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Nitrogen removal from an AAO pilot plant with nitrifier bioaugmentation after seasonal deterioration

Liying Pei*, Dangcong Peng, Jilin Wei, Binbin Wang, Xinyan Zhang, Lifang Yu

Key Lab of Water Resources, Environment and Ecology in Northwest China, Xi'an University of Architecture and Technology, Xi'an 710055, P.R. China
Tel. +86 2982201354; Fax: +86 2982201354; email: l.y.pei@163.com

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ABSTRACT

Nitrogen removal was studied in a pilot-scale anaerobic–anoxic–oxic (AAO) system that was bioaugmented with nitrifiers cultivated from reject water after seasonal deterioration. The process of nitrogen removal was evaluated with increased temperature from 11 to 24℃. Nitrification efficiency rapidly recovered with increased temperature from 12 to 15℃, and the nitrification rates at 18℃ were 3.1 times that at 12℃, higher than the report in ASM1. Bioaugmentation may shorten the recovery time of nitrification activity in WWTPs. The nitrification activity and microbial ecology of the full-scale system operating parallel with the pilot-scale were correspondingly studied, and similar community structures were observed. Despite the lower nitrifying bacteria count, the nitrification activity of the bioaugmented pilot-scale plant was still higher than that of the full-scale one.

Keywords: Bioaugmentation; Nitrification; Seasonal deterioration; Nitrifying activity; Community structure

1. Introduction

Implementation of stricter effluent standards, particularly regarding nitrogen removal, requires large expansions of WWTPs. The nitrification potential of activated sludge systems becomes considerably less at lower temperatures (<15℃) [1]. A decrease in nitrification rate has often been reported at WWTPs in northern China during the winter months. Reject water produced in sludge processing has an important role in increasing the nitrogen loading in WWTPs. Reject water comprises less than 1% of the total flow [2], but generally accountable for 20% of the total influent ammonia loading [3]. Therefore, both increasing the nitrification efficiency at low temperatures and reducing the nitrogen loading caused by reject water are crucial for effective nitrogen removal at WWTPs.

Bioaugmentation is one of the most favorable technologies for improving the nitrogen removal efficiency of WWTPs. Bioaugmentation consists of adding selected strains of microorganisms, with known capabilities, to a biological process, to improve the process performance [4]. For nitrification enhancement in WWTPs, bioaugmentation can increase the efficiency of nitrification in the biological process by decreasing

*Corresponding author.

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the required aerobic solids retention time (SRT), thereby reducing the working volume devoted to nitrification [5]. As nitrifying bacteria increases, bioaugmentation can maintain the high nitrification capability of the biological system in low temperatures. In addition, bioaugmentation from sidestream also reduces the nitrogen loading of the mainstream system by preventing the reject water from mixing with the mainstream influent.

Many studies have been conducted to evaluate the benefits of bioaugmentation with nitrifying bacteria. Kos [6] has reported that the apparent SRT of a nitrifying wastewater treatment system can be decreased from a range of 13 to 18 d, down to between 7 and 10 d, by nitrifying NH_3 from the centrate from a sidestream and then recycling the excess biomass into the main bioreactors. Rittmann et al. [7] has stated that the apparent SRT can be decreased from 15 to 1.5 d to achieve effluent with NH_4^+-N concentrations less than 1 mg/L, when at least 15 mg/L of active nitrifying biomass was added into the influent stream of a chemostate that was treated with 33 mg NH_4^+-N/L d. Daigger et al. [8] found that nitrification occurred in an aerobic bioreactor tank as a result of sloughing of nitrifying biomass from an upstream trickling filter. Abeysinghe et al. [4] compared the effects of bioaugmentation with 5 d SRT in 4℃ to 2 d SRT in 22℃ in a laboratory, with the application of a bioaugmentation product (INOC 8166) containing highly enriched ammonia-oxidizing bacteria. The experimental results indicated that doses, temperature, and SRT, and each had an impact on the effectiveness of bioaugmentation, of which, temperature had a greater impact than the SRT.

Among all the operational factors affecting nitrification, temperature has the most significant influence on the growth of nitrifying bacteria and on the rate of nitrification [9]. A significant reduction in the rate of nitrification is observed as temperatures decrease, and conversely, a significant acceleration in the rate of nitrification as temperatures increase. Several researches have been carried out on the contemporary effects of temperature and bioaugmentation [1,10–12]. These studies have focused on synthetic municipal wastewater, or *in situ* bioaugmentation or modeling. However, the effects of seasonal deterioration for an *ex situ* bioaugmentation system with real wastewater have not been discussed. The objective of this research was to evaluate the nitrogen removal from a bioaugmented anaerobic–anoxic–oxic (AAO) pilot-scale WWTP after seasonal deterioration due to reduced wastewater temperatures.

2. Materials and methods

2.1. Experimental settings

2.1.1. Mainstream reactor and operations

A pilot-scale AAO reactor with a working volume of 3.7 m^3 was operated as a mainstream treatment system (Fig. 1). The working volumes of the anaerobic, anoxic, and aerobic zones in the AAO system were 0.73, 1.2, and 1.7 m^3, respectively. The sludge recycle ratio of the system was 40%. The aerobic zone was aerated by passing pressurized air through the microporous diffusers. The mixed liquor from the AAO system settled in a sedimentation tank with a total volume of 0.96 m^3. The excess sludge was continuously withdrawn from the aerobic zone. The reactor was installed at the 4th WWTP in Xi'an, China, and was used to treat the effluent of the primary clarifier. The typical characteristics of the influent are shown in Table 1. The experiment can be divided into three stages, with the operation parameters of the influent flow, SRT, and temperature, as shown in Table 2.

2.1.2. Sidestream reactor and operation

A continuous stirred-tank reactor with a working volume of 0.25 m^3 was employed as a sidestream system to treat reject water. The characteristics of the reject water are shown in Table 1. The average carbon to nitrogen (C/N) ratio of the reject water was between 0.32 and 0.70. The reactor was operated at 20℃ when the ambient temperature was below 20℃ and kept at ambient when the temperature was higher than 20℃. The hydraulic retention time (HRT) was controlled at 32 h, and the SRT was maintained at 10 d by pumping 8.3 L of mixed liquor from the sidestream reactor to the mainstream reactor for bioaugmentation, three times a day.

2.1.3. Full-scale system WWTP

The 4th full-scale WWTP was a continuous anoxic/anaerobic/aerobic system for simultaneous C, N, and P removal. The influent flow rate was approximately 250,000 m^3/d, and the pollutant load corresponds to approximately 1,200,000 population equivalents. Industrial sources contribute to about 30% of the total pollutant load. The biological step consists of eight circular secondary clarifiers and four parallel bioreactors. The treated wastewater was directly discharged to the Weihe River, which is part of the Yellow River.

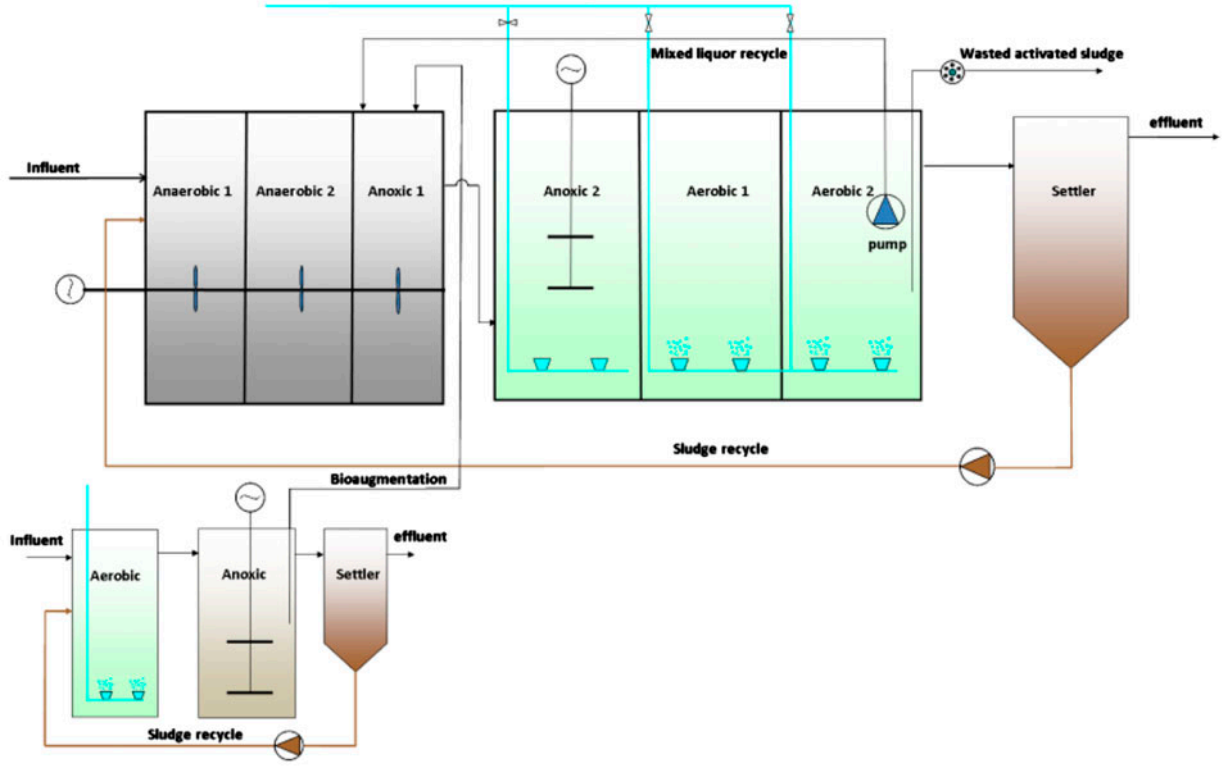

Fig. 1. Schematic of the pilot-scale system.

Table 1
Major characteristics of the influent in the main- and sidestream reactor

Reactors	Mainstream	Sidestream
Temperature, ℃	11–24	20–22
Alkalinity ($CaCO_3$), (mg/L)	230–320	2,458–2,571
pH	6.5–7.5	8.5–9.5
TCOD, mg/L	177–303	386–825
NH_4^+-, mg/L	25–44	321–375
TKN, mg/L	37–49	417–467
TP, mg/L	3.3–5.5	–
PO_4^3—P, mg/L	2.0–4.3	–

Table 2
Operation parameters during three stages of the experiments

Parameters	I	II	IV
Flow, m^3/d	8.4	7.2	9.6
HRT, h	10	12	9
SRT, d	15	20	10
Temperature, ℃	11–15	12–19	18–24

2.2. Nitrification activity measurement

The specific ammonia utilizing rate (SAUR) and specific nitrite utilizing rate (SNUR) (linear correlation coefficient $R^2 > 0.97$) of the activated sludge were determined in batch experiments by measuring the consumption of NH_4^+-N and NO_2^--N at the temperature in line with the reactors. Oxygen concentration was automatically monitored and maintained at approximately 2 to 3 mg/L. The pH value was controlled at 7–8 through the addition of $NaHCO_3$. The average MLVSS of the main-stream was 1803 mg/L, whereas that of the sidestream was 1,270 mg/L.

The initial ammonium and nitrite concentration used for the test was 40 mg/L for the sidestream reactor and 20 mg/L for the mainstream reactor. Samples of 10 mL of mixed liquor were drawn off at 8 min intervals for the sidestream and 15-min interval for mainstream reactor. Eight samples were taken over time.

2.3. FISH analysis

To investigate the relationship of nitrification activity, community structure, and populations, several oligonucleotide probes, targeted for 16SrRNA sequence (NSO1225, Nmv, Nsv443, NIT3, Ntspa662) (Table 3), were used for quantifying the nitrifiers by FISH analysis. Samples were obtained and fixed in 4% paraformaldehyde. Ultrasonification (Vibra cell,

Sonics, USA) was applied to break down large flocs prior to hybridization. All samples were stained with 4′,6-diamidino-2-phenyliddole (DAPI) and the probe selected. The hybridization and washing procedures were carried out according to the method described by Amann et al. [13]. Microscopy was performed using an Olympus BX51, with an Olympus DP72 camera. About 10 to 20 views were obtained of each sample. Image-Pro Plus software was used for counting the target populations in relation to the total microbial population of the sample.

2.4. Physicochemical analyses

All other components, such as COD, MLSS, MLVSS, NH_4^+-N, NO_2^--N, NO_3^--N, and TKN were determined according to standard methods [14]. The dissolved oxygen (DO) and temperature were monitored online by the probe (Hach, USA) that was connected to a data acquisition program.

3. Results and discussion

3.1. Removal of organic compounds at different stages

The removal of COD at different stages is shown in Fig. 2. All three stages indicated that the average effluent COD concentration was maintained at 44 mg/L, which met the discharge standards requirements of < 60 mg/L and the average removal efficiency of COD kept at 80% despite the changes in the parameters, such as SRT, temperature, and influent flow. However, higher effluent COD concentrations were also observed, which were mainly attributed to the high SS concentration in the effluent caused by the denitrification during settlement.

3.2. Removal of NH_4^+-N and nitrification activity

The removal of NH_4^+-N and nitrification activity at different stages are shown in Fig. 3, and some details extracted from the figures are shown in Table 4. The influent concentration of NH_4^+-N during the entire experiment was 35 ± 6 mg/L. However, the average effluent concentration of NH_4^+-N was 18, 9.5, and 1 mg/L and the removal efficiency was 41, 71, and 98% for stages I, II, and III, respectively.

The nitrification activity correlated well with temperature as shown in Fig. 3(b). The SAUR and SNUR were 1.8 mg NH_4^+-N/g VSS h and 1.9 mg NO_2^--N/gVSS h at a temperature of 12℃ in stage II (59 d), as shown in Table 4. The ratio of MLVSS/MLSS in the reactor was 74–76%. When the temperature increased from 12 to 15℃, the MLSS was stable with the same SRT of 20 d. The SAUR and SNUR increased by 40 and 51%, respectively, and the removal efficiency increased from 35 to 84%. The rapid increase in nitrification activity was consistent with the removal efficiency of NH_4^+-N. You et al. have reported that the NH_4^+-N removal efficiency and ammonia-utilizing rate were 64% and 1.43 mg NH_4^+-N/gSS h for an AAO system operated at 10 d SRT and 20℃, which were much lower than the values obtained in the present work [15].

In ASM1, the maximum specific growth rate for autotrophic biomass at 20 and 10℃ are 0.8 and 0.3 d^{-1}, respectively. The former is 2.7 times that of the latter [16]. Head et al. have stated that the percentage increase in nitrification rate is the same as the percentage increase in growth rate [12]. In this work, the nitrification rate increased by 3.1 times when the temperature increased from 12 to 18℃, as shown in Table 4, which was slightly higher than the report in ASM1. Consequently, the potential benefit of bioaugmentation to municipal wastewater treatment is the faster recovery times of nitrification, following a seasonal deterioration, consistent with Smith's findings [17].

3.3. Evaluation of the effects of temperature and bioaugmentation on nitrification rates

The van't Hoff–Arrhenius equation (Eq. (1)) was adopted to describe the dependence of NH_4^+-N and NO_2^--N oxidation kinetics on temperature [18]. The SAUR and SNUR over temperature were analyzed by

Table 3
List of 16S rRNA-targeted oligonucleotide probes used in the study

Probe	Sequence(5′–3′)	Specificity	Concentration[a]
NSO 1225	CGCCATTGTATTACGTGTGA	Ammonia-oxidizing beta-proteobacteria	35
Nmv	TCCTCAGAGACTACGCGG	Nitrosomonas	35
Nsv443	CCGTGACCGTTTCGTTCCG	Nitrosospira	30
NIT 3	CCTGGCTCCATGCTCCG	Nitrobacter	40
Ntspa662	GGAATTCCGCGCTCCTCT	Nitrospira	35

[a]Concentrations presented as percentage of formamide in hybridization buffer.

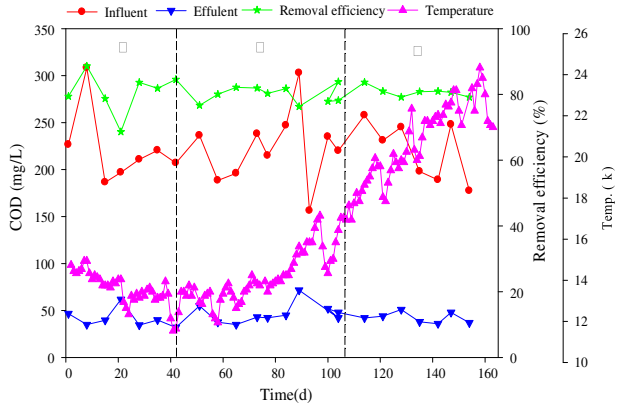

Fig. 2. Variations of COD profiles in each stage.

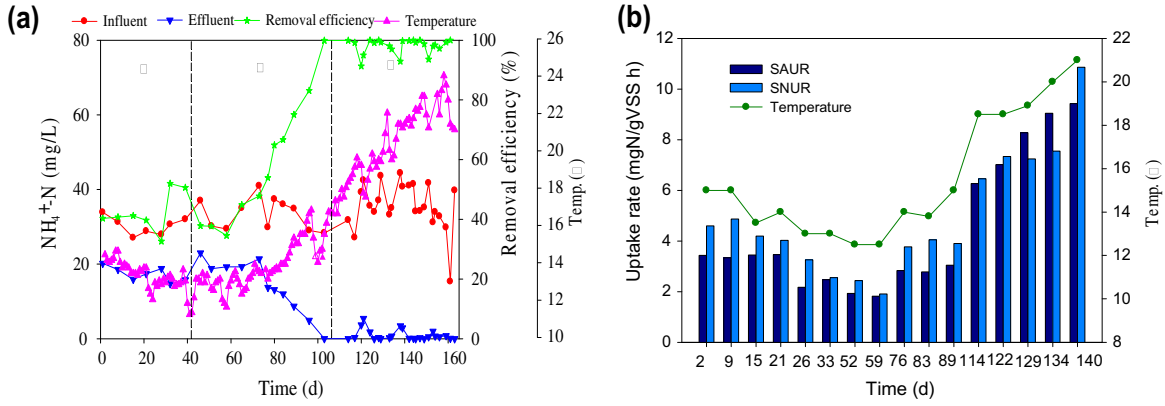

Fig. 3. Nitrogen removal and nitrification activity during the entire experiment. (a) Variations of NH_4^+-N profiles in each stage; (b) Variations of nitrification activity with temperatures.

Table 4
Nitrogen removal and nitrification activity in several typical days

Time, d	59	90	114
Temperature, ℃	12	15	18
SRT, d	20	20	10
MLSS, mg/L	2,906	3,026	2,230
MLVSS, mg/L	2,156	2,211	1,650
SAUR, mg NH_4^+-N/gVSS h	1.8	3.1	6.3
SNUR, mg NO_2^--N/gVSS h	1.9	3.8	6.5
Removal efficiency, %	35	84	99

exponential regression using the SigmaPlot 10.0 software. The temperature dependency factors, K_T of 0.0634/℃ and 0.0522/℃, were obtained for AOB and NOB, and the corresponding temperature coefficients θ_A and θ_B were 1.065 and 1.054.

$$\mu^T = \mu_{20} \times e^{K_T(T-20)} = \mu_{20} \times \theta^{T-20} \quad (1)$$

In this study, the temperature impacts on NH_4^+-N and NO_2^--N oxidation kinetics can be expressed by the following equation:

$$\mu_A^T = \mu_{A20} \times e^{0.0634(T-20)} = \mu_{A20} \times \theta_A^{T-20} = 5.9840 \times 1.065^{T-20} \quad (2)$$

$$\mu_B^T = \mu_{B20} \times e^{0.0522(T-20)} = \mu_{B20} \times \theta_B^{T-20} = 6.1208 \times 1.054^{T-20} \quad (3)$$

where μ_{A20} and μ_{B20} are the SAUR and SNUR at 20℃; and θ_A and θ_B are the temperature coefficients for AOB and NOB.

The dependence of nitrification kinetics on temperature has been widely investigated. The temperature

Table 5
Temperature coefficients observed under different experimental conditions

Process	Ammonia or nitrite concentration (mg/L)	Temperature (℃)	θ_A	References
Suspended-growth systems	5	10–30	1.056	[20]
	100	12–30	1.054	[21]
	30	10–35	1.065	This work
Attached-growth systems	0.59–6.08	10.2–23.3	1.098	[22]
	0–10	8–27	1.043	[19]

coefficient ranged from 1.043 to 1.127 [19–24] and several are shown in Table 5. As shown in Table 5, the temperature coefficient of 1.065 for θ_A obtained in this study is comparable with the values reported by others.

For suspended-growth systems operated under oxygen-limiting conditions, Groeneweg et al. [20] and Wang and Yang [21] found the temperature coefficients of 1.056 and 1.054, which were both lower than the values observed in the present study. The increased population or activity of the nitrifier caused by bioaugmentation may be the reason for the higher temperature coefficients for AOB in the mainstream reactor. The obvious differences among the temperature coefficients (1.043–1.127) cannot be explained because little information was given concerning whether or not the nitrification rates were tested under rapid or gradual changes in temperature [12], which was different [25].

For the attached growth systems, diffusion mass transport has an important role in the nitrification processes. Consequently, the effect of temperature on nitrification rate may have some differences compared with that of suspended growth processes. The temperature coefficients reported for attached growth systems changed between 1.043 and 1.098 [19,22]. The discrepancies indicated that the temperature coefficients varied between different treatment process, oxygen or ammonia limiting conditions, and the proportion or activity of the nitrifiers. The temperature coefficients for NOB (θ_B) resemble the results of Nitrospira and Nitrobacter [26].

The contribution of bioaugmentation to nitrification in the mainstream reactor can be calculated using the following description. We assume that the retention time of the nitrifier bioaugmented from the sidestream was decided by the SRT of the mainstream reactor. The *seed source* had a SAUR of 16.7 mg NH_4^+-N/gVSS h and a SNUR of 19.6 mgNO_2^--N/gVSS h. and a SNUR of 19.6 mgNO_2^--N/gVSS h. Approximately, 25 L of the *seed source* was added to the mainstream reactor (i.e. an 146-fold dilution) each day. The SAUR due to the *seed source* in the mainstream can be calculated as follows.

$$\mathrm{SAUR}_1 = \frac{\mathrm{SAUR}^* \times \left(1 - \frac{1}{\mathrm{SRT}}\right)}{M}$$

where SAUR^* is the SAUR of the side-stream reactor (16.7 mg NH_4^+–N/gVSS h) and M was the fold dilution (146).

SAUR^* was determined as r and $\left(1 - \frac{1}{\mathrm{SRT}}\right)$ as a (the proportion of nitrifiers in the main-stream after one day's bioaugmentation).

Then,

$\mathrm{SAUR}_1 = \frac{a \times r}{M}$

$$\mathrm{SAUR}_2 = \frac{(\mathrm{SAUR}_1 \times M + \mathrm{SAUR}^*) \times \left(1 - \frac{1}{\mathrm{SRT}}\right)}{M} = \frac{(a^2 + a) \times r}{M}$$

$$\mathrm{SAUR}_3 = \frac{(\mathrm{SAUR}_2 \times M + \mathrm{SAUR}^*) \times \left(1 - \frac{1}{\mathrm{SRT}}\right)}{M} = \frac{(a^3 + a^2 + a) \times r}{M}$$

……

$$\mathrm{SAUR}_N = \frac{(\mathrm{SAUR}_{N-1} \times M + \mathrm{SAUR}^*) \times \left(1 - \frac{1}{\mathrm{SRT}}\right)}{M} = \frac{(a^N + a^{N-1} + \ldots + a^2 + a) \times r}{M}$$

The SNUR caused by the *seed source* in the mainstream can be calculated using the same method. The results of different temperature range are listed in Table 6. The nitrification rates of the mainstream are the synactic of the indigenous nitrifiers and the bioaugmented biomass. The greatest contribution of bioaugmentation to the mainstream nitrification rate ranged from 9.3 to 32%. However, the proportion may be overstated for the ignorance of the differences between temperatures, substrate concentration of the reactors, and the growth and decay of the bioaugmented nitrifiers.

Table 6
Effects of bioaugmentation on the nitrification rates

Temperature (℃)	15	18	20
SRT (d)	15	20	10
$SAUR_{cal}$ (mg NH_4^+-N/gVSS h)	1.0	1.4	0.7
$SAUR_{obs}$ (mg NH_4^+-N/gVSS h)	3.1	6.3	7.5
$SAUR_{cal}/SAUR_{obs}$ (%)	32	22	9.3
$SNUR_{cal}$ (mg NO_2^--N/gVSS h)	1.2	1.6	0.8
$SNUR_{obs}$ (mg NO_2^--N/gVSS h)	3.9	6.5	7.7
$SNUR_{cal}/SNUR_{obs}$ (%)	31	25	10

Notes: $_{cal}$ –Nitrification rates of the mainstream caused by side-stream bioaugmentation.

$_{obs}$ –Nitrification rates of the mainstream observed at 15, 18, and 20℃.

3.4. Comparisons of the nitrification activity and nitrifying bacteria from the pilot-scale and the full-scale WWTPs

To further discuss the nitrogen removal process in the pilot-scale WWTP, FISH was used to confirm the nitrifying bacterial numbers and their community structure. The SAUR and SNUR of the full-scale WWTP at 15℃ were 2.0 mgNH_4^+-N/gVSS h and 2.4 mgNO_2^--N/gVSS h, which were lower than the nitrification activity of the mainstream, as shown in Table 4. The total AOB and NOB number in the pilot-scale WWTP were $10^{8.15}$ and $10^{8.0}$ cells/mL, whereas those in the full-scale WWTP were $10^{8.40}$ and $10^{8.39}$ cells/mL. The pilot-scale WWTP had a higher nitrifying activity (Table 4) and lower bacterial numbers (Table 7) than the full-scale WWTP, indicating that the activity of single bacteria in the pilot-scale (bioaugmented) WWTP was higher than the activity in the full-scale WWTP. The lower bacterial number of the pilot-scale WWTP can be attributed to shorter HRT of 10 h compared with the full-scale one with a HRT of 14 h. As reported by Li et al. [27], both AOB and NOB populations were reduced at short HRT. In addition, the short SRT of 15 d compared with 20 d of the full-scale one may be another reason for the lower nitrifier number because a longer SRT may have certain impact on the physiological state of the AOB community [28]. Finally, the differences of the oxic to aerobic ratio (O/A), 0.88 for the pilot-scale WWTP and 1.7 for the full-scale one may have certain contribution to the lower nitrifier number of the pilot-scale WWTP. The proportion of *Nitrosomonas europaea/Nitrosococcus mobilis* lineage (Probe Nmv) to total AOB were 70% and 67%, and the proportion of *Nitrobacter* (Probe NIT3) to total NOB were 75% and more than 99% for the pilot- and full-scale WWTP, respectively. Consequently, *N. europaea/N. mobilis* lineage and *Nitrobacter* were the dominant AOB and NOB, which were consistent with other studies [29].

Table 7
Nitrifying bacteria in pilot-scale WWTP and the 4th full-scale WWTP (AOB and NOB population percentage relative to DAPI)

	Pilot-scale WWTP (%)	Full-scale WWTP (%)
AOB		
Ammonia-oxidizing beta-proteobacteria (NSO1225)	5.43 ± 0.78	6.72 ± 1.03
Nmv	3.80 ± 0.52	4.53 ± 0.10
Nsv443	0.19 ± 0.03	0.97 ± 0.09
Total AOB	5.43	6.72
Nmv/Total AOB	70	67
NOB		
Nitrobacter (NIT3)	2.08 ± 0.28	5.38 ± 0.81
Nitrospira (Ntspa662)	0.69 ± 0.04	<0.1
Total NOB	2.77	5.38
NIT3/Total NOB	75	>99
AOB + NOB	8.20	12.10
AOB/NOB	1.96	1.25

4. Conclusions

The removal efficiency of the NH_4^+-N increased with the temperature and was consistent with the nitrification activity changes. Bioaugmentation may shorten the recovery time of the nitrification activity in WWTPs. The temperature correction factor was equal to 1.065/℃ for AOB and 1.054/℃ for NOB. The pilot-scale WWTP and the full-scale WWTP had the same nitrifying community structure. *N.europaea/N. mobilis* lineage (Probe Nmv) and *Nitrobacter* (Probe NIT3) were the dominant AOB and NOB, whereas the pilot-scale had a higher nitrification activity and lower bacterial count than the full-scale WWTP. Despite of the changes of operation parameters, the average removal efficiency of COD was maintained at 80%.

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