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Greenhouse gases emission from aerobic methanotrophic denitrification (AeOM-D) in sequencing batch reactor

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Abstract. This study presents the effect of hydraulic retention time (HRT) on the characteristics of emission of three major greenhouse gases (GHGs) including CH_4, CO_2 and N_2O during operation of a sequencing batch reactor for aerobic oxidation of methane with denitrification (AeOM-D SBR). Dissolved N_2O concentration increased, leveled-off and slightly decreased as the HRT increased from 0.25 to 1d. Concentration of the dissolved N_2O was higher at the shorter HRT, which was highly associated with the lowered C/N ratio. A longer HRT resulted in a higher C/N ratio with a sufficient carbon source produced by methanotrophs via methane oxidation, which provided a favorable condition for reducing N_2O formation. With a less formation of the dissolved N_2O, N_2O emission rate was lower at a longer HRT condition due to the lower C/N ratio. Opposite to the N_2O emission, emission rates of CH_4 and CO_2 were higher at a longer HRT. Longer HRT resulted in the greater total GHGs emission as CO_2 equivalent which was doubled when the HRT increased from 0.5d to 1.0 d. Contribution of CH_4 onto the total GHGs emission was most dominant accounting for 98 - 99% compared to that of N_2O (< 2%).

Keywords: aerobic oxidation of methane with denitrification (AeOM-D); hydraulic retention time (HRT); methane (CH_4); carbon dioxide (CO_2); nitrous oxide (N_2O); greenhouse gases (GHGs)

1. Introduction

Denitrification in wastewater treatment is significant since discharge of nitrate at a high concentration to water environment can potentially cause eutrophication which eventually deteriorates quality of water resources (Kim *et al*. 2005). In addition to damages on water environmental quality, nitrate is known to be forming carcinogenic compounds such as nitrosamines and nitrosamides (Ono *et al*. 2000, Forman *et al*. 1985). A typical method for enhancing denitrification is to add external carbon sources such as methanol and acetate into either biological nutrient removal (BNR) process or post-denitrification process as a tertiary step (Costa *et al*. 2000). However, the excess addition of the external carbon sources often generates concerns

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such as increase in operating cost, excess biological growth and deterioration of effluent quality due to the residual organic carbon (Lee *et al*. 2014).

As an alternative, denitrification using CH_4 (or H_2) has been recently attemped at a laboratory level study aiming water and wastewater treatment (Daelman *et al*. 2014, Cuba *et al*. 2011, Modin *et al*. 2010). It can be attractive due to its some benefits with less potential of biomass production and organic pollutants remaining in the effluent (Sun *et al*. 2013). The denitrification using CH_4 as a sole carbon source under aerobic condition is known as aerobic oxidation of methane with denitrification (AeOM-D). Despite some benefits in AeOM-D, its applicability is still limited due to low mass transfer rate of CH_4 gas into water and utilization rate of the dissolved CH_4 by the relevant microorganisms. As for the latter, CH_4 in AeOM-D is firstly metabolized by methanotrophs with methane monooxygenase (MMO) oxidizing methane to methanol. The rate of MMO activity has not been clearly explained in AeOM-D for water treatment system but the denitrification rate is strongly subject to the methanotrophic activity (Houbron *et al*. 1999). This means that methane oxidation is the rate-liming step and AeOM-D might have a higher potential of emission of unutilzed CH_4 into the atmosphere. Waki *et al*. (2005) pointed out that the risk of explosion during operation if CH_4 is not sufficiently transformed or utilized and its concentration in the off-gas may exceed 5-14%. In addition to the risk problems, CH_4 is one of major greenhouse gases (GHGs) of which emission should be carefully managed. In order to understand or control the emission of GHGs, many studies have been done focusing on various factors or design and operating parameters affecting on emission of GHGs from nature such as sediment and soil or from engineering systems such as water/wastewater treatment plants and landfill. The emission of GHGs during methane oxidation in soil has largely been affected by soil physical factors (Smith *et al*. 2003). Emission of CH_4 from AeOM-D could be also affected by many factors such as hydraulic retention time (HRT) and it could be reduced by increase of retention time due to enhanced CH_4 oxidation (Petersen *et al*. 2005). In spite of few studies, it is necessarily studied in depth for reducing GHGs emission from engineering AeOM-D system.

In addition to CH_4, there are concerns for emission of the other GHGs including CO_2 and N_2O from AeOM-D. Fig. 1 shows the hypothetical mechanisms for emission of GHGs from AeOM-D system. Emission of CH_4 was associated with unused CH_4 before methanotrophic oxidation. CO_2 could be generated through the metabolisms by methanotrophs and denitrifiers, while N_2O can be generated by denitrification process. It has been reported that production of N_2O in denitrification was increased in some condition such as low pH, and presence of O_2 (Knowles 1982). Global warming impacts of CH_4 and N_2O are 21 and 298 times as strong as carbon dioxide (CO_2), respectively (IPCC 2013). Similar to common denitrification process, nitrous oxide (N_2O) could be also produced from AeOM-D (Kits *et al*. 2015). In spite of low production potential of N_2O compared to CH_4 and CO_2, it is very seriously considered due to its higher global warming impact. Mechanisms for N_2O production are still under study but in many studies, N_2O could be formed through various biological reaction related to nitrogen transformation: denitrification, autotrophic and heterotrophic nitrification, nitrifier-denitrification (Hu *et al*. 2011, Wrage *et al*. 2001). N_2O production in denitrification is more significant when nitrate is highly loaded (Hu *et al*. 2012, Wunderlin *et al*. 2012). It is important to point out that the most possible conditions for greater N_2O emission were associated with unfavorable conditions to nitrifiers and denitrifiers such as low DO, short solids retention time (SRT), low C/N ratio, and low temperature (Zheng *et al*. 1994, Her and Huang 1995, Thoern and Soerensson 1996, Noda *et al*. 2003, Tallec *et al*. 2008, Hu *et al*. 2013, Paudel *et al*. 2015). The N_2O production in the full-scale conventional wastewater treatment plant is estimated from 0 to 14.6% of input nitrogen (Kampschreur *et al*. 2009) accounting for up

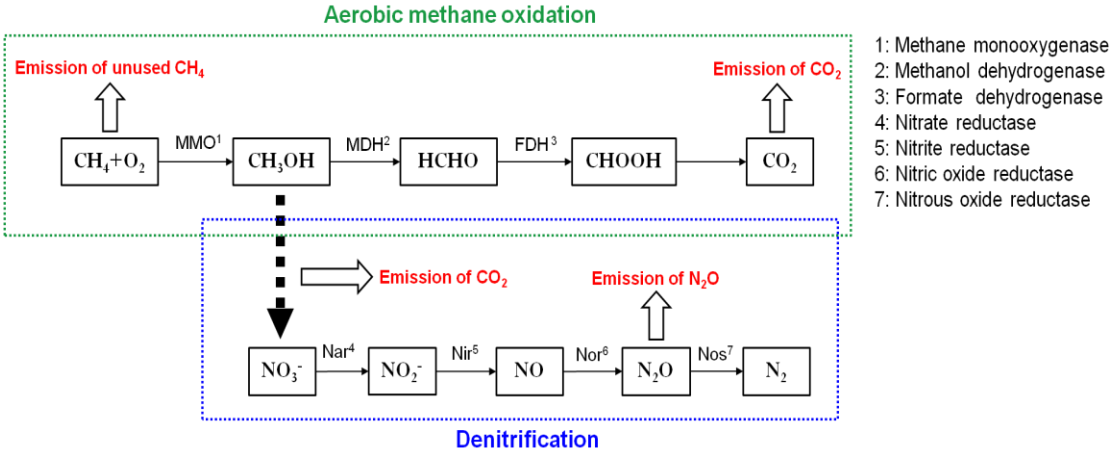

Fig. 1 Hypothetical mechanisms for GHGs emission in AeOM-D process

to 26% of total GHGs emission from wastewater plant (Frijns *et al*. 2008). However, to our knowledge, N_2O emission from biological denitrification using methane oxidation has not been intensively studied yet. Some relevant studies attained so far are more likely related to anammox and denitrifying anaerobic methane oxidation (DAMO) in wastewater and soil area (Shi *et al*. 2013, Ding *et al*. 2014).

Generally, HRT is a key designing parameter to determine the size of the bioreactor which in turn primarily governs the performance of the system by affecting important rates such as mass transfer, reaction and growth rate (Zonoozi *et al*. 2014). Hence, the HRT could also affect the emission of GHGs from bioreactor as well as treatment performance since the GHGs are obviously dependent on those rates. The overarching goal of this study was to characterize the emission potential of GHGs from AeOM-D in a sequencing batch reactor (SBR) operated under different hydraulic retention time (HRT) conditions. Specific objectives were 1) to understand the transformation of CH_4 and nitrogen 2) to investigate the N_2O formation and emission during AeOM-D, 3) to calculate overall emission potential of GHGs equivalent to CO_2 depending on HRT condition in AeOM-D SBR system via abiotic and biotic track studies.

2. Materials and methods

2.1 Mixed culture consortium and medium

Mixed culture consortium was prepared by mixing activated sludge and anaerobic digested sludge, obtained from 'J' wastewater treatment plant (Seoul, Korea) at 1:1 (w/w) ratio, and was cultivated for 1 month until methanotrophs and denitrifiers became enriched in terms of their reaction activity (Lee *et al*. 2014). Growth medium was consisted with the following composition (Modin *et al*. 2010): $MgSO_4 \cdot 7H_2O$, 1000mg/L; $CaCl_2 \cdot 2H_2O$, 270mg/L; $FeSO_4 \cdot 7H_2O$, 9.1mg/L; and KNO_3, 144.4mg/L (20mg N/L). 1mL of trace element and 2mL of phosphate buffer solution were also contained in growth medium. The composition of phosphate buffer contained (mg/L): KH_2PO_4 24,400; Na_2HPO_4 10,200, and that of trace metal contained (mg/L): $FeSO_4 \cdot 7H_2O$ 2486;

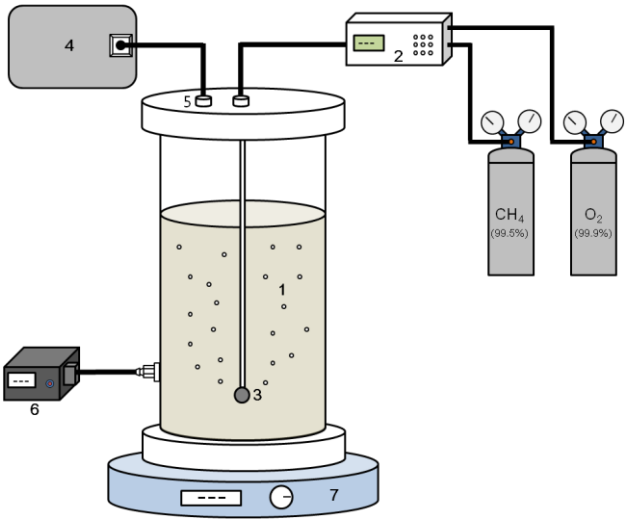

Fig. 2 Schematic diagram of system; (1) reactor, (2) mass flow controller, (3) diffusing stone, (4) gas bag, (5) gas sampling port, (6) fill and draw pump, (7) magnetic stirrer

$MnCl_2 \cdot 4H_2O$ 500; $ZnCl_2$ 50; $NiSO_4 \cdot 6H_2O$ 101; $CoCl_2 \cdot 6H_2O$ 50; $Na_2MoO_4 \cdot 2H_2O$ 26; H_3BO_3 50; $CuSO_4 \cdot 5H_2O$ 310; and 35% HCl 5 mL.

2.2 Track study for AeOM-D

In order to investigate the effect of HRT on nitrate nitrogen (NO_3^--N) transformation and GHGs emission during AeOM-D, a cylindrical acrylic sequencing batch bioreactor (SBR) (10 cm I.D. ×60 cm L.; 2.54 L actual working volume) was prepared as shown in Fig. 2. The SBR was operated under solid retention time (SRT) of 50 d and three different HRT conditions of 0.25, 0.5 and 1 d. Acclimation period with at least three weeks was given whenever the operation condition for HRT changed. The reaction time of 2, 5 and 11 hr was assigned to the equivalent HRT of 0.25, 0.5 and 1 d, respectively. The time for fill (0.25 hr), settle (0.5 hr) and withdraw (0.25 hr) was identically fixed in all bioreactor regardless of HRT condition. The SBR was operated in a temperature controlled chamber maintaiend at 20±1℃. CH_4 (99.5%) and O_2 (99.9%) in the pressurized cylinder were separately supplied into the SBR through a porous diffuser. Flow rate of CH_4 and O_2 were set to be identically 5 mL/min (1:1 ratio) by mass flow controller (MFC, TSC-200, NFSystem, Korea). In order to provide a homogeneous condition inside the reactor, continuous mixing was given at 300 rpm using magnetic stirrer. Initial concentration of the inoculum mixture was set to be 1,000 mg MLVSS/L (identically MLSS/L=1,270 mg/L) whenever the HRT condition for the SBR was changed. Mixed liquor samples were periodically taken for track study. They were immediately transferred into a 10 mL serum bottle for analyses of dissolved CH_4 and N_2O by using a headspace method (Lee *et al*. 2010). Acetylene gas was added to headspace of serum bottle for ceasing methane oxidation (Chan and Parkin 2000). While, dissolved nitrogenous compounds (NO_3^--N, NO_2^--N) were analyzed after filtrating the mixed liquor samples by membrane filters (0.45 μm pore-size). Gaseous samples emitted through the head space of AeOM-D SBR were also periodically collected in a 1L of tedlar bag (SKC, USA) for analyses of three different GHGs of CH_4, CO_2, N_2O. All samples were analyzed in duplicate.

Denitrification efficiency and specific denitrification rate (SDNR) was calculated as follows

$$\text{Denitrification efficiency (\%)} = \frac{C_{in}V_{in} + C_fV_f - C_{out}V_{tot}}{C_{in}V_{in}} \times 100 \quad (1)$$

where, C_{in} is the influent NO_3^- concentration (mmol N/L), C_f is the residual NO_3^- concentration after withdrawl in the previous cycle (mmol N/L), C_{out} is the NO_3^- concentration at the end of reaction before withdrawl (mmol N/L), V_{in} is influent volume every cycle (L), V_f is the residual volume after withdrawl in the previous cycle (L), V_{tot} is total working volume of the SBR (L).

$$\text{SDNR (mg N/g MLVSS·hr)} = \frac{\text{Amount of } NO_3^- \text{ reduced during reaction period (mg N)}}{\text{Amount of inoculum (g MLVSS)} \times \text{reaction time (hr)}} \quad (2)$$

2.3 CH_4 dissolution test

Abiotic CH_4 dissolution test was conducted to estimate the variation of the dissolved CH_4 concentration in water as a function of time under various HRT conditions without biological reaction. The same bioreactor for AeOM-D was used for it. To calculate theoretical amount of CH_4 dissolved under abiotic condition, the dissolution test was performed by supplying CH_4 and O_2 at 5 mL/min which was the same condition to that of biotic experiments. Dissolved concentraiton of CH_4 in liquid phase was also analyzed at every 30 min by using headspace method (Lee *et al*. 2010).

2.4 Analytical methods

The CH_4 concentration was analyzed by using gas chromatography (GC, DS6200, Donam, Korea) equipped with a thermal conductivity detector (TCD) using Hayesep Q column (8', 1/8'', 0.085'', alltech, USA). The temperatures of oven, injector and detector were 40, 120 and 120℃, respectively. The concentration of N_2O was also measured by GC equipped with a pulsed discharge detector (PDD) using Hayesep D column (80/100, 18'', 8ft, Alltech, USA). Oven temperature was programmed from 40℃ kept for 4 min, ramped to 120℃ at 20℃/min with injector temperature 120℃ and detector temperature 170℃.

Volatile suspended solids (VSS) concentrations in the mixed liquor samples were measured according to standard method (APHA, 2005). Liquid samples were also prepared after filtration with a 0.45 μm membrane filter to analyze nitrate nitrogen (NO_3^--N), and nitrite nitrogen (NO_2^--N) of which concentration were analyzed by ion chromatography (ICS-900, Dionex, Sunnyvale, CA, USA).

3. Results and discussion

3.1 Denitrification rate in AeOM-D

Track study was conducted for investigating denitrification performance in AeOM-D SBR operated with different HRT and the variations of nitrate concentration are shown in Fig. 3. Initial concentration of NO_3^--N was 1.13, 0.8 and 0.77 mmol/L for HRT of 0.25, 0.5 and 1.0 d, respectively, and the difference in the initial concentration was attributed to the difference in denitrification performances in the previous cycle. Denitrification using CH_4 as a sole carbon

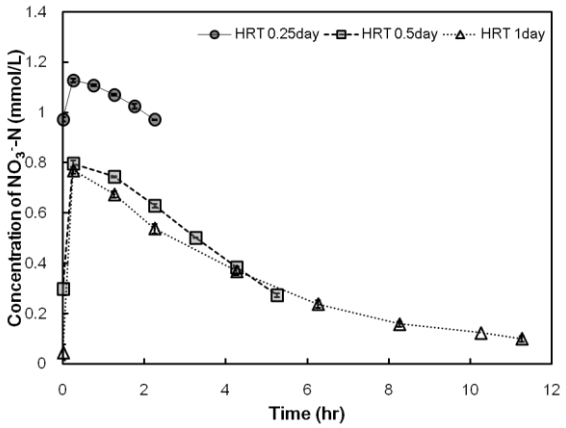

Fig. 3 Behaviour of nitrate depending on HRT of AeOM-D SBR in track study

Table 1 Specific denitrification rate (SDNR, mg NO_3^--N/g MLVSS·hr) of AeOM-D with different HRT

SDNR	HRT (d)		
	0.25	0.5	1
Overall SDNR	0.82	1.02	0.58
Maximum SDNR	1.12 (2h*)	1.24 (3hr*)	1.59 (4hr*)

*The number in the parenthesis means the time that the maximum SDNR took place

source was well achieved in the AeOM-D SBRs despite a relatively low CH_4 flow rate (5 mL/min). Denitrification efficiency was increased as the HRT increased and it was varied in the range between 33% and 93%. Build-up of NO_2^--N was not observed in any bioreactor. NO_3^--N concentration began to decline as soon as the reaction period started switching from fill period. The overall specific denitrification rate (SDNR) during the entire reaction period for each condition was calculated and the results are shown in Table 1. The condition of 0.5 d HRT resulted in the higher overall SDNR (1.02 mg NO_3^--N/g MLVSS·hr) than the other HRT conditions. The longer HRT condition (1.0 d) did not exhibit the maximum rate because the nitrate to be reduced was deficient in the middle of reaction period. The maximum SDNR occurred at 2, 3, 4 hr for HRT of 0.25, 0.5, 1.0 d, respectively. The maximum SDNR was also increased from 1.12 to 1.59 mg NO_3^--N/g MLVSS·hr as the HRT increased from 0.25 to 1.0 d (Table 1). The maximum SDNR obtained in this study was almost comparable to those of the previous studies ranging from 0.37 to 1.96 mg NO_3^--N/g MLVSS·hr (Khin and Annachhatre 2004, Sánchez *et al*. 2004, Lee *et al*. 2001).

3.2 CH_4 utilization and N_2O formation in AeOM-D

Concentration of the dissolved CH_4 was monitored in AeOM-D SBR under either abiotic or biotic condition (Fig. 4). The profile of the dissolved CH_4 concentration under abiotic and biotic condition means the total available CH_4 concentration supplied to methanotrophs and the CH_4 concentration remaining after methanotrophic denitrification, respectively. Thus, the difference between two profiles represented the concentration of CH_4 oxidized by methanotrophs. In this study, the oxidized CH_4 was hypothetically identical to CH_3OH formed by MMO in

Table 2 Characteristics of N_2O formation depending on HRT

Parameters	HRT (d)		
	0.25	0.5	1
Nitrate loading rate (mmol NO_3^--N/d)	22.9	8.1	3.9
C/N ratio*	0.5	5.4	17.4
DO (mg/L)	1.35 ± 0.104	0.61 ± 0.147	0.59 ± 0.105
Maximum concentration of dissolved N_2O (μmol N_2O-N /L)	21.1	17.2	11.6
N_2O discharge rate in effluent (mmol N_2O-N/d)	0.2	0.1	0

*Total amount of CH_4 oxidized product (mol)/initial amount of NO_3^- (mol) in each cycle

methanotrophs as shown in Eq. (3). It has been known that a part of CH_4 is also directly utilized by methanotrophs for their synthesis but we assumed that all the CH_4 was converted to CH_3OH due to difficulty in fractionation of those CH_4 utilization as similar to the previous studies (Mancinelli 1995, Modin *et al*. 2007). Total amount of dissolved CH_4 was calculated as 1.21, 7.66, 23.6 mmol for HRT of 0.25, 0.5 and 1.0 d, respectively. On the other hands, total amount of the oxidized product of CH_4 (e.g., CH_3OH) was calculated as 0.58, 4.32 and 13.38 mmol for HRT of 0.25, 0.5 and 1.0 d, respectively. Once the concentration of the CH_4 oxidized by methanotrophs was determined, the molar C/N ratio (CH_4 oxidized product/NO_3^--N) for denitrification could be estimated at each sampling time in the track study. The theoretical molar C/N ratio for denitrification without consideration of cell growth is 0.83 according to the following equations (Lee *et al*. 2014).

$$5CH_4 + 2.5O_2 \rightarrow 5CH_3OH \quad (3)$$

$$5CH_3OH + 6NO_3^- \rightarrow 5CO_2 + 3N_2 + 6OH^- + 7H_2O \quad (4)$$

Combining Eq. (3) and (4)

$$5CH_4 + 2.5O_2 + 6NO_3^- \rightarrow 5CO_2 + 3N_2 + 6OH^- + 7H_2O \quad (5)$$

The C/N ratio based on the total oxidized CH_4 to initial NO_3^- in each cycle was 0.5, 5.4 and 17.4 for HRT of 0.25, 0.5 and 1.0 d, respectively, which indicated that the C/N ratio at HRT of 0.25 d was insufficient for denitrification (Table 2).

N_2O, one of major GHGs, could be formed in biological denitrification, especially under unfavorable condition for denitrification such as high nitrate loadings (Wunderlin *et al*. 2012) or low C/N ratio (Hu *et al*. 2013). The reason for greater N_2O emission under such conditions was not clearly explained, but one of possible explanations was associated with deactivation of N_2O reductase compared to N_2O producing enzymes (Holtan-Hartwig *et al*. 2002). N_2O formation could be highly influenced by the C/N ratio available for denitrification. Fig. 5 shows the concentration of the dissolved N_2O and C/N ratio for AeOM-D with different HRT. The dissolved N_2O concentration under 0.25 d HRT condition increased and levelled off until the end of the reaction time. However, under higher HRT condition of 0.5 and 1.0 d, it was increased and then decreased during reaction period. The maximum concentration of the dissolved N_2O for 0.25, 0.5, 1.0 d of HRT was 21.1, 17.2, and 11.6 μmol/L, respectively (Table 2). The C/N ratio appeared to

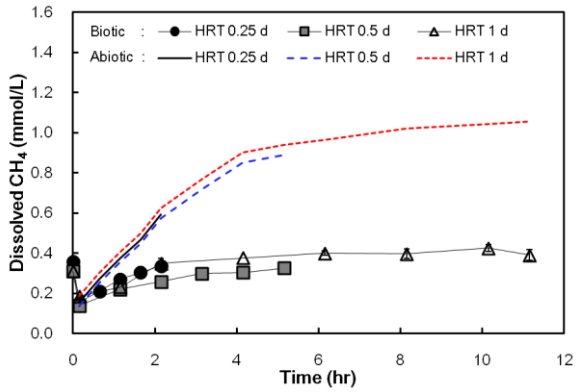

Fig. 4 Change of CH_4 concentration under abiotic condition and its concentration in effluent

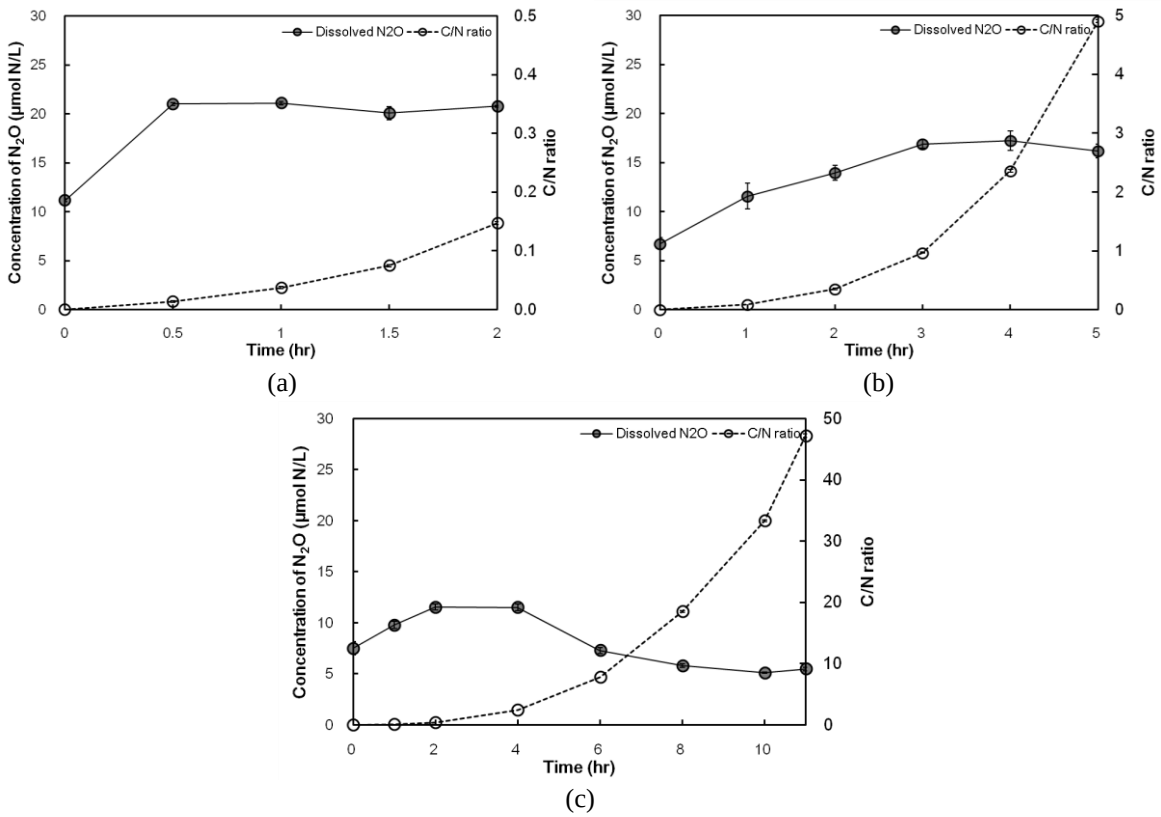

Fig. 5 Profile for C/N ratio and concentration of dissolved N_2O and ; HRT (a) 0.25 d, (b) 0.5 d, (c) 1.0 d

exponentially increase as the reaction time increased, higher C/N ratio induced reduction of N_2O concentration. When the dissolved N_2O concentration started to decrease, the C/N ratio was greater than 2.4 in the HRT higher than 0.5 d (Fig. 5 (b)-(c)). In many studies for denitrification, formation of N_2O was most likely significant when the C/N ratio was low probably due to the lowered activity of nitrous oxide reductase (Itokawa *et al*. 2000, Noda *et al*. 2003).

Another possible reason for N_2O formation in AeOM-D was associated with dissolved oxygen (DO) concentration. Activity of nitrous oxide reductase (*Nos*) is sensitive to DO concentration. Nitrate and nitrite reductase can maintain their activity at relatively high level of DO up to 4 and 2 mg/L, respectively, whereas *Nos* was strongly inhibited at a higher DO concentration than 0.25 mg/L (Bonin *et al*. 1992). In this study, the DO concentration at the end of reaction was higher when the HRT of AeOM-D was shorter (Table 2). The higher DO at the shorter HRT was probably attributed the lowered oxidation rate of methane. Even though the DO concentration for all HRT did not reach the inhibitory level for *Nos* activity, the higher DO concentration under shorter HRT condition might provide unfavorable condition to *Nos* activity. The results revealed that shorter HRT (0.25 d) did not lead to complete denitrification and consequently formed a more N_2O due to low C/N ratio and high DO concentration.

3.3 Greenhouse gases (GHGs) emission in AeOM-D SBR

Fig. 6 shows the concentrations of the GHGs of CH_4, CO_2 and N_2O emitted from AeOM-D SBR depending on HRT condition. The concentration of the emitted CH_4 appeared to drastically increase in 2 hr and then leveled off until the end of reaction period describing that most of CH_4 conversion, i.e., methanotrophic reaction, occurred during the earlier reaction period. The maximum concentration CH_4 in the emitted gas was similarly 20.5 mmol/L regardless of the HRT condition and it was almost close to the input CH_4 concentration supplied to the AeOM-D SBR. The CH_4 concentrations limitedly increased for the latter 5 hr of the reaction time in the AeOM-D under 1 d HRT condition, which means that the input CH_4 was not further transformed or utilized due to the deficiency of nitrate (Fig. 6(a)).

Similar to CH_4, concentrations of the emitted CO_2 for all HRT conditions were rapidly increased in the early 2 hr reaction time indicating that denitrification took place mainly during the earlier reaction time period (Fig. 6(b)). Once the CO_2 concentration rapidly increased and then slowly increased during the rest of the reaction time until the end of reaction time. This result illustrated that CH_4 oxidation by methanotrophs into CO_2 kept occurring even after the denitrification completed despite a lowered rate before coupling with denitrification.

Concentration of the emitted N_2O showed a similar pattern to that of the dissolved N_2O. Different from the other GHGs, dependence of the emitted N_2O concentration on HRT was significant. The maximum concentration of N_2O was higher in the AeOM-D under shorter HRT condition. The N_2O concentration in the emitted gas from AeOM-D under 0.25 d of HRT kept increasing until the end of reaction time, however it was leveled-off or decreased at the longer HRT of 0.5 or 1.0 d, respectively. The maximum N_2O concentration for 0.25 and 0.5 d was 26.5 and 22.0 μmol/L respectively, and those took place at the end of reaction time. The concentration of N_2O reached a maximum value of 15.6 μmol/L at 6hr and then decreased to 8.0 μmol/L at the end of reaction in case of 1 d HRT.

Emission rate of CH_4, CO_2 and N_2O in AeOM-D SBR was calculated by using the following equation

$$\text{Emission rate (mg/d)} = \Sigma[C_g \times Q_g] \times t_R/d \quad (6)$$

where, C_g is concentration of emitted GHGs (CH_4, CO_2 and N_2O) measured at periodic interval (mg/L; Q_g is flow rate of CH_4 and O_2 with 1:1 ratio (mL/min); t_R is reaction time in a day

The overall emission rates of GHGs from AeOM-D SBR are shown in Table 3. Emission rates of CH_4 and CO_2 increased as the HRT increased due to the extended reaction time. The reason for

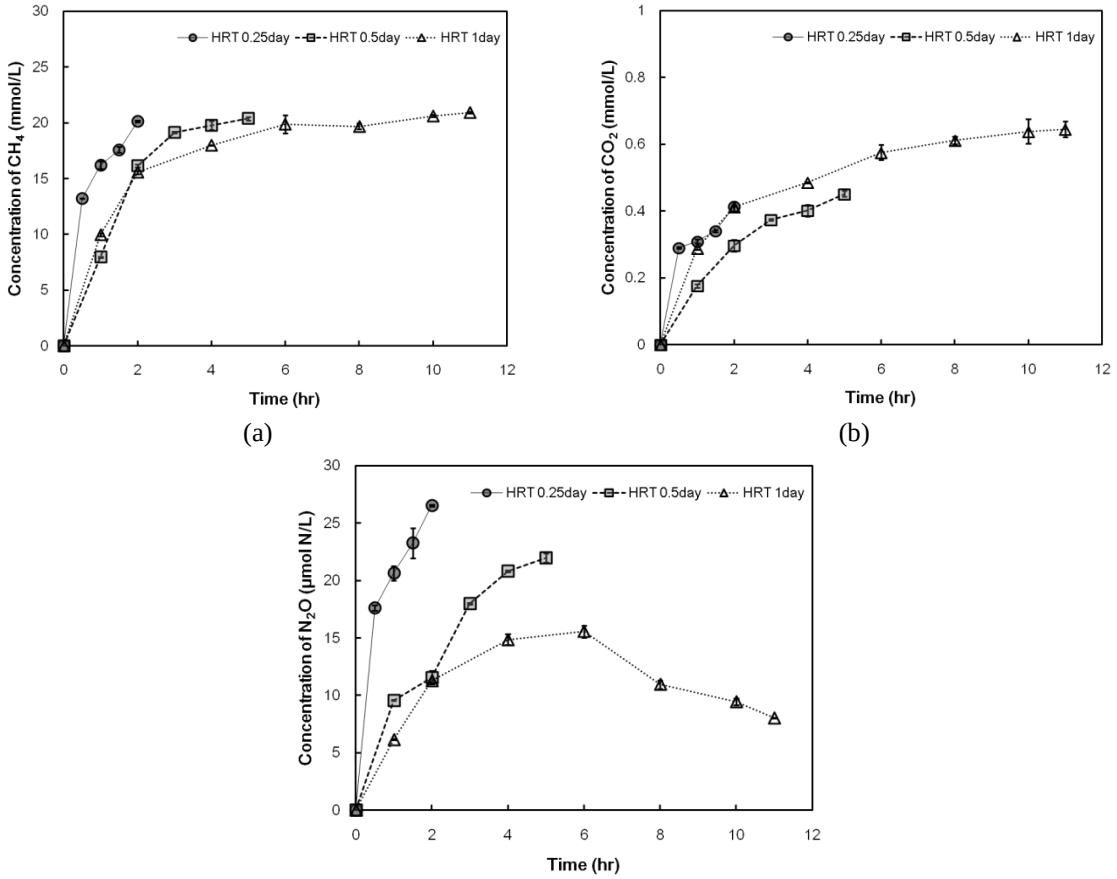

Fig. 6 Concentration of emitted GHGs with different HRT; (a) 0.25 d, (b) 0.5 d, (c) 1.0 d

Table 3 Emission rate of GHGs and conversion ratio of NO_3^--N to N_2O with different HRT

Parameters		HRT (d)		
		0.25	0.5	1
Emission rate (mmol/d)	CH_4	161.1	200.2	243.3
	CO_2	1.0	4.1	7.2
	N_2O	0.21	0.20	0.15

high emission rate of CH_4 and CO_2 was attributed to more unused CH_4 and continuous methane oxidation by methanotrophs even after nitrate was exhausted. However, the emission rate of N_2O showed an opposite result to those of CH_4 and CO_2. As discussed above, the shorter HRT was a condition leading to higher N_2O formation, so the emission rate of N_2O was increased as the HRT decreased. N_2O emission rate for HRT 1.0 d was 71% of that for HRT 0.25 d. Total generation rate of N_2O, which is the sum of the emission rate and discharge rate into effluent, showed a more evident dependence on HRT in AeOM-D: 0.41, 0.30 and 0.15 mmol/d for 0.25, 0.5 and 1.0 d, respectively. Hence, the conversion ratio of the reduced NO_3^--N into N_2O was estimated at 12.8,

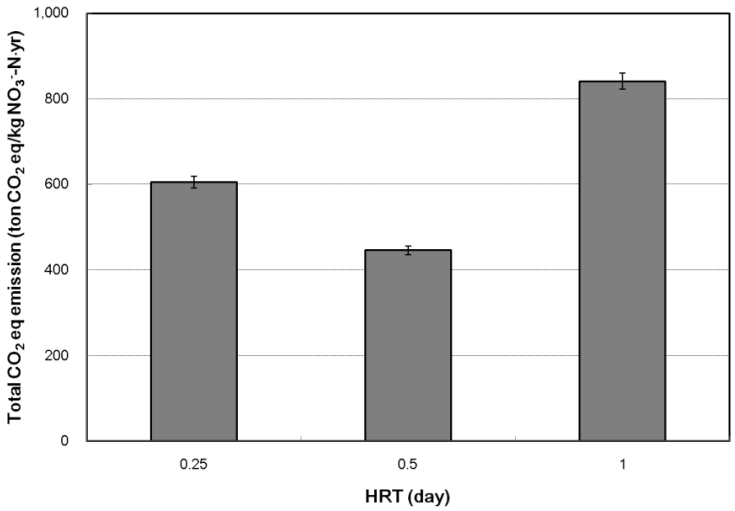

Fig. 7 Annual total GHGs emission equivalent to CO_2 from AeOM-D SBR under different HRT

5.5, 4.5 % for HRT of 0.25, 0.5, 1.0 d, respectively. Interestingly, the results for GHGs emission demonstrated that both CH_4 and CO_2 have higher emission rate but N_2O has a lower rate in the AeOM-D SBR under a longer HRT condition.

3.4 Impact of AeOM-D on climate change

A better denitrification performance in the AeOM-D could be achieved by supplying CH_4 for a longer reaction time in a higher HRT. However, in operation of the AeOM-D, N_2O and CO_2 were produced and emitted as a result of metabolisms of methanotrophs and denitrifiers. Furthermore, a greater amount of unused CH_4 was emitted to atmosphere without sufficient dissolution into water and utilization by the relevant microorganisms. Thus, it is inevitable to avoid emission of GHGs from AeOM-D and it is important to estimate the impact of these on climate change. In order to estimate the impact of GHGs emission from AeOM-D SBR on climate change, the annual total GHGs emission as CO_2 equivalent for denitrification was calculated for each HRT condition as follows:

Annual emission rate of total GHGs as CO_2 equivalent (ton CO_2 Eq./kg NO_3^--N·yr)

$$= \sum \text{Emission rate of each GHG} \times \frac{\text{Global warming potential (GWP)}}{\text{Total amount of the reduced } NO_3^- \text{-N/d}} \times \frac{365\text{d}}{\text{yr}} \quad (7)$$

where, GWP is the global warming potential as CO_2 equivalent and GWP for CH_4, N_2O and CO_2 are 28, 265 and 1, respectively (IPCC 2013).

Annual total GHGs emission rate for denitrification was minimized at HRT of 0.5 d in spite of linear increase of emission rate of GHGs except N_2O with decrease of HRT in AeOM-D (Fig. 7). Thus, HRT of 0.5 d turned to be an optimal condition if considering both denitrification performance and GHGs emission rate. It was reported that the emission factor (EF) of N_2O in domestic wastewater treatment was ranged from 0.005 to 0.025 kg N_2O-N/kg TN (IPCC, 2006). The EF of N_2O obtained in this study was ranged from 0.04 to 0.13 kg N_2O-N/kg NO_3^--N indicating that the AeOM-D SBR potentially produces more N_2O during denitrification. Recently,

in spite of smaller emission, more attention was laid upon N_2O in wastewater treatment due to its large contribution to global warming with a higher GWP (Kampschreur *et al*. 2009). Even though the EF of N_2O was remarkably high compared to that of wastewater treatment, CH_4 was the most representative GHG enormously contributing to the total GHGs emission rates. Contribution of N_2O on the total GHGs emission was 2.0 and 1.0 and 0.5% in AeOM-D SBR at HRT of 0.25, 0.5 and 1.0 d, respectively, whereas that of CH_4 was in the range from 98 to 99%. Contribution of CO_2 was lower than that of N_2O. This study suggested that AeOM-D has a relatively high GHGs emission potential with a dominant contribution of CH_4 emitting to atmosphere as the unused form. Furthermore, N_2O could be also potentially emitted more than that of typical wastewater treatment. Consequently, it should be carefully considered to enhance the efficiency of CH_4 dissolution and utilization in operation of AeOM-D system in the future.

4. Conclusions

Recently, methanotrophic denitrification is not limited to scientific studies occurring in nature but is recently expanded to applications for water and wastewater treatment. Denitrification performance was successfully achieved in AeOM-D SBR when a relatively enough HRT was given. A longer HRT leads to a relatively higher denitrification performance with a lower emission rate of N_2O. Formation of N_2O in AeOM-D was strongly dependent on C/N ratio which was increased with increase in HRT. On the contrary, emission rate of CH_4 and CO_2 was proportionally increased as the HRT increased. The total GHGs emission as CO_2 equivalent was dominantly high at HRT of 1 d due to the excess emission of unused CH_4. Thus, extension of HRT in AeOM-D system might be a good strategy for denitrification performance including N_2O emission, but it might be ineffective for controlling total GHGs emission. Considering both denitrification performance and GHGs emission, the optimal condition in this study was HRT of 0.5 d. In engineering aspect, there is still remained concern related to GHGs emission in particular CH_4. Thus, it may be necessary to enhance the efficiency of CH_4 utilization as well as to optimize the operating conditions in order to successfully apply the AeOM-D for water treatment in the future. Our suggestion is to use of microporous membrane diffuser for enhancing the mass transfer of CH_4 with less input or to recirculate the head space gas into the reactor for enhancing the utilization of the emitted CH_4.

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