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Research Article

Shifts in Nitrification Kinetics and Microbial Community during Bioaugmentation of Activated Sludge with Nitrifiers Enriched on Sludge Reject Water

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This study used two laboratory-scale sequencing batch reactors (SBRs) to evaluate the shifts in nitrification kinetics and microbial communities of an activated sludge sewage treatment system (main stream) during bioaugmentation with nitrifiers cultivated on real sludge reject water (side stream). Although bioaugmentation exerted a strong influence on the microbial community and the nitrification kinetics in the main stream, there was 58% of maximum ammonia uptake rate (AUR) and 80% of maximum nitrite uptake rate (NUR) loss of the seed source after bioaugmentation. In addition, nitrite accumulation occurred during bioaugmentation due to the unequal and asynchronous increase of the AUR (from 2.88 to 13.36 mg N/L·h) and NUR (from 0.76 to 4.34 mg N/L·h). FISH results showed that ammonia oxidizing bacteria (AOB) was inclined to be washed out with effluent in contrast to nitrite oxidizing bacteria (NOB), and *Nitrosococcus mobilis* lineage was the dominant AOB, while the dominant NOB in the main stream gradually transferred from *Nitrospira* to *Nitrobacter*. *Nitrospina* and *Nitrococcus* which existed in the seed source could not be detected in the main stream. It can be inferred that nitrite accumulation occurred due to the mismatch of NOB structure but washed out with effluent.

1. Introduction

Biological nitrification and denitrification are key processes to remove nitrogen from wastewater and have become more important due to stringent discharge regulations. Research has shown that nitrifier's population (ammonia oxidizing bacteria (AOB) + nitrite oxidizing bacteria (NOB)) should be more than 5–8% of the biomass for good nitrification [1]. However, nitrifiers have slow growth rates and they are also believed to be sensitive to environmental changes such as toxic shocks, pH, and temperature changes [2]. Due to these characteristics, it is difficult to obtain and maintain sufficient nitrifiers in wastewater treatment plants (WWTPs), if solids retention time (SRT) gets shorter [3]. And as a consequence, it is difficult to maintain nitrification in municipal and industrial wastewater treatment plants (WWTPs) [4].

In conventional activated sludge processes, a long SRT is necessary to maintain sufficient nitrifiers for nitrification. The long SRT increases the concentration of mixed liquor suspended solids (MLSS) which requires large tanks and clarifiers to accommodate the accumulation of solids inventory [5].

In wastewater treatment plants a significant source of ammonia is generated within the plant in anaerobic digesters. Dewatering operations on anaerobic digester sludge and supernatant produces a stream of filtrate and centrate called reject water which contains up to 15~25% of the total nitrogen load to the plant [6, 7]. This is a problematic recycle stream for municipal biological WWTPs.

A number of side-stream reject water treatment processes have emerged in recent years, such as oxygen-limited autotrophic nitrification/denitrification (OLAND)

process [8] and anaerobic ammonium oxidation (ANAMMOX) process [9]. In addition to reducing the impact of recycle nitrogen loadings, some processes promote the potential for cultivating a stable source of nitrifying microorganisms to bioaugment the main stream, such as mainstream autotrophic recycle enabling enhanced N removal (MAUREEN process) [10], bioaugmentation batch enhanced technology (BABE) [11].

Bioaugmentation uncouples nitrification in the mainstream wastewater treatment processes from the aerobic SRT. The amount of nitrifiers needed to be grown in the main treatment stream is reduced by the amount of new nitrifiers supplied from the reject water side-stream treatment system. Since a smaller amount of nitrifiers need to be grown in the mainstream treatment plant, the nitrification section (oxic section) can be smaller and the MLSS concentration decreases since higher SRT is not required [12].

Considering the nitrifier's diversity and kinetic differences, even what might seem to be a small environmental difference seemingly can impact nitrifier's activity [13]. The addition of enriched nitrifiers from reject water to municipal wastewater with its unique microbial communities may have limited success if the added nitrifiers are not adaptive to the conditions inherent to a sewage treatment system, which can lead to the reduction of nitrifier's activity or even to a decay of nitrifiers [12], or nitrifiers washout with effluent Head et al. [5]. Therefore, the community survivability and functional stability of the seed source are common problems in bioaugmentation applications, which may determine the results of bioaugmentation. So, it is important to investigate the enriched nitrifier's population diversity, fate, and functional variation after bioaugmentation to the main stream.

Berends et al. [14] have pointed out that *Nitrosomonas* and *Nitrobacter* were the dominant AOB and NOB in the seed source, respectively. So far, many studies have measured structural diversity of AOB and stability of ammonia oxidization in main stream activated sludge systems bioaugmented from side-stream reject water treatment systems [5, 15]. Smith and Oerther [16] have also theorized that increased population diversity from input of microorganisms possessing desirable properties from side-stream processes to a mainstream process creates a more robust system that is less susceptible to inhibition or upsets.

However, there is a lack of information for the variation of nitrifier's community structure and function during bioaugmentation. Furthermore, there is also a lack of information about the diversity of NOB in order to determine which species are successfully bioaugmented and to understand the mechanisms that enable them to be incorporated into main-stream biomass. This information will provide useful insights into the second step of the nitrification process since the nitrite oxidation step may be less stable than the ammonia oxidation step for side-stream reject water treatment systems.

This study focuses on investigating functional stability (ammonia uptake rate (AUR) and nitrite uptake rate (NUR)) of the inoculated nitrifiers after being bioaugmented from side-stream reject water treatment systems by measuring the shifts in nitrification ability in the main stream. Furthermore, the fate of the seed source was analyzed by comparing the shifts in the population and structure of nitrifiers in the activated sludge and that in the effluent of the main-stream reactor by using fluorescent in situ hybridization (FISH).

2. Materials and Methods

2.1. Reactors Operation. Side-stream reactor, A 5-L sequencing batch reactor (SBR), was operated with a hydraulic retention time (HRT) of 2.5 d and SRT of 7 d at 20°C. Feeding consisted of adding 500 mL of sludge reject water four times per day, every 6 h (feed, 1 min; anoxic, 59 min; oxic, 240 min; settle, 50 min; decant, 1 min; idle, 9 min). Wasting occurred once per day, at the end of the third recycle. The pH was controlled at 7~8 by addition of sodium bicarbonate ($NaHCO_3$) to the sludge reject water. Oxygen concentration was monitored automatically and maintained approximately 2.0 mg/L in the oxic phase.

The sludge reject water used in the experiment was the supernatant of the anaerobic digestion tank of the Dangjiacun waste water treatment plant in Xi'an, China. It was delivered to the laboratory once per week and stored in a closed container at 4°C. Sludge processing includes cothickening of primary sludge and waste activated sludge and anaerobic digestion for about 14~17 d at ambient temperature, followed by rethickening and dewatering by centrifuge. The sludge reject water contained 1949 ± 562 mg/L total chemical oxygen demand (TCOD), 455 ± 131 mg/L soluble chemical oxygen demand (SCOD), 812 ± 135 mg/L total Kjeldahl nitrogen (TKN), and 3035 ± 395 mg/L alkalinity ($CaCO_3$) (mean value of fresh sludge reject water during the experiment).

Main-stream reactor, A 4-L SBR, was operated to treat a synthetic domestic wastewater, containing 300 mg/L glucose-COD, 50 mg/L NH_4^+-N, and 5 mg/L phosphorous, 50×10^{-3} mg/L EDTA, 5×10^{-3} mg/L $ZnSO_4 \cdot 7H_2O$, 5.06×10^{-3} mg/L $MnCl_2 \cdot 4H_2O$, 4.99×10^{-3} mg/L $FeSO_4 \cdot 7H2O$, 1.1×10^{-3} mg/L $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 1.57×10^{-3} mg/L $CuSO_4 \cdot 5H_2O$. The pH was controlled at 7~8 by addition of sodium bicarbonate ($NaHCO_3$) to the synthetic domestic water. Oxygen concentration was monitored automatically and maintained approximately 2.0 mg/L in the oxic phase.

The reactor was operated with a hydraulic retention time (HRT) of 8 h and sludge retention time (SRT) of 5 d at 20°C, and the nitrogen load was 150 mg N/L/d, about half of the side stream (812 mg N/L/2.5 d = 324.8 mg N/L/d). The reactor was fed 2 L of synthetic wastewater six times daily, every 4 h (feed, 1 min; anoxic, 89 min; oxic, 90 min; settle, 50 min; decant, 1 min; idle, 8 min).

The main-stream reactor was run for about three months before it was seeded once daily for 50 d with 50 mL seed source. The volume of the seed source for bioaugmentation was calculated as follows: for the side stream reactor, the nitrogen load was 812 mg N/L × 2 L/d = 1624 mg N/d, and the wasted sludge (enriched nitrifiers) was equivalent to the amount of MLVSS in 700 mL mixed liquor per day (the

SRT was about 7 days), so the yield of enriched nitrifiers was 700 mL/1624 mg N = 0.43 mL/mg N. For the main-stream reactor, the nitrogen load was 50 mg N/L × 12 L/d = 600 mg N/d, and accordingly the nitrogen load was about 20% of the main stream, so the volume of seed source matched with main-stream reactor should be 600 mg N × 20%× 0.43 mL/mg N = 50 mL.

2.2. Batch Experiments. To evaluate the activity of nitrifiers, nitrification rates were measured in batch scale (400 mL) outside of the reactors. The 400 mL biomass for these tests originated from the reactors and was maintained at 20°C. Oxygen concentration was monitored automatically and maintained approximately 2.0 mg/L.

Considering the nitrifier's kinetic difference between the side stream and main stream, the initial ammonia and nitrite concentration used in the test was about the maximum concentration in the reactors. The initial ammonium concentration used for the test was 85 mg/L for the side stream and 50 mg/L for the main-stream reactor, and the initial nitrite concentration was 50 mg/L for the side stream and 20 mg/L for the main stream. The MLVSS of side stream was 2.86 mg/L, and the MLVSS of main stream was 1.73∼1.81 mg/L. The pH value was controlled at 7∼8 by addition of $NaHCO_3$. 8 samples were taken over time, the AUR or NUR (linear correlation coefficient $R^2 > 0.98$) was determined by measuring the consumption of NH_4^+-N and NO_2^--N [22].

2.3. Chemical Analysis. Mixed liquor volatile suspended solids (MLVSS), NH_4^+-N, NO_2^--N, NO_3^--N, and chemical oxygen demand (COD) were analyzed in accordance with standard methods [23]. Dissolved oxygen (DO) was measured with HANNA HI9143 dissolved oxygen meters; oxidation-reduction potential (ORP) and pH were measured with HANNA HI9025 ORP/pH meter.

2.4. Fluorescence In Situ Hybridization (FISH) Analysis. Samples (2 mL) were taken at days 30th (the day before bioaugmentation), 38th, 57th, and 77th and fixed in 4% paraformaldehyde. Direct ultrasonification (20 kHz, five minutes) was applied to break up large flocs prior to hybridization. The FISH analysis was performed according to the protocol previously described by Amann et al. [24] with fluorescently labeled probes listed in Table 1. A 3 μL sample was applied to each well of the slide and then dried at 46°C. The samples were then dehydrated in 50%, 80%, and 98% for 3 minutes and dried at 46°C. 8 μL of hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl, 0.01% (w/v) SDS) with *X*% (v/v) deionized formamide ("*X*" is listed in Table 1) and 1 μL of fluorescently labeled probe (50 ng/μL) were added to each well. The sample was then hybridized at 46°C for 2 hours. The slide then washed in 50 mL prewarmed washing buffer (0.3 M NaCl, 20 mM Tris-HCl, 0.01% (w/v) SDS). Washing buffer was removed by serial washing in deionized water. Slides were then stained with 10 μL of 1 μg/mL DAPI for 5 minutes. The slides were rinsed again by serial washing in deionized water and allowed to natural air drying in dark.

All samples hybridized with oligonucleotide probes were embedded in Citifluor prior to microscopic observation. Epifluorescence microscopy was done on an Olympus BX51 microscope at 1000× magnification. Photographs were taken using a high resolution Microscopy Olympus DP72. The software Image pro-plus 7.0 was used for counting the targeted population in relation to the total microbial population in the sample. 10∼20 microscopic fields were analyzed per sample and per probe. The cell counts were performed in triplicate using independently prepared fluorescence in situ hybridization (FISH) slides for each probe [25].

3. Results

3.1. Side-Stream Reactor. The side-stream reactor was operated about two years before the bioaugmentation experiment, the reactor performance was stable, and full nitrification was achieved during the experiment. The mean value of MLVSS was 2.85 ± 0.87 g/L, the effluent NH_4^+-N concentration was lower than 10 mg/L and the conversion efficiency was greater than 98%, and the effluent NO_2^--N concentration was lower than 1 mg/L (Figure 1).

The maximum ammonia uptake rate (AUR) was 66.9 mg NH_4^+-N/L·h, and the maximum nitrite uptake rate (NUR) was 34.4 mg NO_2^--N/L·h of the activated sludge in the side-stream reactor (seed source or inoculums).

FISH analysis showed that the AOB/DAPI was about 18.8% ± 2.7% in the seed source, AOB consisted of 83% *Nitrosococcus mobilis* lineage, and no *Nitrosospira* spp. were detected. The NOB/DAPI was about 14.4% ± 2.8%, NOB consisted of 65% *Nitrobacter*, 19% *Nitrospina gracilis*, 10% *Nitrospira,* and 6% *Nitrococcus mobilis* (mean values for four samples, Table 2). And the kinetics characterization of the enriched nitrifiers was shown in Yu et al.'s [26].

3.2. Main-Stream Reactor. The main-stream reactor was operated with an apparent SRT near design SRT_{min} in engineering (5 days) as demonstrated by high NH_4^+-N concentration and low NO_x^--N concentration in the effluent for about three months and achieved a stable condition before the start of bioaugmentation. During the stable stage before bioaugmentation, the MLVSS was 1.75 ± 0.08 g/L (mean value), and the NH_4^+-N, NO_2^--N and NO_3^--N concentration in the effluent was 40∼42 mg/L, 0.4∼0.8 mg/L, and 1∼2 mg/L, respectively.

Figure 2 presents the effluent profiles of the main-stream reactor after bioaugmentation. It showed that the NH_4^+-N concentration in the effluent decreased gradually after bioaugmentation, and at day 79, the NH_4^+-N cannot be detected in the effluent, while the concentration of NO_2^--N, NO_3^--N in the effluent increased gradually. At the same time, the sum of total inorganic nitrogen concentration (NH_4^+-N + NO_2^--N + NO_3^--N) in the effluent of main-stream reactor decreased gradually after bioaugmentation, and about half of the removed ammonia is not retrieved in the total oxidized metabolites (sum of nitrite and nitrate). Because the fill ratio of the reactor is 50%, and the reactor went to an anoxic phase after fill, then about 50% of the

Table 1: List of 16S rRNA-targeted oligonucleotide probes used in the study.

Probe	Sequence (5′-3′)	Specificity	Concentration*(%)	Reference
NSO1225	CGCCATTGTATTACGTGTGA	Ammonia oxidizing beta-proteobacteria	35	Mobarry et al. [17]
Nsv443	CCGTGACCGTTTCGTTCCG	*Nitrosospira* spp.	30	Mobarry et al. [17]
Nmv (Ncmob)	TCCTCAGAGACTACGCGG	*Nitrosococcus mobilis* lineage	35	Juretschko et al. [18]
Ntspa662	GGAATTCCGCGCTCCTCT	*Nitrospira*	35	Daims et al. [19]
NIT3	CCTGTGCTCCATGCTCCG	*Nitrobacter*	40	Wagner et al. [20]
Ntcoc206	CGGTGCGAGCTTGCAAGC	*Nitrococcus mobilis*	10	Juretschko et al. [21]
Ntspn693	TTCCCAATATCAACGCATT	*Nitrospina gracilis*	20	Juretschko et al. [21]

* Concentrations presented as percentage of formamide in hybridization buffer (v/v).

nitrite and nitrate in a cycle will be denitrified in next cycle, and the denitrification ability increased gradually along with the increase of nitrification ability. In addition, the MLVSS slightly increased to 1.79 ± 0.12 g/L.

In the main-stream reactor, the shifts in nitrification performance had a strong relationship with the chemical analysis of nitrogen parameters (Figure 3). The AUR increased from 2.88 to 13.36 mg NH_4^+-N/L·h and the NUR increased from 0.76 to 4.34 mg NO_2^--N/L·h for the main-stream reactor. The AUR increased much more than the NUR, corresponding to the evident nitrite accumulation in the main-stream reactor after bioaugmentation (Figure 2).

The shifts in AOB and NOB composition in the main-stream reactor during bioaugmentation were shown in Table 3. Before bioaugmentation, the AOB/DAPI and NOB/DAPI were 3.7 ± 1.2% and 0.11 ± 0.1%, respectively (day 30, samples pre-bioaugmentation). Once bioaugmentation started, the percentage of AOB/DAPI and NOB/DAPI increased quickly at the beginning of bioaugmentation (days 0∼8), the AOB/DAPI and NOB/DAPI increased to 7.0 ± 2.2% and 3.4 ± 1.1%, respectively, while during days 57∼77, the fractions of AOB and NOB relative to the total microbial population in the study did not exhibit an evident difference.

Table 3 also showed the percentage of nitrifiers in the (suspended solids) SS flow out with the effluent after bioaugmentation. Both the percentage of AOB and NOB tend to decrease with the bioaugmentation time. In the initial time of bioaugmentation (day 38), the percentage of AOB/DAPI in the effluent was about 2.5 times of that in activated sludge, but the ratio was close at day 57 and day 77. It was surprising that the percentage of NOB/DAPI in the effluent was lower than that in the activated sludge at day 57 and day 77.

Figure 4 showed the community structure shifts in AOB and NOB in the main-stream reactor during bioaugmentation according to the detection of nitrifiers in the activated sludge by FISH techniques.

AOB were dominated by members related to *Nitrosococcus mobilis* lineage (Probe Nmv), which made up about 60∼80% of all detected AOB (Probe Nso1225). No ammonia-oxidizing bacteria stainable with Nsv443 (*Nitrosospira* spp.) were presented. This was quite similar with the AOB species in the side stream.

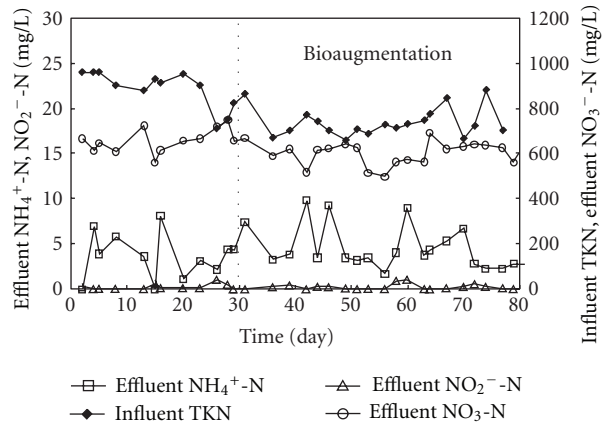

Figure 1: Influent and effluent N concentrations for the side-stream reactor.

Table 2: AOB and NOB composition in seed source ("—" no signals were detected).

Species (probe)	Percentage relative to DAPI
AOB	
Nitrosococcus (Nmv)	13.1% (±3.4%)
Nitrosospira spp. (Nsv 443)	—
Total AOB (Nso1225)	18.8% (±2.7%)
NOB	
Nitrobacter (NIT 3)	9.4% (±1.6%)
Nitrospira (Ntspa 662)	2.7% (±0.47%)
Nitrococcus (Ntcoc206)	0.86% (±0.11%)
Nitrospina (Ntspn693)	1.2% (±0.62%)
Total NOB	14.4% (±2.8%)
AOB + NOB	33.2
AOB/NOB	1.31

The NOB in the main-stream reactor belonged to the genus *Nitrospira* (Probe Ntspa662), and no *Nitrobacter* (Probe NIT3), *Nitrococcus mobilis* (Probe Ntcoc206), and *Nitrospina gracilis* (Probe Ntspn693) appeared before bioaugmentation. However, after bioaugmentation, the percentage of *Nitrobacter* in NOB increased and that of *Nitrospira* decreased gradually, the dominant NOB transferred

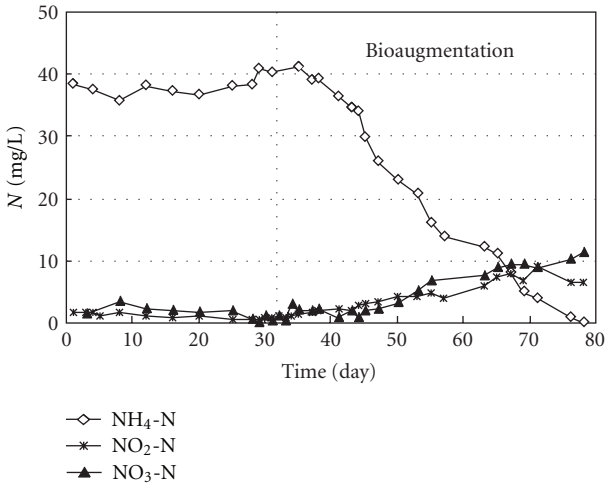

Figure 2: Effluent profiles of main-stream reactor.

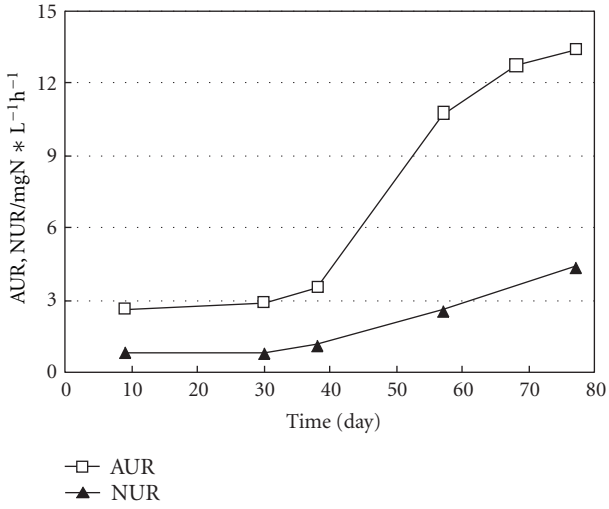

Figure 3: The ammonia uptake rate and nitrite uptake rate of the activated sludge in main-stream reactor.

gradually from *Nitrospira* to *Nitrobacter* (the dominant NOB in the side-stream reactor), and the *Nitrospina gracilis* and *Nitrococcus mobilis* were not detected.

4. Discussion

4.1. Nitrifiers Enrichment with Sludge Reject Water. The low concentration of ammonia and nitrite detected in the effluent of the side-stream reactor (Figure 1) reflected that stable and full nitrification was achieved. And the biomass in the side-stream reactor achieved high nitrification rates (66.9 mg NH_4^+-N/L·h of AUR and 34.4 mg NO_2^--N/L·h of NUR), Wett et al. [27] also reported a high nitrification rate of 50~58.3 mg NH_4^+-N/L·h in an SBR treating sludge reject water at 20~25°C. FISH analysis showed that AOB/DAPI and NOB/DAPI in the seed source averaged 18.8% and 14.4%, respectively (Table 2), and the average ratio of nitrifiers to total bacteria (DAPI) was about 33.2%. Head and Oleszkiewicz [5] detected 9.3~17.9% of AOB/DAPI in an SBR treating sludge reject water (631 ± 47 mg NH_4^+-N/L, lower than the ammonia concentration in the research). These data were much higher than data in the activated sludge of the normal WWTP with full nitrification in which nitrifiers account for approximately 5~8% [1]. The big difference of nitrifier's content in the activated sludge between the main-stream reactor and the side-stream reactor is due to the difference of C/N ratio in the feedings. High ammonium concentration and low C/N ratio stimulates a high fraction of nitrifiers content in the sludge. Therefore, sludge reject water is an effective source for nitrifier's enrichment.

4.2. Impact of Bioaugmentation on the Performance of Main-Stream Reactor. The significant increases of the AUR and the NUR in the main stream suggests that bioaugmentation with nitrifiers enriched by sludge reject water is a promising approach to enhance the nitrification performance in sewage treatment. However, the advantages of the bioaugmentation should not be overstated. Some problems have to be pointed out, such as nitrite accumulation in the main-stream and the decrease of nitrification ability of the seed source.

For the main stream reactor, nitrite could not be detected before bioaugmentation. Nitrite concentration accumulated gradually after bioaugmentation and reached 10 mg NO_2^--N/L at the end of experiment. The nitrite concentration at the end of the experiment was due to the unequal and asynchronous increase of the AUR and NUR. The increase in the AUR was 10.48 mg NH_4^+-N/L·h and the increase in the NUR was just 3.58 mg NO_2^--N/L·h.

Bioaugmentation indeed enhances nitrification in the main-stream reactor, but cannot function fully. That is to say, not all nitrifiers added to the main stream work well and part of their nitrification capability is lost during bioaugmentation. For AOB, AUR of the seed source was 66.9 mg NH_4^+-N/L·h. 50 mL per day of the "seed source" was added to 4 L mainstream bioreactor volume/day, that is, an 80-fold dilution. SRT was 5 d.

The AUR due to the seed source was calculated to be 0.67 mg NH_4^+-N/L·h per day ($(1 - 1/5) * 66.9/80 = 0.67$), but the slope of the AUR curve (Figure 4) was about 0.28 mg NH_4^+-N/L·h. The NUR due to the seed source should have been 0.34 mg NO_2^--N/L·h per day ($(1 - 1/5) * 34.4/80 = 0.34$), but the slope of the NUR curve (Figure 3) was about 0.07 mg NO_2^--N/L·h. The experimental results were much smaller than the calculated values. This difference indicated that most of the nitrification ability (58% of AUR and 80% of NUR) introduced by the seed source was lost in the main-stream reactor. Therefore, the nitrite oxidation step may be less stable than ammonia oxidation for main-stream systems after bioaugmentation.

4.3. Impact of Bioaugmentation on the Microbial Community of Main-Stream Reactor. Compared to side stream, the anoxic/aerobic phase ratio in the main-stream reactor was much higher and relativly lower SRT leading to a lower nitrifier growth. But the bioaugmentation exerted a strong

Table 3: AOB and NOB percentages relative to DAPI in the main stream reactor (%).

	Time (day)	30	38	57	77
Sludge	AOB/DAPI	3.7 ± 1.2	7.0 ± 2.2	9.1 ± 3.1	8.7 ± 2.7
	NOB/DAPI	0.1 ± 0.1	3.4 ± 1.1	5.0 ± 1.7	5.0 ± 2.5
	ΔAOB/ΔNOB	—	0.97	1.1	1.0
Effluent	AOB/DAPI	—	18.2 ± 4.6	11.3 ± 2.8	10.0 ± 3.4
	NOB/DAPI	—	3.9 ± 2.0	3.6 ± 1.5	1.4 ± 1.2

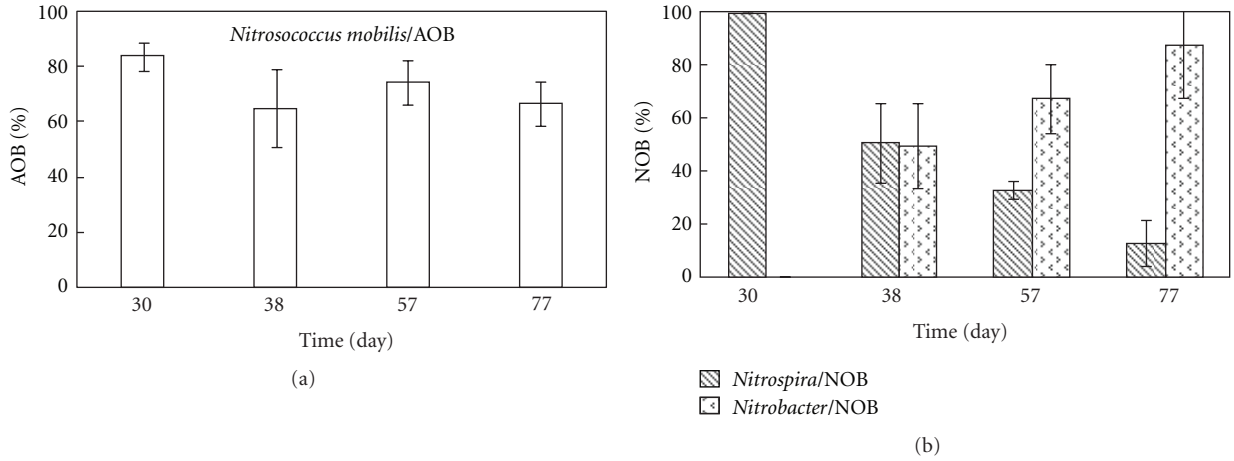

Figure 4: (a) Community structure shifts in AOB in the main-stream reactor. (b) Community structure shifts in NOB in the main-stream reactor.

influence on the microbial community. The percentage of nitrifiers to total biomass increased quickly and reached a stable level at day 38 (8 days after bioaugmentation), and the AOB+NOB/DAPI was approximate 14.0 ± 5.2%, which was much higher than the pre-bioaugmentation value of 3.8 ± 1.3%. The ratio of AOB/NOB was 1.7–2.1 in the main-stream reactor after bioaugmentation due to the longer generation time of NOB. However, $\Delta AOB_i/\Delta NOB_i$ ($\Delta AOB_i = AOB_i - AOB_0$, $\Delta NOB_i = NOB_i - NOB_0$, i, the day number after bioaugmentation. AOB_0, NOB_0, the content of AOB or NOB in the activated sludge before bioaugmentation) in the main-stream reactor was approximate 1.0, lower than the ratio of AOB/NOB in the seed source (= 1.3). These results indicate that much more of the population of AOB in the seed source was lost than the population of the NOB. In addition, the NUR decrease was much more than the AUR decrease of the seed source after bioaugmentation. Apparently, the nitrifier's loss with the decant liquor could not be accounted for by the nitrite accumulation.

Presumably the nitrifier's washout was one of the reasons for bioaugmentation failure [12]. The AOB/DAPI in the effluent was about 2.5 times of that in the activated sludge in the initial stage of bioaugmentation, while it approached to the AOB/DAPI in the activated sludge during the course of the experiment. As to NOB, a similar shift profile was discovered. It is interesting that the percentage of NOB/DAPI was much lower than the percentage of AOB/DAPI in the effluent, and it also was lower than the NOB/DAPI in the activated sludge at day 57 and day 77. These results also indicated that washout with effluent should not be the main reason for nitrification loss of the seeded nitrifiers. The adhesion characteristics of nitrifiers in activated sludge also contributed to the nitrifier's behavior. Larsen et al. [28] discovered that nitrifiers can form relatively dense and strong microcolonies in activated sludge and remain almost intact even under extreme physical and chemical conditions so that NOB are much more difficult to deflocculate than AOB.

Comparing the microbial structure in two reactors revealed that the community structure of AOB in the side-stream reactor is similar with that in the main-stream reactor, while a significant difference is observed for the NOB community. *Nitrobacter* was the dominating NOB in the seed source, which exhibits a low affinity to the substrate and a high maximum growth rate [29–31]. However, after being added to the main-stream reactor, in which the nitrite concentration was relativly low and the *Nitrospira* was the dominating NOB, the *Nitrobacter* cannot surpass the *Nitrospira* in competing for nitrite. Therefore, NOB structure mismatch resulting from substrate concentration may be one of the main reasons for more NUR loss than AUR loss during the course of bioaugmentation.

5. Conclusions

In conclusion, bioaugmentation exerted a strong influence on the microbial community and activity of the nitrifiers.

(1) After bioaugmentation, the ammonia uptake rate (AUR) increased from 2.88 to 13.36 mg NH_3-N/L·h, and the nitrite uptake rate (NUR) increased from 0.76 to 4.34 mg NO_2^--N/L·h for main-stream reactor.

(2) FISH analysis showed that *Nitrosococcus mobilis* lineage was the dominant AOB which matches with the main-stream reactor, while the NOB in the seed source was *Nitrobacter*, and mismatches the main-stream reactor in which the dominant NOB was *Nitrospira* spp. before bioaugmentation; however, it gradually transferred to *Nitrobacter* spp. (the dominant NOB in the seed source) after bioaugmentation.

(3) Although AOB was inclined to wash out with effluent compared with NOB, the NUR of the seed source lost more than that of AUR due to the mismatch of NOB structure.

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