water

MDPI

Article

Partial Nitrification Characteristics of an Immobilized Carrier in Municipal Wastewater under Low-Temperature Shock: The Role of the Nitrifying Bacterial Community Structure

Jiawei Wang [1], Lixinrui Yang [1], Yan Zhang [1], Haiping Zhang [1] and Jiaju Liu [2,3,*]

[1] Hebei Key Laboratory of Water Quality Engineering and Comprehensive Utilization of Water Resources, Hebei University of Architecture, Zhangjiakou 075000, China
[2] Research Center for Integrated Control of Watershed Water Pollution, Chinese Academy of Environmental Sciences, Beijing 100012, China
[3] College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China
* Correspondence: ljj19910213@pku.edu.cn; Tel.: +86-139-0322-5989

Abstract: To analyze the effects of the nitrifying bacterial community structure on the partial nitrification (PN) characteristics in a PN-immobilized carrier in municipal wastewater under low-temperature shock, two PN-immobilized carriers with different nitrifying bacterial communities were investigated. The E1-immobilized carrier contained a high abundance of ammonia-oxidizing bacteria (AOB; 38.59%), and the E2-immobilized carrier had a low AOB abundance of 4.78%. The results of experiments with different dissolved oxygen (DO) concentrations showed that the oxygen-limited environment inside the immobilized carrier, generated by the high AOB abundance, was critical for achieving PN. The nitrite accumulation rate (NAR) decreased from 90.0–93.9% to 84.2–88.3% for the E1-immobilized carrier and from 86.0–90.4% to 81.7–85.8% for the E2-immobilized carrier under low-temperature shock (the temperature suddenly decreased from 25 ± 1 °C to 15 ± 1 °C). The decrease in the ammonia oxidation rate due to the decreased AOB activity led to a decrease in NAR. Moreover, NOB abundance in the E2-immobilized carrier increased because of the destruction of the oxygen-limiting region in the immobilized carrier due to the low AOB abundance. Increasing the abundance of AOB in the PN-immobilized carrier could reduce the adverse effects from the low-temperature shock. The results of this study can be used to further develop immobilization technology for efficient PN in mainstream wastewater treatment.

Keywords: immobilized carrier; low-temperature shock; municipal wastewater; nitrifying bacteria structure; partial nitrification

check for updates

Citation: Wang, J.; Yang, L.; Zhang, Y.; Zhang, H.; Liu, J. Partial Nitrification Characteristics of an Immobilized Carrier in Municipal Wastewater under Low-Temperature Shock: The Role of the Nitrifying Bacterial Community Structure. *Water* **2023**, *15*, 1714. https://doi.org/10.3390/w15091714

Academic Editor: Christos S. Akratos

Received: 29 March 2023
Revised: 20 April 2023
Accepted: 25 April 2023
Published: 28 April 2023

1. Introduction

The process of biological denitrification around anaerobic ammonium oxidation (Anammox) is an efficient and energy-saving process that can replace traditional nitrification and denitrification in mainstream wastewater treatment [1,2]. However, the widespread use of anammox in the treatment of municipal wastewater is limited because of the lack of substrate NO_2^--N [3–5]. Partial nitrification (PN, NH_4^+-N→NO_2^--N) can achieve a stable NO_2^--N output by maintaining the activity of ammonia-oxidizing bacteria (AOB) while inhibiting that of nitrite-oxidizing bacteria (NOB) during nitrification [6,7]. Stable PN under municipal wastewater conditions has been achieved via the control of dissolved oxygen (DO) and free ammonia/free nitrite acid (FA/FNA) levels in side-stream suppression in activated sludge systems [8–11]. However, these approaches have low efficiency and are complex, and further research is needed before large-scale application.

Recently, scientists reported that immobilization technology can effectively promote the application of PN in wastewater treatment [12–15]. Compared with the traditional activated sludge system, the DO gradient in the immobilized carrier can effectively inhibit

NOB activity without affecting AOB activity, thereby achieving stable PN [16,17]. It is assumed that the formation of an oxygen-limiting environment in immobilized carriers is the key to achieving PN [16,18]. The growth and metabolism of nitrifying bacteria in immobilized carriers will consume DO, thus affecting the formation of an oxygen-limited environment. This indicates that nitrifying bacteria in immobilized carriers can greatly affect PN.

Physical or chemical methods have been employed to immobilize bacteria [19]. Physical immobilization involves cell adsorption and attachment. However, for chemical methods, the cells are fixed into a matrix using irreversible covalent bonds, so chemically immobilized cells are not easily released from the matrix. Many raw materials and synthetic polymers, such as sodium alginate, polyacrylamide, agar, and polyvinyl alcohol (PVA), have been extensively applied in immobilization [20]. However, synthetic polymers such as PVA can provide the advantages of higher mechanical strength and chemical resistance. Therefore, when immobilizing nitrifying bacteria, chemical immobilization using PVA as the carrier can effectively maintain the stability of the immobilized carrier under aeration conditions.

Although some studies have shown that immobilized carriers can achieve PN in municipal wastewater [17,21], the components of municipal wastewater are complex. Partial nitrification is not only affected by the structure of the nitrifying bacterial community but also by temperature [22,23] and organic matter [24,25]. At low temperatures, the growth rate difference between AOB and NOB decreases, especially in the case of a sudden temperature drop, which is unfavorable for the maintenance of PN [26]. When the influent contains organic matter, on the one hand, the heterotrophic bacteria in the immobilized carrier will compete with nitrifying bacteria for DO, thereby affecting PN [25]. On the other hand, when the environment is anoxic, the denitrifying bacteria will use the organic matter in the influent for denitrification, which also affects PN. In this context, analyzing the effects of the nitrifying bacterial community structure and low temperatures on the PN characteristics of municipal wastewater can effectively promote the practical application of immobilization technology used for municipal wastewater.

In this study, using polyvinyl alcohol (PVA) as a carrier, the PN characteristics of two PN-immobilized carriers with different nitrifying bacterial community structures in municipal wastewater were investigated using a continuous flow reactor. The objectives were as follows: (1) to investigate the effects of the nitrifying bacterial community structure on the PN characteristics in an immobilized carrier with synthetic and municipal wastewater; (2) to compare the PN characteristics and organic matter removal performance of immobilized carriers with different nitrifying bacteria under low-temperature shock; and (3) to study the effects of low temperatures on the microbial community structure of the immobilized carrier in municipal wastewater. The results can be used to further develop immobilization technology for efficient PN in mainstream wastewater treatment.

2. Materials and Methods

2.1. Preparation of the Partial-Nitrification-Immobilized Carrier with Different Nitrifying Bacterial Communities

The PN-immobilized carriers with different nitrifying bacterial communities were prepared separately from our previous studies [17,18]. According to the determination of the ammonia oxidation rate of activated sludge at different stages of the partial nitrification sludge culture, inoculated sludge with different AOB abundances can be obtained. The immobilized carrier was a cylinder with a size of 15 mm in diameter, 5 mm in height, and 1–2 mm in thickness. The E1-immobilized carrier was a PN-immobilized carrier with a high AOB abundance (*Nitrosomonas*, belonging to AOB, accounted for 38.59% of the total bacteria), and the E2-immobilized carrier was a PN-immobilized carrier with a low AOB abundance (*Nitrosomonas* accounted for 4.78% of the total bacteria). The production processes and biomass amounts of the two immobilized carriers were similar.

2.2. Partial-Nitrification-Immobilized Carrier Reactor Operation

Two lab-scale reactors (Figure 1) with a working volume of 15 L each were operated at a filling rate of 10% (v/v). The temperature was controlled at 25 ± 1 °C and 15 ± 1 °C, respectively, using a temperature probe (WTW, Munich, Germany) in conjunction with a heat exchanger to heat the water via a programmable logic controller (PLC). The DO concentration was controlled using a DO probe (WTW, Munich, Germany) in conjunction with an OFF/ON air pump via a PLC.

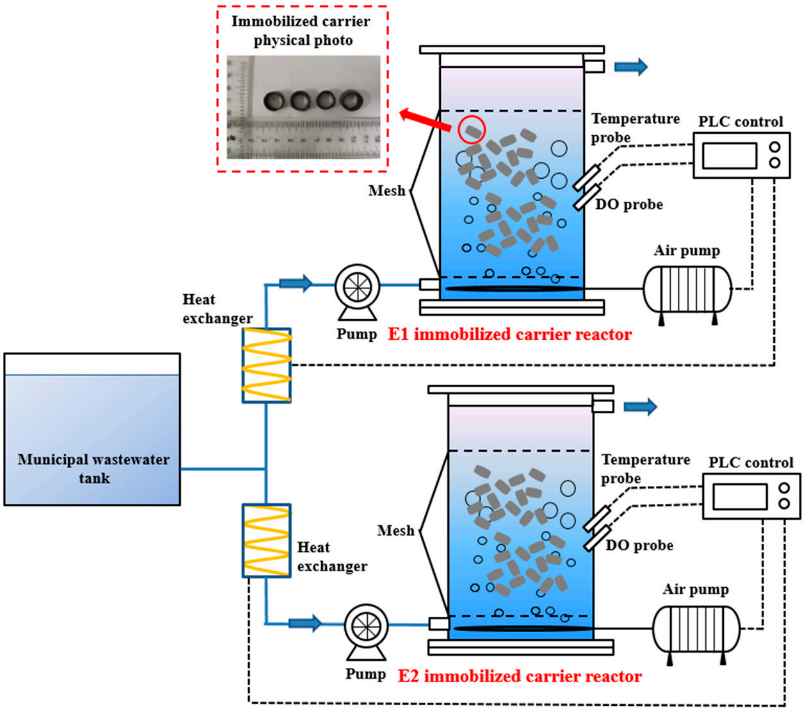

Figure 1. Schematic representation of the immobilized carrier reactor.

2.2.1. Batch Experiments

Batch experiments were divided into synthetic wastewater and municipal wastewater batch tests. Synthetic wastewater batch tests were conducted to investigate the effects of the nitrifying bacterial community structure on the activities of AOB and NOB. Approximately 100 mg/L NH_4^+-N was added to the reactor, with aeration for 2.0 h at DO levels of 3, 4, 5, and 6 mg/L. The temperature was 25 ± 1 °C, and the pH was adjusted to 7.6–7.8 using a PLC equipped with a pH probe and pH buffer. The NH_4^+-N and NO_2^--N levels were determined every 20 min.

Municipal wastewater batch tests were conducted to investigate the PN characteristics of the immobilized carriers. At DO levels of 3, 4, 5, and 6 mg/L, the municipal wastewater was added to the reactor with aeration for 1.5 and 3.0 h at 25 and 15 °C, respectively. The ammonia oxidation rate (AOR) and the nitrite oxidation rate (NOR), as well as the COD and TN removal rates of E1- and E2-immobilized carriers, were determined. The optimal DO concentration in the continuous flow condition was determined according to the test results.

2.2.2. Low-Temperature Shock Experiment

After the batch experiments, the low-temperature shock was run for 60 days. The initial temperature was 25 ± 1 °C, with a hydraulic retention time (HRT) of 1.5 h from days 1 to 30, followed by 15 ± 1 °C, with an HRT of 3.0 h from days 31 to 60.

2.3. Wastewater Characteristics

The municipal wastewater used in this study was collected from the effluent of the primary sedimentation tank of the Gao Bei Dian municipal wastewater treatment plant (Beijing, China).

2.4. Analytical Methods

2.4.1. Wastewater Quality Analysis

The concentrations of COD, total nitrogen (TN), NH_4^+-N, NO_2^--N, and NO_3^--N were analyzed according to the APHA standard methods [25].

2.4.2. High-Throughput Sequencing

The Illumina MiSeq high-throughput sequencing platform (Illumina, San Diego, CA, USA) was used to analyze the microbial community composition of the initial E1-immobilized carrier (E1a) and the initial E2-immobilized carrier (E2a), as well as the E1-immobilized carrier (E1b) and E2-immobilized carrier (E2b) after the operation. The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified with the primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC). The obtained bacterial 16S rRNA gene sequences were compared with those in the National Center for Biotechnology Information (NCBI) database. Using the MEGAN software, the 16S rRNA gene sequences were analyzed and classified according to a certain threshold to obtain operational taxonomic units (OTUs). Diversity analysis was performed according to the OTUs, and, finally, the results were visualized.

2.5. Calculations

The nitrite accumulation ratio (NAR) was calculated as follows:

$$\mathrm{NAR} = \frac{\mathrm{NO}^-_{2,eff} - \mathrm{NO}^-_{2,\mathrm{inf}}}{\left(\mathrm{NO}^-_{2,eff} - \mathrm{NO}^-_{2,\mathrm{inf}}\right) + \left(\mathrm{NO}^-_{3,eff} - \mathrm{NO}^-_{3,\mathrm{inf}}\right)} \times 100\%,$$

where $\mathrm{NO}^-_{2,\mathrm{inf}}$ and $\mathrm{NO}^-_{2,eff}$ are the NO_2^- concentrations of the influent and the effluent, respectively, and $\mathrm{NO}^-_{3,eff}$ and $\mathrm{NO}^-_{3,\mathrm{inf}}$ are the NO_3^- concentrations of the effluent and the influent, respectively.

The ammonia oxidation rate (AOR) was calculated as follows:

$$\mathrm{AOR} = \frac{\mathrm{NH}^+_{4,start} - \mathrm{NH}^+_{4,end}}{\mathrm{T}} \times 100\%$$

where $\mathrm{NH}^+_{4,start}$ and $\mathrm{NH}^+_{4,end}$ are the NH_4^+-N concentrations at the beginning and end of the batch experiments, respectively, and T is the duration of the batch experiment.

The nitrite oxidation rate (NOR) was calculated using the following equation:

$$\mathrm{NOR} = \frac{\mathrm{NO}^-_{3,end} - \mathrm{NO}^-_{3,start}}{\mathrm{T}} \times 100\%$$

where $\mathrm{NO}^-_{3,start}$ and $\mathrm{NO}^-_{3,end}$ are the NO_3^--N concentrations at the beginning and end of the batch experiments, respectively, and T is the duration of the batch experiment.

3. Results and Discussion

3.1. Effects of the Nitrifying Bacterial Community Structure on Partial Nitrification in the Immobilized Carrier

At an influent NH_4^+-N concentration of approximately 100 mg/L and a temperature of 25 °C, the changes in the NH_4^+-N and NO_3^--N concentrations in E1- and E2-immobilized carriers under different DO concentrations are shown in Figure 2. The changes in NH_4^+-N and NO_3^--N concentrations reflected the activity changes of AOB and NOB, respectively, in the immobilized carrier. As shown in Figure 2a, when the DO concentration increased from 3 to 4 and 5 mg/L, the NH_4^+-N removal rates of the E1- and E2-immobilized carriers increased. This indicates that increasing the DO concentration in the reactor could increase the activity of AOB in the immobilized carrier. However, when the DO concentration reached 6 mg/L, the NH_4^+-N removal rate of the E1- and E2-immobilized carriers did not increase further, suggesting that there is a threshold for the influence of DO on AOB activity. In addition, at the same DO level, the NH_4^+-N removal rate of the E1-immobilized carrier was higher than that of the E2-immobilized carrier, indicating that the AOB abundance in the immobilized carrier directly affected its ammonia oxidation performance. This leads us to infer that increasing the concentration of DO in the reactor and the abundance of AOB in the immobilized carrier can effectively increase AOB activity.

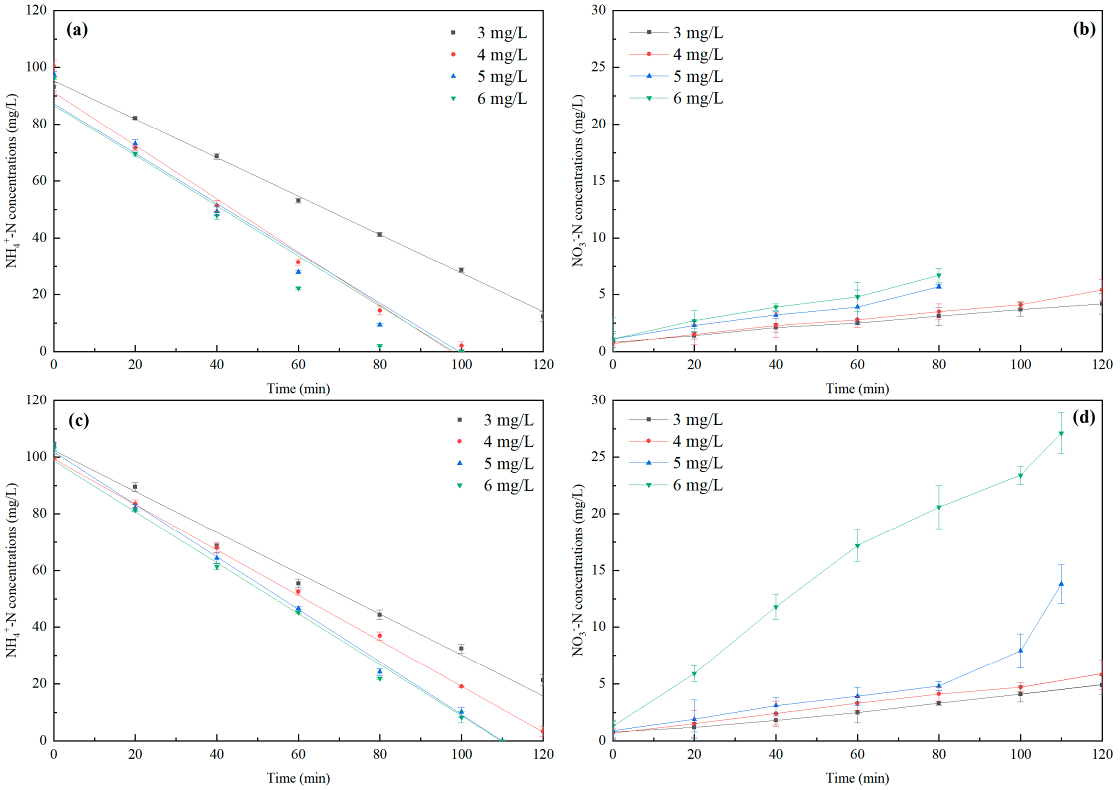

Figure 2. Performance of partial nitrification at different DO levels in synthetic wastewater: NH_4^+-N concentration changes in the E1-immobilized carrier (**a**) and the E2-immobilized carrier (**c**); NO_3^--N concentration changes in the E1-immobilized carrier (**b**) and E2-immobilized carrier (**d**).

Figure 2b,d show the changes in the NOB activity in E1- and E2-immobilized carriers under different DO concentrations. With increasing DO concentration, the NO_3^--N production in the E1-immobilized carrier did not increase significantly, indicating that the NOB activity in this carrier did not increase. This can be explained by the increase in the DO concentration, along with the increased oxygen consumption of AOB in the E1-immobilized carrier. Therefore, the mass transfer gradient of DO in the immobilized carrier and the considerable AOB oxygen consumption formed an oxygen-limited environment, which effectively inhibited NOB activity. Kunapongkiti et al. [13] found that during the operation of the nitrification-immobilized carrier, AOB was observed at the 10–230 μm layer of the gel matrix, preventing DO from reaching the inner area of the gel matrix, which also indicated that the AOB of the immobilized carrier played an important role in the formation of an oxygen-limited environment. As shown in Figure 2d, the NO_3^--N production of the E2-immobilized carrier at a DO concentration of 6 mg/L was 27.1 ± 1.8 mg/L, which surpassed the nitrate production at DO concentrations of 3, 4, and 5 mg/L (4.9 ± 0.8, 5.8 ± 1.3, and 13.8 ± 1.7 mg/L). This was due to the low oxygen consumption because of the low abundance of AOB in the E2-immobilized carrier, which could not maintain the oxygen-limited environment. Figure 2a,c also show that at the same DO level, the AOB activity in the E1-immobilized carrier was higher than that in the E2-immobilized carrier. Therefore, increasing the AOB abundance in the immobilized carrier facilitated the creation of an oxygen-limited environment, thereby improving the effect of PN.

Figure 3a,b show the changes in the ammonia oxidation rate (AOR) and nitrite oxidation rate (NOR) of E1- and E2-immobilized carriers under different DO concentrations in municipal wastewater at 25 and 15 °C. The AOR of the E1- and E2-immobilized carriers at 25 °C was lower in municipal than in simulated wastewater, indicating that the AOB activity in the PN-immobilized carrier was affected by the low ammonia nitrogen and organic matter levels in municipal wastewater. However, when municipal wastewater was used, the AOR of the E1-immobilized carrier was higher than that of the E2-immobilized carrier under different DO conditions, indicating that increasing the AOB abundance of the PN-immobilized carrier could effectively improve PN efficiency. In addition, when the DO concentration of the E2-immobilized carrier was 6 mg/L in municipal wastewater, the environment was no longer oxygen-limited, which resulted in a sudden increase in NOR from 2.6 ± 0.3 to 5.8 ± 0.2 mg/(L·h). When the municipal wastewater temperature was reduced to 15 °C, the AOR levels of the E1- and E2-immobilized carriers were reduced by 31.2 ± 1.5% and 34.2 ± 4.1%, respectively. This indicated that increasing the AOB abundance in the PN-immobilized carrier cannot effectively mitigate the effect of low temperature on AOB activity. However, the NOR of the E2-immobilized carrier increased from 2.3 ± 0.4 to 2.6 ± 0.2 mg/(L·h) when the DO concentration increased from 5 to 6 mg/L when the temperature was reduced to 15 °C. This may be due to the low NOB activity in the PN-immobilized carrier because of the low temperature. Even if the DO concentration was high due to the destruction of the oxygen-limited environment in PN-immobilized carriers, the activity of NOB could not be effectively improved. According to the above phenomenon, the DO concentration of the E1- and E2-immobilized carriers was controlled at 5 mg/L under continuous flow conditions.

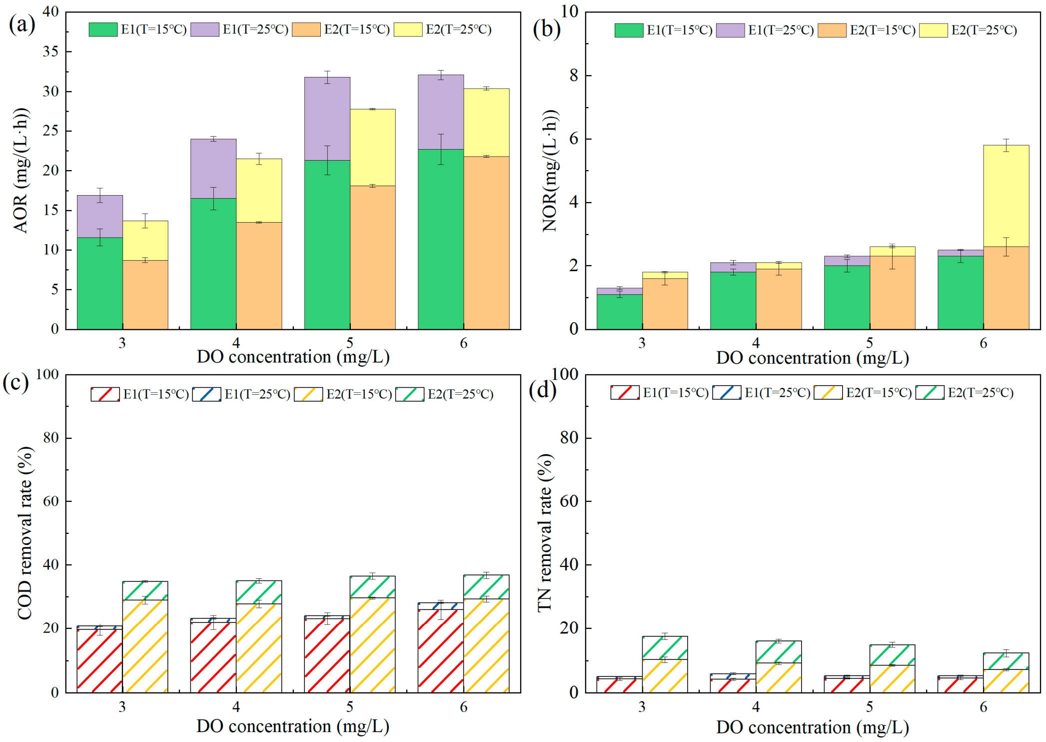

Figure 3. Performance of the E1- and E2-immobilized carriers at 25 and 15 °C in municipal wastewater: (**a**) AOR; (**b**) NOR; (**c**) COD removal; (**d**) TN removal.

The removal of COD and TN from municipal wastewater by both carrier types is shown in Figure 3c,d. Under municipal wastewater, both carriers showed COD and TN removal, with the E2-immobilized carrier having a greater removal effect, most likely because of the high abundances of denitrifying and heterotrophic bacteria in the E2-immobilized carrier (Section 3.3). In the E2-immobilized carrier, the TN removal rate decreased with increasing DO concentration, which indicated that higher DO levels inhibit the denitrification in this carrier. In addition, TN removal was also observed in the E1-immobilized carrier under municipal wastewater, which indicated that COD could promote simultaneous nitrification and denitrification in PN carriers. Figure 3c,d show that the influence of low temperature on the removal rates of COD and TN in the E2-immobilized carrier was greater than that of the E1-immobilized carrier. This was because the activity of denitrifying bacteria decreased when the temperature decreased [27–29], whereas the denitrifying bacteria in the E1 carrier had a lower proportion and a weaker activity. Therefore, the decrease in temperature had a greater impact on the TN removal rate of the E2-immobilized carrier.

3.2. Effects of Low-Temperature Shock on Partial Nitrification

3.2.1. Pollutant Removal Performance and Partial Nitrification Characteristics

Figure 4a shows the PN characteristics of municipal wastewater under a low-temperature shock for E1- and E2-immobilized carriers. When the temperature of the municipal wastewater was 25 ± 1 °C, the NH_4^+-N concentrations of both carriers were below 1.5 and 5.0 mg/L, respectively, and the NAR levels were 90.0–93.9% and 86.0–90.4%, respectively. When the temperature of the municipal wastewater suddenly dropped to 15 ± 1 °C, both carriers maintained an ammonia effluent level above the standard by extending the HRT to 3.0 h, but the NAR levels of both carriers decreased to 84.2–88.3% and 81.7–85.8%. Jones et al. [30] assumed that AOB are inactivated when they are directly transferred from normal to low-temperature conditions. However, with a gradual decrease in temperature, AOB could adjust the types of fatty acids in their cell membranes, partly converting saturated long-chain fatty acids into short-chain unsaturated fatty acids to prevent "freezing". Therefore, when the temperature dropped suddenly, the AOB in the PN-immobilized carrier were poorly adapted to the low-temperature environment, which led to a significant decrease in their activity and, in turn, affected the oxygen-limited environment inside the immobilized carrier, thereby decreasing the NAR. The above phenomenon shows that although the low-temperature shock could lead to a decrease in the NAR of the PN-immobilized carrier, increasing the AOB abundance in the PN-immobilized carrier could effectively increase the NAR, thus maintaining a high NAR. In addition, increasing the AOB abundance in the PN-immobilized carriers could effectively reduce the effluent NH_4^+-N concentration to meet more stringent discharge standards.

Figure 4b,c show the COD and TN removal characteristics of both carriers under a low-temperature shock. After the low-temperature shock, the COD and TN removal rates of the E2-immobilized carrier decreased more than those of the E1-immobilized carrier, which was due to the lower abundance of nitrifying bacteria and the higher abundance of heterotrophic and denitrifying bacteria in the E2-immobilized carrier. At the lower temperature, the activities of heterotrophic and denitrifying bacteria were more affected than those of autotrophic bacteria. However, the abundances of heterotrophic and denitrifying bacteria in the E1-immobilized carrier were low, which decreased the effect of the low temperature on the COD and TN removal rates.

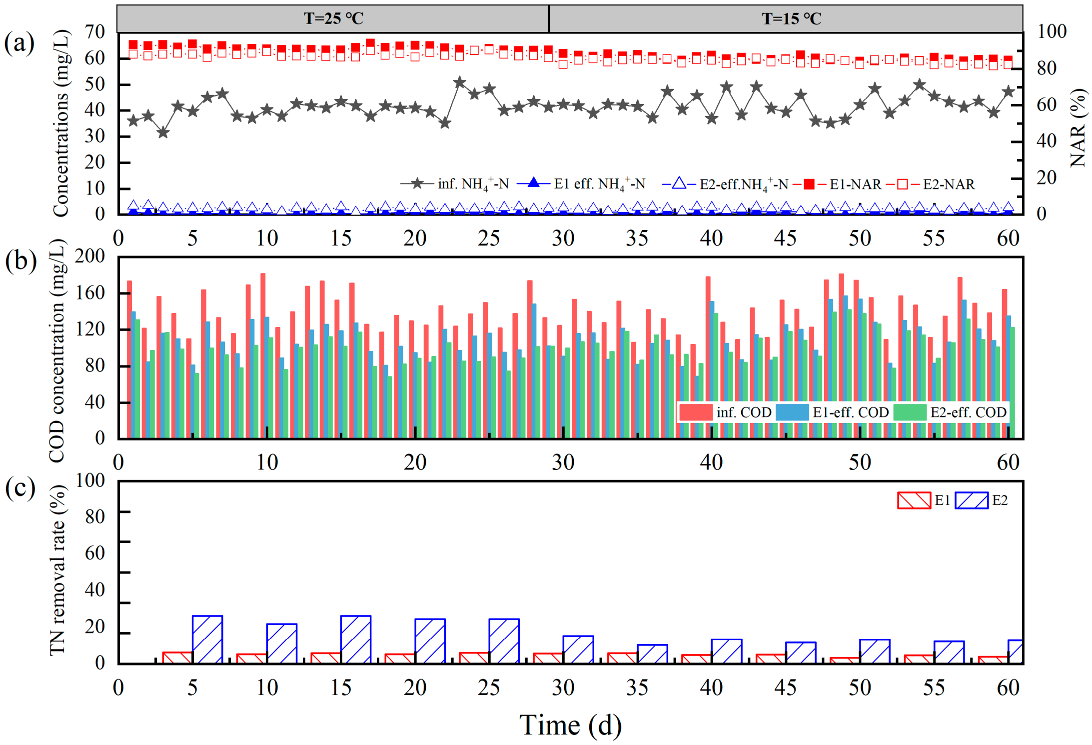

Figure 4. Partial nitrification characteristics and pollutant removal performance of the E1- and E2-immobilized carriers: (**a**) influent and effluent NH_4^+-N, NAR; (**b**) influent and effluent COD; (**c**) TN removal rate.

3.2.2. Typical Operating Cycle Tests

Figure 5a,b show the PN processes of the E1- and E2-immobilized carriers in municipal wastewater at a DO concentration of 5 mg/L and a temperature of 25 °C. Within 0–30 min, the NH_4^+-N concentration in the E1- and E2-immobilized carriers decreased from 47.7 ± 4.6 to 40.5 ± 2.7 and 43.5 ± 2.1 mg/L, respectively, whereas the NO_3^--N level increased from 0.5 ± 0.3 to 1.1 ± 0.8 and 1.6 ± 0.5 mg/L, respectively. The COD concentration decreased from 144.5 ± 7.5 to 119.7 ± 8.0 and 110.8 ± 6.2 mg/L, respectively, whereas the TN concentration remained constant. The NH_4^+-N degradation rates and the NO_3^--N generation rates of the E1- and E2-immobilized carriers in the first 30 min were significantly lower than those observed from 60 to 90 min, whereas the COD degradation rate was high in the first 30 min. This indicates the preferential removal of organics from municipal wastewater in the immobilized carrier. In municipal wastewater, the rapidly biodegradable organic matter (dissolved COD) accounts for 10–30% of the COD of the effluent [31]. When rapidly biodegradable organic matter is present, the heterotrophic bacteria have a stronger oxygen competing ability than the nitrifying bacteria [32]. Therefore, at the early stage of the reactor operation, the DO concentration in the immobilized carrier decreased due to the large consumption of DO by the heterotrophic bacteria. On the one hand, the ammonia degradation rate by AOB was reduced, but, at the same time, the inhibition effect of NOB in the oxygen-limited environment of the immobilized carrier was also promoted. Since the rapidly biodegradable organic matter in municipal wastewater was removed, and most of the remaining organic matter was slowly biodegradable organic matter, the ability of heterotrophic bacteria to degrade organic matter decreased; consequently, the recovery of nitrifying bacterial activity led to an increased ammonia removal rate. This phenomenon indicated that increasing the abundance of AOB in PN-immobilized carriers could effectively increase the activity of AOB in the later stage of the nitrification process, thereby increasing the ammonia removal rate. In addition, the decrease in TN concentration was observed at 60–90 min, indicating that the COD of municipal wastewater was removed by heterotrophic bacteria in the early stage but by denitrifying bacteria in the later stage.

The removal of COD and TN in the E2-immobilized carrier was higher than that in the E1-immobilized carrier from 0 to 90 min, mainly because the denitrification efficiency of the E2-immobilized carrier was higher than that of the E1-immobilized carrier during this period.

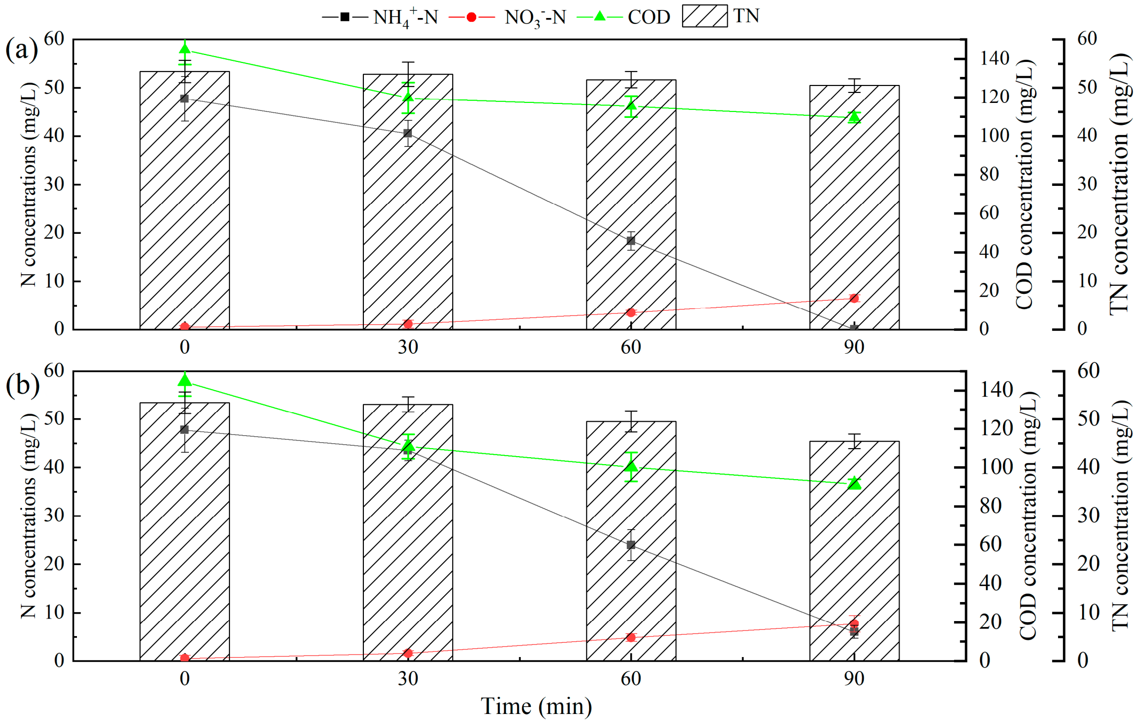

Figure 5. Variations in NH_4^+-N, NO_3^--N, TN, and COD during a typical cycle of the E1-immobilized carrier (**a**) and the E2-immobilized carrier (**b**) at 25 °C.

3.3. Microbial Community Structure of the Partial-Nitrification-Immobilized Carrier under Low-Temperature Shock

To investigate the changes in the microbial community structure in both carriers after the low-temperature shock, the microbial structure at the genus level was examined (Figure 6). *Nitrosomonas*, affiliated with AOB, is the main genus in municipal wastewater treatment plants, although *Nitrospira* and *Nitrobacter*, affiliated with NOB, can also be found [33,34]. After the low-temperature shock, the relative abundance of *Nitrosomonas* in the E2-immobilized carrier increased from 4.78% to 5.03%, whereas that of *Nitrosomonas* in the E1-immobilized carrier decreased from 38.59% to 15.91%. The relative abundances of *Nitrospira* and *Nitrobacter* in the E2-immobilized carrier increased from below the detection limit and 2.15% to 0.01% and 4.01%, respectively, whereas those of *Nitrospira* and *Nitrobacter* in the E1-immobilized carrier decreased from 0.01% and 0.11% to below the detection limit and 0.04%, respectively. Although it is widely believed that the ability of nitrifying bacteria to reproduce is inhibited at low temperatures [35], the above phenomena indicate that PN-immobilized carriers with nitrifying bacteria undergo different changes in bacterial relative abundance after a low-temperature shock. Some studies found that low temperatures do not reduce the NAR of PN reactors [22,23], but, in this study, the low-temperature shock reduced the NAR of the PN carriers. Most likely, this occurred because of a decrease in AOB abundance, which led to a weakened oxygen-limited environment in the E1-immobilized carrier, whereas an increase in NOB abundance led to a higher NOB activity in the E2-immobilized carrier.

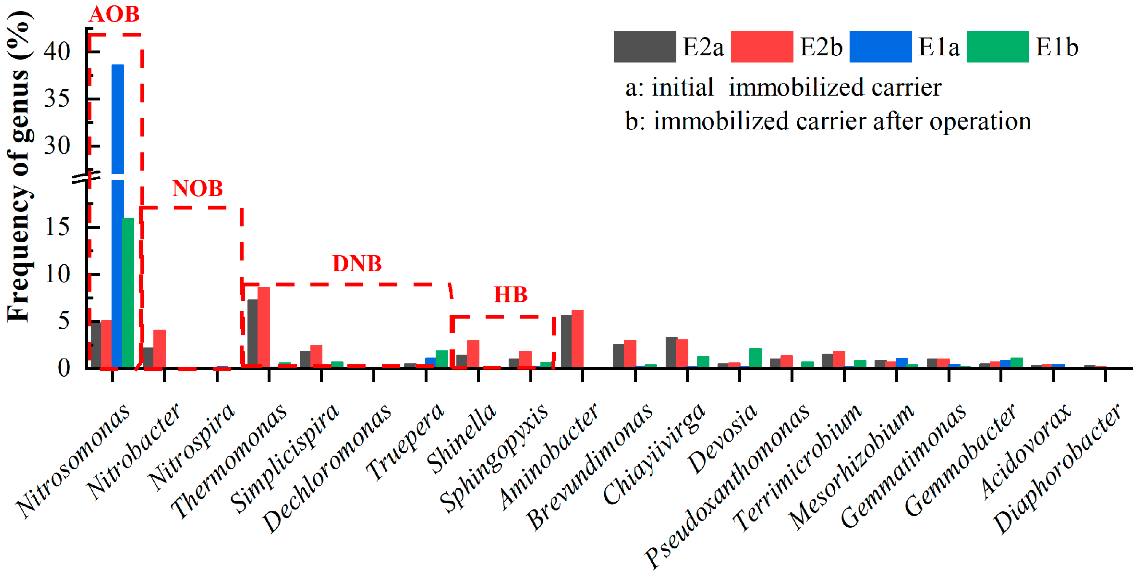

Figure 6. Diversity of the microbial communities from the E1- and E2-immobilized carriers at genus level.

Heterotrophic bacteria (HB) and denitrifying bacteria (DNB) are also functional bacteria that affect PN [36]. *Shinella* [37] and *Sphingopyxis* [38] are considered HB as they can degrade organic matter. The proportions of these two types of HB in the E1- and E2-immobilized carriers increased during the operation in municipal wastewater, indicating that organic matter in the municipal wastewater promoted the growth of HB. The abundance of DNB in the E2-immobilized carrier was higher than that in the E1-immobilized carrier, before and after the operation, which was the main reason for the higher COD and TN removal rates of the E2-immobilized carrier in municipal wastewater. Notably, *Thermomonas* is considered DNB that perform partial denitrification (PDN, NO_3^--N→NO_2^--N) [39]. The relative abundance of *Thermomonas* in the E2-immobilized carrier increased from 7.24% to 8.54%, indicating that the reduction of NO_3^--N to NO_2^--N via PDN may have contributed to NO_2^--N accumulation. In addition, the abundance of *Dechloromonas* [40], a denitrifying phosphorus-accumulating organism, in the E1-immobilized carrier increased, indicating that the reduction of NO_3^--N may also have contributed to NO_2^--N accumulation.

4. Conclusions

This study identified the key role of the nitrifying bacterial community structure in PN-immobilized municipal wastewater under a low-temperature shock. Increasing the abundance of AOB in the immobilized carrier to promote the formation of an oxygen-limited environment is the key to achieving high NAR levels. Since the low-temperature shock had a greater effect on the activity of AOB than on the activity of NOB, the decrease in AOR was greater than that in NOR under the low-temperature shock, resulting in a decrease in NAR of the PN-immobilized carrier. However, increasing the abundance of AOB in the PN-immobilized carrier could reduce the adverse effects of the low-temperature shock on PN. The results of the microbial diversity analysis showed that the low-temperature shock had different effects on the nitrifying bacterial community structure in the two PN-immobilized carriers. The high relative AOB abundance of the E1-immobilized carrier decreased, whereas the relative NOB abundance of the E2-immobilized carrier with a low AOB abundance increased after the low-temperature shock. The results of this study promote the use of immobilization technology for efficient PN in mainstream wastewater treatment.

Author Contributions: Methodology, J.W. and J.L.; software, L.Y.; data curation, Y.Z.; writing—original draft preparation, H.Z.; writing—review and editing, J.W. and J.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Science and Technology Project of Hebei Education Department, grant numbers BJK2023052, and the Natural Science Foundation of Heilongjiang Province of China, grant number LH2022E110.

Data Availability Statement: All the data have been provided in the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nsenga Kumwimba, M.; Lotti, T.; Senel, E.; Li, X.; Suanon, F. Anammox-based processes: How far have we come and what work remains? A review by bibliometric analysis. *Chemosphere* **2020**, *238*, 124627. [CrossRef] [PubMed]
2. Deng, S.; Peng, Y.; Zhang, L.; Li, W. Advanced nitrogen removal from municipal wastewater via two-stage partial nitrification-simultaneous anammox and denitrification (PN-SAD) process. *Bioresour. Technol.* **2020**, *304*, 122955. [CrossRef] [PubMed]
3. Ma, B.; Wang, S.; Cao, S.; Miao, Y.; Jia, F.; Du, R.; Peng, Y. Biological nitrogen removal from sewage via anammox: Recent advances. *Bioresour. Technol.* **2016**, *200*, 981–990. [CrossRef] [PubMed]
4. Du, R.; Cao, S.; Zhang, H.; Li, X.; Peng, Y. Flexible Nitrite Supply Alternative for Mainstream Anammox: Advances in Enhancing Process Stability. *Environ. Sci. Technol.* **2020**, *54*, 6353–6364. [CrossRef] [PubMed]
5. Jin, P.; Li, B.; Mu, D.; Li, X.; Peng, Y. High-efficient nitrogen removal from municipal wastewater via two-stage nitritation/anammox process: Long-term stability assessment and mechanism analysis. *Bioresour. Technol.* **2019**, *271*, 150–158. [CrossRef] [PubMed]
6. Wang, J.L.; Yang, N. Partial nitrification under limited dissolved oxygen conditions. *Process Biochem.* **2004**, *39*, 1223–1229.
7. Sinha, B.; Annachhatre, A.P. Partial nitrification—Operational parameters and microorganisms involved. *Rev. Environ. Sci. Bio/Technol.* **2007**, *6*, 285–313. [CrossRef]
8. Miao, Y.; Zhang, L.; Yang, Y.; Peng, Y.; Li, B.; Wang, S.; Zhang, Q. Start-up of single-stage partial nitrification-anammox process treating low-strength swage and its restoration from nitrate accumulation. *Bioresour. Technol.* **2016**, *218*, 771–779. [CrossRef]
9. Bian, W.; Zhang, S.; Zhang, Y.; Li, W.; Kan, R.; Wang, W.; Zheng, Z.; Li, J. Achieving nitritation in a continuous moving bed biofilm reactor at different temperatures through ratio control. *Bioresour. Technol.* **2017**, *226*, 73–79. [CrossRef]
10. Wang, Q.; Duan, H.; Wei, W.; Ni, B.; Laloo, A.; Yuan, Z. Achieving Stable Mainstream Nitrogen Removal via the Nitrite Pathway by Sludge Treatment Using Free Ammonia. *Environ. Sci. Technol.* **2017**, *51*, 9800–9807. [CrossRef]
11. Duan, H.; Ye, L.; Lu, X.; Yuan, Z. Overcoming Nitrite Oxidizing Bacteria Adaptation through Alternating Sludge Treatment with Free Nitrous Acid and Free Ammonia. *Environ. Sci. Technol.* **2019**, *53*, 1937–1946. [CrossRef] [PubMed]
12. Isaka, K.; Kimura, Y.; Matsuura, M.; Osaka, T.; Tsuneda, S. First full-scale nitritation-anammox plant using gel entrapment technology for ammonia plant effluent. *Biochem. Eng. J.* **2017**, *122*, 115–122. [CrossRef]
13. Kunapongkiti, P.; Limpiyakorn, T.; Sonthiphand, P.; Rongsayamanont, C. Partial nitrification in entrapped-cell-based reactors with two different cell-to-matrix ratios: Performance, microenvironment, and microbial community. *J. Environ. Sci. Health Part A* **2019**, *54*, 874–883. [CrossRef] [PubMed]
14. Kunapongkiti, P.; Rongsayamanont, C.; Nayramitsattha, P.; Limpiyakorn, T. Application of cell immobilization technology to promote nitritation: A review. *Environ. Eng. Res.* **2020**, *25*, 807–818. [CrossRef]
15. Liu, C.; Yu, D.; Wang, Y.; Chen, G.; Tang, P.; Huang, S. A novel control strategy for the partial nitrification and anammox process (PN/A) of immobilized particles: Using salinity as a factor. *Bioresour. Technol.* **2020**, *302*, 122864. [CrossRef]
16. Rongsayamanont, C.; Limpiyakorn, T.; Khan, E. Effects of inoculum type and bulk dissolved oxygen concentration on achieving partial nitrification by entrapped-cell-based reactors. *Bioresour. Technol.* **2014**, *164*, 254–263. [CrossRef]
17. Wang, J.; Yang, H.; Liu, X.; Wang, J.; Chang, J. The impact of temperature and dissolved oxygen (DO) on the partial nitrification of immobilized fillers, and application in municipal wastewater. *RSC Adv.* **2020**, *1*, 33721–37194. [CrossRef]
18. Wang, J.; Yang, H.; Zhang, F.; Su, Y.; Wang, S. Activated sludge under free ammonia treatment using gel immobilization technology for long-term partial nitrification with different initial biomass. *Process Biochem.* **2020**, *99*, 282–289. [CrossRef]
19. Le, C.; Wu, J.; Li, P.; Wang, X.; Zhu, N.; Wu, P.; Yang, B. Decolorization of anthraquinone dye Reactive Blue 19 by the combination of persulfate and zero-valent iron. *Water Sci. Technol.* **2011**, *3*, 64. [CrossRef]
20. Lin, Q.; Ding, J.; Yang, Y.; Sun, F.; Shen, C.; Lin, H.; Su, X. Simultaneous adsorption and biodegradation of polychlorinated biphenyls using resuscitated strain Streptococcus sp. SPC0 immobilized in polyvinyl alcohol-sodium alginate. *Sci. Total Environ.* **2023**, *868*, 161620. [CrossRef]
21. Wang, J.; Zhang, S.; Li, J.; Yang, H. Municipal wastewater treatment via the two-stage partial nitrification-anammox (PN/A) process with gel immobilization. *J. Water Process Eng.* **2022**, *50*, 103267. [CrossRef]
22. Gu, S.; Wang, S.; Yang, Q.; Yang, P.; Peng, Y. Start up partial nitrification at low temperature with a real-time control strategy based on blower frequency and pH. *Bioresour. Technol.* **2012**, *112*, 34–41. [CrossRef] [PubMed]

23. Guo, J.; Peng, Y.; Huang, H.; Wang, S.; Ge, S.; Zhang, J.; Wang, Z. Short and long-term effects of temperature on partial nitrification in a sequencing batch reactor treating domestic wastewater. *J. Hazard. Mater.* **2010**, *179*, 471–479. [CrossRef] [PubMed]
24. Bao, R.; Yu, S.; Shi, W.; Zhang, X.; Wang, Y. Aerobic granules formation and nutrients removal characteristics in sequencing batch airlift reactor (SBAR) at low temperature. *J. Hazard. Mater.* **2009**, *168*, 1334–1340. [CrossRef] [PubMed]
25. Bassin, J.P.; Abbas, B.; Vilela, C.L.S.; Kleerebezem, R.; Muyzer, G.; Rosado, A.S.; van Loosdrecht, M.C.M.; Dezotti, M. Tracking the dynamics of heterotrophs and nitrifiers in moving-bed biofilm reactors operated at different COD/N ratios. *Bioresour. Technol.* **2015**, *192*, 131–141. [CrossRef] [PubMed]
26. Paredes, D.; Kuschk, P.; Mbwette, T.S.A.; Stange, F.; Müller, R.A.; Köser, H. New Aspects of Microbial Nitrogen Transformations in the Context of Wastewater Treatment—A Review. *Eng. Life Sci.* **2007**, *7*, 13–25. [CrossRef]
27. APHA. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association: Washington, DC, USA, 2005.
28. Banerjee, A.; Elefsiniotis, P.; Tuhtar, D. The effect of addition of potato-processing wastewater on the acidogenesis of primary sludge under varied hydraulic retention time and temperature. *J. Biotechnol.* **1999**, *72*, 203–212. [CrossRef]
29. Maharaj, I.; Elefsiniotis, P. The role of HRT and low temperature on the acid-phase anaerobic digestion of municipal and industrial wastewaters. *J. Biotechnol.* **2001**, *3*, 191–197. [CrossRef]
30. Jone, D.; Morit, Y.; Koops, H. A new marine ammonium-oxidizing bacterium, *Nitrosomonas cryotolerans* Sp. nov. *Can. J. Microbiol.* **1988**, *10*, 1122–1128. [CrossRef]
31. Cao, Y.; van Loosdrecht, M.C.M.; Daigger, G.T. Mainstream partial nitritation–anammox in municipal wastewater treatment: Status, bottlenecks, and further studies. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 1365–1383. [CrossRef]
32. Perez, J.; Lotti, T.; Kleerebezem, R.; Picioreanu, C.; van Loosdrecht, M.C. Outcompeting nitrite-oxidizing bacteria in single-stage nitrogen removal in sewage treatment plants: A model-based study. *Water Res.* **2014**, *66*, 208–218. [CrossRef] [PubMed]
33. Ge, S.; Wang, S.; Yang, X.; Qiu, S.; Li, B.; Peng, Y. Detection of nitrifiers and evaluation of partial nitrification for wastewater treatment: A review. *Chemosphere* **2015**, *140*, 85–98. [CrossRef] [PubMed]
34. Xu, H.; Wang, C.; Liang, Z.; He, L.; Wu, W. The structure and component characteristics of partial nitrification biofilms under autotrophic and heterotrophic conditions. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 3673–3683. [CrossRef] [PubMed]
35. Li, X.; Lu, M.; Huang, Y.; Yuan, Y.; Yuan, Y. Influence of seasonal temperature change on autotrophic nitrogen removal for mature landfill leachate treatment with high-ammonia by partial nitrification-Anammox process. *J. Environ. Sci.* **2021**, *102*, 291–300. [CrossRef]
36. Liu, X.; Kim, M.; Nakhla, G.; Andalib, M.; Fang, Y. Partial nitrification-reactor configurations, and operational conditions: Performance analysis. *J. Environ. Chem. Eng.* **2020**, *8*, 103984. [CrossRef]
37. Hailin Tian, I.A.F.E.; Mercedes Ballesteros, C.G.I.A. Acclimation to extremely high ammonia levels in continuous biomethanation process and the associated microbial community dynamics. *Bioresour. Technol.* **2018**, *247*, 616–623. [CrossRef]
38. Mavriou, Z.; Alexandropoulou, I.; Melidis, P.; Karpouzas, D.; Ntougias, S. Biotreatment and bacterial succession in an upflow immobilized cell bioreactor fed with fludioxonil wastewater. *Environ. Sci. Pollut. Res. Int.* **2021**, *28*, 3774–3786. [CrossRef]
39. Mcilroy, S.J.; Starnawska, A.; Starnawski, P.; Saunders, A.M.; Nierychlo, M.; Nielsen, P.H.; Nielsen, J.L. Identification of active denitrifiers in full-scale nutrient removal wastewater treatment systems. *Environ. Microbiol.* **2016**, *18*, 50–64. [CrossRef]
40. Zhao, W.; Bi, X.; Peng, Y.; Bai, M. Research advances of the phosphorus-accumulating organisms of Candidatus Accumulibacter, Dechloromonas and Tetrasphaera: Metabolic mechanisms, applications and influencing factors. *Chemosphere* **2022**, *307*, 135675. [CrossRef]