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Effect of long-term bioaugmentation on nitrogen removal and microbial ecology for an A^2O pilot-scale plant operated in low SRT

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ABSTRACT

The effect of long-term bioaugmentation on nitrogen removal, nitrification activity, and microbial ecology for an anaerobic/anoxic/aerobic (A^2O) pilot-scale plant operated in low sludge retention time (SRT) to treat municipal wastewater was investigated. Reject water from sludge treatment was used as a feed to cultivate the nitrifier contained in activated sludge for bioaugmentation. Under the conditions of 10 h hydraulic retention time, 8 d of SRT, and 14℃ water temperature ammonia removal efficiency increased by 25%, and specific ammonia utilizing rate and specific nitrite utilizing rate increased by 1.86 and 1.90 times, respectively. The percentages of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) in the total number of bacteria increased from 2.4 and 2.1% to 6.8 and 7.8%, respectively. The dominant AOB and NOB were transformed from *Nitrosospira* and *Nitrospira* to *Nitrosomonas europaea* and *Nitrobacter.*

Keywords: Bioaugmentation; Municipal wastewater; Nitrogen removal; Nitrification activity; Nitrifier community structure; Reject Water

1. Introduction

The removal of nitrogen from municipal wastewater is highly important because nitrogen emissions have a negative effect on lakes, rivers, coasts, and other receiving bodies of water. Biological nitrogen removal in wastewater is affected by factors such as temperature and nitrogen load. Nitrogen removal efficiency rapidly decreases when the temperature drops to 15℃ and below or when the nitrogen load is extremely high [1]. The traditional, highly efficient, and stable nitrogen removal method entails changing ambient conditions, such as increasing the volume of reactors or extending the sludge retention time (SRT). However, this method causes other problems simultaneously, including bad phosphorus removal effect and high infrastructure costs [2]. Bioaugmentation is a new method that involves the addition of micro-organisms with special functions into a waste treatment station or bioreactor to accelerate the degradation of harmful

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substances [3–6]. Bioaugmenting nitrifiers cultivated in a sidestream reactor with reject water can enhance the nitrification capability in the mainstream system [1,7–11].

A number of researchers have conducted theoretical model [7,12–14] and experimental studies to shorten the SRT by performing bioaugmentation at a low temperature [9,10,15–18]. Results show that bioaugmentation can significantly reduce the SRT of the nitrifying process. As most studies have been conducted in the laboratory [2,10,17,19–23], investigations on the effect of bioaugmentation on nitrification in pilot-scale and full-scale plants are limited [7–9,15,16,18].

In their study of bioaugmentation-strengthening nitrification, Plaza et al. [8] found that the addition of excess sludge from the sludge treatment system to the wastewater treatment system could significantly shorten the aeration period. However, the flow ratio of reject water to wastewater was 45%, which was significantly higher than the percentage in a general sewage treatment plant (1–3%) [9]. The higher addition of sludge may be the main factor behind the shortened aeration period of the wastewater treatment system.

ScanDeNi and BABE are well-known experimental studies using enriched nitrifying bacteria in reject water to perform bioaugmentation. Their common feature is the ability to lead the reject water directly into the reactor that receives returned sludge to culture the nitrifying bacteria.

In the ScanDeNi process, all of the returned sludge is led into the reject water treatment reactor. The practical operation result shows that through this process, the quality of effluent can be standard at 8–9℃ water temperature [24]. However, this process does not include conducting microbiological analysis to monitor changes in the nitrifying bacteria.

In the BABE process, partial returned sludge is led into the BABE reactor to enrich the nitrifying bacteria. The growth of nitrifiers in the original returned sludge ensures the consistency of the enriched nitrifying bacteria in the BABE reactor, with the dominant nitrifying bacteria in the water treatment system. In addition, short retention time and low returned sludge ratio can ensure the operation of the reactor in higher temperature, thereby obtaining higher nitrification efficiency [18,25,26]. Fluorescence *in situ* hybridization (FISH) is used to qualitatively measure the amount of ammonia-oxidizing bacteria (AOB) in the BABE reactor and all reactors of the wastewater treatment system. However, this method neither conducts the tracking analysis of nitrite-oxidizing bacteria (NOB) nor monitors the changes in the number of nitrifying bacteria.

This paper deals mainly with the use of a continuous flow reactor to treat reject water, and conducts long-term bioaugmentation in the mainstream system for treating municipal wastewater, monitoring nitrification efficiency, nitrification activity, and changes in community structure and number of nitrifying bacteria to provide references for the design and operation of bioaugmentation systems.

2. Materials and methods

2.1. Pilot plant

A pilot plant was built in the fourth municipal wastewater treatment plant in Xi'an, China. The process employed in the pilot plant is shown in Fig. 1. Anaerobic/anoxic/aerobic (A^2/O) process is used for mainstream treatment and aerobic/anoxic is used for sidestream (reject water) treatment. The wasted sludge in sidestream, in which nitrifier is enriched, is pumped back to the mainstream for bioaugmentation.

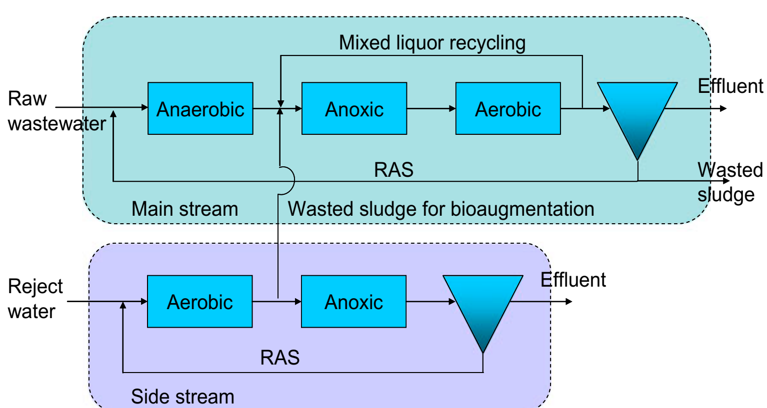

Fig. 1. Experimental system employed in the pilot plant.

The total net volume for the mainstream is 3.6 m^3 in which the volume ratio of anaerobic/anoxic/aerobic is 1/1.7/2.4; and that for sidestream is 0.25 m^3 in which the volume ratio of anoxic/aerobic is 1/5. The flow rate in the mainstream is 0.36 m^3/h, which is equivalent to a hydraulic retention time (HRT) of 10 h; and the flow rate in the sidestream is 0.0072 m^3/h (2% of flow rate in the mainstream), which is equal to HRT of 34 h. The sidestream reactor is operated at 20℃ when the ambient temperature is below 20℃ and kept at ambient when the temperature is higher than 20℃.

2.2. Wastewater

Wastewater used in the mainstream is the same as that used in full-scale plants. The characteristics of wastewater and reject water in the sidestream are shown in Table 1.

2.3. Analytical methods

2.3.1. Physicochemical analyses

Nitrate, nitrite, and phosphate were analyzed simultaneously by ion chromatography. Ammonia, COD, SS, and VSS were conducted by following the Standard Methods [27].

2.3.2. FISH test

For FISH test, samples were pre-treated with 4% paraformaldehyde for fixing and ultrasonic (Vibra cell, Sonics, USA) in order to break up the large flocs prior to hybridization. All samples were stained by DAPI (4,6-diamidino-phenylindole) for the total bacteria. *In situ* hybridizations of cells were performed with fluorescently labeled rRNA-targeted oligonucleotide probes (Table 2). The microscopy was performed using an Olympus BX51 with an Olympus DP72 camera. Ten to twenty views were obtained of each sample. The software Image-Pro Plus software 7.0 was used for counting target populations in the sample.

2.3.3. Nitrification activity measurements

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 a temperature in accordance with the reactors. Oxygen concentration was automatically monitored and maintained at approximately 2–3 mg/L. The pH value was controlled at 7–8, by adding $NaHCO_3$. The average MLVSS of the mainstream was 1,626 mg/L, whereas that of the sidestream was 1,933 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, respectively. Samples of 10 mL of mixed liquor were drawn off at 8-min intervals for the sidestream and at 15-min intervals for the mainstream reactor. Eight samples were taken over time.

Table 1
Characteristics of municipal wastewater and reject water used in the experiment

Components	pH	Alkalinity ($CaCO_3$) (mg/L)	SS (mg/L)	TCOD (mg/L)	SCOD (mg/L)	NH_4^+-N (mg/L)	TKN (mg/L)	PO_4^{3-}-P (mg/L)
Municipal wastewater	6.5–7.5	230–320	140–280	220–580	80–210	22–53	34–62	1.8–8.4
Reject water	8.5–9.5	2,458–2,571	212–606	288–989	147–670	120–480	140–620	13.2–40

Table 2
Probes used for FISH and the corresponding hybridization conditions

Probe	Sequence(5´–3´)	Specificity	Conc[a]	Reference
NSO 1,225	CGCCATTGTATTACGTGTGA	Ammonia-oxidizing beta-proteobacteria	35	[28]
Nmv	TCCTCAGAGACTACGCGG	Nitroso-coccus	35	[29]
Nsv443	CCGTGACCGTTTCGTTCCG	Nitroso-spira, -lobus, -vibrio	30	[28]
NIT 3	CCTGTGCTCCATGCTCCG	Nitrobacter	40	[30]
Ntspa662	GGAATTCCGCGCTCCTCT	Nitrospira	35	[31]

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

3. Results and discussion

3.1. Mainstream system performance and nitrification activity

The mainstream system had been operating for 876 d. Nitrifiers from the sidestream reactor were bioaugmented from the 214 d. After bioaugmentation, ammonia removal efficiency of the mainstream system increased under the conditions of 14℃ water temperature and 8 d of SRT. The experiment was divided into six stages, but this study focused only on three stages: before bioaugmentation (II), early stage of bioaugmentation (III), and long-term bioaugmentation (V). Ammonia content in influent and effluent, removal efficiency, and temperature change are shown in Fig. 2.

Influent ammonia of the mainstream system ranged from 22 to 53 mg/L. Effluent ammonia, removal efficiency, and nitrification activity are affected by the seasonal temperature. Table 3 summarizes the effect of bioaugmentation on nitrification. At approximately 14℃, the efficiency of ammonia removal increased from 27.5% (II) before bioaugmentation to 31.4% (III) in the early stages of bioaugmentation. After long-term bioaugmentation, the efficiency of ammonia removal increased by nearly 25% (V) with the enrichment of nitrifying bacteria and adaptation of nitrifiers to the environment of the mainstream system. The absolute removal quantity of ammonia increased by 5.81 mg/L. Nitrification is good in summer when the mean temperature is approximately 22℃. Bioaugmentation has no significant effect on nitrogen removal at a temperature of more than 20℃ [22].

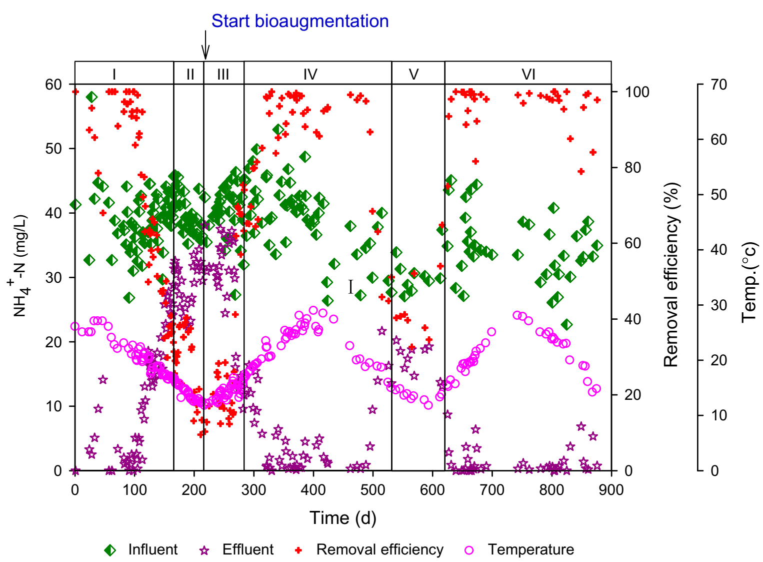

Fig. 2. Changes of ammonia in influent and effluent as well as removal efficiency and temperature.

Table 3
Summary of the influent/effluent and removal efficiency as well as nitrifying activity

	Before bioaugmentation (II)	In early stage of bioaugmentation (III)	After long-term bioaugmentation (V)
Temperature (℃)	13.7 ± 1.2	13.9 ± 1.3	13.8 ± 1.1
Influent (mg/L)	39.6 ± 2.9	40.1 ± 4.0	31.7 ± 4.7
Effluent (mg/L)	28.7 ± 3.6	27.5 ± 9.0	15.1 ± 4.9
Absolute removal quantity (mg/L)	10.9	12.6	16.7
Removal efficiency (%)	27.5	31.4	52.6
SAUR (mg N/gVSS h)	1.50 ± 0.21	2.29 ± 0.29	2.79 ± 0.59
SNUR (mg N/gVSS h)	1.90 ± 0.41	2.98 ± 0.27	3.62 ± 0.90

Table 3 shows the change in nitrification activity of the mainstream system. After bioaugmentation for 320 d (V), when SAUR increases from 1.50 mg NH_4^+-N/L•h to 2.79 mg NH_4^+-N/L•h, the absolute ammonia removal quantity increases from 10.9 to 16.7 mg/L; SNUR increases from 1.90 mg NO_2^--N/L•h to 3.62 mg NO_2^--N/L•h; and SAUR and SNUR increase by 1.86 and 1.90 times, respectively. Yu et al. observed an asynchronous increase of ammonia utilizing rate (AUR) and nitrite utilizing rate (NUR) in their research on laboratory-scale bioaugmentation-enhanced nitrification. Nitrite accumulation was observed [19]. However, in this study, the number and nitrification activity of AOB and NOB increased synchronously. Therefore, the accumulation of nitrite was not determined.

3.2. Sidestream system performance and nitrification activity

The sidestream system adopts the continuous stirred-tank reactor (CSTR) to treat reject water. SRT is controlled at 10 d. Figs. 3 and 4 show the ammonia concentrations in influent and effluent, nitrite in effluent, and changes in SAUR and SNUR. After the reactor is started for 53 d, ammonia in influent is increased from 45 to 400 mg/L, and ammonia in effluent is kept below 10 mg/L, with better sidestream nitrification activity. After the reactor runs for 53 d, SAUR and SNUR are increased from 2.7 mg NH_4^+-N/g VSS/h and 2.3 mg NO_2^--N/g VSS/h at the beginning, to 11.8 mg NH_4^+-N/g VSS/h and 11.2 mg NO_2^--N/g VSS/h, respectively. Subsequently, SAUR and SNUR increase to 22.8 mg NH_4^+-N/g VSS/h and 23.1 mg NO_2^--N/g VSS/h, respectively. At that time, SAUR and SNUR of the sidestream reactor are 7.2 and 5.9 times the corresponding activity of the mainstream at 14℃. The experimental result shows that the reject water generated from the sludge treatment can be used to culture the nitrifying bacteria to conduct the bioaugmentation of the mainstream system. This result is in agreement with the findings of Kos [7] and Plaza et al. [8]. In the reject water treatment process, nitrite increases suddenly, as shown in Fig. 5. Smith and Oerther show that nitrite is accumulated temporarily, if a CSTR is used to treat wastewater with high ammonia concentrations [22]. However, the concentration reaches a balanced state rapidly. The foregoing finding is similar to that obtained in the current study. However, Yu et al. adopted a plug flow reactor (PFR) to treat the reject water and it was observed that the activity of NOB was significantly lower than that of AOB. Similarly, the accumulation of nitrite occurred [2]. According to Chudoba et al., PFR is not easily inhibited by a high concentration of ammonia with a higher nitrification efficiency [32]. However, Smith and Oerther showed that nitrite accumulates easily when a PFR is used to treat high concentration of ammonia in wastewater [22]. Therefore, the nitrifying bacteria cultured by PFR are more diverse [22], but a CSTR may be more suitable to treat the sludge water based on stable treatment.

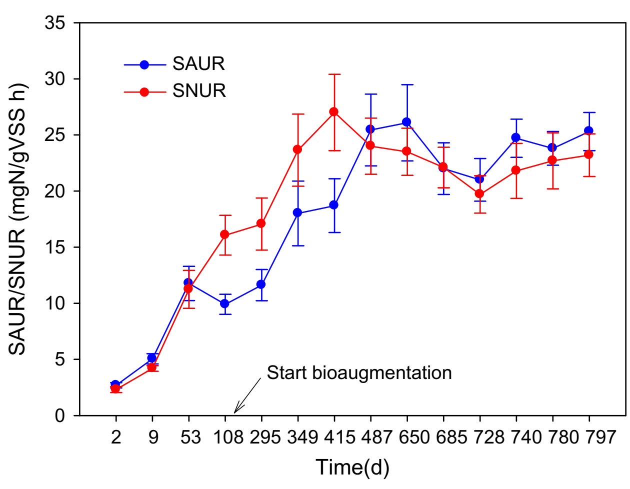

Fig. 3. Changes of SAUR /SNUR in sidestream reactor (Error bars indicate ± one standard error).

3.3. FISH test result

The FISH test result shows that the ratio of AOB + NOB to DAPI is 22.7% in the stable operation stage of the sidestream reactor, and in operation stage V of the mainstream reactor. The percentage of nitrifying bacteria (by NSO1225 + NIT3 + Ntspa662) in the total number of bacteria (by DAPI) is 14.6%. This percentage is higher than the percentage of nitrifying bacteria in a general wastewater treatment plant (5–8%) [12]. Bioaugmentation may increase the share of the nitrifying bacteria in the mainstream system [2]. The difference in the number of nitrifying bacteria between the mainstream and sidestream systems is mainly caused by the different influent C/N ratios. High concentration of ammonia nitrogen and low C/N ratio (0.51) cause a high percentage of nitrifying bacteria in the sidestream system. Meanwhile, high A/O ratio (1.13) and low SRT in the mainstream system are the reasons for the lower number of nitrifying bacteria.

Before bioaugmentation, the nitrifying bacteria in the mainstream system are fewer, and the corresponding nitrification activity is extremely low, with higher concentration of ammonia nitrogen in effluent. After

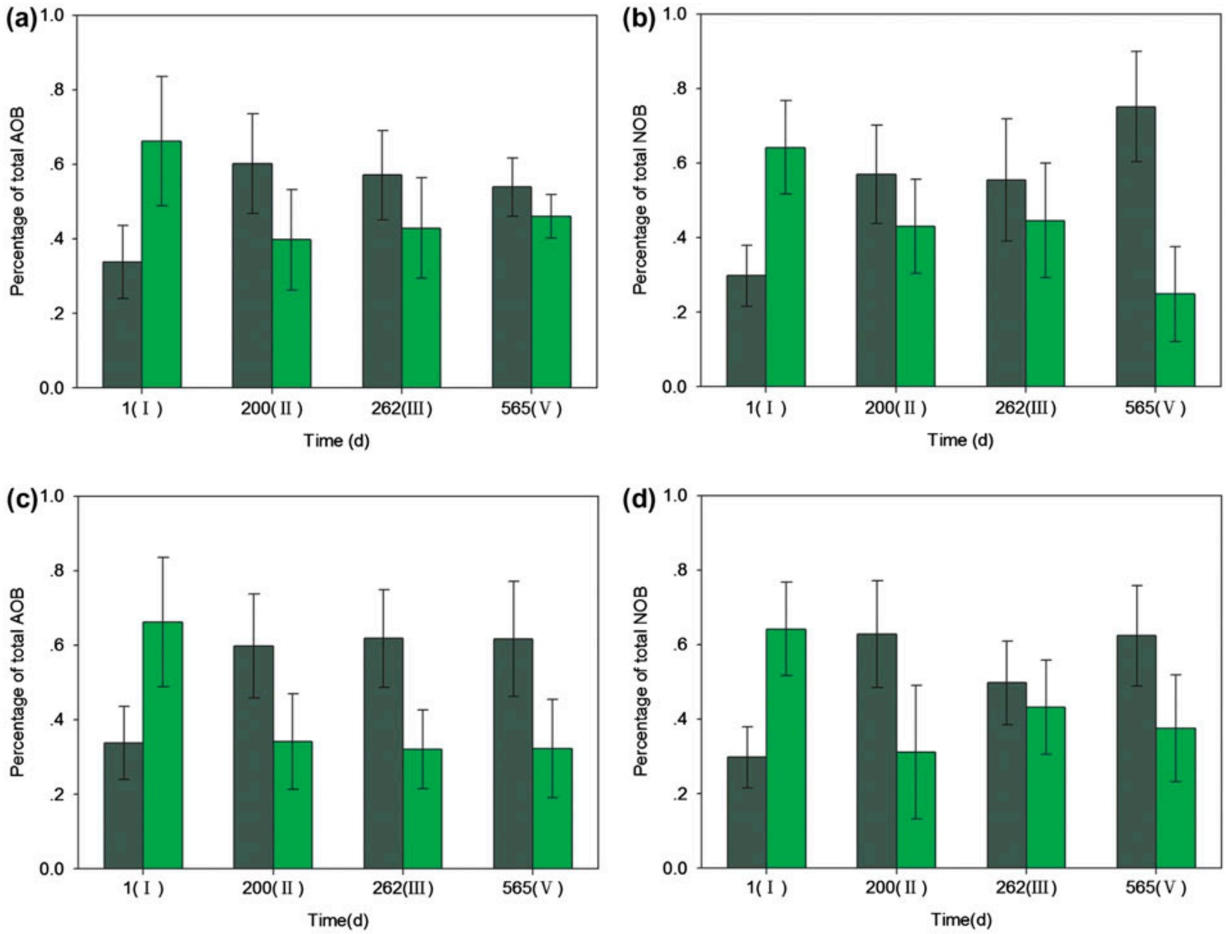

Fig. 4. Community structure of nitrifiers in main- and side stream reactor. Error bars indicate ± one standard deviation ofeach group of AOB and NOB (n = 3). The total number of AOB stained by the probe of NSO1225 and the total number of NOB calculated by the sum of *Nitrobacter* and *Nitrospira*.

nitrifier organisms are bioaugmented for 60 d, the percentages of AOB (by NSO1125) and NOB (by NIT3 + Ntapa662) in the total number of bacteria increased from 2.4 ± 0.2% and 2.1 ± 0.2% (200 d) before bioaugmentation to 4.0 ± 0.6 and 2.8 ± 0.3%. Finally, they are kept (at stage V) at 6.8 ± 1.2 and 7.8 ± 2.2%. Changes in the number and activity of the nitrifying bacteria indicate that nitrifiers bioaugmented from the sidestream can stay in the mainstream system to grow continuously. This result is further verified in Fig. 4, which shows the community structure of nitrifying bacteria. As Fig. 4(a) and (b) indicate, at the beginning, *Nitrosospira* (Probe Nsv443) and *Nitrospira* (Probe Ntspa662) are dominant AOB and NOB of the mainstream system. Subsequently, they are changed to *Nitrosomonas europaea* (Probe Nmv) and *Nitrobacter* (Probe NIT3) to maintain a relatively stable community structure. The community structure of the nitrifying bacteria in the sidestream system is shown in Fig. 4(c) and (d). The sidestream and mainstream systems are inoculated with activated sludge from the same wastewater treatment plant. Therefore, at the beginning stage, the community structure of nitrifying bacteria in the sidestream system is similar to that in the mainstream system. Dominant AOB are changed from *Nitrosospira* to *N. europaea* when the system has operated for approximately 50 d, and dominant NOB are changed rapidly from *Nitrospira* to *Nitrobacter* after the reactor is started. The dominant AOB and NOB are kept for the next 2 years of operation. Under stable conditions of operation, the community structure of nitrifying bacteria in the mainstream system is similar to that in the sidestream system. NOB cultured by laboratory-size PFR treating reject water is different from those in the mainstream reactor. The community of nitrifying bacteria cultured by the pilot-scale CSTR is similar to that in the mainstream system [19]. From the point of flow regime reactor adopted, the

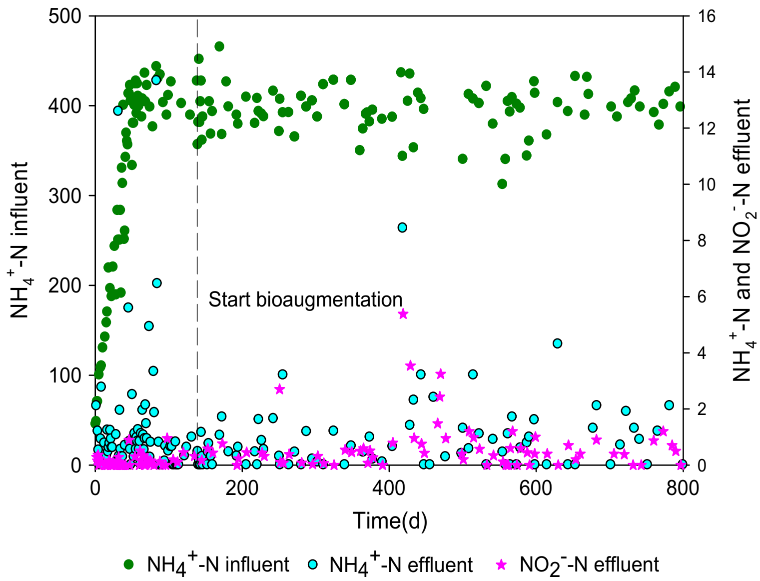

Fig. 5. Change of ammonium in sidestream reactor.

nitrifying bacteria generated by using the CSTR to treat reject water may be better matched with the state of nitrifying bacteria in the mainstream system. This condition, which may be caused by a higher concentration of the substrate in the mainstream system, disagrees with the findings of Smith and Oerther [20].

The changes in the AOB community structure of the mainstream system can be explained by the *K*–*r* hypothesis. *K*-strategists have higher affinity with the substrate and lower maximum growth rates. By contrast, the *r*-strategists have lower affinity with the substrate and higher maximum growth rates [33–36]. *N. europaea* is an *r*-strategist, which is a dominant bacterium in high concentration of the substrate [37]. Therefore, when the concentration of ammonia in the reactor is high, the dominant bacterium is changed from *Nitrosospira* to *N. europaea*. The variety of nitrifying bacteria in the active sludge may affect the nitrifying rate [38]. High nitrification activity of the activated sludge in the mainstream reactor is consistent with that obtained when the *r*-strategist is in a dominant position.

4. Conclusions

(1) Under the conditions of short SRT and low temperature, the ammonia nitrogen removal efficiency and nitrification activity exhibited an almost twofold increase after bioaugmentation. Moreover, the FISH result shows that the percentage of AOB and NOB in the total number of bacteria increased to 2.83 and 3.71 times after bioaugmentation, which is higher than the increase in nitrification activity.

(2) After long-term bioaugmentation, the dominant AOB in the mainstream system changed from *Nitrosospira* to *N. europaea*, and the dominant NOB changed from *Nitrospira* to *Nitrobacter*. The sidestream reject water treatment and mainstream system exhibited similar community structures of nitrifying bacteria.

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