Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2019.23921

147 (2019) 326–333
April

Effects of aeration partition ratio on nitrogen removal performance and the microbial community in the A²/O process

Yi Rong[a], Haidong Chang[a], Chunlei Wu[a], Wushou Zhang[b], Pengkang Jin[a,*]

[a]*Key Laboratory of Environmental Engineering, School of Environmental and Municipal Engineering, Xi'an University of Architecture and Technology, Xi'an, Shaanxi Province, Shaanxi 710055, China, Tel. +86 13379217572; email: pkjin@hotmail.com (P. Jin), Tel. +86 15596899631; email: 270947119@qq.com (Y. Rong), Tel. +86 13193347077; email: 735311493@qq.com (H. Chang), Tel. +86 15829008726; email: 1009750256@qq.com (C. Wu)*
[b]*Shaanxi Lang Zheng Environmental Protection Technology LTD. CO., Shaanxi 710075, China, Tel. +86 13609208593; email: LZ-zjb@sxlzhb.com*

Received 16 March 2018; Accepted 30 January 2019

ABSTRACT

The partition of the aeration zone is the key factor to control the A²/O process, and it directly affects nitrogen removal efficiency. In this study, a pilot-scale A²/O plant was operated for the treatment of real domestic wastewater, which has an effective volume of 10 m^3. The nitrogen removal efficiency and variations in the microbial community were studied on four different proportions of the aeration zone. Optimal denitrification performance with removal efficiency of 82.7% was achieved with the ratio of anoxic zone (A) to aerobic zone (O) = 5.7/6.6, and the effluent total nitrogen concentration of 9.3 mg L^{-1}. The denitrification rate was the highest at this ratio, and the activated sludge exhibited good settleability. The microbial community structure was detected by high-throughput sequencing, the results demonstrated that a large proportion of the anoxic zone provided a more suitable environment for denitrifying bacteria. Thus, the diversity and relative abundance of denitrifying bacteria at the ratio of A/O = 5.7/6.6 (15, 1.54%) were higher than that of A/O = 2.6/6.6 (13, 1.01%) that resulted in overall higher total nitrogen removal efficiency.

Keywords: Aeration zone; A²/O; Nitrogen removal performance; High-throughput sequencing; Microbial community structure

1. Introduction

With the implementation of national water management policies in China, municipal wastewater treatment plants are complying with more stringent water quality standards, particularly for reductions in total nitrogen. A²/O is a traditional biological nutrient removal (BNR) process that is also facing the challenge of improvement. In the traditional A²/O process, nitrogen in wastewater is converted to nitrogen gas (N_2) as the final product, by both aerobic autotrophic nitrification and anoxic heterotrophic denitrification [1]. Over the past decades, various new BNR processes have been proposed, researched, and applied [2], including the modified UTC (University of Cape Town process) [3], A²N-SBR (anaerobic-anoxic/nitrification-sequencing batch reactor) [4], the Dephanox process [5], and the AAO-BAF (anaerobic/anoxic/oxic-biological aerated filter) system [6]. However, all these new BNR processes used for different purposes are configured with anoxic and aerobic phases [7].

The nitrogen compounds of most wastewater exist in the form of ammonium (NH_4^+). Therefore, ammonium removal or conversion in the aeration zone is a key point for nitrogen removal [8]. Nevertheless, traditional BNR processes utilized in wastewater treatment plants involve the oxidation of ammonium to nitrate (NO_3^-) (nitrification) followed by nitrate reduction, with an organic carbon source, to nitrogen gas

* Corresponding author.

(denitrification) in the anoxic zone. Thus, the total nitrogen (TN) in sewage is synergized by nitrification in the aerobic zone and denitrification in the anoxic zone, and the ratio of the aeration zone to the anoxic zone plays an important role in TN removal efficiency. Zhang et al. [2] enhanced the average removal efficiency of TN to 83% ± 3% by optimizing the volume ratio of the anoxic zone to the aerobic zone in the AAO-BAF system. Meanwhile, many reports have been investigated for improving the efficiency of A^2/O process and reducing operation cost [9,10]. However, few systematic studies focus on the effect of the ratio of anoxic zone volume to aerobic zone volume on nutrient removal in the A^2/O process [2].

Changes in the aeration partition ratio affect the microorganisms' environment, thereby affecting nitrogen removal efficiency in the A^2/O process. Jin et al. [11] found that the relative abundance of each species in the microorganism community was affected by the aeration mode in the oxidation ditch. The diversity and relative abundance of denitrifying bacteria under step aeration were better and achieved higher nitrogen removal efficiency. However, the effect of the aeration partition ratio on nitrogen removal performance and the microbial community in the A^2/O process have not yet been thoroughly investigated.

In this study, the nitrogen removal mechanism of the A^2/O process was investigated with different aeration partitions. In addition, the microbial community distribution was utilized to discuss the mechanism for high-performance nitrogen removal under the optimal ratio of A/O. The objective of this research is to lay a theoretical and technical foundation to municipal wastewater treatment plants for strengthening the removal of nitrogen.

2. Materials and methods

2.1. Plant and its operation

A pilot-scale plant (Fig. 1) with anaerobic/anoxic/aerobic(A/A/O) process was established. The plant was made of square steel plate with the thickness of 10 mm, and it had an approximate working volume of 10 m^3 ($L \times W \times H$ = 3.75m × 1.5 m × 2.0 m). Baffles were used to divide the plant into 11 compartments, and the last eight compartments were equipped with independently adjustable, microporous aerator pipes on the bottom. Therefore, the volume of the aeration zone could be changed at any time.

The A^2/O process was operated for a 120 d as a period, and this period was divided into four phase: Phase 1 (days 0–29) with an aeration partition ratio of A/O = 2.6/6.6, phase 2 (days 30–59) with an aeration partition ratio of A/O = 3.9/6.6, phase 3 (days 60–89) with an aeration partition ratio of A/O = 5.7/6.6, and phase 4 (days 90–119) with an aeration partition ratio of A/O = 8.1/6.6. During the entire operation, the influent flow was maintained at 0.5 ± 0.03 m^3 h^{-1}, the hydraulic retention time (HRT) was 20 h, and the operation temperature was maintained at 20°C ± 1°C by a thermostat. The sludge retention time (SRT) was maintained for 20 d by controlling sludge wastage. The resulting mixed liquor suspended solids (MLSS) concentration was within the range of 3,800–4,200 mg L^{-1}. The returned sludge from the secondary clarifier (external recycle) and nitrate recirculation from the last aerobic compartment (internal recycle) were controlled by peristaltic pumps. The internal and external recycle ratios were set at 2 and 1, respectively. In each anaerobic, anoxic, and aerobic zone, the aeration was controlled by the gas flow meter, the dissolved oxygen (DO) concentration was maintained at less than 0.2 mg L^{-1}, within the ranges of 0.2–0.5 mg L^{-1} and 2 ± 0.5 mg L^{-1}, respectively. A detailed description of conditions is shown in Table 1.

2.2. Wastewater and sludge

Raw wastewater was collected from an aerated grit chamber of a municipal wastewater treatment plant in Xi'an, China. Raw wastewater was first pumped into an intermediate tank (5 m^3) before being pumped into the bioreactor. A summary of the influent characteristics is listed in Table 2.

The seed sludge was taken from the returned sludge of the municipal wastewater treatment plant in Xi'an, China. The experiments reported below were carried out 20 d after seeding, when the pilot plant reached a steady state, as indicated by the relatively constant MLSS and mixed liquor volatile suspended solids (MLVSS) concentrations as well as stable plant performance.

2.3. Analytical methods

The concentrations of ammonium (NH_4^+–N), nitrate (NO_3^-–N), TN, MLSS, MLVSS, and the sludge volume index (SVI)

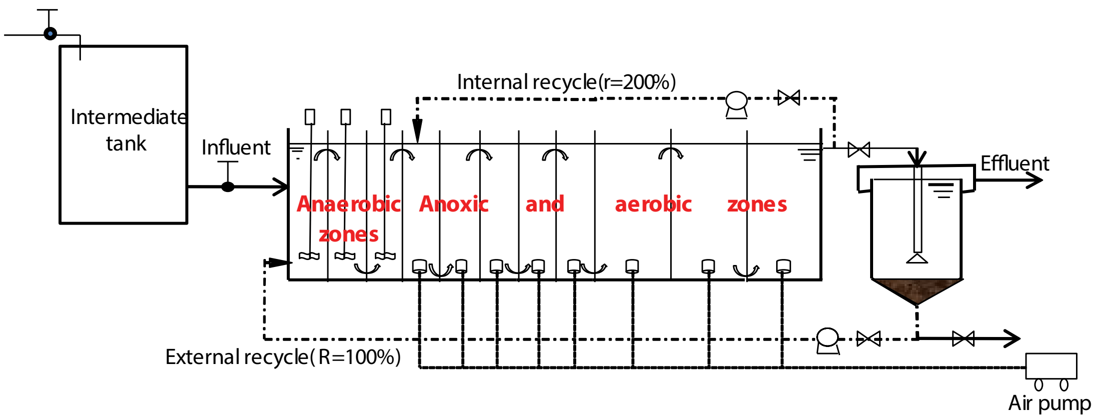

Fig. 1. Schematic plan of the pilot-scale plant.

Table 1
Operational conditions for this study

Items	Phase 1	Phase 2	Phase 3	Phase 4
Time (d)	0–29	30–59	60–89	90–119
Aeration partition ratio	A/O = 4/10	A/O = 5.25/8.75	A/O = 6.5/7.5	A/O = 7.75/6.25
HRT (h)	20			
SRT (d)	20			
MLSS (mg L^{-1})	3,792–4,213			
Anaerobic/anoxic DO (mg L^{-1})	≤0.5			
Aerobic DO (mg L^{-1})	2 ± 0.5			
Internal recycle ratio	1			
External recycle ratio	2			

Table 2
Influent characteristics of the pilot-scale A^2/O plant

Parameter	Range	Mean
pH	7.2–7.6	7.5
COD (mg L^{-1})	275.3–499.1	384.1
TN (mg L^{-1})	43.7–88.4	56.7
TP (mg L^{-1})	2.8–21.9	10.3
NH_4^+–N (mg L^{-1})	24.3–44.6	35.6
NO_3^-–N (mg L^{-1})	0.17–3.3	1.1

were measured by the standard Chinese State Environmental Protection Administration (SEPA) methods [12].

To determine the denitrification rate, 2 L of active sludge was taken from the anoxic zone in a 4 L organic glass barrel for testing; the experiments were conducted at room temperature (22°C). 2 L of domestic sewage was added after injecting nitrogen into the reactor to expel air, and potassium nitrate was then added to reach a nitrate concentration of 25 mg L^{-1} in the reactor; 1 mL of allylthiourea was also added, to inhibit the nitrification reaction in the activated sludge. After the mixture was uniformly mixed by magnetic stirrer, the first sample was extracted and filtered over a 0.45 μm filter for nitrate determination. This procedure was repeated at 0, 5, 10, 15, 20, 30, 40, 50, 60, 80, 100, 120, 150, 180, 210, and 240 min. At the end of the experiment, the MLVSS value of the mixture was determined. A curve of nitrate concentration varied with time was drawn after each determination of nitrate concentration, and the rate of denitrification was calculated on the basis of the slope (r) of the curve.

Total genome DNA from samples was extracted using the CTAB/SDS method [13]. The DNA concentration and purity were monitored on 1% agarose gels. Based on the concentration, the DNA was diluted to 1 ng μL^{-1} by sterile water. 16S rRNA/18S rRNA/ITS genes of distinct regions (16S V4/16S V3/16S V3–V4/16S V4–V5, 18S V4/18S V9, ITS1/ITS2, and Arc V4) were amplified by specific primers (e.g., 16S V4: 515F-806R, 18S V4: 528F-706R, 18S V9: 1380F-1510R) with the barcode. All PCR amplification was completed by Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Hitchin Herts SG4 0TY, UK) [14].

3. Results and discussion

3.1. Nitrogen removal efficiency with different aeration partition ratios

The aeration partition ratio had a direct effect on nitrogen removal efficiency in the A^2/O process. An insufficient aerobic zone would result in a high concentration of ammonium in the effluent, while an excessive aerobic zone would cause low denitrification efficiency [2]. Fig. 2 shows the nitrogen removal efficiency of the A^2/O system in terms of four different aeration partition ratios. A high nitrification effect was obtained from phase 1 (A/O = 2.6/6.6) to phase 3 (A/O = 5.7/6.6) when ammonium was completely oxidized to nitrate and the average effluent ammonium concentration was lower than 1 mg L^{-1}. The average TN concentration of the effluent reduced from 12.9 to 9.3 mg L^{-1}, and the TN removal efficiency increased from 77.4% to 82.7%. The results indicated that the denitrification effect of the A^2/O system gradually increased with the increase of anoxic zone. However, in phase 4, the average effluent ammonium concentration increased to 3.44 mg L^{-1} and the effluent's average TN concentration rapidly increased to 12.1 mg L^{-1} when the aeration partition ratio was set as A/O = 8.1/6.6. A large anoxic zone led to insufficient nitrification in the A^2/O system, which affected the efficiency of effluent TN removal. The optimal ratio was phase 3 (A/O = 5.7/6.6) in which the TN removal efficiency increased to 82.7% and the effluent's average TN concentration reduced to 9.3 mg L^{-1}.

3.2. Denitrification rate with different aeration partition ratios

Fig. 3 shows the denitrification rate curve of the A^2/O system with four different aeration partition ratios. It is observed that the denitrification could be divided into three stages: Stage 1 (0–30 min) represents the initial reaction, stage 2 (30–120 min) represents a gradual decrease in reaction rate, and stage 3 (120–240 min) represents a slow reaction rate. The calculation results about denitrification rate are shown in Table 3. The stage 1 denitrification rate for the four aeration partition ratios was all greater than 4 mg (NO_3^-–N)/g(MLVSS)·h and the stage 1 had a significantly higher denitrification rate than stages 2 and 3. It is possible that denitrifying bacteria may preferentially utilize easily biodegradable organics as carbon source in wastewater during

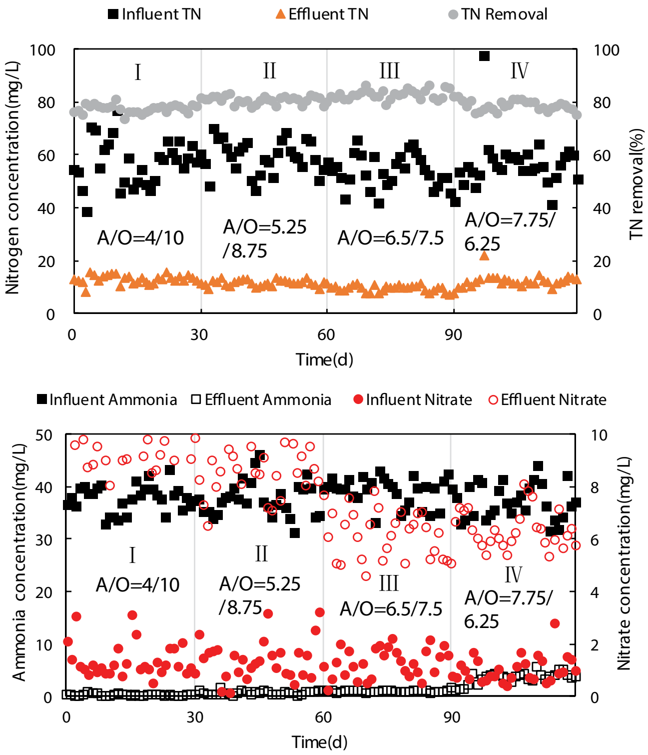

Fig. 2. Nitrogen removal efficiency at different aeration partition ratio.

denitrification in stage 1, and the denitrification rate was independent of the concentration of NO_3^-–N in stage 1, but this is only related to the population of denitrifying bacteria in the system [15].

The denitrification rate in stage 2 was lower than that of in stage 1 by 1.59–2.17 mg (NO_3^-–N)/g(MLVSS)·h. It is likely that easily biodegradable organics had been depleted by the denitrifying bacteria in stage 1, and the residual slowly biodegradable organics reduced the denitrification efficiency. Enwall et al. [16] have demonstrated that denitrifying bacteria require high-quality carbon source, and have a low efficiency with inferior carbon source. It is observed that the carbon source was the key factor limiting the denitrification rate in addition to the low NO_3^-–N concentration in stage 2. The lowest denitrification rate in stage 3 was within the range of 0.58–0.87 mg (NO_3^-–N)/g(MLVSS)·h. After stage 2, the denitrifying bacteria could only utilize endogenous respiration metabolites as carbon source for denitrification because the biodegradable carbon source and nitrate had been exhausted.

The comparisons of the denitrification rate of the A^2/O system among the four different ratios demonstrated that the denitrification effect of the A^2/O system was improved significantly with the increase of the A/O ratio. The denitrification rate increased from 4.14 mg (NO_3^-–N)/g(MLVSS)·h to 6.72 mg (NO_3^-–N)/g(MLVSS)·h. We inferred that the variations in the aeration partition ratio would affect the abundance of denitrifying bacteria in the A^2/O system, and the abundance of denitrifying bacteria in activated sludge would increase with the A/O ratio. The enrichment of denitrifying bacteria would enhance the intensity of denitrification and affected the overall nitrogen removal efficiency of the A^2/O system.

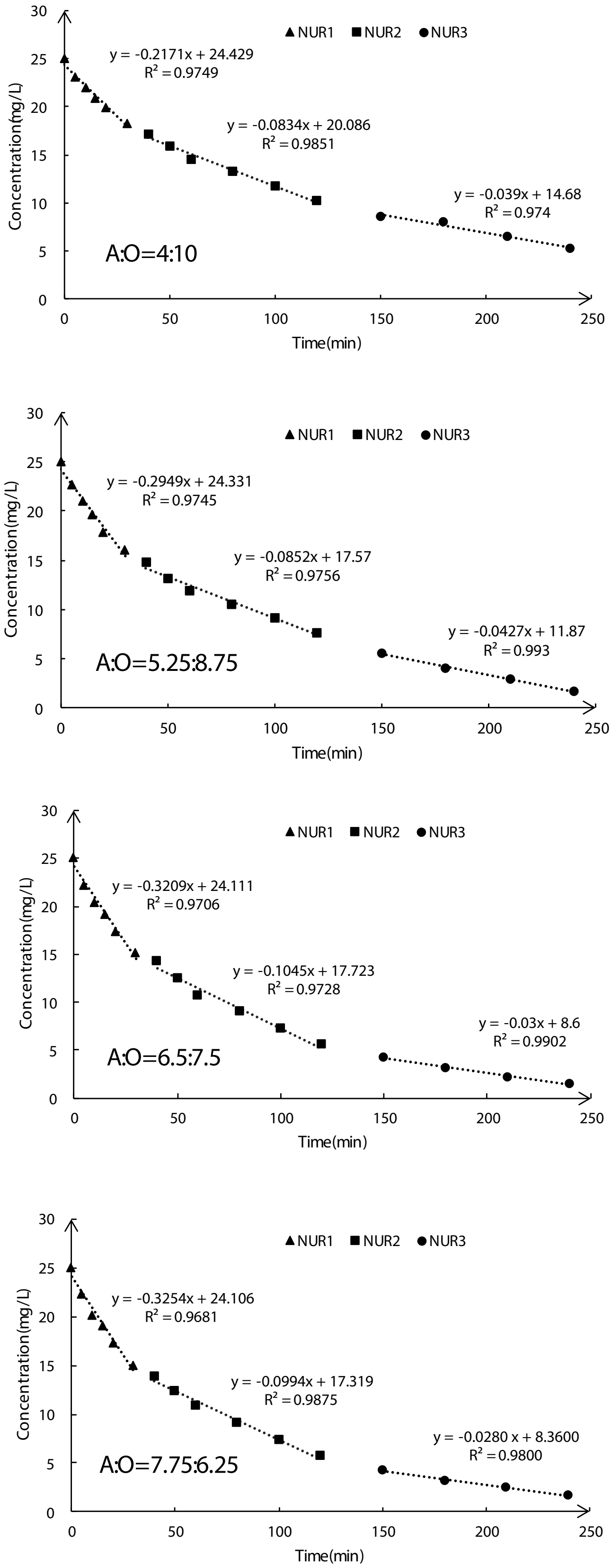

Fig. 3. Denitrification rate of the A^2/O system with different A/O ratios.

Table 3
Calculation of the denitrification rate with different A/O ratios

Aeration partition ratio	Stage	Slope/mg(NO_3^-–N)·(L·h)$^{-1}$	MLVSS/mg L^{-1}	Denitrification rate/ mg(NO_3^-–N)·(g[MLVSS]·h)$^{-1}$
A/O = 4/10	NUR1	13.03	3,150	4.14
	NUR2	5.00	3,150	1.59
	NUR3	2.34	3,150	0.74
A/O = 5.25/8.75	NUR1	17.69	2,958	5.98
	NUR2	5.11	2,958	1.73
	NUR3	2.56	2,958	0.87
A/O = 6.5/7.5	NUR1	19.25	2,894	6.65
	NUR2	6.27	2,894	2.17
	NUR3	1.8	2,894	0.62
A/O = 7.75/6.25	NUR1	19.52	2,905	6.72
	NUR2	5.96	2,905	2.05
	NUR3	1.68	2,905	0.58

3.3. Performance of activated sludge with different aeration partition ratios

Table 4 shows the SVI and MLVSS/MLSS of the anoxic zone sludge at four different aeration partition ratios. It is observed that SVI increased from 97 ± 12 mL g^{-1} of phase 1 (A/O = 2.6/6.6) to 159 ± 14 mL g^{-1} of phase 4 (A/O = 8.1/6.6). According to Richard [17], a well SVI value of activated sludge is generally maintained between 80 and 120 mg L^{-1}. However, the SVI of the anoxic zone activated sludge reached 159 mL g^{-1} approximately in phase 4 (A/O = 8.1/6.6). Some filamentous sludge bulking occurred in phase 4 during operation. The filamentous sludge bulking may have been caused by an insufficient aeration zone where led the A^2/O system to low DO levels, resulting in excessive growth of filamentous bacteria in the anoxic zone [18,19]. Therefore, the aeration partition ratio may affect the characteristics of activated sludge in the A^2/O system. The MLVSS/MLSS of the anoxic zone sludge increased gradually with the extension of the anoxic zone. It implies that the diversity and population of the biological community had changed.

3.4. Microbial community under different aeration partition ratios

3.4.1. Analysis of microbial composition

To further investigate the mechanisms of the aeration partition ratio on nitrogen removal efficiency in the A^2/O process, the bacterial community was measured and analyzed. Four samples were collected from the anoxic zone (A1), aeration zone (A3) in phase 3 (A/O = 5.7/6.6), the anoxic zone (A2) and aeration zone (A4) in phase 1 (A/O = 2.6/6.6), respectively. Fig. 4 shows the microbial DNA dilution curve of the four samples. The curves of the four samples tend to be flat, indicating that the sequencing data were reasonable [20].

Fig. 5 shows the sequence number of the four samples at different classification levels. The sequence number of the four samples was different at the kingdom, phylum, class, order, family, genus, and species levels, respectively, this is to say, the four samples had different microbial community abundances. The microbial abundance of sample in phase 3 slightly exceeded that in phase 1, and the differences between the two samples from the anoxic zone were greater than that from the aeration zone.

Table 4
SVI and MLVSS/MLSS of the anoxic zone sludge at different A/O ratios

A/O	SVI (mL g^{-1})	MLVSS/MLSS
Phase 1 (4/10)	97 ± 12	0.72
Phase 2 (5.25/8.75)	114 ± 10	0.73
Phase 3 (6.5/7.5)	136 ± 17	0.75
Phase 4 (7.75/6.25)	159 ± 14	0.76

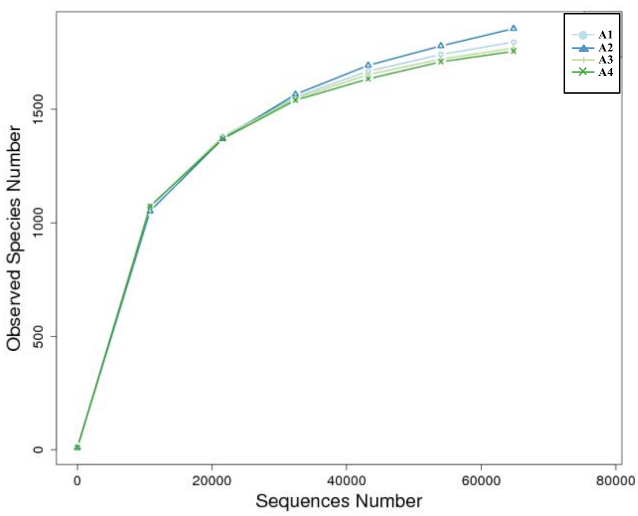

Fig. 4. Microbial DNA dilution curves of the four samples.

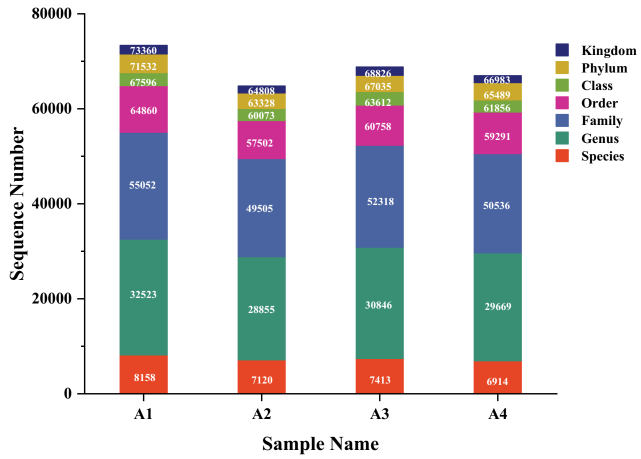

Fig. 5. Sequence number of the four samples at different classification levels.

3.4.2. Analysis of microbial diversity

The various functions of A^2/O process are achieved through the metabolic activities of microorganisms, and the strength of the A^2/O system function is directly related to the abundance of corresponding microbial species. That is the higher the abundance of certain microorganisms, the stronger the corresponding function. Therefore, it is important to analyze the species and abundance of microorganisms in the reactor for the exploration of reactor function. Fig. 6 shows the relative abundance of the four samples at the phylum level. The main microbial populations of four samples were basically the same. Proteobacteria, Bacteroidetes, Firmicutes, and Chloroflexi were the main bacterial phyla in both aeration partition ratios. This result is consistent with the most reported results [21–23]. Jaenicke et al. [24] pointed out that Proteobacteria were the main microorganisms in sludge fermentation, and they play an important role in sludge hydrolysis. Firmicutes and Bacteroidetes are also two important fermentative bacterial phyla, which could convert protein and polysaccharide into acetic acid and propionic acid [25,26].

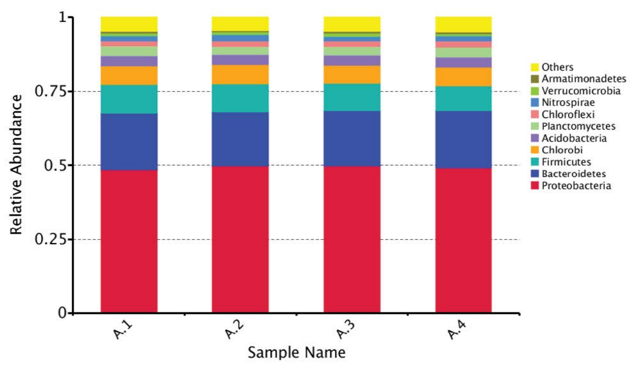

Fig. 6. Relative abundance of microorganisms in the four samples at the phylum level.

Fig. 7 shows the operational taxonomic units (OTUs) Venn diagrams for the four samples. The OTUs of the four samples were 1,797; 1,856; 1,770; and 1,756, respectively. The differences between the samples from both aeration partition ratios were not significant. The similarity between the samples may be their identical operating parameters (e.g., SRT, DO, influent flow rate, and temperature). This implies that the aeration partition ratio had a slight influence on the microbial species.

3.4.3. Microbial population distribution

To further compare the microbial communities, the species and relative abundances of denitrifying bacteria from the anoxic zone at both aeration partition ratios are shown in Table 5. There were 15 and 13 genera of denitrifying bacteria in phase 3 (A/O = 5.7/6.6) and phase 1 (A/O = 2.6/6.6), respectively. Denitrifying bacteria species in phase 3 (A/O = 5.7/6.6) were richer than in phase 1 (A/O = 2.6/6.6). The relative abundance of denitrifying bacteria in phase 3 (A/O = 5.7/6.6) and phase 1 (A/O = 2.6/6.6) were 1.54% and 1.01%, respectively. There were more denitrifying bacteria in phase 3 (A/O = 5.7/6.6) than in phase 1 (A/O = 2.6/6.6).

Thauera, *Zoogloea*, and *Hyphomicrobium* were the three main denitrifying bacteria in terms of both aeration partition ratios, accounting for more than 70% of the total denitrifying bacteria that corresponds to Zhang et al's report [2]. The higher relative abundance and diversity of denitrifying bacteria in phase 3 (A/O = 5.7/6.6) led to higher denitrification activity. The maximum denitrification rates in phase 3 (A/O = 5.7/6.6) and phase 1 (A/O = 2.6/6.6) were 6.65 mg $(NO_3^-–N)$/g(MLVSS)·h and 4.14 mg $(NO_3^-–N)$/g(MLVSS)·h, respectively. These results correspond to the TN removal efficiency of the two aeration partition ratios. This indicates that the aeration partition ratio in the A^2/O process could affect the microbial environment and the enrichment of denitrifying bacteria.

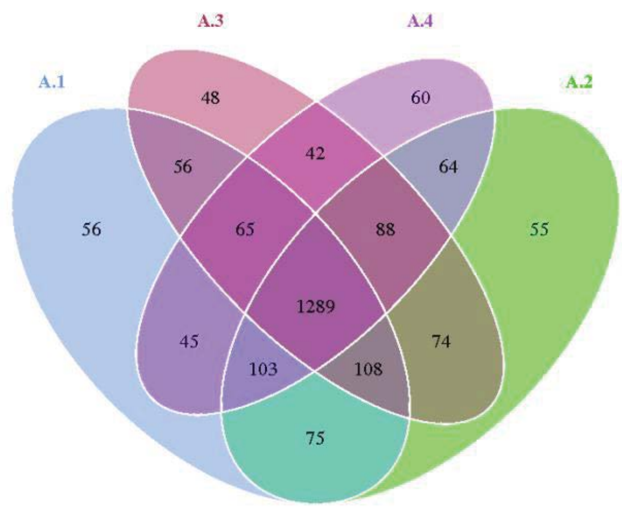

Fig. 7. OTUs Venn diagram of the four samples.

Table 5
Species and relative abundances of denitrifying bacteria at the two aeration partition ratios

Denitrifying bacterial	Relative abundance (%)	
	A/O = 6.5/7.5	A/O = 4/10
Thauera	0.575068	0.240785
Hyphomicrobium	0.305214	0.276021
Zoogloea	0.22509	0.14386
Thiobacillus	0.10728	0.11596
Paracoccus	0.080421	0.0632
Methylobacter	0.02251	0.03482
Lactobacillus	0.011825	0.023256
Bacillus	0.02075	0.01053
Microbacterium	0.04758	0.01126
Comamonas	0.05286	0.03658
Eubacterium	0.05017	0.03241
Acinetobacter	0.01157	0
Diaphorobacter	0.01096	0.01258
Flavobacterium	0.01175	0
Stenotrophomonas	0.01108	0.01142
Total	1.535128	1.012682

4. Conclusions

The aeration partition ratio had a significant impact on the nitrogen removal efficiency in the A^2/O process for real domestic wastewater treatment, and the denitrification rate of the A^2/O process would increase with the extension of the anoxic zone. The optimal A/O ratio was 5.7/6.6. A large anoxic zone had an adverse effect on sedimentation performance of the activated sludge and resulted in excessive growth of filamentous bacteria in the anoxic zone. Compared with phase 1 (A/O = 2.6/6.6), phase 3 (A/O = 5.7/6.6) provided a suitable environment for denitrifying bacteria. Thus, the diversity and relative abundance of denitrifying bacteria were found to be higher to achieve better nitrogen removal efficiency.

Acknowledgements

This study is supported by National Key Research and Development Program (2016YFC0400701), Shaanxi Science & Technology Co-ordination & Innovation Program (No. 2016TZC-S-19-3), the Program for Innovative Research Team in Shaanxi (Grant No. 2013KCT-13).

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