Korean J. Chem. Eng., **29**(9), 1196-1202 (2012)
DOI: 10.1007/s11814-012-0004-5

Deterioration of denitrification by oxygen and cost evaluation of electron donor in an uncovered pre-denitrification process

Seung Joo Lim†, Tak-Hyun Kim, Tae-Hun Kim, and In Hwan Shin

Research Division for Industry and Environment, Korea Atomic Energy Research Institute,
1266, Sinjeong, Jeongeup, Jeonbuk 580-185, Korea
(*Received 11 September 2011 • accepted 16 January 2012*)

Abstract–Specific nitrate uptake rates (SNURs) under two test conditions were measured to evaluate effects of oxygen inhibition on denitrification. A test condition was that activated sludge was completely prevented from contacting of oxygen ($SNUR_{closed}$), the other was that activated sludge was contacted to free air ($SNUR_{open}$). Municipal wastewater and acetate were used as electron donors. $SNUR_{closed}$ was 2.42 mg NO_3-N/g VSS-hr and $SNUR_{open}$ was 1.09 mg NO_3-N/g VSS-hr when municipal wastewater was used as electron donor. Meanwhile, when acetate was used as electron donor, $SNUR_{closed}$ was 24.65 mg NO_3-N/g VSS-hr and $SNUR_{open}$ was 18.00 mg NO_3-N/g VSS-hr. The operating costs for electron donors were calculated based on the unit price of acetate to remove nitrate. When municipal wastewater was used as electron donor the ratio of $cost_{open}$ to $cost_{closed}$ was 0.45. Cost evaluation showed the adverse impacts on denitrification and explained why an anoxic reactor should be sequestered from oxygen.

Key words: Specific Nitrate Uptake Rate, Denitrification, Oxygen Inhibition, C/N Ratio, Cost Evaluation

INTRODUCTION

Nitrate in the aqueous or soil environment is usually denitrified by various chemoheterotrophic/chemoautotrophic bacteria [1-3]. Also, denitrification is carried out by denitrifying phosphate accumulating organisms (dPAOs) and/or denitrifying glycogen accumulating organisms (dGAOs) under anoxic conditions [4,5]. Several investigators reported that electron donors such as methanol, ethanol, acetate, glucose, municipal wastewater, and industrial wastewaters can be efficiently used for denitrification [1,3,6-9]. It was shown that the greatest nitrate uptake rate (NUR) was associated with acetate because acetate is a very biodegradable substrate [1,9-10]. Ekama and Marais [11] reported that a theoretical chemical oxygen demand (COD) was $2.86/(1-Y_H)$ to denitrify 1 mg NO_3-N (Y_H: heterotrophic yield coefficient). After IWAQ model No. 1 assumed that Y_H was 0.67, a theoretical C/N ratio was considered 8.67 g COD/g NO_3-N [12]. However, in many cases, industrial wastewaters are usually discharged with low C/N ratios because of the high nitrogen concentration or the low fraction of biodegradable organic matter in a wastewater treatment plant (WWTP) effluent [6]. In other words, the denitrification efficiency in most biological nutrient removal (BNR) processes is significantly affected by the C/N ratios of various substrates.

Specific nitrate uptake rate (SNUR) is commonly used for evaluating the denitrification capacity in a given anoxic condition. SNUR may be affected by several parameters. Kujawa and Klapwijk [13] reported that the configuration of a WWTP can have a significant impact on denitrification. You et al. [14] showed heavy metals such as cadmium, lead, and nickel can decrease SNUR. Specifically, the effects of oxygen inhibition on denitrification have been argued for decades [3,8,15-19]. As a matter of fact, even though all of anoxic reactors are designed to be covered, pilot- and/or full-scale anoxic reactors in WWTPs are commonly operated without any cover as an anoxic reactor operator is not carefully operated or an operator is not well informed of his or her duty. Choi et al. [20] reported that massive heat losses were observed at an uncovered sequencing batch reactor (SBR) treating organic matter and nitrogen in piggery waste. To biologically remove nitrogen in a WWTP, pre-denitrification processes using wastewater or post-denitrification processes using external carbon sources such as methanol, ethanol, and acetate are usually considered. One of the greatest advantages in a pre-denitrification process is that it can simultaneously/cheaply remove organic matter and nitrogen because wastewater contains an electron donor. Meanwhile, unlike a post-denitrification process, a pre-denitrification process takes the pumping cost of nitrate internal circulation into consideration. On the other hand, nitrogen can almost be removed in a post-denitrification process [21]. However, one of main disadvantages in post-denitrification processes is that each external carbon source for denitrification is very expensive. MacDonald [22] reported the estimated annual operation and maintenance cost for denitrification in a typical facility. According to his research, 70% of total cost for denitrification was caused by external carbon source (methanol). In this study, the effects of oxygen inhibition on denitrification were evaluated by SNUR measurements. In addition, the cost evaluation for electron donors used in this study (municipal wastewater and acetate) was performed based on the unit price of acetate for denitrification. When oxygen in air dissolves into aqueous phase, several physicochemical parameters such as pH, dissolved oxygen (DO), and oxidation reduction potential (ORP) in water are severely affected. Given that most BNR facilities are operated without any cover, this investigation evidently showed the importance why an anoxic reactor should be sequestered from oxygen to be more stable and economical denitrification.

†To whom correspondence should be addressed.
E-mail: seungjoolim@gmail.com

MATERIALS AND METHODS

1. Specific Nitrate Uptake Rate Measurement

SNUR measurement was performed in two types of batch reactors at room temperature (23°C) as described in Kristensen et al. [23]. The active volume of each batch reactor was 1 L. Since the ratio of cross-sectional area to volume in a full-scale anoxic reactor is usually 0.2-0.3 (typical depth: 4 m), a theoretical cross-sectional area was 2.6×10^{-4} m^2 in this study [24]. However, as this was not a feasible dimension, a graduated volumetric mass cylinder of which each volume was 1 L was used in this study. The height and cross-sectional area in a graduated volumetric mass cylinder were 0.23 m and 4.3×10^{-3} m^2, respectively. The ratio of cross-sectional area to volume in a graduated volumetric mass cylinder was 4.35. The ratio of cross-sectional area to volume increased within a factor of 1 different due to both the decrease in depth and increase in cross-sectional area. To evaluate the effects of oxygen inhibition on denitrification, two SNUR test conditions were set. One test condition was that activated sludge was completely prevented from contacting of oxygen ($SNUR_{closed}$), the other was that activated sludge was contacted to free air ($SNUR_{open}$). SNUR measurement under two test conditions was performed as described in Kristensen et al. [23]. To completely prevent contacting of oxygen, electron donor was added and then a graduated volumetric mass cylinder was immediately sealed using parafilm (Pechiney Plastic Packaging, Chicago, IL, USA). The used parafilm has a small hole (Approx.: 1 mm) for exhausting nitrogen gas off. Diluted municipal wastewater or acetate was used as electron donor for measuring SNUR. The initial concentration of nitrate (electron acceptor) was 15.0 mg/L, and nitrate (electron acceptor) and electron donor were simultaneously/slowly mixed using a low-speed mixer (magnetic stirrer). Activated sludge was inoculated from an anoxic reactor in a municipal WWTP located at Jeongeup, Jeollabuk-do, Korea. The average concentration of the inoculated activated sludge was 1,750.4±4.5 mg VSS/L. The sample at each condition was collected and measured at 5, 10, 15, 30, 45, 60, 75, 90, 120, 150, and 180 minutes after blending electron donor with electron acceptor. To calculate SNUR, volatile suspended solids (VSS) were also measured at each sample.

2. Effects of C/N Ratio on Denitrification

To investigate the adverse effects of oxygen on denitrification during the denitrification process, two C/N ratio test conditions were set. The test set was the same as that of SNUR measurement. The evaluation of the C/N ratio was performed for only municipal wastewater since municipal wastewater is commonly used for electron donor in a pre-denitrification system. The concentration of electron acceptor (nitrate) was 15.0 mg/L. The initial C/N ratio was 2.8 at each NUR measurement since the effect of inhibition by oxygen on denitrification at low C/N ratio was significant [17]. Such as the SNUR measurement, the sample at each condition was collected and measured at 5, 10, 15, 30, 45, 60, 75, 90, 120, 150, and 180 minutes after blending electron donor with electron acceptor.

3. Characteristics of Electron Donors

When considering pre-denitrification processes such as Bardenpho process, raw municipal wastewater can be efficiently used as electron donor because the C/N ratio of municipal wastewater is approximately 5-6. In addition, municipal wastewater contains sufficient buffer (bicarbonate and/or phosphate) and macro- and micro-nutrients required for synthesizing cells [24]. The raw municipal wastewater used in this study was collected at a municipal WWTP located at Jeongeup, Jeollabuk-do, Korea. The characteristics of raw municipal wastewater used in this study are shown in Table 1. For the purpose of the rapid SNUR measurement, raw municipal wastewater was diluted with tap water and the dilution ratio was approximately 0.27 (raw municipal wastewater : tap water=1 : 3.7). Acetate, which is one of the external carbon sources, was prepared for representing a very easily biodegradable electron donor. The initial COD concentrations of municipal wastewater and acetate were 78.0 mg/L and 83.5 mg/L, respectively. When measuring SNUR with acetate, $(NH_4)_2SO_4$ 0.0472 g/L and K_2HPO_4 0.0283 g/L were added to prevent the limitation of macro nutrients during the experiments.

Table 1. Characteristics of municipal wastewater used in this study

Parameter	Value (Ave.±Std.)
pH	6.6-8.2 (7.2±0.5)
Alkalinity (mg $CaCO_3$/L)	282.0-365.0 (320.8±32.0)
BOD (mg/L)	216.0-256.0 (233.1±32.5)
COD (mg/L)	267.0-321.0 (282.5±50.3)
TKN (mg/L)	34.0-68.0 (58.2±11.2)
NH_3-N (mg/L)	31.2-52.1 (37.6±12.2)
NO_2-N (mg/L)	0.0-0.0 (0.0±0.0)
NO_3-N (mg/L)	0.0-0.2 (0.1±0.0)

4. Analytical Methods

To evaluate the effects of oxygen inhibition on denitrification, samples for pH, oxidation reduction potential (ORP), dissolved oxygen (DO), alkalinity, COD, solids, and nitrate were taken from each batch reactor at the same time. All analyses were performed according to Standard Methods for the Examination of Water and Wastewater [25].

5. Statistical Analyses

One-way analysis of variance (ANOVA) was performed at significance ($p<0.05$) and Pearson correlation between denitrification indicators was performed using SPSS (ver. 17) to statistically and more accurately evaluate denitrification parameters such as pH, ORP, and DO.

RESULTS AND DISCUSSION

1. Effects of Oxygen Inhibition on Denitrification

The ratio of cross-sectional area to volume in a full-scale anoxic reactor is usually 0.2-0.3. Meanwhile, the ratio of cross-sectional area to volume in this study was 4.35. This implies that the effects of oxygen on denitrification may be different between a full-scale reactor and this study. Several investigators showed the values of oxygen mass transfer coefficient in lab-, pilot-, full-scale anoxic reactors [8,17,43]. According to their research, however, the oxygen mass transfer coefficient was not the main parameter for determining the deterioration of denitrification by oxygen in an anoxic reactor. Alves et al. [44] clearly showed that Kla is not a function of cross-sectional area of a bioreactor. They concluded that Kla is determined by the physical properties of the liquid media. Lemoine and Morsi [45] stated that the values of Kl and Kla in a con-

Table 2. SNUR measurement results for each electron donor

Electron donor	Municipal wastewater		Acetate	
	Closed[a]	Open[b]	Closed[a]	Open[b]
SNUR (mg NO_3-N/g VSS-hr)	2.42±0.00	1.09±0.02	24.65±0.05	18.00±0.04

[a]Activated sludge was completely prevented from contacting of oxygen
[b]Activated sludge was contacted to free air

tinuous stirred tank reactor such as an anoxic reactor were proportional to mixing speed and temperature. Meanwhile, those were inversely proportional to the height of a reactor. This shows that the biochemical reactions of microorganisms are important parameters rather than the configuration of a reactor. In addition, Plósz et al. [17] showed that the effects of oxygen uptake in an anoxic reactor on denitrification are not negligible when the amount of electron donor is not sufficient. In other words, for the case of supplying sufficient exogenous substrate, even though oxygen in air is dissolved in an anoxic reactor, microorganisms usually use substrate as electron donor and oxygen as electron acceptor using aerobic respiration. However, when an insufficient amount of electron donor is supplied for denitrification, the adverse effect by oxygen on the denitrification rate is significant [17]. Thus, a graduated volumetric mass cylinder was used in this study since the depth is more important than the cross-sectional area to simulate a full-scale anoxic reactor. SNUR measurement results for each electron donor are shown in Table 2. As shown in Table 2, when municipal wastewater was used as electron donor $SNUR_{closed}$ was 2.42 mg NO_3-N/g VSS-hr. In the meantime, $SNUR_{open}$ decreased to 1.09 mg NO_3-N/g VSS-hr compared to $SNUR_{closed}$. De Lucas et al. [6] reported that $SNUR_{closed}$ was 2.75 mg NO_3-N/g VSS-hr when municipal wastewater was used as electron donor. On the other hand, when acetate was used as electron donor SNUR sharply increased, irrespective of test conditions. When municipal wastewater was used as electron donor the ratio of $SNUR_{closed}$ to $SNUR_{open}$ was 2.22, while when acetate was used as electron donor that of $SNUR_{closed}$ to $SNUR_{open}$ decreased 1.37. It shows whether acetate is a more efficient electron donor under anoxic conditions or municipal wastewater is a more vulnerable substrate than acetate for oxygen. Unlike acetate, as some fraction of municipal wastewater is composed of fermentable and/or biodegradable solids, it is necessary that it be fermented to be used as electron donor. Also, municipal wastewater contains various non-biodegradable organic matter, inert solids, and inorganic matter [24]. In contrast, simple monomers, acids, or alcohols are very easily degradable and then can be very effectively used as electron donor (e.g., acetate, methanol, ethanol, or sugar). Thus, it is possibly explained why SNURs of acetate were much greater than those of municipal wastewater. Since the 1990s, several investigators showed Y_H values under (anoxic/aerobic) conditions. Orhon et al. [26] reported Y_H values from several industrial wastewaters such as confectionery waste (0.61/0.72), dairy waste (0.52/0.65), meat processing waste (0.51/0.64), and domestic sewage (0.50/0.63). Spérandio et al. [27] concluded and compared various Y_H values using synthetic electron donors such as acetic acid+starch (0.54/0.66), glucose (0.57/0.67), and acetate (0.45/0.54). On the other hand, Mokhayeri et al. [28] stated that SNUR and yield coefficient obtained from acetate were greater than those resulting from other external electron donors. It shows that acetate can be quickly and efficiently used as electron donor in an anoxic reactor because heterotrophic assimilation (biosynthesis using acetate and nitrate) is completely different from denitrification (Madigan et al., 2006). In other words, it is necessary to discriminate assimilation and denitrification by denitrifying bacteria. Several investigators already reported aerobic denitrification (co-respiration of O_2 and NO_3-N) and little effects of oxygen on denitrification [29, 30]. However, it was shown that the effects of oxygen inhibition on denitrification were significant in this study (Table 2). NADH is used for electron donor and oxygen or nitrate can be used for electron acceptor. In an aerobic condition, NADH is usually oxidized under the sequence: flavoprotein→Fe-S protein→ubiquinone→cyt b_{562}→cyt o→O_2. Meanwhile, in an anoxic condition, additional oxidoreductases, located at a periplasmic membrane (nitrite/nitrous oxide reductase) and an integral membrane proteins (nitrate/nitric oxide reductase), are needed to denitrify nitrate [2]. Several investigators presented that oxygen is able to inhibit the activity of nitrate reductase, thereby decreasing the denitrification efficiency [2,3,31]. Thomas et al. [3] showed a dissimilatory denitrification by *Pseudomonas* sp. was severely inhibited by oxygen since oxygen can inhibit their genes and the activity of nitrate enzyme (nitrate reductase). Wu et al. [31] showed that nitrite reductase is much more sensitive to oxygen than other reductases. Oxygen appears to regulate synthesis of nitrate reductase and inhibit the activity of its enzyme [19,21].

2. pH, ORP, and DO Changes During the SNUR Measurement

The pH, ORP, and DO are key parameters and can be efficient operation indicators in an anoxic process [21,32,33]. The changes of pH, ORP, and DO during the SNUR measurement are shown in Fig. 1, Fig. 2, and Fig. 3, respectively. As shown in Fig. 1, pH ranged 6.2 to 8.0, irrespective of electron donors. The obvious change or trend of pH was not shown during the SNUR measurement. When measuring SNUR under the condition of preventing the contact of

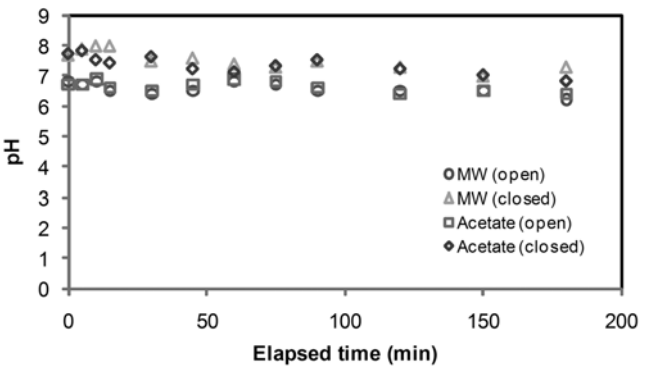

Fig. 1. Variation of pH during the SNUR measurement.

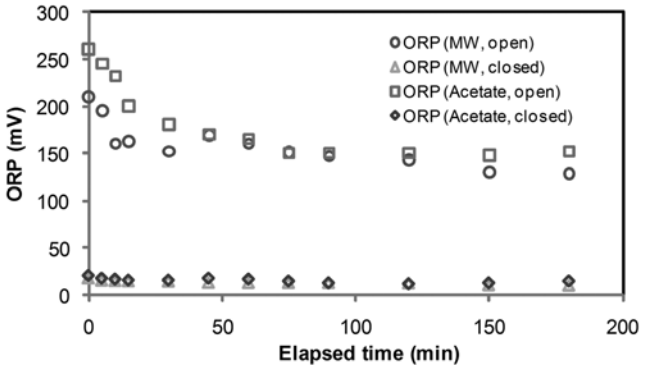

Fig. 2. Variation of ORP during the SUNR measurement.

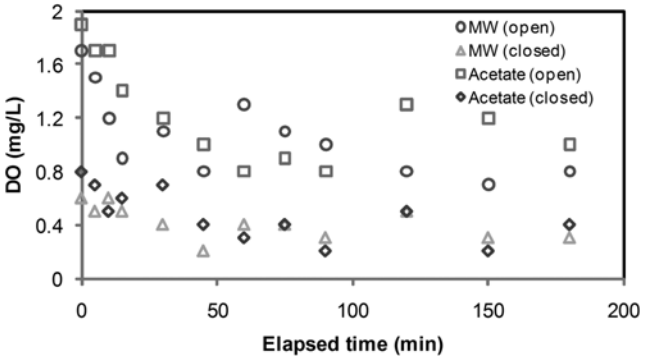

Fig. 3. Variation of DO during the SUNR measurement.

free air (closed), ORP was maintained low (below 21 mV), irrespective of electron donors. The maximum concentration of DO was 0.8 mg/L (Ave.: municipal wastewater: 0.42±0.13 mg/L; acetate: 0.48±0.20 mg/L). For the case of contacting to free air, the maximum concentration of DO was 1.7 mg/L (municipal wastewater) and 1.9mg/L (acetate) and average DO concentration was 1.1±0.3 mg/L (municipal wastewater) and 1.2±0.4 mg/L (acetate). The concentration of DO was not decreased below 0.7 mg/L after 90 minutes since free air can be continuously dissolved through the surface during the SNUR measurement. The trend of ORP was associated with the concentration of DO during the SNUR measurement. This evidently shows that DO concentration (oxygen) causes ORP increase, deteriorating the denitrification efficiency. When considering the maximum ORP was 260 mV and the maximum concentration of DO was 1.9 mg/L (acetate, open), the effects of oxygen inhibition on denitrification were significant. According to the manual of the United States Environmental Pollution Agency (US EPA) [21], the concentration of DO in an anoxic reactor significantly affects the denitrification efficiency. The concentration of DO at 0.2 mg/L can cease denitrification in pure cultures. In activated sludge systems, the concentration of DO between 0.3 and 1.5 mg/L causes the diffusion limitation in microbial floc. On the other hand, the effect of pH variation on the denitrification efficiency is not significant. At below pH 6.0 or above pH 8.0, the denitrification efficiency is affected [21,34,38]. Glass and Silverstein [38] reported that denitrification was completely inhibited below pH 7.0 and free ammonia in water adverse influences on denitrification is severely affected by pH [40,41]. The denitrification efficiency, even at psychrophilic conditions (approximately 15 °C), is not severely decreased [2,21,24]. In other words, the concentration of DO is more sensitive than other operation indicators such as pH or temperature.

Table 3. Pearson correlations between denitrification indicators

Electron donor	SNUR	pH	ORP	DO
Municipal wastewater (open)				
SNUR	1	−0.176	−0.242[a]	−0.071[a]
pH	−0.176	1	0.564	0.688
ORP	−0.242[a]	0.564	1	0.729[a]
DO	−0.071[a]	0.688	0.729[a]	1
Municipal wastewater (closed)				
SNUR	1	−0.075	−0.034[b]	−0.493[a]
pH	−0.075	1	0.815[a]	0.640
ORP	−0.034[b]	0.815[a]	1	−0.750[a]
DO	−0.493[a]	0.640	−0.750[a]	1
Acetate (open)				
SNUR	1	0.220	−0.159[a]	−0.161[a]
pH	0.220	1	0.129	0.029
ORP	−0.159[a]	0.129	1	0.971[a]
DO	−0.161[a]	0.029	0.971[a]	1
Acetate (closed)				
SNUR	1	0.804	−0.881[a]	−0.978[a]
pH	0.804	1	0.427	0.661
ORP	−0.881[a]	0.427	1	−0.961[a]
DO	−0.978[a]	0.661	−0.961[a]	1

[a]$p<0.05$; [b]$p<0.01$

From the result of ANOVA, it was shown that all of denitrification indicators (pH, ORP, and DO) were significant ($p<0.05$). F-values of pH, ORP, and DO were 45.9, 175.6, and 29.2, respectively. At the 5% significance level, the data provided sufficient evidence to conclude that a difference exists in each test condition. Peason correlations between denitrification indicators such as pH, ORP, and DO are shown in Table 3. As shown in Table 3, DO and ORP were negatively correlated with SNUR, irrespective of test conditions. It evidently shows the adverse effects of oxygen on denitrification mainly due to the increase of ORP in an anoxic condition. Li and Irvin [32] reported that ORP can be one of excellent indicators for monitoring denitrification in an anoxic reactor. According to their research, the T-N (NH_3-N+NO_3-N) removal efficiency in an SBR was significantly dependent upon ORP. The T-N removal efficiency sharply decreased at ORP of 250 mV. Given that ORP was 210 mV (municipal wastewater, open) and 260 mV (acetate, open) after blending electron donor with electron acceptor in this study, a severe deterioration of denitrification might be expected in an uncovered full-scale anoxic reactor. This supports that ORP values were not decreased after substrate was exhausted (Fig. 2).

3. Effects of C/N Ratio on Denitrification

In a dissimilatory denitrification, the C/N ratio (as COD/NO_3-N ratio) is one of key parameters to efficiently remove nitrogen. Mateju et al. [34] suggested the following equation, which shows a required carbon (acetate) to remove nitrogen:

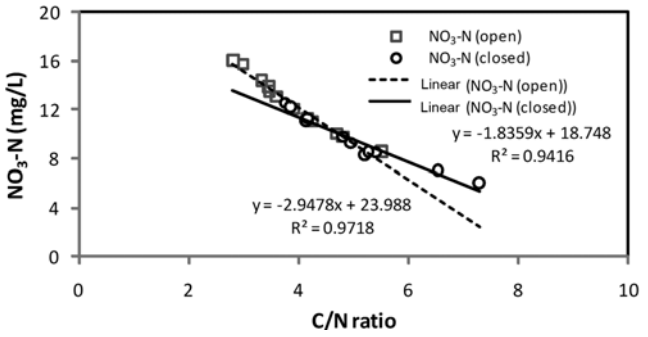

Fig. 4. Effects of C/N ratio on denitrification circle: activated sludge was completely prevented from contacting of oxygen (closed), square: activated sludge was contacted to free air (open).

$$0.819CH_3COOH+NO_3^- \rightarrow 0.068C_5H_7NO_2 + HCO_3^- + 0.301CO_2 + 0.902H_2O + 0.466N_2 \quad (1)$$

On the basis of Eq. (1), 1 g NO_3-N theoretically requires 3.74 g COD (3.51 g acetate). However, several investigators reported different optimal C/N ratio at each denitrification test condition. The C/N ratios ranged approximately from 2.5 to 5.5 when acetate, molasses, methanol, ethanol, glucose, and benzoate were used as electron donor [7,28,34-37]. In this study, the effects of the C/N ratio on denitrification are shown in Fig. 4. Municipal wastewater was used as electron donor and the initial C/N ratio was 2.8 at each NUR measurement. The C/N ratios under the test condition where activated sludge was completely prevented from contacting of oxygen ranged 3.7 to 7.3. Meanwhile, the C/N ratios under the test condition where activated sludge was contacted to free air ranged 2.8 to 5.5. It suggests that the high C/N ratios (low NO_3-N concentrations) were due to the non-inhibitory effects of nitrate reductase. In other words, when activated sludge is sequestered from oxygen in an anoxic reactor, the optimal denitrification can be expected. The US EPA reported the optimal C/N ratio for denitrification is approximately 6.0 to obtain high nitrogen removal efficiency [21]. In addition, the ratio of slopes between two test conditions (closed and open) was 1.61. It definitely shows that denitrifying bacteria were severely inhibited by oxygen, and nitrate reductase of denitrifying bacteria should be protected from oxygen to obtain the high nitrogen removal efficiency in a pre-denitrification system.

4. Case Study of Cost Evaluation for Electron Donors Used in this Study

Mokhayeri et al. [28] reported the unit prices of some external carbon sources usually used for denitrification (Table 4). As shown in Table 4, the highest unit price was acetate and the unit price of acetate ($1.03/kg) was 312.12% compared to methanol. If nitrate is removed with acetate, which is used as an external carbon source in a pre-denitrification system, the unit price of acetate ($8.15/kg NO_3-N) to remove nitrate would be 714.91% compared to methanol. The unit price for acetate to remove nitrate was used to evaluate and compare the cost of each electron donor used in this study, since there exists no unit price for municipal wastewater. The operating cost for electron donor used in this study in a pre-denitrification system can be calculated as follows:

$$\$\ \text{Operating cost for electron donor} = \frac{\$8.15}{\text{kg}NO_3\text{-N}} \frac{\text{mg}\ NO_3\text{-N}}{\text{g VSS hr}} \times \frac{\text{kg}NO_3\text{-N}}{10^6\ \text{mg}\ NO_3\text{-N}} \times \frac{\text{g VSS hr}}{10^3\ \text{mg VSS hr}} \times \frac{24\ \text{hr}}{\text{day}} \times \text{HRT} \times \text{MLVSS} \times \text{V} \quad (2)$$

where,

$\frac{\$8.15}{\text{kg}NO_3\text{-N}}$: unit price of acetate for denitrification

$\frac{\text{mg}\ NO_3\text{-N}}{\text{g VSS hr}}$: specific nitrate uptake rate

HRT: hydraulic retention time (day)

MLVSS: mixed liquor volatile suspended solids (mg VSS/L)

V: reactor volume (L)

A municipal WWTP, located in Junbuk, Korea, consists of an anoxic/oxic reactor (a pre-denitrification) system to control nitrogen, subsequently adding polymer to remove phosphorus. This WWTP operates an anoxic reactor without a cover. A municipal WWTP, located in Chungnam, Korea, treats nutrients using an uncovered SBR. The SBR is followed by anaerobic digestion, and the effluent is directly discharged into a river after recalcitrant organic matter is removed by using an advanced oxidation process. Operation condition of each WWTP to calculate operating cost for electron donors is shown in Table 5. Although each BNR system was operated without any cover, the nitrogen removal efficiency was maintained at approximately 50-60%. The cost evaluation of electron donors was performed using Eq. (2), and operating costs of electron donors are shown in Table 6. The operating cost of each electron donor was based on the unit price of acetate ($8.15/kg NO_3-N; Table 4) to remove nitrate in order to compare the results from the calculation. Some investigators showed that nitrite is accumulated when the high nitrate concentration water/wastewater is denitrified [38,40,41]. Francis and Mankin [42] stated that the biological denitrification process can be inhibited when the concentration of nitrate is above 1,350 mg/L. However, when the concentration of nitrate is not too high, nitrite is not accumulated [17,39]. Beccari et al. [39] reported that the complete denitrification was achieved with the concentration of 500-1,000 mg VSS/L in an activated sludge process. As shown in

Table 4. Unit prices of external carbon sources usually used for denitrification

External carbon source	Unit price ($/kg)	Unit price for denitrification ($/kg NO_3-N)
Methanol	0.33	1.14
Ethanol	0.84	2.43
Acetate	1.03	8.15

Table 5. Operation conditions of two municipal WWTPs

Parameter	Junbuk municipal WWTP	Chungnam municipal WWTP
HRT (day)[a]	0.5	1
MLVSS (mg/L)	4010	1730
Reactor volume (L)	1500×10^3	1000×10^3

[a]Anoxic reaction time (day)

Table 6. Operating costs of electron donors used in this study

External carbon source	Operating cost of electron donor ($ for acetate)					
	Junbuk municipal WWTP			Chungnam municipal WWTP		
	Closed[a]	Open[b]	Open[b]/Closed[a]	Closed[a]	Open[b]	Open[b]/Closed[a]
Municipal wastewater	1423.61	641.21	0.45	818.90	368.84	0.45
Acetate	14500.78	10588.81	0.73	8341.26	6090.98	0.73

[a]Activated sludge was completely prevented from contacting of oxygen
[b]Activated sludge was contacted to free air

Table 6, when municipal wastewater was used as electron donor the ratio of cost under the open test condition where activated sludge was contacted to free air ($cost_{open}$) to that of under the closed test condition where activated sludge was completely prevented from contacting of oxygen ($cost_{closed}$) was 0.45. In the meantime, when acetate was used as electron donor the ratio of $cost_{open}$ to $cost_{closed}$ was 0.73. This difference between the two electron donors is mostly due to the biodegradability of acetate. It implies that effects of oxygen inhibition on denitrification in a municipal WWTP can be more significant because the operating cost of municipal wastewater was much more affected. In Eq. (2), the cost for denitrification is a function of unit price of acetate for denitrification ($8.15/kg NO_3-N), SNUR, HRT, MLVSS, and a reactor volume. Higher values of $cost_{open}$ were caused by those of $SNUR_{open}$. Plósz et al. [17] reported that the effects of inhibition by oxygen on denitrification were significant when denitrifying bacteria use exogenous substrate such as municipal wastewater. In addition, Table 6 shows the absolute cost different between two facilities since the concentration of MLVSS in the Junbuk municipal WWTP is 2.32 greater than that in the Chungnam municipal WWTP. Consequently, the decrease in SNUR in an uncovered anoxic reactor causes the increase in a reactor volume or the hydraulic retention time increase in a municipal WWTP. The trend of results in this study was consistent with that according to Plósz et al. [17]. Given that most carbon sources for denitrification are in municipal wastewater in a pre-denitrification process, the adverse effects of oxygen on denitrification must be considered. As shown in Eq. (2) and Table 6, the SNUR of electron donor in a municipal WWTP determines the volume of anoxic reactor in a pre-denitrification system and it is severely affected by electron acceptor.

CONCLUSIONS

The results of this research have yielded the following conclusions.

- Oxygen causes adverse impacts on denitrification, irrespective of electron donors. It is necessary to be maintained at anoxic conditions in order efficiently denitrify nitrate.
- Oxygen dissolved air into aqueous phase poses adverse impacts to denitrification. DO and ORP can be efficiently used for monitoring indicators of denitrification in an anoxic reactor.
- When denitrificans are not inhibited, higher C/N ratios and lower nitrate concentrations could be obtained.
- The cost for denitrification is a function of unit price of acetate for denitrification, SNUR, HRT, MLVSS, and reactor. The decrease in SNUR in an uncovered anoxic reactor causes an increase in a reactor volume or the hydraulic retention time increase in a municipal WWTP.

ACKNOWLEDGEMENTS

This research was supported by the nuclear R&D program and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology.

REFERENCES

1. M. Hagman, J. L. Nielsen, P. H. Nielsen and J. la. C., Jansen, *Water Res.*, **42**, 1539 (2008).
2. M. T. Madigan, J. M. Martinko, P. V. Dunlap and D. P. Clark, *Brock biology of microorganisms*, 12th Ed., Pearson, New York (2006).
3. C. M. Thomas, E. M. H. Wellington, R. Diaz-Orejas and M. Espinosa, *Microbiol.*, **140**, 1799 (1994).
4. S. H. Chuang, C. F. Ouyang and Y. B. Wang, *Water Res.*, **30**, 2961 (1996).
5. R. J. Zeng, Z. Yuan and J. Keller, *Biotechnol. Bioeng.*, **81**, 397 (2003).
6. A. De Lucas, L. Rodríguez, J. Villaseòor and F. J. Fernández, *Water Res.*, **39**, 3715 (2005).
7. J. J. Her and J. S. Huang, *Bioresour. Technol.*, **54**, 45 (1995).
8. J. Oh and J. Silverstein, *Water Res.*, **33**, 1925 (1999).
9. N. Y. F. Tam, Y. S. Wong and W. G. Leung, *Water Res.*, **26**, 1229 (1992).
10. I. Somiya, H. Tsuno and M. Matsumoto, *Water Res.*, **22**, 49 (1998).
11. G. A. Ekama and G. v. R. Marais, in theory, design and operation of nutrient removal activated sludge processes. A collaborative information document prepared for the Water Research Commission by the University Cape Town, City Council of Johannesburg and the National Institute for Water Research of the CSIR, PRETORIA, South Africa (1984).
12. M. Henze, C. P. L. Grady, W. Gujer, G. v. R. Mararis and T. Matsuo, Activated sludge model No. 1, Scientifical and Technical Report No. 1, IWAPRC, London (1987).
13. K. Kujawa and B. Klapwijk, *Water Res.*, **33**, 2291 (1999).
14. S. J. You, Y. P. Tsai and R. Y. Huang, *Environ. Eng. Sci.*, **26**, 1207 (2009).
15. A. Jobbágy, J. Simon and B. Plósz, *Water Res.*, **34**, 2606 (2000).
16. E. V. Münch, P. Lant and J. Keller, *Water Res.*, **30**, 277 (1996).
17. B. G. Plósz, A. Jobbágy and C. P. L. Grady Jr., *Water Res.*, **37**, 853 (2003).
18. L. A. Robertson and J. G. Kuenen, *Arch. Microbiol.*, **139**, 351 (1984a).

19. A. J. B. Zehnder, Biology of anaerobic microorganisms, 1st Ed., Wiley, New York (1988).
20. E. Choi, D. Kim, Y. Eum, Z. Yun and K. Min, *Water Environ. Res.*, **77**, 381 (2005).
21. US EPA, Manual: Nitrogen control, EPA/625/R-93/010, Washington, DC (1993).
22. D. V. MacDonald, *J. Water Pol. Cont. Fed.*, **62**, 796 (1990).
23. G. H. Kristensen, P. E. Jørgensen and M. Henze, *Water Sci. Technol.*, **25**, 43 (1992).
24. Metcalf and Eddy, Wastewater engineering: treatment and reuse, 4th Ed., McGraw Hill, New York (2003).
25. APHA, Standard Methods for the Examination of Water and Wastewater, 20th Ed., APHA, Washington DC (1998).
26. D. Orhon, S. Sözen and N. Artan, *Water Sci. Technol.*, **34**, 67 (1996).
27. M. Spérandio, V. Urbain, J. M. Audic and E. Paul, *Water Sci. Technol.*, **39**, 139 (1999).
28. Y. Mokhayeri, R. Riffat, S. Murthy, W. Bailey, I. Takacs and C. Bott, *Water Sci. Technol.*, **60**, 2485 (2009).
29. L. C. Bell, D. J. Richardson and S. J. Ferguson, *FEBS Lett.*, **265**, 85 (1990).
30. L. A. Robertson and J. G. Kuenen, *Antonie Van Leeuwenhoek*, **50**, 525 (1984b).
31. Q. Wu, E. Knowles and D. F. Niven, *Can. J. Microbiol.*, **40**, 916 (1994).
32. B. Li and S. Irvin, *Biochem. Eng. J.*, **34**, 248 (2007).
33. S. Saby, M. Djafer and G. H. Chen, *Water Res.*, **37**, 11 (2003).
34. V. Mateju, S. Cizinska, J. Krejci and T. Janoch, *Enzyme Microb. Technol.*, **14**, 170 (1992).
35. D. Y. Bang, Y. Watanbe and T. Noike, *Water Sci. Technol.*, **32**, 235 (1995).
36. F. Cecen and E. Gionenc, *Water Sci. Technol.*, **26**, 2225 (1992).
37. Y. C. Chiu and M. S. Chung, *Int. Biodeterioration Biodeg.*, **51**, 43 (2003).
38. C. Glass and J. Silverstein, *Water Res.*, **32**, 831 (1998).
39. M. Beccari, R. Passion, R. Ramadori and V. Tandoi, *J. Water Pollt. Control Fed.*, **55**, 58 (1983).
40. C. Glass, J. Silverstein and J. Oh, *Water Environ. Res.*, **69**, 1086 (1997).
41. J. Ma, Q. Yang, S. Wang, L. Wang, A. Takigawa and Y. Peng, *J. Hazard. Mater.*, **175**, 518 (2010).
42. C. Francis and J. Mankin, *Water Res.*, **11**, 289 (1977).
43. G. Koch, M. Kühni, W. Gujer and H. Siegriest, *Water Res.*, **34**, 3580 (2000).
44. S. S. Alves, C. I. Maia and J. M. T. Vasconcelos, *Chem. Eng. Proc.*, **43**, 823 (2004).
45. R. Lemoine and B. I. Morsi, *Int. J. Chem. React. Eng.*, **3**, 1 (2005).