toward functional redundancy and enriching psychrotolerant genera such as

Flavobacterium to prevent functional deterioration. This study reveals the structural advantages and ecological resilience of immobilised carriers against low-temperature stress, providing a solid theoretical foundation and technical support for the practical application of partial nitrification-denitrification processes in wastewater treatment plants in cold regions.

Subject Areas

Bioengineering

Keywords

Immobilised Carriers, Partial Nitrification-Denitrification, Low Temperature, Microbial Community, Process Resilience

1. Introduction

Low temperature severely inhibits microbial activity and disrupts the functional stability of biological nitrogen removal systems, especially partial nitrification-denitrification, which relies on the stable competition between Ammonia-Oxidising Bacteria (AOB) and Nitrite-Oxidising Bacteria (NOB) [1]-[3]. With the promotion of low-carbon development strategies, energy-efficient and high-resilience wastewater treatment technologies have become an urgent demand for urban water treatment systems, especially in seasonally cold areas.

Partial nitrification-denitrification is regarded as a high-efficiency and energy-saving nitrogen removal pathway, which can reduce oxygen consumption by 25% and carbon source demand by 40% compared with traditional full nitrification-denitrification [4] [5]. Nevertheless, low temperature (below 15˚C) easily breaks the delicate balance between AOB and NOB, resulting in the failure of nitrite accumulation and even system collapse.

Immobilised carrier technology provides a feasible solution to low-temperature bottlenecks owing to three prominent merits: high biomass retention, stable internal microenvironment with natural DO gradients, and strong resistance to environmental fluctuations [6]-[9]. However, most previous studies focused on mesophilic conditions or short-term low-temperature exposure. Systematic investigations on long-term operational performance, microbial adaptive succession mechanisms, and performance boundaries under gradually decreasing temperature down to 6˚C are still insufficient [10]-[12].

To fill these research gaps, this study established an immobilised carrier partial nitrification-denitrification system and conducted a 175-day stepwise cooling experiment. The objectives were:

1) To determine the low-temperature performance limit and resilience of the system;

2) To reveal the stability and evolutionary characteristics of nitrogen conversion pathways under low-temperature stress;

3) To clarify the succession and adaptation mechanisms of functional microbial communities;

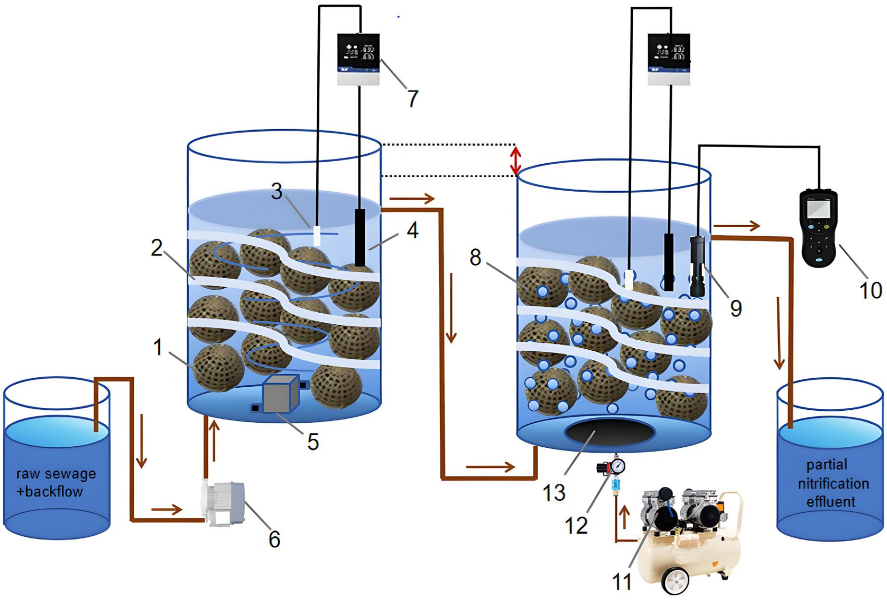
4) To provide theoretical support for engineering application in cold regions.

2. Materials and Methods

2.1. Experimental Setup

The experimental system adopted an Anoxic/Oxic (A/O) configuration, consisting of a denitrification reactor and a partial nitrification reactor with effective volumes of 6.5 L and 5.6 L, respectively. Immobilised carriers were prepared using Polyvinyl Alcohol (PVA), enriched nitrifying sludge, denitrifying sludge, calcium carbonate, and powdered activated carbon (See Figure 1).

A high-precision temperature control system based on passive cooling and active compensation was applied, ensuring temperature fluctuation within ±0.2˚C.



1. Denitrification carriers; 2. Phase-change ice packs; 3. Temperature probes; 4. Heating rods; 5. Miniature circulation pumps; 6. Metering pumps; 7. Temperature controllers; 8. Partial nitrification carriers; 9. Dissolved oxygen probes; 10. Dissolved oxygen meters; 11. Air compressors; 12. Gas flow control valves; 13. Aeration discs.

Figure 1. Schematic diagram of the experimental reactor systems. (a) Denitrification reactor; (b) Partial nitrification reactor.

2.2. Experimental Wastewater

The influent was actual domestic sewage collected weekly from a residential community and stored at 4˚C prior to use to minimize compositional changes. During the 175-day experimental period, a total of 26 grab samples were collected from the influent tank and analyzed to characterize the influent quality. The main water quality characteristics are summarized in Table 1. Organic nitrogen concentrations were consistently below 1 mg/L and were therefore considered negligible for nitrogen mass balance calculations.

Table 1. Influent water quality characteristics ($mg\cdot L^{-1}$).

COD	NH_4^+-N	NO_2^--N	NO_3^--N	C/N
200~220	80~100	0~0.2	0~0.2	2.5~2.85

2.3. Operation Strategy

The initial temperature was 22˚C, with a reflux ratio of 150%. The HRT was 2.5 h for the denitrification reactor and 3.5 h for the partial nitrification reactor, and DO was controlled at 5.0 ± 0.2 mg/L. The temperature was reduced by 1˚C every 10 days until reaching 6˚C, with a total operation period of 175 days. HRT and DO were adjusted in a timely manner to maintain stable effluent quality.

HRT was extended incrementally when ammonia removal efficiency declined by >10% within 24 h, and DO was adjusted to maintain nitrite accumulation rates >90% while avoiding excessive aeration. The specific HRT and DO setpoints applied at each temperature stage are provided in Table 2.

Table 2. Parameters under each temperature range.

Temperature (˚C)	HRT(h)-Denitrification	HRT(h)-Partial Nitrification	DO (mg/L)
22 - 15	2.5	3.5	5.0 ± 0.2
14 - 11	3.0	4.35	4.7 ± 0.2
10 - 8	3.5	5.0	4.2 ± 0.2
7 - 6	4.0	6.0	3.5 ± 0.2

2.4. Analytical Methods

Water samples were filtered through 0.45 μm membranes. NH_4^+-N, NO_2^--N, NO_3^--N, and COD were determined using standard methods. Total Nitrogen (TN) was calculated as the sum of NH_4^+-N, NO_2^--N, and NO_3^--N. Key indicators including Ammonia Removal Efficiency (ARE), Nitrite Accumulation Rate (NAR), Total Nitrogen Removal Efficiency (TNRE), and Ammonia Oxidation Rate (AOR) were calculated.

Organic nitrogen in the domestic sewage was negligible (<1 mg/L, data not shown); therefore, TN was approximated as the sum of inorganic nitrogen species.

2.5. Batch Experiments and Microbial Analysis

Batch experiments were conducted in 500-mL glass reactors at the conclusion of each temperature phase. Reactors were filled with fresh domestic sewage (initial NH_4^+-N ≈ 85 mg/L, NO_2^--N < 0.2 mg/L, NO_3^--N < 0.2 mg/L) and inoculated with carriers collected from the corresponding continuous-flow reactor (approximately 10% v/v). The reactors were stirred at 100 rpm under constant temperature control. Samples were collected at 20-min intervals over a period equivalent to one HRT of the continuous system and immediately filtered through 0.45 μm membranes for nitrogen species analysis. All batch tests were performed in duplicate.

For microbial community analysis, biomass samples were collected in triplicate from the immobilised carriers at 22˚C, 14˚C, and 6˚C after the system had operated stably for at least 10 days at each temperature. High-throughput sequencing of the 16S rRNA gene V3 - V4 region was performed on the Illumina MiSeq platform. Raw sequences were quality-filtered using QIIME2, and Operational Taxonomic Units (OTUs) were clustered at 99% similarity against the SILVA database. Alpha diversity indices were calculated using Mothur, and statistical comparisons between temperature groups were performed using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$ considered significant).

3. Results and Discussion

3.1. Performance of the Partial Nitrification Reactor

The long-term performance of the partial nitrification reactor is presented in Figure 2.

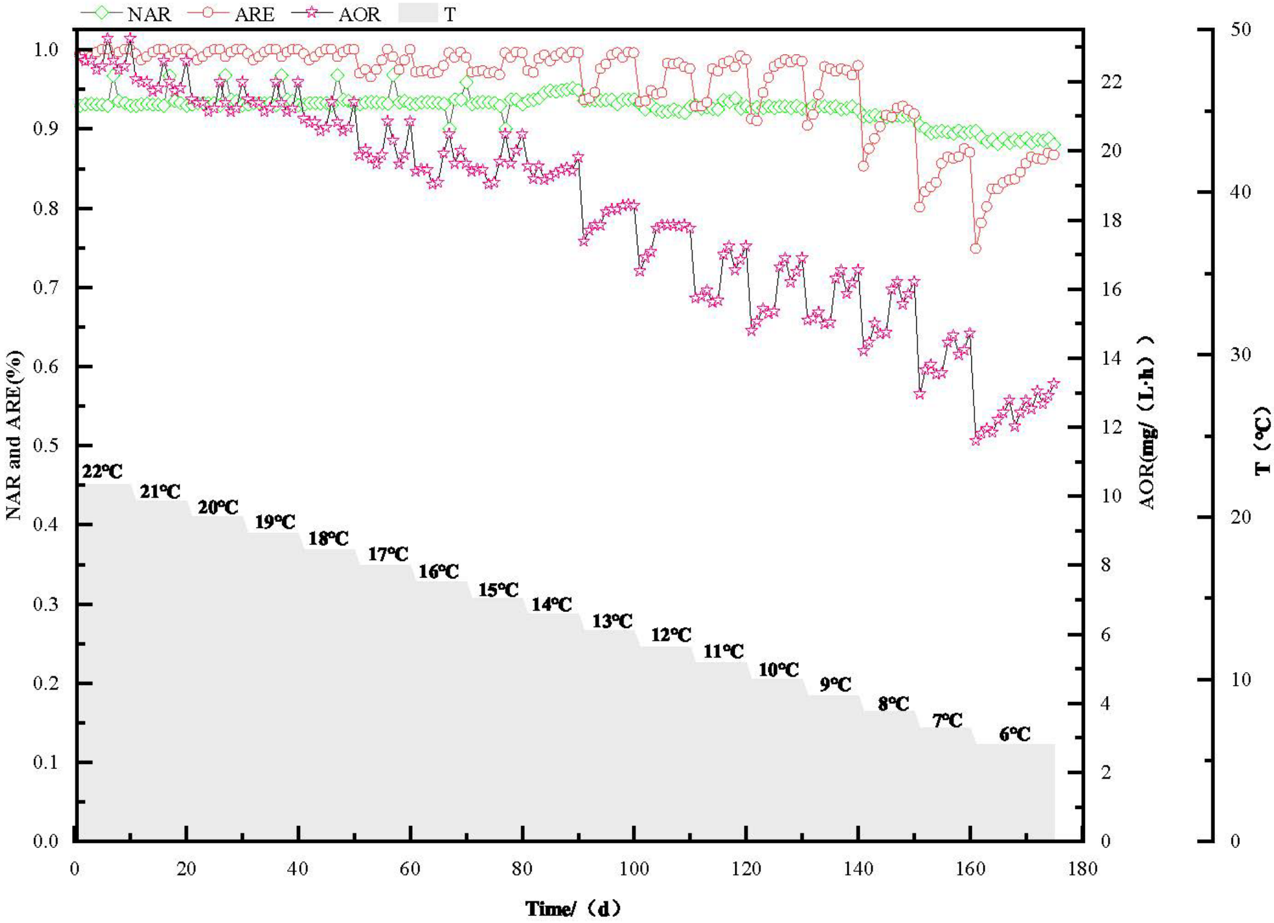


Figure 2. Long-term performance of the partial nitrification reactor.

From 22˚C to 14˚C, ARE remained above 97.6% and NAR was consistently higher than 90%, with a slight upward trend as temperature decreased. Low temperature strengthened the inhibition of NOB and intensified DO gradients inside the carriers, which further limited NOB activity [13] [14].

From 14˚C to 8˚C, ARE decreased instantaneously after each cooling step but recovered within 1 - 2 HRT by adjusting DO and appropriately extending HRT. Throughout the fluctuation, NAR remained above 93%, indicating that the partial nitrification pathway was highly stable. The high biomass in the carriers provided strong functional redundancy and buffer capacity, preventing performance collapse.

When the temperature dropped below 8˚C, ARE decreased significantly to 86.2% at 6˚C, while NAR still remained above 88%. This indicated that the metabolic rate of AOB became the main limiting factor, whereas the partial nitrification pathway remained intact. Therefore, 8˚C was determined as the critical inflection point of the system.

3.2. Performance of the Denitrification Reactor

The denitrification reactor was the most temperature-sensitive unit and the rate-limiting step of the integrated system (Figure 3).

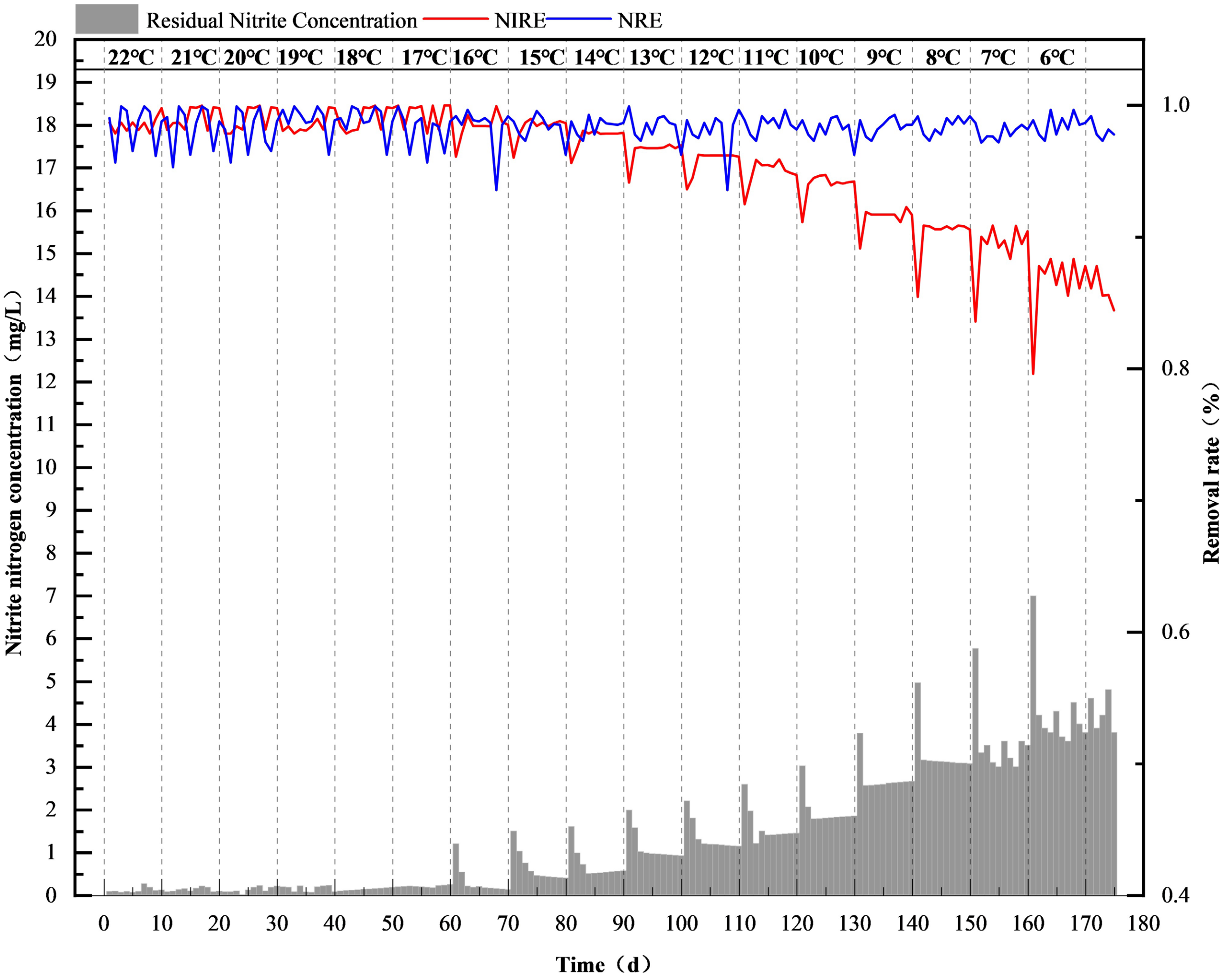


Figure 3. Long-term performance of the denitrification reactor.

At 22˚C, NIRE exceeded 98%. When the temperature decreased to 10˚C, denitrification activity declined sharply. Even with extended HRT, NIRE could only be maintained at approximately 85%, accompanied by obvious nitrite accumulation. Low temperature strongly inhibited nitrite reductase and aggravated mass transfer resistance of carbon sources into the inner biofilm [15] [16].

Despite intensified low-temperature stress, the system recovery time remained stable at 1 - 2 HRT. The carriers provided high biomass storage and a stable microenvironment, enabling rapid functional recovery.

3.3. Overall Performance of the Coupled System

The overall performance of the coupled system is displayed in **Figure 4**.

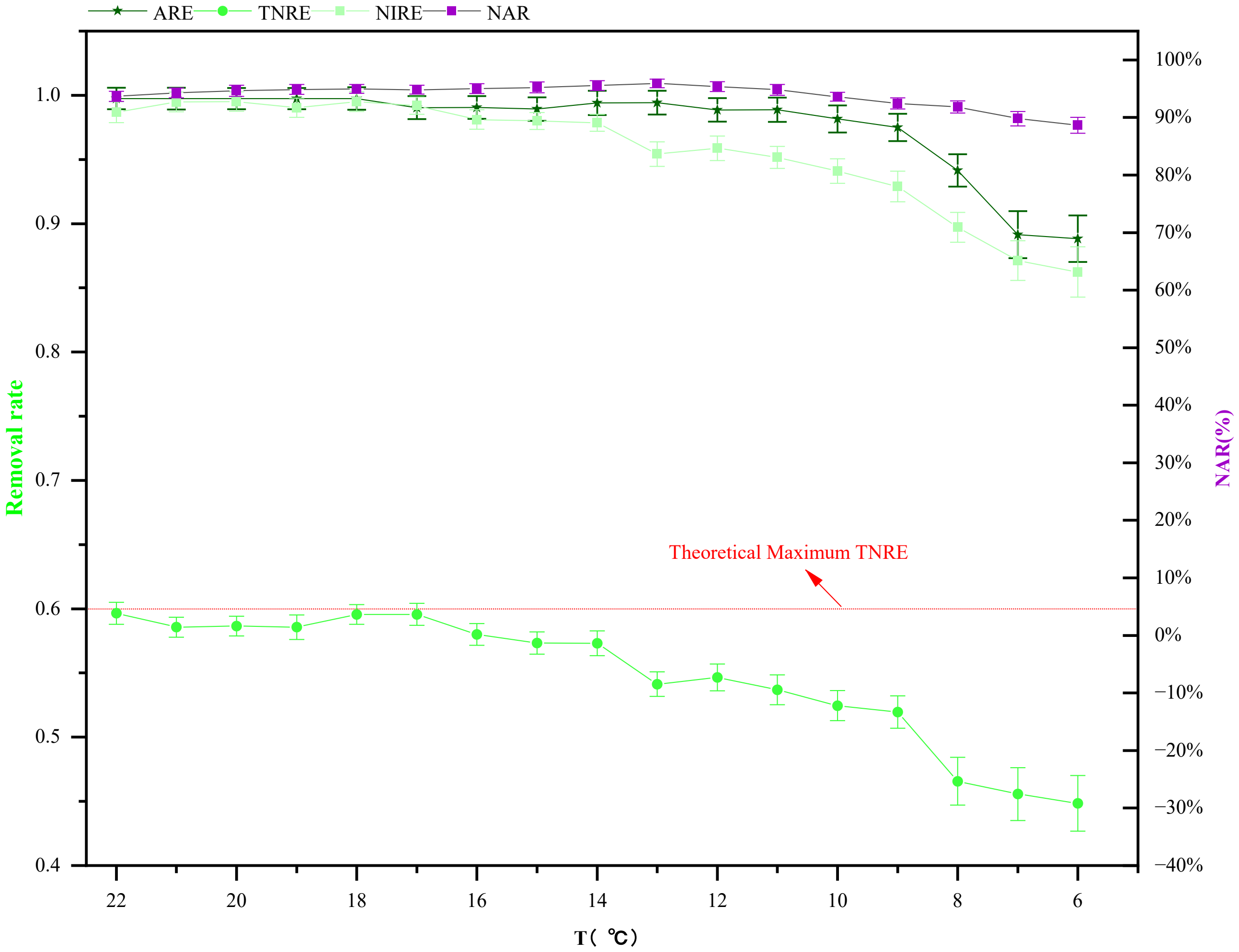


Figure 4. Overall performance of the coupled system.

Above 14˚C, TNRE was stable at 55% - 58%, close to the theoretical maximum of 60% under the fixed reflux ratio. Below 14˚C, TNRE declined synchronously with NIRE, indicating that the system shifted from hydraulic limitation to biological limitation.

Throughout the cooling process, NAR remained above 90%, demonstrating that the partial nitrification function maintained excellent robustness. The deterioration of total nitrogen removal was mainly caused by the decline of denitrification efficiency rather than the failure of partial nitrification [17] [18].

3.4. Dynamic Response and Pathway Stability

The dynamic response of the system during the transition from 14˚C to 13˚C is shown in Figure 5.

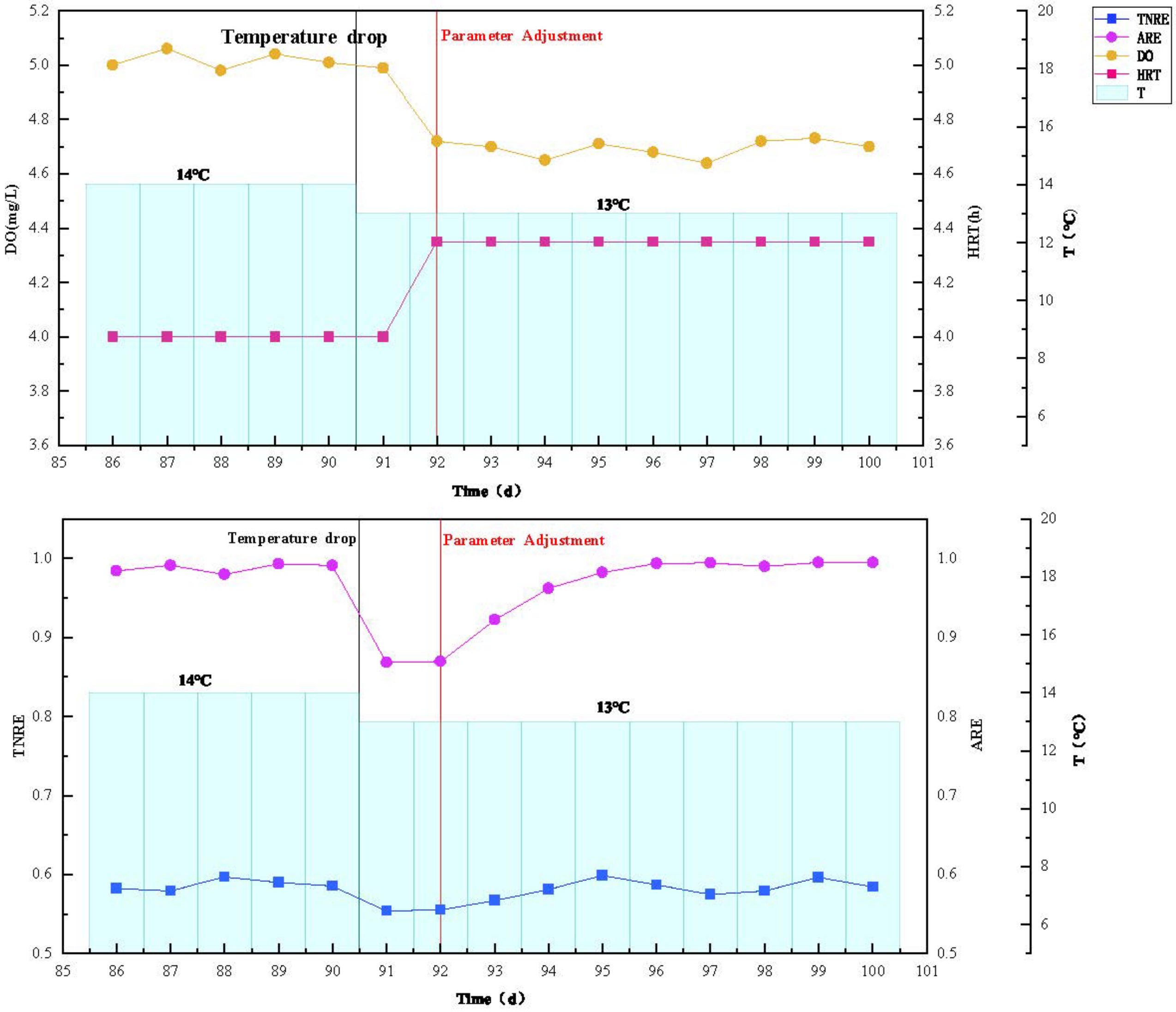


Figure 5. Dynamic response of the system during the transition from 14˚C to 13˚C.

Temperature shock immediately caused a decrease in ARE and TNRE, but the system recovered rapidly through the coordinated regulation of DO and HRT.

Batch experiments at representative temperatures are illustrated in Figure 6.

The results confirmed that low temperature mainly reduced microbial reaction rates without changing the nitrogen conversion pathway. Even at 6˚C, nitrite was still the dominant product, and nitrate production was negligible, verifying the strong pathway robustness of the immobilised carrier system [19] [20].

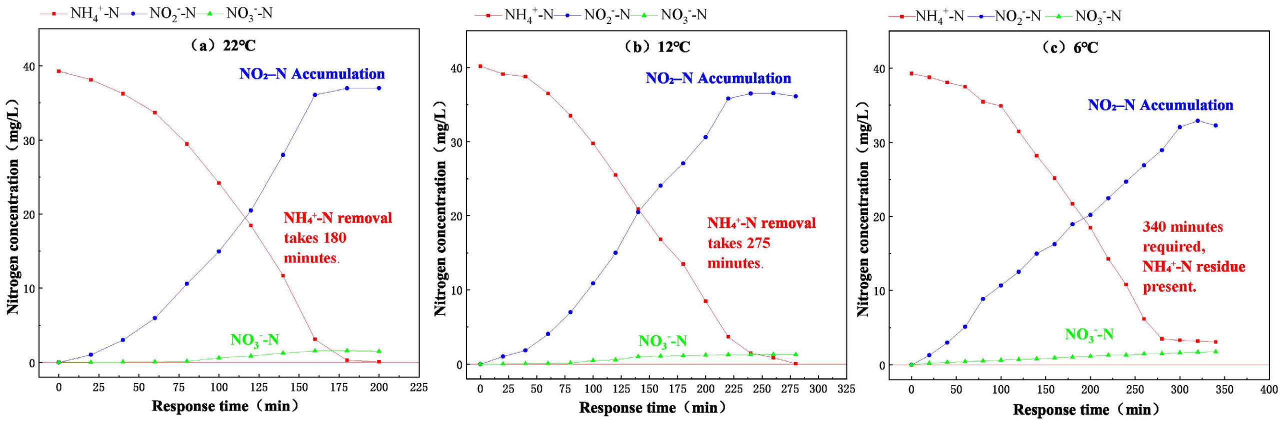
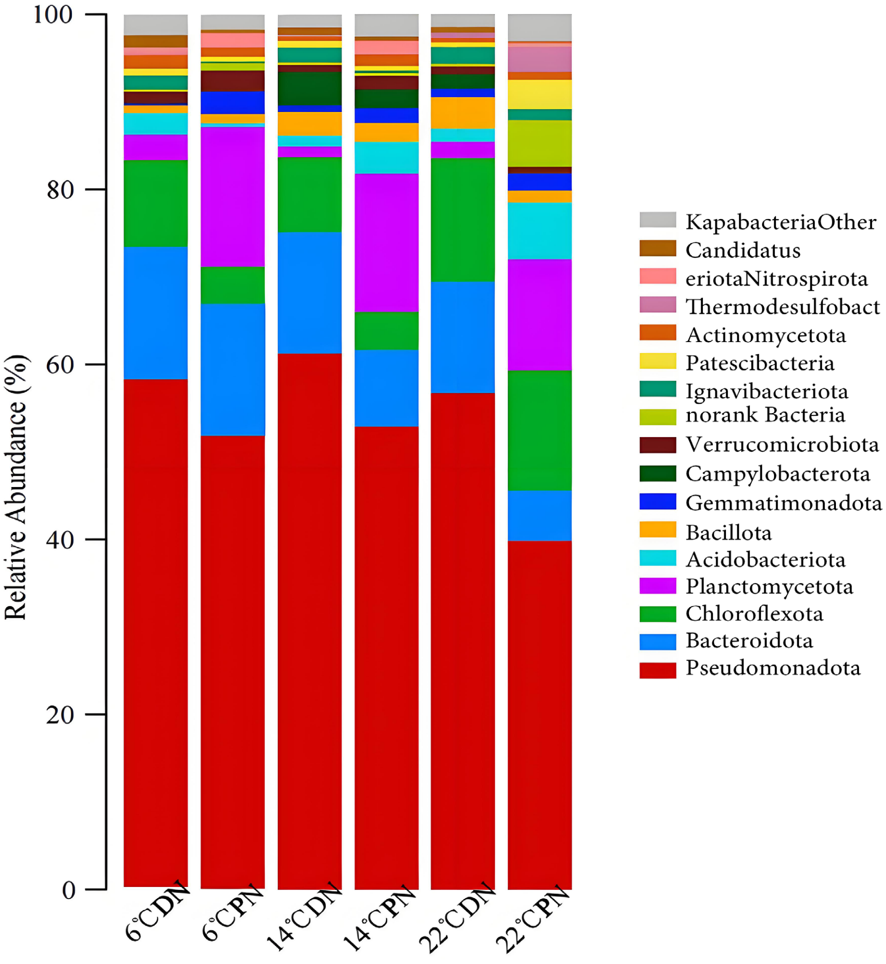


Figure 6. Nitrogen transformation pathways in batch experiments at 22˚C, 12˚C, and 6˚C.

3.5. Microbial Community Succession and Adaptation

Microbial community composition at phylum and genus levels is shown in **Figure 7**.



(a)

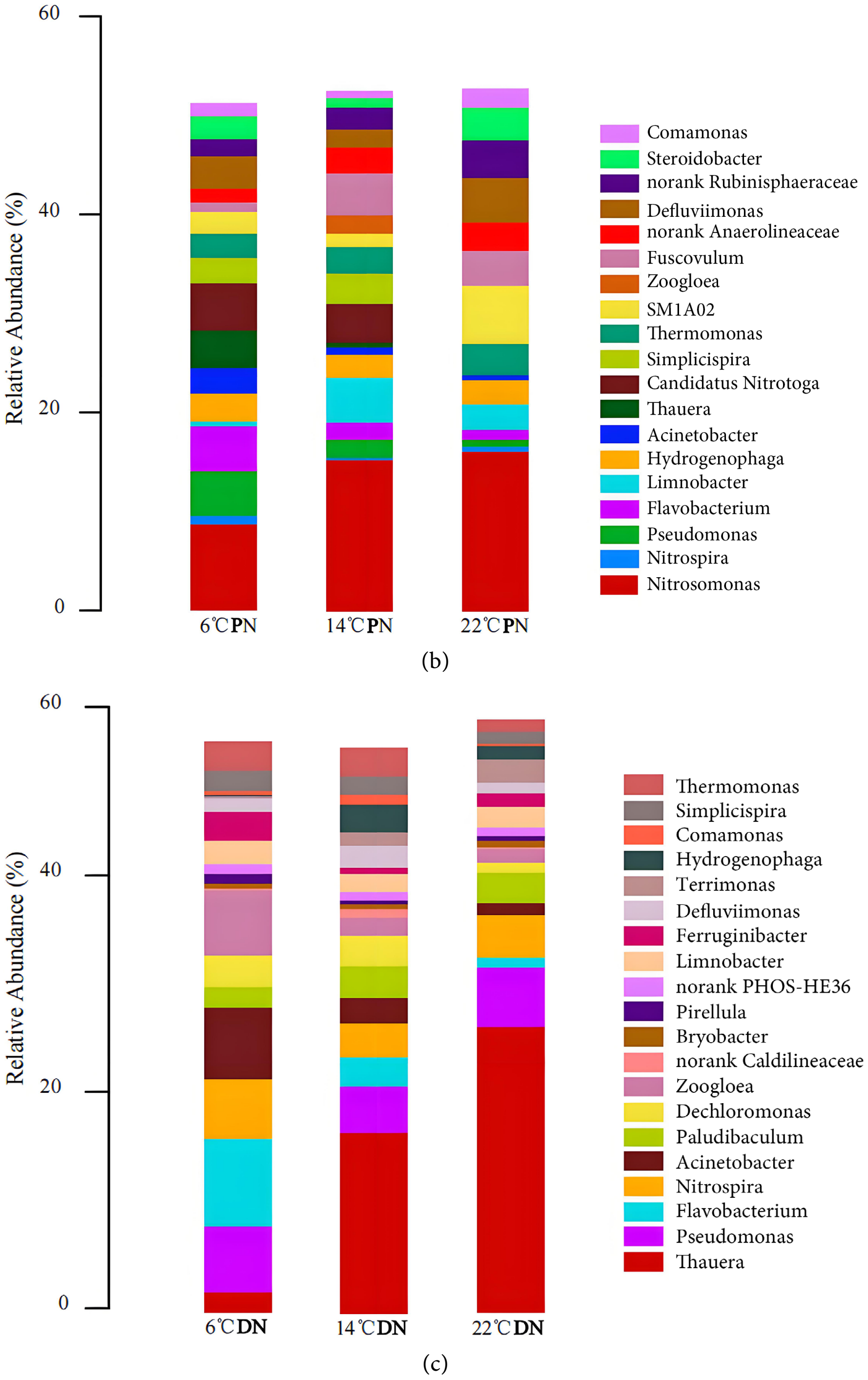


Figure 7. (a) Phylum-level community composition in both reactors at 22˚C, 14˚C, and 6˚C. PN: Partial Nitrification; DN: Denitrification. (b) Relative abundance of key genera in the partial nitrification reactor (only genera with relative abundance >1% in at least one sample are shown). PN: Partial Nitrification; DN: Denitrification. (c) Relative abundance of key genera in the denitrification reactor (only genera with relative abundance > 1% in at least one sample are shown). PN: Partial Nitrification; DN: Denitrification.

At the phylum level, Proteobacteria and Bacteroidetes were dominant. Proteobacteria remained stable, supporting the persistence of core nitrogen removal functions. Bacteroidetes increased at low temperatures, facilitating carbon source utilisation and stress resistance [21] [22].

At the genus level:

- In the partial nitrification reactor, Nitrosomonas (AOB) was stable above 14˚C but decreased below 8˚C. Nitrospira (NOB) was strongly inhibited. Psychrotolerant genera such as Flavobacterium and Pseudomonas were significantly enriched.
- In the denitrification reactor, Thauera and Pseudomonas dominated at 22˚C but decreased markedly at low temperatures. Cold-adapted Flavobacterium and versatile Acinetobacter became dominant, forming a low-temperature-adapted community.

These observations suggest that the immobilised carrier may have functioned as a microbial refuge, potentially facilitating gradual community succession under low-temperature stress and contributing to the avoidance of sudden functional collapse [21] [23].

3.6. Low-Temperature Performance Potential

Supplementary experiments showed that extending HRT to 8.5 h (partial nitrification) and 9.6 h (denitrification) at 6˚C achieved nearly complete nitrogen removal. Low temperature reduced reaction rates but did not destroy microbial activity. The total required HRT was still significantly shorter than that of conventional activated sludge systems under the same conditions, highlighting the superior advantages of the immobilised carrier system [24]-[27]. It should be noted that the comparison with conventional activated sludge systems drawn here is indirect, as a parallel control reactor was not operated simultaneously under identical conditions. The advantage of the immobilised carrier system is therefore inferred from literature-reported HRT requirements for comparable low-temperature nitrogen removal rather than from direct side-by-side experimentation [26] [27].

4. Conclusions

1. The critical temperature inflection point of the immobilised carrier partial nitrification-denitrification system was 8˚C. Above 8˚C, TNRE could approach the theoretical maximum by adjusting HRT and DO.

2. The denitrification reactor was the rate-limiting step under low-temperature conditions, and its temperature sensitivity determined the lower boundary of system performance.

3. Temperature exerted a dual-phase effect on partial nitrification: at ≥8˚C, low temperature enhanced the competitive advantage of AOB and inhibited NOB; below 8˚C, the metabolic rate of AOB became the limiting factor, but the partial nitrification pathway remained stable.

4. Microbial community analysis verified that NOB was effectively inhibited and psychrotolerant genera were enriched under low temperatures, which was

consistent with macroscopic performance.

5. The immobilised carrier system possessed excellent biomass retention and microenvironmental buffering effects, exhibiting strong resilience under low-temperature stress and high feasibility for engineering application in cold regions.

Conflicts of Interest

The authors declare no conflicts of interest.

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