Nitrous oxide emission in an aerobic granulation sequencing batch airlift reactor at ambient temperatures

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Abstract

This study aims to investigate the nitrous oxide (N_2O) emission in an aerobic granulation sequencing batch airlift reactor (SBAR) and the associated microbial community of aerobic granular sludge at ambient temperature (18 ± 3)º C. After 48 days of operation, 1e2 mm granules were obtained and excellent chemical oxygen demand (COD) and ammonium (NH_4^+-N) removal efficiencies were stably O concentration in the off gas was maximal at the beginning of the aerobic period and stabilized at a lower concentration after an initial peak. (0.60 ± 0.17, n = 3) % of the total nitrogen load to the SBAR was emitted as N_2O. A dramatic change in the microbial community structure was noted be- tween the initial seed sludge and the final mature aerobic granular sludge. Nitrosospira was identified to be the dominant ammonium oxidizing bacteria (AOB) which was attributed as the dominant source of N_2O production in aerobic granular sludge by analysis of 16S rDNA sequences

1. Introduction

Aerobic granulation sludge technology, a new and promising environmental biotechnological process, is increasingly drawing interest of researchers engaging in work in the area of biological wastewater treatment. Aerobic granules were considered to be a special case of self-immobilized microbial consortium (Seviour et al., 2010). Compared to conventional activated sludge systems, aerobic granules have the advantages of compact microbial struc- ture, good settling ability, high biomass retention, and have the ability to withstand high organic loading rate (A dav et al., 2008; Xavier et al., 2007).

Aerobic granular sludge was first reported in an aerobic up flow sludge blanket reactor by Mishima and Nakamura (1991). Forma- tion of granules in aerobic conditions has been possible and ap- pears as a promising technique for high strength or highly toxic wastewater treatment. During the last 20 years, aerobic granules have been successfully applied to the treatment of high strength organic wastewater, toxic organic wastewater, heavy metals and dyes, dairy and brewery wastewater, and even low-strength domestic wastewater in laboratory studies (A dav et al., 2008). However, it is not yet established as a large-scale application. Aerobic granulation can be affected by a number of operational parameters, such as seed sludge, substrate composition, organic loading rate, feeding strategy, reactor design, settling time, ex- change ratio, and aeration intensity (hydrodynamic shear force) etc. Inside the granules, aerobic and anoxic zones are present that can simultaneously perform different biological processes in the same system, i.e. simultaneous nitrification-denitrification (Beun et al., 1999; Qin and Liu, 2006).

The research efforts have been focused on the cultivation con- ditions and factors influencing granulation. As aerobic granular sludge is a complex biological system, the emission of nitrous oxide (N_2O) as an intermediate or end product in the metabolism of both nitrification and denitrification can not be neglected. The green- house impact of N_2O is about 300 times of carbon dioxide. N_2O can also contribute to ozone layer depletion. As the reason of N_2O has long estimated half-life (approximately 120 years), even a small amount of N_2O accumulation may cause destructive effects for centuries (Ravishankara et al., 2009). It was reported that about two thirds of the overall N_2O was emitted by microbial processes. Therefore, a better understanding and controlling of N_2O emission are urgently required for minimizing the adverse effect of N_2O. Controlling the emission of N_2O has become an interesting topic of

biological wastewater treatment. However, there is little information available in literature about the emission of N_2O in aerobic granulation.

Microbial processes are the major emission source of the N_2O. Few researches have been focused on the relationship between N_2O emission and microbial community structure, especially in aerobic granulation. With the rapid development of molecular biological techniques, many microbial molecular ecological techniques have been applied to monitor the variance of the bacterial community in activated sludge (Forney et al., 2004). Among these molecular methods, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) seems to be a more practical and useful approach for bio-monitoring the bacterial community (Zhang et al., 2011). In combination with a wide range of other micro-scale techniques such as scanning electron microscopy (SEM), researchers are now able to better investigate microbial evolution and granule morphology during sludge granulation (Li et al., 2008).

In this study, we focused on investigating the N_2O emission in an aerobic granulation sequencing batch airlift reactor and the microbial community of the aerobic granular sludge at ambient temperatures. Aerobic granules were achieved in sequencing batch airlift reactor (SBAR). The physical and chemical properties of the aerobic granules were determined. PCR-DGGE was employed for revealing the microbial community structure. This work offers a detailed investigation on the nitrous oxide emission in aerobic granulation at ambient temperatures. It could be useful for the development of the pilot- and full-scale application.

2. Materials and methods

2.1. Experimental set-up and operation

A double-walled cylindrical column gastight sequencing batch airlift reactor was used with an internal diameter of 8 cm. The reactor contained an internal riser (80 cm high, 5 cm internal diameter, bottom clearance 1.5 cm) with a working volume of 3.6 L. Air was introduced via a fine bubble aerator at the bottom of the reactor with an airflow rate of 3 L/min during the aeration phase. During the experiment temperature was controlled at ambient temperature (18 ± 3)°C.

A 6-h working cycle was applied over the entire experiment. The reactor was operated in a sequencing fed batch mode and the different periods are displayed in Fig. 1. One cycle consisted of five successive phases including: (1) 10 min feeding, (2) 317 min aerobic reaction, (3) 3 min settling, (4) 5 min discharging and (5) 30 idling. The exchange volume was 50% resulting in a hydraulic retention time (HRT) of 12 h. The sludge settling time was reduced gradually from 20 to 3min after 108 cycles in 27 days and the aeration time was increased accordingly from 300 to 317 min.

Typical cycle was performed regularly by measuring the control parameters (pH, DO and ORP), nitrogen compounds (ammonium, nitrite and nitrate) and the emission of N_2O throughout the 6-h cycle. During the aeration reaction phase, the emission gas was directly collected in gas sampling bags at the interval of 30 min. During settling, discharging, idling and feeding phases, N_2 was blown in from one side of the reactor's top cover and the injected N_2 transported the emitted gas into sampling bags (Kong et al., 2013).

2.2. Seed sludge and synthetic wastewater

Activated sludge was used as the seed sludge for the reactors at an initial sludge concentration of 6490 mg/L in mixed liquor volatile suspended solids (MLVSS) collected from a full-scale municipal wastewater treatment plant in Jinan, China.

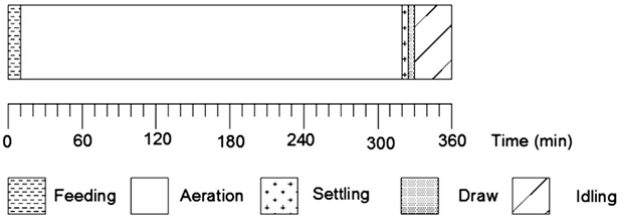

Fig. 1. Sequencing operation of the SBAR.

The comparison of synthetic wastewater were as follows (per liter): 0.52 g glucose; 0.83 g NaAC; 0.24 g NH_4Cl; 0.058 g K_2HPO_4; 0.024 g KH_2PO_4; 0.067 g $CaCl_2$; 0.042 g $MgSO_4\$7H2O$; 0.042 g EDTA; 0.25 g $NaHCO_3$; 1 ml trace element solution. One liter of trace element solution contained: 1.5 g $FeCl_3\$6H_2O$; 0.15 g H_3BO_3; 0.03 g $CuSO_4\$5H_2O$; 0.03 g KI; 0.12 g $MnCl\$4H_2O$; 0.06 g $NaMoO_4\$2H_2O$; 0.12 g $ZnSO_4\$7H_2O$; 0.15 g $CoCl_2\$6H_2O$. $NaHCO_3$ was dosed into the feeding solution to maintain the reactor pH in the neutral range between 7.0 and 7.8.

2.3. Analytical methods

Ammonium ($NH_4^+ - N$), nitrite ($NO_2^- - N$), nitrate ($NO_3^- - N$), sludge MLVSS concentration, zone settling velocity (ZSV), sludge volume index at 10 min (SVI10) and effluent volatile suspended solids (EVSS) were measured regularly according to standard methods (APHA, 2005). On-line data were collected by probes for pH, dissolved oxygen (DO), oxidation-reduction potential (ORP) and temperature (HACH HQ40d, USA).

The morphology of the seed sludge and granules in the reactors was observed under an optical microscope (BX53, Olympus, Japan) equipped with a digital camera (DP72, Olympus, Japan). In addition, the microstructure of mature granules was examined with a scanning electron microscope (SEM) (S-520, Hitachi, Japan) following the sample treatment procedure detailed by Diao et al. (2004). The emission of N_2O was measured by a gas chromatography (SP-3410, China) with an electron capture detector (ECD) and a Poropak Q column, using 30 mL/min high-purity nitrogen as the carrier gas. The temperature of the detector and oven were set at 360 °C and 50 °C, respectively (Wu et al., 2009).

2.4. Calculation of N_2O emission rate and emission quantity

The quantity of N_2O emission with time was calculated from the follo wing equation Eq. (1):

$$m_{N_2O} = \sum_{2}^{n} \left[Q \cdot \left(c_{N_2O,n} + c_{N_2O,n-1} \right) \cdot \Delta t \cdot M_{N_2O} \cdot P / (R \cdot T \cdot 2) \right] \quad (1)$$

where $m_{N_2O\text{-}N}$ is the N_2O emission quantity (g) varying with time; Q is the volumetric flow rate of air during aerobic reaction phase or of N_2 during mixing, settling, discharging and feeding phase (L min^{-1}); c_{N_2O} is the N_2O concentration in the emission-gas (mol/mol); n is the number of sampling points; Dt is the time interval between each sampling point (30 min); M_{N_2O} is the molecular weight of N_2O (44.02 g mol^{-1}); P is the atmospheric pressure (1 atm); R is the gas constant (0.082 L atm/(K mol); T is room temperature (K).

The N_2O-N emission in the aerobic granulation sequencing batch airlift system of the incoming nitrogen load was calculated from the following equation Eq. (2):

$$N_2O - N\ \text{Emitted}\ \% = (m_{N_2O-N}/m_{load-N}) \times 100 \quad (2)$$

where $m_{N_2O\text{-}N}$ is the N_2O emission quantity in cycle operation (mg); $m_{load\text{-}N}$ is the incoming nitrogen load to the aerobic granulation sequencing batch airlift system (mg).

2.5. Microbial community analysis

The seed sludge and granule samples were centrifuged (8000 rmin^{-1} for 5 min). The cell pellets were washed twice with PBS solution (NaCl 137 Mm, KCl 2.7 Mm, Na_2HPO_4 10 Mm, KH_2PO_4 2 Mm; pH 7.4). Total genomic DNA was extracted using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, USA), according to the manufacturer's instructions. The variable V3 region of the bacterial 16S rRNA gene sequence (corresponding to positions 341e534 of the Escherichia coli sequence) was amplified by PCR (Muyzer et al., 1993; Watanabe et al., 1998). PCR reactions were conducted using a PCR Authorized Thermocycler (Bio-rad, USA). Amplified products were separated by DGGE on 8% (w/v) polyacrylamide gels with 35e60% gradient of urea-formadie denaturant. DGGE was conducted at 60 °C in 1 x TAE at 120 V for 7.5 h on a Dcode system (Bio-rad, USA). The gel was stained with ethidium bromide and visualized under UV transillumination. Predominant bands were excised from the DGGE gel for nucleotide sequence determination.

The primer set F338GC/R518 (Muyzer et al., 1993) was used to amplify the bands and the products were again subjected to DGGE to check their migration. The target DNA fragments were then excised and reamplified using the primer set F338/R518 without the GC-clamp, thus obtaining a pure sample for the subcloning and sequencing step. Amplicons were ligated into pMD 18-T Vector (TAKARA, Japan) and then transformed into competent cells JM109 (TAKARA, Japan). Sequencing was carried out with a Sequencing System ABI PRISM 3730 (Applied Biosystems, USA). The obtained sequences were compared with sequences in the GenBank by BLAST Search program.

3. Results and discussion

3.1. Overall performance of the granular SBAR

The SBAR was seeded with activated sludge collected from a full-scale municipal wastewater treatment plant with nearly complete nitrogen removal. The initial MLVSS concentration in the SBAR was 6490 mg L^{-1}. Fig. 1 shows how MLVSS and effluent VSS (EVSS) responded to decreasing the settle time in the SBAR during startup period. Decreasing the settle time from 20 to 15 min on day 4 caused the EVSS to increase from 275 to 675 mg L^{-1} and MLVSS to decrease from over 6400 mg L^{-1} to less than 5400 mg L^{-1}. However, over the next 2 days EVSS decreased and MLVSS stabilized. Decreasing the settle time to 10 min on day 12 and then to 5 min on day 20 caused similar spikes in EVSS, followed by a rapid decrease in EVSS levels 2e3 days thereafter. Decreasing the settle time to 3 min on day 28 caused only slight increase in EVSS.

The startup strategy was designed to gradually decrease the settle time to promote granule formation while avoiding excess washout of biomass. The rapid recovery of EVSS within 2e4 days after each decrease in settle time (Fig. 2) shows that the remaining biomass settled better than the biomass washed out, indicating that decreasing the settle time effectively selected for biomass with enhanced settling properties. The EVSS eventually leveled off near 80 mg L^{-1} after 30 days. Decreasing the settle time from 30 to 3 min caused MLVSS levels in the SBAR to decrease from 6490 mg L^{-1} to nearly 4800 mg L^{-1}, before recovering to over 5200 mg L^{-1} on day 46.

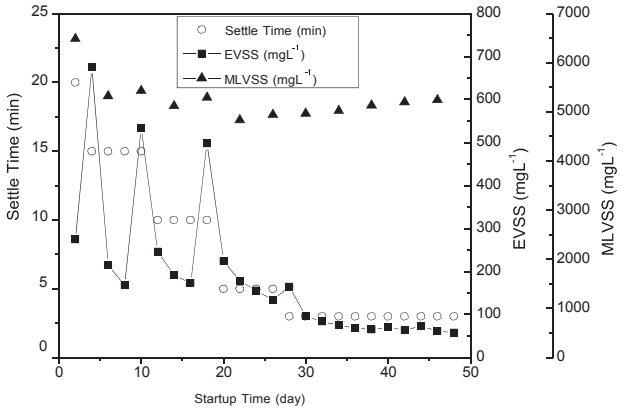

Fig. 2. The variations of MLVSS and EVSS concentrations with decreasing settle time during startup of the granular SBAR.

Table 1 lists the average influent and effluent measurements for the SBAR during steady state operation with granular sludge (day 48eday 72). During this period, the reactor efficiently removed COD and NH_4^+ – N. COD removal averaged over 90%. The residual concentration of total COD was 104 mg L^{-1}. This is consistent with residual COD levels reported by other authors treating meat processing wastewaters (Cassidy and Belia, 2005; Martínez et al., 1995; Thayalakumaran et al., 2003). The granular SBAR removed 100% of NH_4^+ – N from the influent. Removal of TN was lower during this period, with the residual N consisting mostly of nitrate (23 mg L^{-1}). P removal was over 70%. This strongly suggested that phosphorus accumulating organisms (PAOs) were enriched in the SBAR. Thayalakumaran et al. (2003) reported similar COD, N, P, and VSS removals in an aerobic SBR treating a meat processing wastewater with flocculating sludge.

After day 48 a biomass wasting strategy was implemented in the sequencing batch airlift reactor to maintain a MLVSS concentration in the SBAR of 5200 mg L^{-1} with a SRT of 20 days. From an operational standpoint, excessive loss of biomass can upset the balance of the COD, N, and P removing capacity. Recovering treatment capacities after excessive washout is difficult, especially for species with low growth rates (e.g., nitrifiers and P-accumulating microorganisms). Removal of COD, N, and P were efficiently during steady state operation, indicating that the strategy was effective. However, if not done properly, converting to a granular SBAR could result in the reactor failure due to excessive biomass washout. It is also desirable to minimize high EVSS levels from a regulatory standpoint.

3.2. Formation and characteristics of the aerobic granules

In the granular SBAR, small granules became visible about 8 days after the inoculation of the seed sludge. Granulation was fully achieved after 48 days). Aerobic granules formed in the SBAR were round with a clear boundary, different from the loose and irregular flocs of the seed sludge. In addition, the morphology and structure features were different for granules produced on different days. The granules on day 48 were smaller and more tightly packed than the granules on day 72. Granules around 1e2 mm in diameter dominated on day 48, whereas the mature granules grew to 2e3 mm on day 72. Compared to the granules on day 72, those on day 48 appeared to be more irregular in shape and less smooth on surface.

To understand the detailed microstructures, SEM was performed. As shown in Fig. 3a, aerobic granules had a clear, compact

Table 1
Average influent and effluent wastewater measurements during steady state operation (day 48eday 72).

Parameter (mg L^{-1})	Influent value	Effluent value
COD	1047 ± 63 (8)[a]	54 ± 16 (8)
NH_4^+ – N	63 ± 3 (8)	0 (8)
NO_2^- – N	0 (8)	0 (8)
NO_3^- – N	1 ± 0.4(8)	23 ± 5 (8)
Total P	14 ± 2 (8)	4 ± 2 (8)

[a] Mean ± standard deviation (number of measurements)

physical structure. On the surface of the mature granules various filamentous cells and rod-shaped bacteria were predominant and cells were tightly attached (Fig. 3b). Li et al. (2009) also observed the appearance of various rod-shaped bacteria on granule surfaces, which was similar to the surface of granules cultivated in this study.

The average biomass density during steady state operation was 21 g VSS l^{-1}, over 3 times greater than the density of seed sludge. Biomass densities between 8 and 75 g VSS l^{-1} have been reported for aerobic granules (Beun et al., 1999, 2002; Toh et al., 2003). As a result of their larger diameter and higher density, the granules settled much better than the seed sludge. The granules settle ability increased markedly after startup period. The average ZSV during steady state operation was 53 m h^{-1} for granules while only 5 m h^{-1} for the seed sludge. The average SVI was 157 ml g^{-1} for the seed sludge, compared with 21 ml g^{-1} for the granules. The values for ZSV and SVI are consistent with published values for aerobic granules (Beun et al., 2002; Toh et al., 2003), and flocculating sludge (Urbain et al., 1993).

3.3. N_2O emission characteristics in typical cycle

To investigate the degradation process of the main pollutants and N_2O emission during the steady state operation period, cycle studies were carried out on different days and the liquid-phase components were measured (Fig. 4). During the cycle operation, the COD decreased sharply in 60 min at the beginning of the cycle operation. COD and NH_4^+ – N were removed effectively in the aerated period. The NO_2^- – N produced in the aerated period was little. The concentration of NO_2^- – N decreased after 120 min in the aerated period. The NO_3^- – N concentration increased with the aeration time. However, the NO_3^- – N produced in aerated period were majority degraded by denitri cation during non-aerated period.

The average N_2O level in the off-gas during the aerated period of the cycle operation was 1321 ppb and varied significantly within the 5.5-h aerated period. The N_2O level decreased more than 10-fold from the start to the end of the aerated period in the off-gas. The N_2O in the off gas was maximal at the beginning of the aerobic period and stabilized at a low concentration after an initial peak. During the non-aerated period, in which the gas flow was only generated under pressure of the headspace of the reactor, the N_2O concentration decreased to about 49 ppb. In general, (0.60 ± 0.17, n ¼ 3)% of the total nitrogen load to the SBAR was emitted as N_2O. The N_2OeN emission in this study was different with previous study that N_2O was not detected in off gas during the simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge (de Kreuk et al., 2005).

3.4. Identification of dominant species in aerobic granules

Well-resolved DGGE bands were obtained and the different patterns demonstrated that the structure of the microbial communities were completely different between the seed sludge and the aerobic granular sludge (Fig. 5). About 200 nucleotides of partial 16S rDNA sequences were successfully amplified from nineteen

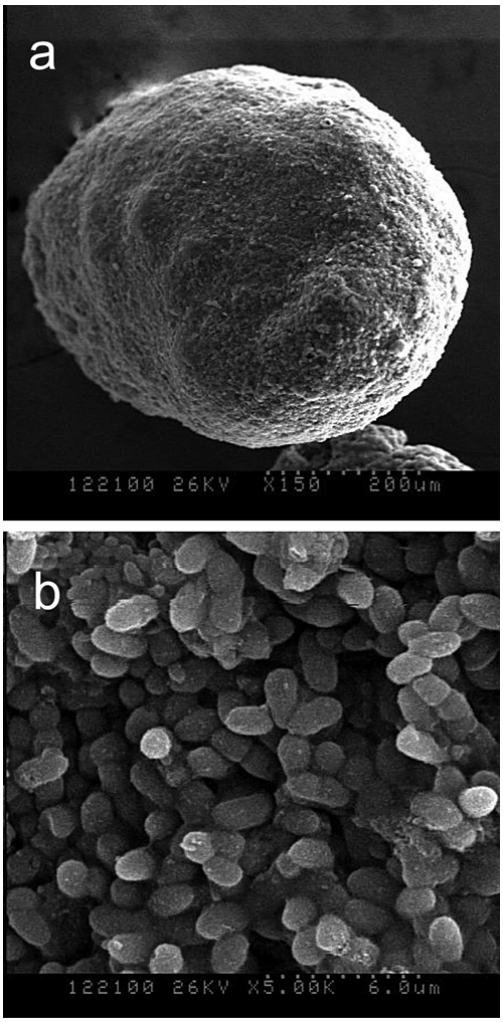

Fig. 3. Scanning electron microscopy images of the mature aerobic granules after 50 days of operation. (a) x50; (b) x5000.

DGGE bands which were dominant microbial species in seed sludge and mature granules (Table 2). In the seed sludge, the majority of the bacterial 16S rDNA sequences grouped with members of Proteobacteria, with four in the b subdivision and one in the ε subdivision. The remaining two clustered with the Flavobacteria, one clustered with the Sphingobacteria, Flavisolibacter and Nitrospira, respectively. Some members, such as Rhodocyclaceae bacterium, Thiobacillus, Zoogloea and Nitrospira, are significant genera of seed sludge bacteria.

In the aerobic granular sludge, the majority of the bactrial 16S rDNA sequences grouped with members of Proteobacteria, with three in the b subdivision, two in the a subdivision and one in the ε subdivision. Some members, such as Pseudomonas and Zoogloea, are significant genera of aerobic granular sludge bacteria (Gerardi, 2006), and they are known for their production of glue-like extracellular polymers and ability to blind cells together. Other members, such as Thauera, are generally present in biological organic oxidation and nitrifying-denitrifying activated sludge. These species were also reported as major populations in other single aerobic granules (De Sanctis et al., 2010; Li et al., 2008). The fragment (i.e. band a) was closely related to the g subclass of the Proteobacteria that are found in various phosphorus-removal

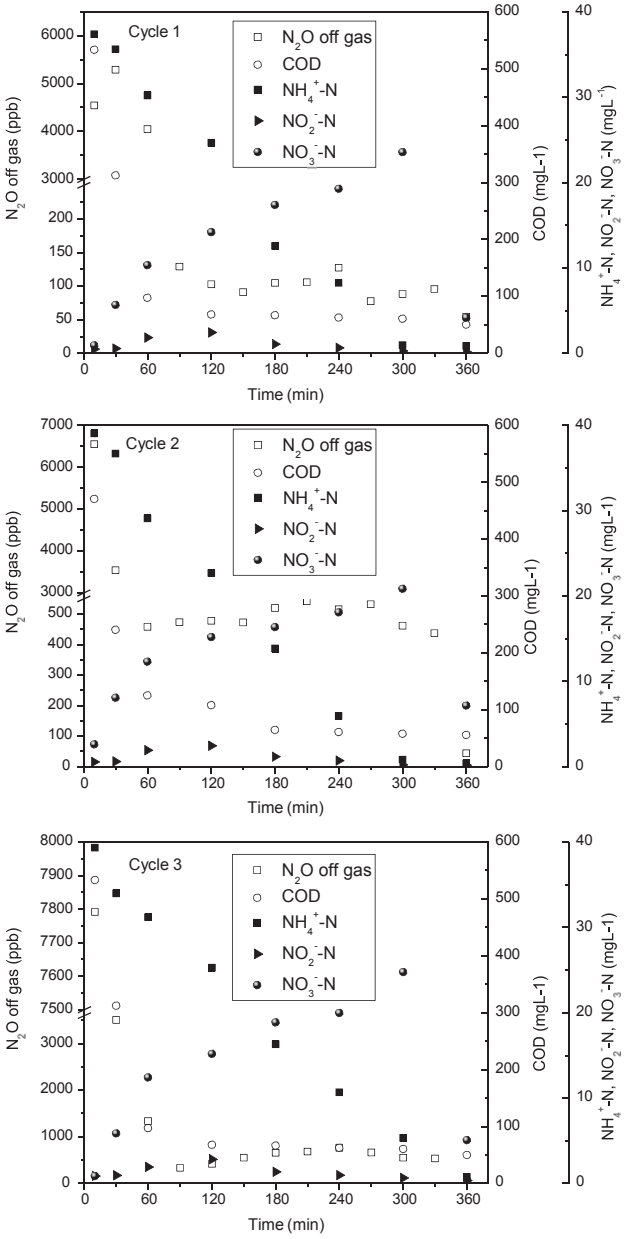

Fig. 4. N_2O in the off gas, nitrogen compounds and carbon concentration profiles during typical cycle of the steady state operation.

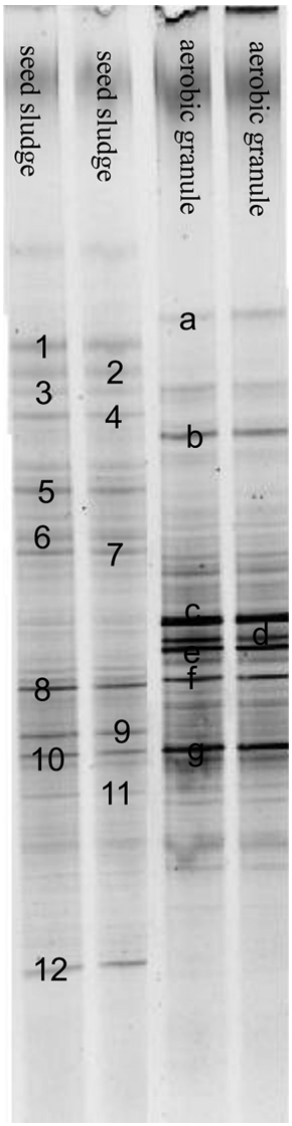

Fig. 5. DGGE profile of the seed sludge (day 1) and aerobic granule (day 50).

ecosystems and sediment. Though the ability of this group to accumulate phosphorus remains unclear, it has been reported that poly (hydroxyalcanoates) (PHA) accumulation can occur simultaneously in enhanced biological phosphorus removal processes (Liu et al., 1996; Dabert et al., 2001).

In biological wastewater treatment, microbial processes such as autotrophic nitrification and heterotrophic denitrification have been identified as major N_2O emission sources; however, the underlying pathways remain unclear (Wunderlin et al., 2012). The individual contribution of the two processes has not been quantified during aerobic granular sludge. The nitrifier denitrification take a prime role in the N_2O emissions from wastewater treatment processes (Kampschreur et al., 2009). N_2O emission in nitrifier denitrification and heterotrophic denitrification is known to be executed and accomplished by certain special bacteria species, mainly ammonia-oxidizing bacteria (AOB) and denitrifiers (Kim et al., 2010). In nitrifier denitrification, the oxidation of NH_3 to NO_2^- is followed by the reduction of NO_2^- to N_2O. This sequence of reactions is carried out by only one group of microorganisms, namely autotrophic ammonia-oxidizing bacteria (Kong et al., 2012). The results in our previous research indicated that nitrifier denitrification was attributed as the dominant source of N_2O production simultaneous nitrification and denitrification process (Jia et al., 2013). In aerobic granular sludge, the nitrogen was removed by SNDprocess(CassidyandBelia,2005).AccordingtothePCR-DGGE results, Nitrosospira (band 11) is the dominant ammonium oxidizing bacteria (AOB) in aerobic granular sludge in this study. Hence, we can conclude that Nitrosospira was attributed as the dominant source of N_2O production in aerobic granular sludge.

Autotrophic nitrification, denitrification, nitrifier denitrification, and coupled nitrification and denitrification may all the possible biochemical pathways of N_2O production in aerobic granular sludge

Table 2
Species identification of selected DGGE bands (the bands are labled in Fig. 5).

Bands	% Identity	Closest relatives	Phylogenetic affiliation
1	93	Arcobacter cryaerophilus (NR025905)	ε-Proteobacteria
2	90	Sediminibacterium salmoneum (NR044197)	Sphingobacteria
3	91	Lutibacter litoralis (NR043301)	Flavobacteria
4	94	Aequorivita Antarctica (NR025639)	Flavobacteria
5	91	Flavisolibacter ginsengisoli (NR041500)	Flavisolibacter
6	93	Uncultured bacterium (GQ336953)	Uncultured bacterium
7	88	Uncultured bacterium (JQ800883)	Uncultured bacterium
8	95	Rhodocyclaceae bacterium (JQ791819)	b-Proteobacteria
9	96	Thiobacillus Q (AJ289884)	b-Proteobacteria
10	95	Zoogloea sp. (HE654688)	b-Proteobacteria
11	92	Nitrosospira sp. (HE654681)	b-Proteobacteria
12	94	Nitrospira sp. (EF019838)	Nitrospira
a	95	Pseudomonas sp. (HM468086)	g-Proteobacteria
b	97	Uncultured bacterium (EU240628)	Uncultured bacterium
c	99	Meganema perideroedes (AY170120)	a-Proteobacteria
d	92	Parvibaculum sp. (GQ351495)	a-Proteobacteria
e	90	beta proteobacterium (JN371484)	b-Proteobacteria
f	93	Thauera sp. (HQ860324)	b-Proteobacteria
g	92	Zoogloea sp. (JN679115)	b-Proteobacteria

(Wunderlin et al., 2012). However, the exact emission mechanisms are related to the specific operating parameters and environmental conditions, and there is little published literature available to distinguish the contribution of each pathway. Further research is needed.

4. Conclusions

Aerobic granular sludge and stable excellent COD and $NH_4^+ - N$ removal efficiencies were successfully obtained in the SBAR operated at ambient temperature $(18 \pm 3)^{\circ}C$. $(0.60 \pm 0.17, n = 3)$% of the incoming nitrogen load was emitted as N_2O. During cycle operation, the N_2O concentration in the off gas increased to a peak value at the beginning of the aerobic period and subsequently decreased and stabilized at a low concentration. DNA analysis showed that Nitrosospira was the dominant AOB which was attributed as dominant source of N_2O production in aerobic granular sludge.

Acknowledgments

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