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Nitrification performance of activated sludge under low dissolved oxygen conditions

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NITRIFICATION PERFORMANCE OF ACTIVATED SLUDGE UNDER LOW DISSOLVED OXYGEN CONDITIONS

by

GUOQIANG LIU

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This dissertation has been prepared in the form of three journal articles for peer review. These articles are presented in Paper I through Paper III. Paper I has been prepared according to the style used by Water Research and pages 15 to 44 have been accepted for publication. Paper II has been prepared according to the style used by Water Research and pages 45 to 130 will be submitted to this publication. Paper III has been prepared in the style used by Water Environment Research and pages 131 to 162 have been submitted to this publication.

NOTE: The sections of Introduction, Goals and Objectives, Materials and Method, Conclusions, Significance and Impacts, Future Research, and Appendices contain supplemental information for the journal articles. They present some information required to complete this dissertation but not necessary for submission to any of the aforementioned journals.

ABSTRACT

The combined effects of solids retention time (SRT) and dissolved oxygen (DO) concentration on nitrification, nitrifying bacterial communities, and aeration needs in the activated sludge process were studied. Nitrification was almost completed in the 10, 20, and 40-day SRT reactors with a minimum DO of 0.37, 0.25, and 0.16 mg/L, respectively. Under long-term low DO conditions, the endogenous decay of nitrifiers was slowed down and then nitirfier biomass concentration increased, thereby reducing the adverse effect of low DO on nitrification. Under long-term low DO conditions, the oxygen affinity of nitrite-oxidizing bacteria (NOB) increased significantly and as a result, NOB became a better competitor for oxygen than ammonia-oxidizing bacteria (AOB). *Nitrosomonas europaea/eutropha*–like AOB were dominant with all tested SRTs and DO levels. *Nitrobacter*-like NOB and *Nitrospira*-like NOB played the main role in the nitrite oxidation in the 5 and 40-day SRT reactors, respectively. In all reactors, *Nitrospira* increased considerably when the DO was reduced to ≤ 0.5 mg/L. Compared to a baseline condition (SRT = 10 days and DO = 2 mg/L), aeration need was reduced by about 20% under these two conditions (SRT = 10 days and DO = 0.37 mg/L; SRT = 40 days and DO = 0.16 mg/L). It was found that the boom of filamentous bacteria inhibited the oxygen transfer efficiency considerably in the 20-day SRT reactor.

In the activated sludge process, a combination of effluent ammonia and nitrite could effectively report insufficient or excessive DO, which was proposed as the indicator for aeration control. A simple control approach can be developed to reduce the aeration when both effluent ammonia and nitrite concentrations are ≤ 0.5 mg-N/L. When either is ≥ 2 mg-N/L, increase the aeration.

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1. INTRODUCTION

Activated sludge processes typically containing an aeration tank and a final clarifier are the most widely used technologies for domestic wastewater treatment. In the aeration tank, organic matter and ammonia are oxidized by heterotrophic bacteria and nitrifying bacteria, respectively, using oxygen as electron acceptor. To maintain the activity of biomass, aeration is needed to maintain the dissolved oxygen (DO) around 2 mg/L (Metcalf & Eddy, 2003). As a result, great amount of energy is used for aeration. In U.S., approximately 3% of total electrical energy consumption is used by wastewater treatment and about half of that is used for aeration (McCarty, et al., 2011). With increasing concerns for climate change associated with fossil fuel consumption and energy demand growing, energy conservation and reducing carbon footprint are emerging topics currently being discussed and targeted.

In the activated sludge process, the aeration need mainly depends on the actual oxygen demand and oxygen transfer efficiency in the aeration tank. Therefore, two directions can reduce aeration needs: improve the oxygen transfer efficiency or reduce the actual oxygen demand. Theoretically, if the aeration tank is running with a DO of 0.5 mg/L instead of 2 mg/L, the oxygen transfer efficiency would be enhanced by about 16% (Metcalf & Eddy, 2003). However, under low DO conditions, incomplete nitrification may occur. Nitrification performance under low DO conditions may be enhanced by extending solids retention time (SRT) (Stenstrom, 1980). In addition, the nitrifying bacterial communities may vary with the change of operational DO and SRT.

1.1. THE EFFECT OF SRT

SRT is a critical control parameter for activated sludge process. To retain a specific group of functional microorganism in the reactor, the minimum operational SRT can be estimated by: $SRT_{\min} \approx \frac{1}{\mu_m - K_d}$ (Metcalf & Eddy, 2003); therefore, the operational SRT for a process will be mainly determined by the functional microorganism with slowest specific growth rate. In activated sludge, BOD is oxidized by heterotrophic bacteria and nitrification is completed by autotrophic nitrifying bacteria through two steps (Metcalf & Eddy, 2003). Ammonia is oxidized into nitrite by ammonia-oxidizing bacteria (AOB) and then into nitrate by nitrite-oxidizing bacteria (NOB). Generally, AOB grow slower than heterotrophic bacteria, while NOB have a lower specific growth rate than AOB (Metcalf & Eddy, 2003; Blackburne et al., 2008). To achieve complete nitrification and avoid nitrite accumulation, the operational SRT will depend on the growth of NOB. The presence of nitrite in the effluent is particularly troublesome for the plants using chlorination for disinfection since 1 g of nitrite-N requires 4 g chlorine (Metcalf & Eddy, 2003). Therefore, understanding the effect of SRT on ammonia and nitrite oxidation in the activated sludge process is important.

In addition, the contaminants removal efficiency in the activated sludge process is mainly determined by the operational SRT. In the aeration tank, the contaminant degradation rate depends on active biomass concentration and its activity, contaminants concentration, DO concentration, and other impact factors, including temperature, pH, nutrients, and toxicants inhibition (Metcalf & Eddy, 2003). In municipal wastewater treatment, generally, pH, and nutrients are sufficient, no temperature control is implemented and inhibition of toxicants is negligible. If the DO is sufficient, the biomass concentration is critical to the contaminant degradation rate and then the effluent quality. For a given wastewater and hydraulic retention time (HRT), the biomass concentration mainly depends on SRT (Metcalf & Eddy, 2003). Therefore, the SRT is the most critical parameter for pollutants removal in the activated sludge process.

On the other hand, a longer SRT with an unlimited DO will result in a lower biomass production, which means a higher oxygen demand. Under a longer SRT, though biomass concentration in the process will increase, the sludge production will decrease due to sludge endogenous decay. As a result, more oxygen will be required for biomass endogenous decay, increasing aeration need.

1.2. EFFECT OF DO CONCENTRATION

In activated sludge, heterotrophic bacteria are responsible for BOD removal, while nitrifying bacteria are responsible for nitrification, using oxygen as the electron acceptors. Therefore, DO is a very important control parameter. A low DO concentration may inhibit contaminants removal and cause sludge bulking. On the other hand, an excessive DO concentration will lead to unnecessary power consumption.

In the aeration tank, the required aeration depends on the actual oxygen demand and the oxygen transfer efficiency. The actual oxygen demand is determined by the amount of pollutants oxidized and biomass produced, while the oxygen transfer efficiency is related to aeration devices, operational DO, temperature, sludge property, and MLSS concentration (Metcalf & Eddy, 2003). Therefore, two directions can reduce aeration need: improve the oxygen transfer efficiency or reduce the actual oxygen demand. Theoretically, if the aeration tank is running with a DO of 0.5 mg/L instead of 2 mg/L, the oxygen transfer efficiency would be enhanced by about 16% (Metcalf & Eddy, 2003).

In activated sludge, nitrifying bacteria are less competitive to utilize DO than heterotrophic bacteria (Metcalf & Eddy, 2003). As a result, when the DO is low, though the oxygen transfer efficiency will increase, incomplete nitrification may occur. Downing and Scragg (1958) reported that a DO of at least 0.3 mg/L was needed for nitrification to occur and nitrification ceased entirely when the DO was below 0.2 mg/L (Downing et al. 1964). Wild et al. (1971) found that the nitrification rates in activated sludge were not affected by a DO concentration above 1.0 mg/L. However, Nagel and Haworth (1969) reported that the nitrification rate in activated sludge doubled when the DO concentration increased from 1.0 to 3.0 mg/L. In a membrane reactor operated for simultaneous nitrification and denitrification, a high effluent ammonia concentration (> 20 mg-N/L) accumulated when the DO was in the range from 0.15 to 0.55 mg/L (Hocaoglu et al., 2011). However, Hanaki et al. (1989) and Bellucci et al. (2011) found that complete nitrification could be achieved when the DO was about 0.5 mg/L. With an even lower DO level (0.24 mg/L), complete nitrification was still achieved in a chemostat reactor (Park et al. 2004). In the text book, a DO of 2 mg/L was recommended for activated sludge processes with nitrification (Metcalf and Eddy, 2003).

Obviously, the reports about the impact of DO on nitrification varied widely in the literature. As discussed previously, a longer SRT will result in a higher nitrifying biomass concentration. It is possible that a reactor with a longer SRT can achieve complete nitrification with a lower DO (Stenstrom, 1980) and the controversy about the DO impact is a result of SRT difference. On the other hand, a longer SRT with an unlimited DO will result in a lower sludge production, meaning a higher oxygen demand for sludge decay. But under a low DO condition, the sludge endogenous decay may be inhibited, thereby increasing the sludge production (Abbassi et al., 1999). Therefore, it is possible that there is no significant difference for the actual oxygen demand under two conditions, a short SRT with a high DO vs. a long SRT with a low DO. Finally, the aeration need under the condition (a long SRT with a low DO) may be less since the oxygen transfer efficiency will be improved by a low DO.

In activated sludge, the nitrite oxidation (the second step of nitrification) was suggested to be more sensitive to low DO concentrations than ammonia oxidation (the first step of nitrification) (Laanbroek and Gerards, 1993; Laanbroek et al., 1994; Metcalf and Eddy, 2003; Sliekers et al., 2005). As a result, nitrite may accumulate under low DO conditions (Li et al., 2011; Blackburne et al., 2008; Park et al., 2010). In these reports, however, the effect of SRT on partial nitrification at low DO concentrations is not evaluated. Possibly, NOB is a better competitor than AOB at low DO concentrations when SRT is long enough.

The effect of DO concentration on nitrifier growth was generally conducted in a short-term batch test and a Monod-based expression was usually used to describe its impact (Stankewich, 1972; Park et al., 2004; Weon et al., 2004; Park and Noguera, 2006; Kaelin et al., 2009). In the short-term DO impact tests, the process of endogenous decay did not play the main role and then the effect of low DO concentration on nitrifier endogenous decay was not examined (Park et al., 2004; Weon et al., 2004; Park and Noguera, 2006). Under long-term low DO conditions, however, the process of endogenous decay will play an important role in nitrifying biomass concentration. Thus it is very important to know the effect of low DO concentration on nitrifier decay. In existing models, the low DO concentration was thought to have no inhibition on nitrifier decay (Stenstrom, 1980; Metcalf and Eddy, 2003) or perform the same inhibition on nitrifier growth and decay (Henze et al., 2000; Manser et al., 2005). However, these hypotheses have not been tested in available literature

1.3. NITRIFIERS IN ACTIVATED SLUDGE

In the groups of AOB and NOB, different nitrifier genera are present and their kinetics performance may be significantly different. Generally, all known AOB belong to the genera *Nitrosomonas*, *Nitrosospira*, or *Nitrosococcus* (Siripong and Rittmann, 2007). In fresh water and soil, usually *Nitrosomonas* and *Nitrosospira* are found, both of which are located in *Betaproteobacteria* (Siripong and Rittmann, 2007). *Nitrosococcus* are mainly found in marine environment, which belong to *Gammaproteobacteria*. Generally, *Nitrosomonas* can be divided into 5 major lineages, including *Nitrosomonas Europaea/eutropha*, *Nitrosomonas Communis*, *Nitrosomonas oligotropha*, *Nitrosomonas Marina*, and *Nitrosomonas Crytolerans* (Koops and pommerening-Röser, 2001; Limpiyakorn et al., 2005; Sonthiphand and Limpiyakorn, 2011). Some unknown lineages may be included into *Nitrosomonas* as well (Limpiyakorn et al., 2005; Lim et al., 2007). NOB are more diverse than AOB. Generally, all known NOB belong to one of the four different genera: genus *Nitrobacter*, genus *Nitrococcus*, genus *Nitrospina*, and genus *Nitrospira*, which belong to *Alphaproteobacteria*, *Deltaproteobacteria*, *Betaproteobacteria*, and *Nitrospirae*, respectively (Teske et al., 1994; Daims et al., 2000; Siripong et al., 2007). In the activated sludge systems, *Nitrosomonas*-like AOB and *Nitrobacter*-like NOB traditionally are considered the most popular nitrifiers (Metcalf &

Eddy, 2003). However, recent findings indicated that *Nitrosospira*-like AOB and *Nitrospira*-like NOB are present in activated sludge as well and may be the dominant nitrifier (Dytczak et al., 2008; Li et al., 2007; Limpiyakorn et al., 2005; Sonthiphand and Limpiyakorn, 2011).

Beside AOB, ammonia-oxidizing archaea (AOA) were found to be present in some wastewater treatment processes as well (Park et al., 2006; Zhang et al., 2009; Limpiyakorn et al., 2011), but no nitrite-oxidizing archaea was found (You et al., 2009).

But the kinetics performance between *Nitrosomonas* and *Nitrosospira*, and between *Nitrobacter* and *Nitrosospira* was significant different. *Nitrobacter* was found to be 10 times more active than *Nitrospira* (Kim and Kim, 2006). Therefore, *Nitrosomonas*, especially *Nitrosomonas europaea/eutropha* lineage and *Nitrobacter*-like NOB, were suggested to be "*r"* strategists, having high specific growth rates but low substrate affinity, while *Nitrosospira* and *Nitrospira* were thought to be "*K*" strategists, having low specific growth rates but high substrate affinity (Dytczak et al., 2008; Schramm et al., 1999; Kim and Kim, 2006). Generally, the "*r"* strategist nitrifiers may outcompete "K" strategist in the situations with short SRTs and high substrate concentrations (Schramm et al., 1999; Kim and Kim, 2006; Yu et al., 2010). However, the "K" strategist nitrifiers may dominate systems with long SRTs and low substrate concentrations (Kim and Kim, 2006; Yu et al., 2010).

In addition, the sublineages in the group of AOB and NOB may have significantly different oxygen affinity (Park and Noguera, 2007), indicating that the DO concentration will impact nitrifying bacterial communities. However, the reports about the impact of DO on nitrifiying bacteria communities are controversial. *Nitrosospira* and *Nitrospira* are believed to be “*K*” strategists, indicating that they may have a higher oxygen affinity (Dytczak et al., 2008). However, in Park et al’s study, *Nitrosomonas europaea* lineage, not *Nitrosospira*, was the dominant AOB in the activated sludge system with a low DO concentration (Park et al., 2004; Park and Noguera, 2007). de Bie et al. observed that *Nitrosomonas oliotropha* lineage were the prevalent AOB under low DO conditions (de Bie et al., 2001). Gieseke et al. (2003) reported that *Nitrosomonas europaea* and *Nitrosomonas oligotropha* lineages dominated the outer layers of a biofilm where the DO concentration was high, while *Nitrosomonas oligotropha* lineages were exclusively found in deeper layers of the biofilm where the DO was low. On the contrary, Li et al. found that *Nitrosospira* outnumbered *Nitrosomonas* under low DO conditions, while the DO did not play important role in the selection of NOB community (Li et al., 2007).

Nitrifying bacterial communities will be impacted by substrate concentration, SRT, and DO concentration. In previous studies, these factors were evaluated individually and so it was difficult to determine which factor would play the main role.

1.4. AERATION CONTROL STRATEGY

As introduced previously, the DO level in the aeration tank is a critical control parameter since it directly relates to both effluent quality and operation cost. Conventionally, it was recommended to control a DO level of 2 mg/L in the aeration tank (Metcalf & Eddy, 2003; Ma et al., 2006). However, in many treatment plants, especially those with long SRTs, the biodegradation can be completed at a DO level of less than 2 mg/L. In this case, maintaining a constant DO of 2 mg/L is unnecessary. On the other hand, insufficient DO could adversely impact organic biodegradation and nitrification. Because the energy used for aeration contributes to the majority of the energy consumption of an entire treatment plant (McCarty et al., 2011), the main goal of aeration control is to minimize aeration while maintaining the required effluent quality. To meet this goal, an optimal operational DO should be used as the control parameter. However, given the large variations in wastewater flow, strength, and temperature, a constant DO level that can result in the required treatment without over aeration does not exist. In addition, due to the large variations in inflow quality and quantity, maintaining a constant DO at all times is very difficult (Phillips and Fan, 2005). Maintaining a dynamic minimum DO level that can achieve the required effluent quality is the key to optimizing the aeration system for energy conservation.

Advanced control strategies, such as model-based predictive control or fuzzy logic control, are currently being investigated to minimize energy use for wastewater treatment (Manesis et al., 1998; Ferrer et al., 1998; Galluzzo et al., 2001; Ma et al., 2006; Holenda et al., 2008). Based on these model-based control strategies, an optimal set-point DO or air flow is determined and tracked through a number of equations and multiple variables, e.g., flow rate, temperature, influent ammonia, and sludge concentration. However, the kinetic parameters used in the equations are very difficult to determine and may vary with time (Cox 2004), which make control extremely difficult. Moreover, model-based control strategies strongly rely on the performance of many sensors, but the maintenance and calibration of these sensors are not simple.

Ammonia removal has become one of the most important goals for municipal wastewater treatment. As introduced previously, both BOD degradation and nitrification need sufficient DO, while nitrifying bacteria are believed to be less competitive in low DO than heterotrophic bacteria (Grady and Lim, 1980; Metcalf & Eddy, 2003).

Nitrification is completed through two steps. The second step of nitrification is more sensitive to low DO level than the first step. As a result, nitrite may accumulate under insufficient DO (Blackbune et al., 2008). Therefore, if the DO in an aeration tank is not sufficient, the effluent ammonia and/or nitrite may provide a faster feedback than the effluent BOD by increasing their concentrations. When the effluent ammonia or nitrite is greater than an upper threshold value, aeration intensity can be increased to improve nitrification. When the effluent ammonia, nitrite, or both of them, are below a lower threshold value, the aeration intensity can be decreased to save energy. Compared to the DO set-point control strategy, the aeration control based on effluent ammonia/nitrite would be more reasonable since it can indicate the effluent quality directly. In addition, aeration can be better controlled since it can indicate the minimum aeration demand more accurately and effectively than the DO set-point control strategy. Compared to the fuzzy control and model based predictive-control strategies, it will be more economical, simple, straight-forward, and accurate since only one parameter need be monitored.

2. GOALS AND OBJECTIVES

In current literature, the combined effects of SRT and DO concentration on nitrification and nitrifying bacterial communities have never been studied. The process of endogenous decay plays an important role in the nitrifier biomass concentration of activated sludge systems, but little is known about the effect of DO concentration on the decay of AOB and NOB. It is possible that the decay of AOB and NOB can be inhibited by a low DO concentration and then more nitrifiers will be enriched, thereby reducing the adverse effect of low DO on nitrification performance of activated sludge. Under long-term low DO conditions, possibly the nitrifiers with a higher oxygen affinity can be selected and finally, the nitrification performance under low DO conditions will be improved. Moreover, the effect of SRT on nitrification performance under long-term low DO conditions is not clear and probably the reactor with a longer SRT can achieve complete nitrification with a lower DO concentration. Though a low DO concentration benefits oxygen transfer, a long SRT has a higher oxygen demand, so that it is interesting to know the oxygen demand and aeration needs under various SRTs and DO levels. Therefore, in this research, the combined effects of SRT and DO concentration on nitrification, nitrifying bacterial community, and aeration needs are studied in the activated sludge process. The specific objectives are as follows:

1. To probe the stoichiometry of the autotrophic nitrification process using a respirometric approach;
2. To quantify the combined effect of SRT and DO on nitrification performance: effluent ammonia and nitrite concentrations and AOB and NOB biomass

concentrations (represented by biomass maximum ammonia and nitrite oxidation rates);

3. To compare the oxygen demand and aeration needs under different SRT and DO levels;
4. To elucidate the effects of DO and SRT on nitrifying bacteria communities; and
5. To develop a potential aeration control strategy: using effluent ammonia or nitrite as the only parameter for aeration control in the activated sludge process.

3. MATERIALS AND METHODS

To fulfill the aforementioned objectives, the following experimental plan has been implemented:

1. Determine the biomass yield coefficients of AOB and NOB.

   Please refer to Paper I.

2. Test nitrification performance under different SRTs and DO levels and do mathematical modeling.

   Please refer to Paper II.

3. Quantify the population size of AOB and NOB and analyze the nitrifying bacterial communities in activated sludge cultivated with various SRTs and DO levels.

   Please refer to Paper II.

4. Analyze the oxygen demand and aeration needs under different SRTs and DO levels. Please refer to Paper II.

5. Develop a potential aeration control strategy: using effluent ammonia or nitrite as the only parameter for aeration control in the activated sludge process.

   Please refer to Paper III.

PAPER

I. Probing the Stoichiometry of the Autotrophic Nitrification Process Using the Respirometric Approach

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Abstract

Quantifying oxygen demand and nitrifier yield are important in the design and operation of advanced wastewater treatment systems. However, the accurate stoichiometry of the autotrophic nitrification process has not been fully developed. In this research, stoichiometric links between nitrifier yield, ammonia and nitrite oxidization, ammonia assimilation, and oxygen uptake for each step of the nitrification process were determined. A pulse-flow respirometer was used to measure the oxygen uptake for complete nitrification and nitrite oxidation reactions. Results indicated that the specific oxygen uptake was 4.23 mg-O_2/mg-N oxidized for complete nitrification, with 3.17 mg-O_2/mg-N oxidized for ammonia oxidation (first step nitrification) and 1.06 mg-O_2/mg-N oxidized for nitrite oxidation (second step nitrification). For complete nitrification, fractions of ammonia used for electron donation, synthesis of ammonia oxidizers, and synthesis of nitrite oxidizers were 97.1%, 2.2%, and 0.7%, respectively. The fractions of electrons transferred into cell synthesis were approximately 7.5% for ammonia oxidation and 7.3% for nitrite oxidation. Biomass yield coefficients for ammonia oxidizers and nitrite oxidizers were 0.18 and 0.06 g-VSS/g-N oxidized, respectively. These parameters are critical when calculating oxygen needs and nitrifier biomass concentrations during the design of advanced wastewater treatment processes.

Keywords

Nitrification, nitrifier yield, ammonia assimilation, oxygen uptake, stoichiometry

Nomenclature

f_e	the fraction of electrons used for energy production (reduce O_2)
f_s	the fraction of electrons used for cell synthesis (CO_2 fixation)
R	the overall balanced reaction
R_a	the half reaction for electron acceptors
R_d	the half reaction for electron donors
R_{cs}	the half reaction for the synthesis of cell tissue
OU	cumulative oxygen uptake in batch respirometric tests
SOU	specific oxygen uptake for complete nitrification, ammonia or nitrite oxidation
OUR	oxygen uptake rate
OUR_{max}	maximum oxygen uptake rate
k	maximum specific substrate uptake rate
μ_{max}	maximum specific growth rate
X	net active nitrifier biomass concentration
T	time
ρ	pure oxygen gas density
AOR_{max}	maximum ammonia oxidation rate
NOR_{max}	maximum nitrite oxidation rate

Subscripts	
NC	refers to variables associated with complete nitrification ($NH_4^+ \rightarrow NO_3^-$)
NH	refers to variables associated with ammonia oxidation ($NH_4^+ \rightarrow NO_2^-$)
NO	refers to variables associated with nitrite oxidation ($NO_2^- \rightarrow NO_3^-$)

1. Introduction

Specific oxygen uptake and nitrifier biomass yield coefficients are very important parameters in the design of activated sludge processes. Theoretically, 4.57 g of oxygen are needed to completely oxidize 1 g of ammonia-nitrogen (ammonia-N) into nitrate, with 3.43 g-O_2/g-N for the first-step nitrification (ammonia oxidation) and 1.14 g-O_2/g-N for the second-step nitrification (nitrite oxidation) (Metcalf and Eddy, 2003). In the autotrophic nitrification process, the energy generated from the above oxidation process was used to fix the CO_2 from the environment to synthesize ammonia oxidizers and nitrite oxidizers. Ammonia is the preferred nitrogen source for biomass synthesis (Rittmann and McCarty, 2001). Therefore, a small amount of ammonia, as well as small fractions of electrons derived from the ammonia oxidation and nitrite oxidation reactions were used for cell synthesis, resulting in less specific oxygen demand than aforementioned theoretical values. When designing a wastewater treatment plant (WWTP), an empirical value of 4.33 g-O_2/g-N consumed is used to calculate oxygen demand for complete nitrification, with 3.22 g-O_2/g-N for ammonia oxidation and 1.11 g-O_2/g-N for nitrite oxidation (Werzernak and Gannon, 1967). However, oxygen used for nitrifier endogenous decay was not subtracted when determining these values (Werzernak and Gannon, 1967), resulting in a slightly over estimation for them.

Activated sludge contains different types of ammonia oxidizers and nitrite oxidizers. Ammonia-oxidizing bacteria were thought to be the main ammonia oxidizers (Metcalf and Eddy, 2003). However, recent findings indicated that ammonia-oxidizing archaea might also be present in some activated sludge samples (Park et al., 2006; Zhang et al., 2009; Sonthiphand and Limpiyakorn et al., 2011). Nitrite-oxidizing bacteria were the only nitrite oxidizers in activated sludge, and no nitrite-oxidizing archaea were reported (You et al., 2009). The kinetics among different nitrifiers were significantly different from each other (Schramm et al., 1999; Kim and Kim, 2006; Dytczak et al., 2008), and it was very difficult to determine these kinetic constants using the pure-culture approach (Blackburne et al., 2007).

Specific oxygen uptake and biomass yield coefficients for nitrification could be estimated based on stoichiometry (Rittmann and McCarty, 2001; Metcalf and Eddy, 2003). To balance a complete biological reaction, the critical step was to determine the fraction of electrons used for cell synthesis. For nitrification, 5% or 10% of electrons were assumed to be used for nitrifier synthesis. By assuming that 5% of electrons would be used for cell synthesis, a complete nitrification reaction was developed (Crites and Tchobanoglous, 1998).

$$NH_4^+ + 1.863\,O_2 + 0.098\,CO_2 \rightarrow 0.0196\,C_5H_7O_2N + 0.98\,NO_3^- + 1.98\,H^+ + 0.094\,H_2O \tag{1}$$

Based on the above equation, for 1 g of ammonia-N removal, 4.26 g of oxygen were consumed and 0.16 g of cells were produced. However, the assumption of a 5% electron for cell synthesis was not validated. Moreover, the complete nitrification process was accomplished by two groups of bacteria, ammonia oxidizers and nitrite oxidizers, through two sequential steps. The detailed relationship within these individual reactions, namely ammonia oxidation and nitrite oxidation, was still unknown.

There is a stoichiometric link between nitrifier yield, ammonia consumption, and oxygen uptake. The use of more electrons for cell synthesis would lead to a higher biomass yield coefficient and a lower oxygen uptake. Based on the stoichiometric link, batch respirometric tests were proposed to estimate nitrifier yield coefficients and nitrification kinetics in activated sludge with minimal analytical effort (Vanrolleghem et al., 1999; Chandran and Smets, 2001; Langergraber et al., 2003). However, the results from the respirometric approach were strongly dependent on the accuracy of the equations expressing the stoichiometric link. In a batch respirometric test, the generated nitrite is normally oxidized into nitrate immediately. Therefore, the total amount of consumed ammonia includes oxidized ammonia and ammonia incorporated into the cells of both ammonia oxidizers and nitrite oxidizers. Unfortunately, ammonia used for synthesizing ammonia oxidizers and/or nitrite oxidizers was not considered in equations used previously (Vanrolleghem et al., 1999; Chandran and Smets, 2001; Langergraber et al., 2003). As a result, errors could occur when estimating nitrifier yield coefficients using these equations.

The primary objective of this research was to determine a more accurate stoichiometric link between nitrifier yield, ammonia consumption, and oxygen uptake for both steps of the nitrification process, with full consideration of the amounts of ammonia incorporated into the cells of ammonia oxidizers and nitrite oxidizers. With this stoichiometric link, practical parameters such as the specific oxygen uptake rate (SOUR) for each step of the nitrification process and the specific bacterial yield coefficients for ammonia oxidizers and nitrite oxidizers are also provided.

2. Theoretical aspects

2.1. Electron transfer during nitrification

Fig. 1 illustrates the mass balance and electron transfer in the autotrophic nitrification process, with values determined from this research. Ammonia oxidizers and nitrite oxidizers require both electrons and energy to convert CO_2 into bacterial cells. If the fraction of electrons used for ammonia oxidizer synthesis is $f_{s,NH}$, the fraction of electrons transferred to oxygen for energy production is $1-f_{s,NH}$. Similarly, for nitrite oxidation, the fractions of electrons used for nitrite oxidizer synthesis and energy production are assumed to be $f_{s,NO}$ and $1-f_{s,NO}$, respectively. Ammonia-N has the same oxidation state as nitrogen in cells; therefore, there is no electron transfer of the fraction of ammonia that is used for cell synthesis.

2.2. The overall nitrification reaction

A biological reaction could be determined using Equation (2) (Rittmann and McCarty, 2001).

$$R = f_e R_a + f_s R_{cs} - R_d \tag{2}$$

In Equation (2), $f_s + f_e = 1$. To balance a biological reaction with known half reactions, the critical step was to determine the value of f_s. Based on the half reactions shown in Table 1, Equation (2), and Fig. 1, overall reactions for ammonia oxidation and nitrite oxidation were developed and are presented as Equations (3) and (4), respectively.

Actually, a small fraction of nitrogen may be released from the systems as N_2O, but under sufficiently aerobic conditions the release of N_2O is very small and can be ignored (Sivret et al., 2008).

$$\begin{aligned}&NH_4^+ + \frac{3}{10} f_{s,NH} NH_4^+ + \frac{3-3f_{s,NH}}{2} O_2 + \frac{6}{5} f_{s,NH} CO_2 + \frac{3}{10} f_{s,NH} HCO_3^- = \\ &\frac{3}{10} f_{s,NH} C_5H_7O_2N + NO_2^- + 2H^+ + (1-\frac{3}{10} f_{s,NH})H_2O\end{aligned} \tag{3}$$

$$\begin{aligned}&NO_2^- + \frac{1}{10} f_{s,NO} NH_4^+ + \frac{1-f_{s,NO}}{2} O_2 + \frac{2}{5} f_{s,NO} CO_2 + \frac{1}{10} f_{s,NH} HCO_3^- \\ &+ \frac{1}{10} f_{s,NO} H_2O = \frac{1}{10} f_{s,NO} C_5H_7O_2N + NO_3^-\end{aligned} \tag{4}$$

Based on Equations (3) and (4), the overall reaction for complete nitrification was developed:

$$\begin{aligned}&NH_4^+ + \frac{3f_{s,NH} + f_{s,NO}}{10} NH_4^+ + (2-\frac{3f_{s,NH} + f_{s,NO}}{2})O_2 + \frac{6f_{s,NH} + 2f_{s,NO}}{5} CO_2 \\ &+ \frac{3f_{s,NH} + f_{s,NO}}{10} HCO_3^- = \frac{3f_{s,NH} + f_{s,NO}}{10} C_5H_7O_2N + NO_3^- + 2H^+ + (1-\frac{3f_{s,NH} + f_{s,NO}}{10})H_2O\end{aligned} \tag{5}$$

2.3. Determining $f_{s,NH}$ *and* $f_{s,NO}$

As shown in Equations (3) and (4), when one unit of ammonia or nitrite is oxidized, $\frac{3}{10} f_{s,NH}$ or $\frac{1}{10} f_{s,NO}$ unit of ammonia is incorporated into the biomass without changing the oxidation state of N. Therefore, for complete nitrification, $\frac{3f_{s,NH} + f_{s,NO}}{10}$ unit of ammonia is incorporated into biomass when one unit of ammonia is completely oxidized, as shown in Equation (5). The specific oxygen uptake for complete nitrification on the basis of total consumed ammonia can be expressed by Equation (6).

$$SOU_{NC} = \frac{OU_{NC}}{NH_{4\,T}^{+}} = \frac{(2 - \frac{3f_{s,NH} + f_{s,NO}}{2}) \times 32}{(1 + \frac{3f_{s,NH} + f_{s,NO}}{10}) \times 14} \tag{6}$$

Rearrange equation (6), $f_{s,NH}$ can be expressed by Equation (7):

$$f_{s,NH} = \frac{4.57 - SOU_{NC} - 1.14 f_{s,NO} - 0.1 f_{s,NO} SOU_{NC}}{3.43 + 0.3 SOU_{NC}} \tag{7}$$

Similarly, according to the relationship of nitrite oxidized and oxygen used in Equation (4), $f_{s,NO}$ can be expressed by Equation (8):

$$f_{s,NO} = \frac{1.14 - SOU_{NO}}{1.14} \tag{8}$$

As shown in Equation (7), to estimate the value of $f_{s,NH}$, the values of $f_{s,NO}$ and SOU_{NC} need to be determined first. SOU_{NC} can be determined in a batch respirometric test for complete nitrification. $f_{s,NO}$ can be calculated based on Equation (8) using SOU_{NO}, and SOU_{NO} can be determined in a separate batch respirometric test for nitrite oxidation. Once the values of $f_{s,NH}$ and $f_{s,NO}$ are determined, detailed stoichiometry of the nitrification process, Equations (3) to (5), can be completed.

In Equation (7), the fraction of ammonia incorporated into the cell synthesis is considered. If this is neglected, Equation (9) is developed to express $f_{s,NH}$ according to the relationship of ammonia oxidized and oxygen used in Equation (3).

$$f_{s,NH} = \frac{3.43 - SOU_{NH}}{3.43} \tag{9}$$

If ammonia incorporated into ammonia oxidizer synthesis is considered, while into nitrite oxidizer synthesis is neglected, then Equation (10) is obtained to express

$f_{s,NH}$ according to the relationship of ammonia consumed and oxygen used in Equation (3).

$$f_{s,NH} = \frac{3.43 - SOU_{NH}}{3.43 + 0.3SOU_{NH}} \qquad (10)$$

Equations (9) and (10) are the same as those developed by Vanrolleghem et al., (1999) and Chandran and Smets (2001), respectively. In Equation (10), the denominator contains an additional term ($0.3SOU_{NH}$) compared with Equation (9), which reflects the effect of ammonia incorporated into ammonia oxidizers (Chandran and Smets, 2001). However, compared to Equation (10), the numerator in Equation (7) has two additional terms ($1.14\,f_{s,NO}$ and $0.1\,f_{s,NO}SOU_{NC}$), which reflect the nitrite oxidation and ammonia incorporated into nitrite oxidizers. Equation (10) could correctly describe the link between $f_{s,NH}$ and SOU_{NH} in batch tests for ammonia oxidizers only. When both ammonia and nitrite oxidizers are contained in the experimental culture, (e.g., activated sludge), results from Equation (10) could include significant error since the amount of ammonia incorporated into the nitrite oxidizer biomass was not included when developing this equation.

2.4. Determining nitrifier yield coefficients

The yield coefficients for ammonia oxidizers are commonly expressed as unit of biomass produced per unit of ammonia-N oxidized rather than per unit of ammonia-N consumed (Rittmann and McCarty, 2001). Based on the link between biomass synthesis and ammonia oxidized in Equation (3), Equation (11) is obtained to calculate the ammonia oxidizer yield coefficient.

$$Y_{NH} = 2.42\,f_{s,NH} \qquad (11)$$

Similarly, Equation (12) is obtained based on Equation (4) to calculate the nitrite oxidizer yield coefficient.

$$Y_{NO} = 0.81 f_{s,NO} \tag{12}$$

When the values of $f_{s,NH}$ and $f_{s,NO}$ are determined, yield coefficients for ammonia and nitrite oxidizers can be calculated.

2.5. Determining nitrification rates

For a given activated sludge sample, the net concentrations of ammonia oxidizers and nitrite oxidizers are difficult to detect. However, the maximum ammonia and nitrite oxidation rates in activated sludge can be conveniently measured, which can be used as indicators of sludge nitrification capacity. In a batch respirometric test, ammonia oxidation can be considered as a zero order reaction when the ammonia concentration is sufficient (Metcalf and Eddy, 2003). Based on Equation (3), the maximum ammonia oxidation rate ($k_{NH} X_{NH}$) can be estimated through OUR:

$$AOR_{\max} = k_{NH} X_{NH} = \frac{OUR_{\max,NH}}{3.43 - 3.43 f_{s,NH}} = \frac{OUR_{\max,NH}}{3.43 - 1.42 Y_{NH}} \tag{13}$$

Similarly, in a batch respirometric test, the maximum nitrite oxidation rate ($k_{NO} X_{NO}$) can be estimated by:

$$NOR_{\max} = k_{NO} X_{NO} = \frac{OUR_{\max,NO}}{1.14 - 1.14 f_{s,NO}} = \frac{OUR_{\max,NO}}{1.14 - 1.42 Y_{NO}} \tag{14}$$

As presented in Equations (13) and (14), yield coefficients are important parameters for accurate estimation of the nitrification rates.

3. Materials and methods

3.1. Activated sludge samples

Two lab-cultured sludges with different solids retention times (SRTs) and one field sludge from an operating oxidation ditch in the Rolla Southeast Wastewater Treatment Plant (WWTP) were used for the experiment. Two bench scale complete-mix reactors of an identical size (31.5 L) were continuously fed synthetic wastewater at the same rate, to achieve a hydraulic retention time of 12 hours. Influent COD and ammonia-N concentrations were 180 mg/L and 48 ± 2 mg/L, respectively. The COD was provided with glucose while ammonia-N was provided with ammonium carbonate. In addition, trace elements were provided as nutrients in the influent, with concentrations of Mn^{2+} = 0.2 mg/L (from $MnCl_2 \cdot 4H_2O$), Mo^{5+} = 0.12 mg/L (from $MoCl_5$), Co^{2+} = 0.001 mg/L (from $CoCl_2$), Zn^{2+} = 0.05 mg/L (from $ZnCl_2$), and Fe^{2+} = 0.005 mg/L (from $FeSO_4 \cdot 7H_2O$). Tap water, with soluble Ca^{2+} and Mg^{2+} concentrations higher than 20 mg/L, was used to make the influent. The pH in the aeration tank, in the range of 7.0-7.5, was controlled using a buffer solution made of KH_2PO_4 and $NaHCO_3$. Operational temperature was approximately 20 ˚C, and dissolved oxygen (DO) was approximately 2 mg/L. The SRT was controlled for 10 days at one reactor and for 20 days at the other. The effluent COD, ammonia-N, nitrite-N, and nitrate-N were monitored regularly. When reactors reached a steady state, batch respirometric tests were conducted using the mixed liquor from the reactor.

The field sludge sample used in the experiment was taken from an oxidation ditch in the Rolla Southeast WWTP. The oxidation ditch achieved complete nitrification during the time of sampling.

3.2. Pulse-flow respirometer

A pulse-flow PF-8000 aerobic/anaerobic respirometer (Respirometer System and Application, Fayetteville, Arkansas, USA) was used to conduct batch respirometric tests. Fig. 2 shows a schematic of the experimental system that consisted of a pure oxygen supply unit, a control module, eight bioreactors, a computer, and a water bath. The control module was also set in an incubator with a constant temperature at 20 ˚C and all reactors were set in a water bath at the same temperature. In a reactor DO was used by the biomass to generate carbon dioxide, nitrate, and nitrite. The carbon dioxide was then adsorbed by a KOH scrubber. The same volume of oxygen was then supplied to the reactor to compensate for the pressure decrease in the headspace. At equilibrium, the oxygen supply rate was equal to the oxygen uptake rate (OUR) of the biomass. The oxygen supply rate was recorded through the control module and a computer.

3.3. Batch respirometric tests

Batch respirometric tests were conducted to determine the total oxygen uptake and OUR for complete nitrification and nitrite oxidation of all sludge samples. To each batch reactor, 500 mL of sludge sample were added. After approximately 5 hours, the endogenous respiration rate in the reactor became constant. Five mL of ammonium bicarbonate stock solution (NH_4HCO_3, NH_4^+-N = 3052 ± 45 mg/L) or sodium nitrite stock solution ($NaNO_2$, NO_2^--N = 3150 ± 14 mg/L) were added into each reactor. The pH in all reactors was controlled at approximately 7.5 using a buffer solution containing KH_2PO_4 and $NaHCO_3$. All reactors were intensively mixed with a magnetic mixer at 1,450 rpm to achieve a DO level greater than 4 mg/L. The OUR of each reactor was recorded every 6 minutes. After all of the added substrate was consumed, the respiration rate decreased to the same level as it was before the substrate addition, and the batch test was terminated. The final concentrations of ammonia-N, nitrate-N, and nitrite-N were measured.

In batch respirometric tests, ammonia oxidation and nitrite oxidation rates could be calculated based on OUR. To confirm the calculated nitrification rates, conventional batch kinetic tests were also conducted for comparison. The comparison experiments were only conducted using the sludge with a 10-day SRT. During the test, concentrations of ammonia-N or nitrite-N as a function of time were measured.

3.4. Chemical analysis

The mixed liquor suspended solid concentration (MLSS) and the mixed liquor volatile suspended solid concentration (MLVSS) were determined using a gravimetric method following standard methods SM 2540 D and SM 2540 E (Clesceri et al., 1998). An Orion model 370 pH meter with a PerpHecT pH electrode (Orion 9206BN) was used to measure the pH. A YSI 58 DO meter with a YSI 5239 probe was used to measure DO. A HACH test kit TNT 832 was used to measure ammonia-N concentration when it was higher than 2 mg-N/L and a TNT 830 was used when it was lower than 2 mg-N/L. A HACH test kit TNT 840 was used to measure the nitrite-N concentration when it was higher than 0.6 mg-N/L and a TNT 839 was used when it was lower than 0.6 mg-N/L. A HACH test kit TNT 835 was used to measure the nitrate-N concentration.

4. Results and discussion

4.1. Determination of oxygen uptake for nitrification

Fig. (3a) shows the OUR change in complete nitrification process for the 10-day SRT sludge. At the 5th hour, ammonia was added. Before the 5th hour, the oxygen was consumed for biomass endogenous respiration only and the OUR was a constant value. After the addition of ammonia at the 5th hour, the OUR increased significantly because oxygen was also used for ammonia and nitrite oxidation, in addition to that for endogenous respiration. After approximately 6 more hours, the OUR curve dropped sharply due to the depletion of added ammonia. At the 12th hour from the beginning, the OUR curve became flat again, which was nearly equal to its value before the ammonia addition, indicating that almost all ammonia and produced nitrite were completely oxidized. Fig. (3b) shows the OUR change in a typical nitrite oxidation batch. Nitrite was added into the reactor at the 5th hour, resulting in a significant OUR increase. After about 7 hours, the OUR dropped sharply due to the depletion of the nitrite.

In Figs. (3a) and (3b), the areas above the endogenous respiration curve (dotted line) were the oxygen used for complete nitrification and for nitrite oxidation, respectively. In the batch respirometric test, the cumulative oxygen uptake was also recorded, as shown in Fig. (3c). Before the addition of substrate, the oxygen was used for biomass endogenous respiration only. Oxygen used between the 5th to the 12th hour included both biomass endogenous respiration and exogenous respiration.

The following equation was used to estimate the oxygen uptake for complete nitrification shown in Fig. (3a) and for nitrite oxidation shown in Fig. (3b).

$$OU = \left(OU_{T_2} - OU_{T_1} - (T_2 - T_1) \times \frac{OUR_{T_1} + OUR_{T_2}}{2} + 0.63 \right) \times \rho \qquad (15)$$

where OU is the oxygen uptake for ammonia or nitrite oxidation, OU_{T_1} is the cumulative oxygen uptake at time T_1 right before substrate addition, OU_{T_2} is the cumulative oxygen uptake at time T_2 after added substrate is totally oxidized, OUR_{T_1} is the endogenous respiration rate at time T_1, OUR_{T_2} is the endogenous respiration rate at time T_2, 0.63 is an empirical correction factor for the impact of cap opening on the recording of oxygen uptake, meaning that 0.63 mL of oxygen is lost during cap opening for the substrate addition, and ρ is oxygen gas density at normal temperature and pressure (1.331 mg-O_2/mL-O_2 at 20˚C).

Based on the calculation, the total oxygen uptake by ammonia and nitrite oxidation was 62.78 and 16.68 mg-O_2 in Figs. (3a) and (3b), respectively.

4.2. Specific oxygen uptake, fractions of electron transfer, and nitrifier yield coefficients

In all respiration tests, concentrations of ammonia-N and nitrite-N in the mixed liquor were lower than 0.1 mg-N/L before ammonia or nitrite addition and after complete oxidation of added ammonia or nitrite. Therefore, the amounts of ammonia consumed (including both oxidized and assimilated) or nitrite oxidized in batch tests were equal to the amount of ammonia or nitrite added. In these batch tests, 15.26 mg of ammonia-N or 15.75 mg of nitrite-N were added to different reactors. Based on the previously determined oxygen uptakes, the specific oxygen uptakes for ammonia consumption and nitrite oxidation were 4.11 and 1.06 mg-O_2/mg-N, respectively. Using Equation (8), the calculated value of f_{NO} was 7.1%. Therefore, f_{NH} was calculated to be 7.4%, based on Equation (7) and using the set of data shown in Fig. 3. Equations (11) and (12) were then used to calculate biomass yield coefficients for ammonia oxidizers and nitrite oxidizers, which were 0.18 g-cell/g ammonia-N oxidized and 0.06 g-cell/g nitrite-N oxidized, respectively. Note that in the nitrite oxidation test, a very low concentration of ammonia, approximately 0.05 mg-N/L, was present in the reactor. It was from the endogenous decay of the heterotrophic bacteria. While this concentration was negligible compared to added nitrite concentration, it was sufficient for the growth needs of the nitrite oxidizers. Therefore, the entire amount of added nitrite was oxidized to nitrate.

If the value of $f_{s,NH}$ was calculated using Equation (9), where the assimilated amount of ammonia into both ammonia oxidizers and nitrite oxidizers was ignored, it was 11.1%. Similarly, if the value of $f_{s,NH}$ was calculated using Equation (10), where the assimilated amount of ammonia into ammonia oxidizers was considered but the assimilated amount of ammonia into nitrite oxidizers was ignored , it was 8.7%. These values were 47% or 16% greater than the 7.4% value determined based on full consideration of the ammonia assimilation.

Equation (10) is valid if only the first step nitrification occurs in the system (only ammonia oxidizers were presented or the second step of nitrification was selectively inhibited by sodium azide) (Aleem and Sewell, 1981; Ginestet et al., 1998; Chandran and Smets, 2000). However, it is necessary to determine the correct chemical dosage for a given sludge sample to make sure that it only inhibits nitrite oxidation but not ammonia oxidation. Vanrolleghem et al. (1999) recommended that Equation (9) could be effective if nitrate production instead of ammonia consumption was used in the calculation for specific oxygen uptake, which, indeed, would exclude the amount of ammonia used for cell synthesis. However, errors could occur because nitrate could also be generated during the endogenous decay.

The batch respirometric tests for the 10-day SRT sludge were repeated six times to reduce measurement and calculation errors. In addition, to validate the results obtained from the 10-day SRT sludge, the 20-day SRT sludge and the field sludge were also tested for the specific oxygen uptake and nitrifier yield coefficients, using the same approach. The results are summarized in Table 2. The average specific oxygen uptake for the complete nitrification and nitrite oxidation by the 10-day SRT sludge was 4.11 mg-O_2/mg-N consumed and 1.06 mg-O_2/mg-N consumed, respectively. For the 10-day SRT sludge, the fractions of electrons used for cell synthesis in ammonia oxidation and nitrite oxidation were approximately 7.5% and 7.3%, respectively. Note that the values of 5% or 10% for the entire nitrification process are commonly cited in benchmark text books (Rittmann and McCarty, 2001; Metcalf and Eddy, 2003). Values for each step of the nitrification process were not provided in literature. In the sludge with 10-day SRT, the yield coefficient for ammonia oxidizers was 0.18 g-cell/g-N oxidized, which was close to the value determined by some researchers (Blackburne et al., 2007; Ahn et al., 2008), but significantly lower than the value provided by Furumai and Rittmann (1994). As listed in Table 2, nitrite oxidizers in the sludge with 10-day SRT had a much lower yield coefficient than ammonia oxidizers. It was 0.06 g-cell/g-N oxidized, which was also in agreement with the yield coefficient provided by Blackburne et al. (2007). If the calculation for yield coefficients is based on total ammonia consumed instead of ammonia oxidized, the yield coefficient for ammonia oxidizers is also close to 0.18 g-cell/g ammonia-N consumed. The total consumption-based value is more convenient to use during biomass yield calculations. Similar results were obtained for the 20-day SRT sludge and the field sludge, as shown in Table 2.

In Werzernak and Gannon’s work (1967), slightly higher specific oxygen uptake values, 3.22 g-O_2/g-N for ammonia oxidation and 1.11 g-O_2/g-N for nitrite oxidation, were reported. However, oxygen used for nitrifier endogenous respiration was not subtracted in their tests, which might cause a higher specific oxygen uptake. If these values are used to calculate yield coefficients for ammonia oxidizers and nitrite oxidizers, large relative errors can be expected.

4.3. Balanced reactions for nitrification

Based on the average value of f_{NH} and f_{NO} for 10-day SRT in Table 2, balanced reactions for ammonia oxidation, nitrite oxidation, and complete nitrification are developed:

$$\begin{aligned}&NH_4^+ + 0.0225NH_4^+ + 1.3875O_2 + 0.0900CO_2 + 0.0225HCO_3^- \\ &= 0.0225C_5H_7O_2N + NO_2^- + 2H^+ + 0.9775H_2O\end{aligned} \tag{16}$$

$$\begin{aligned}&NO_2^- + 0.0073NH_4^+ + 0.4635O_2 + 0.0292CO_2 + 0.0073HCO_3^- \\ &+ 0.0073H_2O = 0.0073C_5H_7O_2N + NO_3^-\end{aligned} \tag{17}$$

$$\begin{aligned}&NH_4^+ + 0.0298NH_4^+ + 1.851O_2 + 0.1192CO_2 + 0.0298HCO_3^- \\ &= 0.0298C_5H_7O_2N + NO_3^- + 2H^+ + 0.9702H_2O\end{aligned} \tag{18}$$

Equations (16) to (18) show that when 1 unit of ammonia is oxidized into nitrite and then into nitrate, 0.0225 and 0.0073 units of ammonia nitrogen would be incorporated into ammonia oxidizers and nitrite oxidizers, respectively. This means that, in complete nitrification, 97.1%, 2.2%, and 0.7% of ammonia-N are used for electron donation, synthesis of ammonia oxidizers, and synthesis of nitrite oxidizers, respectively. These values are marked in Fig. 1.

The specific oxygen uptake for ammonia oxidation (Equation 16) was 3.10 g-O_2/g of ammonia-N consumed (including ammonia oxidized and ammonia assimilated into ammonia oxidizers). Note that the sum of the specific oxygen uptake for ammonia oxidation and for nitrite oxidation is little higher than that for complete nitrification, as shown in Table 2, because when 1 unit of ammonia-N is consumed, less than 1 unit of nitrite will be produced. When only oxidized ammonia was considered, the specific oxygen uptake for complete nitrification and ammonia oxidation was 4.23 and 3.17 g-O_2/g-N oxidized, based on Equation (18) and (16), respectively. For nitrite oxidation, there was no difference in the base of nitrite oxidized or nitrite consumed because nitrite is not directly used to synthesize biomass.

Based on Equation (18), the overall biomass yield coefficient for nitrifiers in complete nitrification was 0.24 g-cell/g-N oxidized. This value was significantly higher than the value reported in the Metcalf and Eddy (2003) (0.1 to 0.15 g-cell/g-NH_4^+-N). Note that Metcalf and Eddy (2003) do not provide separate yield values for ammonia oxidizers and nitrite oxidizers. If that value refers only to the ammonia oxidizers, it is still much smaller than the values determined in this study, which were 0.18 g-cell/g ammonia-N oxidized.

As shown in Equations (16) and (17), the alkalinity required for ammonia oxidation is 7.22 g as $CaCO_3$/g N oxidized. This value includes the amount of alkalinity needed to neutralize the acid (hydrogen nitrite) produced and that needed to synthesize ammonia oxidizers. The alkalinity needed for nitrite oxidation is 0.03 g as $CaCO_3$/g N oxidized. This is the amount of alkalinity needed to synthesize nitrite oxidizers only because there is no acid production during the nitrite oxidation process. For the complete nitrification process, the alkalinity requirement is 7.04 g as $CaCO_3$/g N consumed, after considering the fraction of ammonia-N used for cell synthesis that does not require alkalinity, and the alkalinity needed for cell synthesis during the nitrification process.

It was reported that a faction of AOB and NOB could grow mixotrophically by utilizing organic as a carbon source (Hommes et al., 2001; Daims et al., 2001; Schmidt, 2009). Hommes et al.'s (2001) study indicated that the chemolithoheterotrophic growth of AOB (ammonia as an energy source, organic as a carbon source and oxygen as an oxidant) could occur in presence of pyruvate and fructose, while glucose, glycerol, acetate, mannose, mannitol, and citrate could not be utilized as a carbon source. In Daims et al.'s study (2001), under aerobic conditions, some NOB could utilize pyruvate but no acetate, butyrate, and propinonate. In our batch respirometric tests, pyruvate and other organics were not added. Therefore, the AOB and NOB in the batch respirometric tests mainly grow autographically and the developed Equations (16-18) and parameters (Table 2) are valid for autotrophic nitrification.

4.4. Ammonia and nitrite oxidation rates

Activated sludge is a mixed culture and it is very difficult to measure the net active nitrifier concentration. However, it would be much easier to measure the maximum ammonia and nitrite oxidation rates, which could be used as an integrated parameter for modeling and to indicate the sludge nitrification capacity. Compared with conventional batch kinetics tests, no chemical reagents would be used to determine the nitrification rate using the respirometric method. However, correct equations and accurate stoichiometric parameters are needed to convert OUR to the nitrification rate.

The OUR used for nitrification could be obtained by subtracting the OUR used for endogenous respiration from the total OUR. As shown in Figs. (3a) and (3b), the average OUR_{max} for complete nitrification was 20.86 mg-O_2/h-0.5L and it was 4.78 mg-O_2/h-0.5L for nitrite oxidation. The OUR_{max} for ammonia oxidation only was calculated to be 16.08 mg-O_2/h-0.5L. According to Equations (13) and (14) and associated parameters in Table 2, ammonia and nitrite oxidation rates were 5.06 mg-N/h-L and 4.51

mg-N/h-L, respectively. Ammonia and nitrite profiles in conventional batch kinetic tests are also shown in Figs. (3a) and (3b), respectively. When concentrations of ammonia or nitrite were more than 2 mg/L, both ammonia and nitrite oxidation reactions followed zero-order kinetics. The estimated ammonia and nitrite oxidation rates were 5.28 and 4.33 mg-N/h-L. These values are close to those determined based on a respirometric assay.

The above experiments were conducted in triplicate using the same sludge. The average nitrification rates are shown in Table 3. The estimated ammonia and nitrite oxidation rates in both the respirometric assay and conventional batch tests were very close, with a relative difference of 2 - 3%, indicating that the respirometric assay was an accurate approach for biomass nitrification rate estimation.

5. Conclusions

Stoichiometric links between biomass yield, ammonia and nitrite oxidized, ammonia assimilated, and oxygen uptake for each step of the nitrification process were developed. Results indicated that the specific oxygen uptake for complete nitrification was approximately 4.23 mg-O_2/mg-N oxidized, including 3.17 mg-O_2/mg-N oxidized for ammonia oxidation and 1.06 mg-O_2/mg-N oxidized for nitrite oxidation. For complete nitrification, 97.1%, 2.2%, and 0.7% of the total consumed ammonia were used for electron donation, synthesis of ammonia oxidizers, and synthesis of nitrite oxidizers, respectively. The fractions of electrons used for ammonia oxidizer synthesis and nitrite oxidizer synthesis were 7.5% and 7.3%, respectively. The yield coefficients for ammonia oxidizers and nitrite oxidizers were approximately 0.18 and 0.06 g-VSS/g-N oxidized, respectively. For 1 g of ammonia-N consumption in complete nitrification, 7.04 g of alkalinity as $CaCO_3$ were used. These values are consistent for lab-cultured sludges and the field sludge from an operating oxidation ditch. These values are very important for the design of an advanced wastewater treatment plant when calculating oxygen demands, nitrifier biomass yield, and alkalinity demand.

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REFERENCES

Ahn, J.H., Yu, R., Chandran, K., 2008. Distinctive microbial ecology and biokinetics of autotrophic ammonia and nitrite oxidation in partial nitrification bioreactor. Biotechnology & Bioengineering 100(6), 1078 – 1087.

Aleem, M.I.H., Sewell, D.L., 1981. Mechanism of nitrite oxidation and oxidoreductase systems in *Nitrobacter agilis*. Current Microbiology 5(5), 267–272.

Alleman, J.E., 1984. Elevated nitrite occurrence in biological wastewater treatment systems. Water Science and Technology 17(2-3), 409-419.

Beccari, M., Marani, D., Ramadori, R., 1979. A critical analysis of nitrification alternatives. Water Research 13(2), 185-192.

Blackburne, R., Vadivelu, V.M., Yuan, Z.G., Keller, J., 2007. Determination of growth rate and yield of nitrifying bacteria by measuring carbon dioxide uptake rate. Water Environment Research 79(12), 2437-2445.

Chandran, K., Smets, B.F., 2000. Single-step nitrification models erroneously describe batch ammonia oxidation profiles when nitrite oxidation becomes rate limiting. Biotechnology and Bioengineering 68(4), 376-406.

Chandran, K., Smets, B., 2001. Estimating biomass yield coefficients for autotrophic ammonia and nitrite oxidation from batch respirograms. Water Research 35(13), 3153-3156.

Clesceri, L.S., Greenberg, A.E., Eaton, A.D., 1998. Standard methods for examination of water and wastewater, 20th edition. American Public Health Association, Washington, DC.

Crites, R., Tchobanoglous, G., 1998. Small and decentralized wastewater management systems, McGraw-Hill, New York.

Daims, H., Nielsen, J. L., Nielsen, P.H., Schleifer, K. H., Wagner, M., 2001. In situ characterization of Nitrospira-like nitrite-oxidizing bacteria active in wastewater treatment plants. Applied Environmental Microbiology 67(11): 5273-84.

Dytczak, M., Londry, K.L., Oleszkiewicz, J.A., 2008. Activated sludge operational regime has signifcant impact on the type of nitrifying community and its nitrification rates. Water Research 42(8-9), 2320-2328.

Furumai, H., Rittmann, B.E., 1994. Evaluation of multiple-species biofilm and floc processes using a simplified aggregate model. Water Science and Technology, 29(10-11), 439-446.

Ginestet, P., Audic, JM., Urbain V., Block, JC., 1998. Estimation of nitrifying bacterial activities by measuring oxygen uptake in the presence of the metabolic inhibitors allylthiourea and azide. Applied Environmental Microbiology 64(6), 2266–2268.

Hommes, N. G., Sayavedra-Soto, A. L., Arp, D. J., 2003. Chemolithoorganotrophic Growth of *Nitrosomonas europaea* on Fructose. Journal of Bacteriology 185(23): 6809-6814.

Kim, D.J., Kim, S.H., 2006. Effect of nitrite concentration on the distribution and competition of nitrite-oxidzing bacteria in nitratation reactor systems and their kinetics characteristics. Water Research 40(5), 887-894.

Langergraber, G., Wuchty, N, Fleischmann, N., Lechner, M., 2003. Rapid automated detection of nitrification kinetics using respirometric. Water Science and Technology 47(2), 149-155.

Metcalf and Eddy Inc., 2003. Wastewater Engineering: Treatment and Reuse. 4th ed. McGraw-Hill, New York.

Park, H.D., Wells, G.F., Bae, H., Criddle, C.S., Francis, C.A., 2006. Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. Applied and Environmental Microbiology 72(8), 5643-5647.

Rittmann, B.E., McCarty, P., 2001. Environmental biotechnology: principles and applications. Boston: NcGraw-Hill.

Schmidt, I., 2009. Chemoorganoheterotrophic Growth of *Nitrosomonas europaea* and *Nitrosomonas eutropha*. Current Microbiology 59(2): 130-138.

Schramm A., de Beer D., van den Heuvel J.C., Ottengraf S., Amann, R., 1999. Microscale distribution of populations and activities of Nitrosospira and Nitrospira spp. along a macroscale gradient in a nitrifying bioreactor: quantification by in situ hybridization and the use of microsensors. Applied Environmental Microbiology 65(8), 3690–3696.

Sivret, E.C., Peirson, W.L., Stuetz, R.M., 2008. Nitrous oxide monitoring for nitrifying activated sludge aeration control: a simulation study. Biotechnology and Bioengineering 101(1), 109-118.

Sonthiphand, P., Limpiyakorn, T., 2011. Change in ammonia-oxidizing microorganisms in enriched nitrifying activated sludge. Applied Microbiology & Biotechnology 89(3), 843-853.

Vanrolleghem, P.A., Spanjers, H., Petersen, B., Ginestet, P., Takacs, I., 1999. Estimation (combinations of) activated sludge model No. 1 parameters and components by respirometry. Water Science and Technology 39(1), 195-214.

Werzernak, C.T., Gannon, J.J., 1967. Oxygen-nitrogen relationships in autotrophic nitrification. Applied Microbiology, 15(5), 1211-1215.

You J., Das, A., Dolan, E.M., Hu, Z.Q., 2009. Ammonia-oxidizing archaea involved in nitrogen removal. Water Research 43(7), 1801-1809.

Zhang, T., Jin, T., Yan Q., Shao, M., Wells G., Criddle C., Fang, H.H.P., 2009. Occurrence of ammonia-oxidizing Archaea in activated sludges of a laboratory scale reactor and two wastewater treatment plants. Journal of Applied Microbiology 107(3), 970-977.

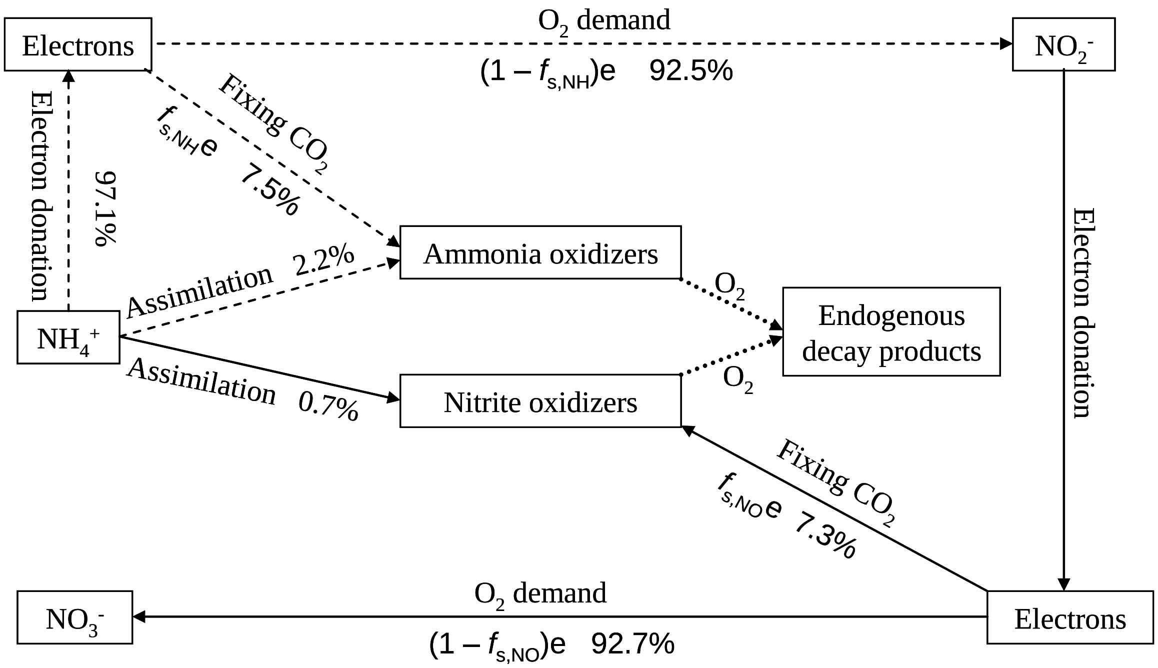

Fig. 1 – Material and energy correlation in the autotrophic nitrification process. Dash lines, solid lines, and round dotted lines indicate the first step nitrification, second step of nitrification, and nitrifier endogenous decay, respectively (modified based on Rittmann and McCarty, 2001, and Chandran and Smets, 2001).

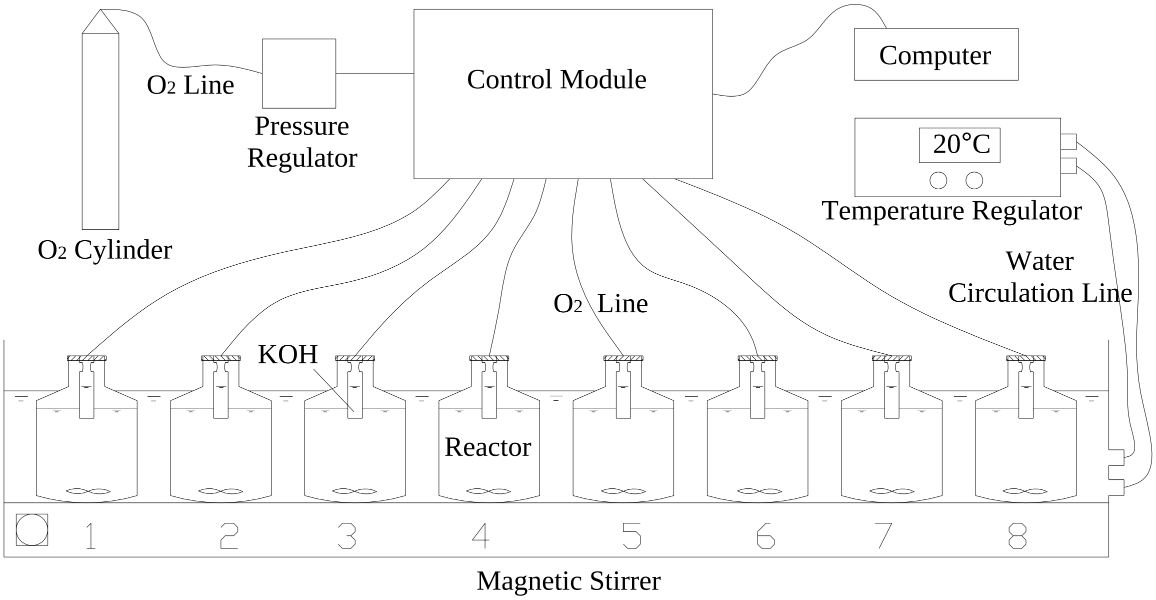

Fig. 2 – The schematic of a pulse-flow respirometer.

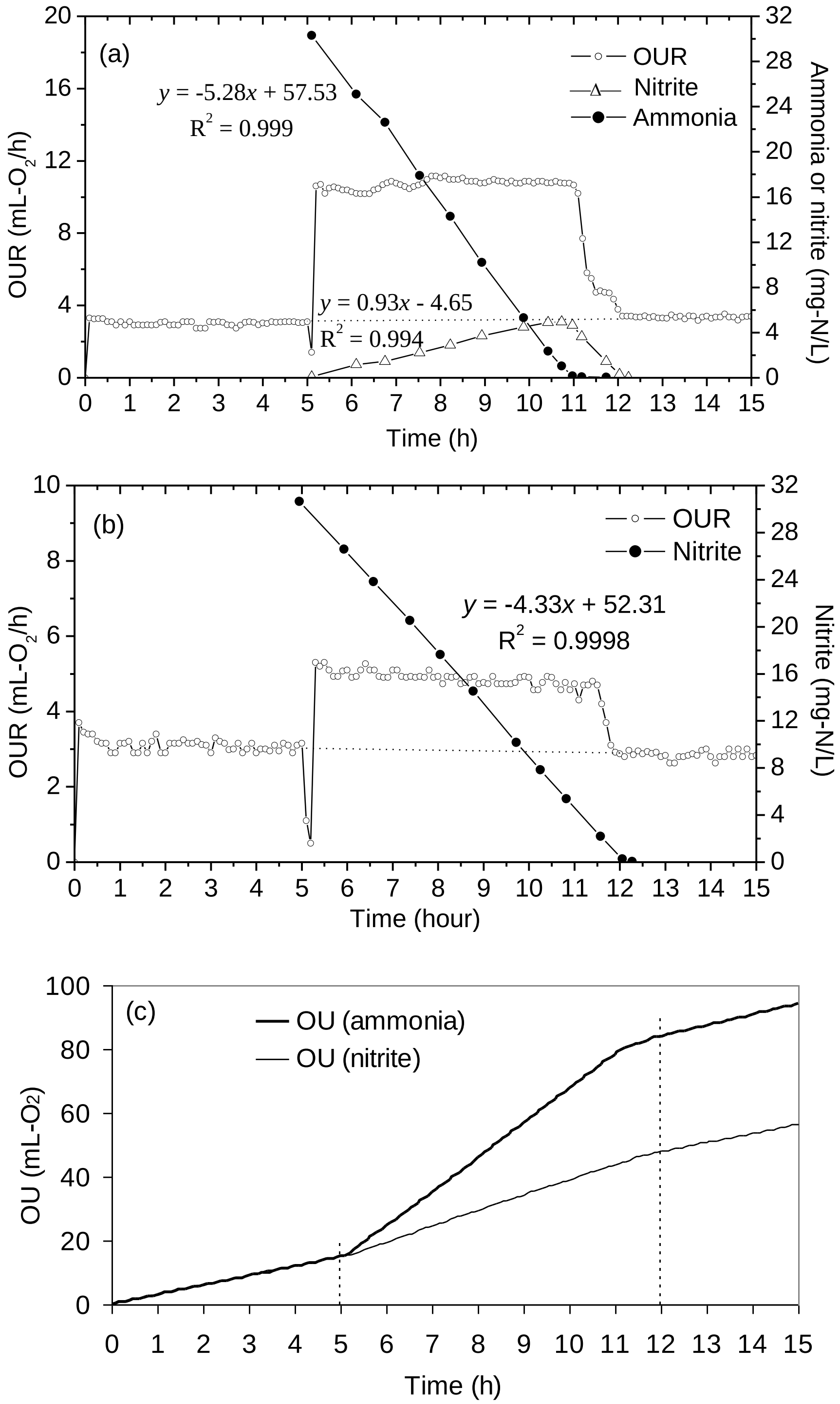

Fig. 3 – Oxygen uptake rate (OUR), ammonia and nitrite concentration change in a typical batch respirometric test for (a) complete nitrification and (b) nitrite oxidation; (c) the corresponding cumulative oxygen uptake for complete nitrification and nitrite oxidation.

Table 1 – Half reactions associated with nitrification.

R_a	$\frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O$
$R_{d,NH}$	$\frac{1}{6}NH_4^+ + \frac{1}{3}HO_2 = \frac{1}{6}NO_2^- + \frac{4}{3}H^+ + e^-$
$R_{d,NO}$	$\frac{1}{2}NO_2^+ + \frac{1}{2}HO_2 = \frac{1}{2}NO_3^- + H^+ + e^-$
R_{cs}	$\frac{1}{5}CO_2 + \frac{1}{20}NH_4^+ + \frac{1}{20}HCO_3^- + H^+ + e^- = \frac{1}{20}C_5H_7O_2N\,\frac{9}{20}H_2O$

Table 2 – Specific oxygen uptake, fs and biomass yield rate in ammonia and nitrite oxidation for the 10-day and 20-day SRT biomass, and sludge from an oxidation ditch (Mean ± stdev, n ≥ 6).

Sludge	Bioprocess	Specific oxygen uptake (g-O_2/g-N consumed)	Specific oxygen uptake (g-O_2/g-N oxidized)	f_s	Yield coefficient (g-cell/g-N oxidized)
10-day	$NH_4^+ \rightarrow NO_3^-$	4.11 ± 0.05	4.23	N/A	N/A
	$NH_4^+ \rightarrow NO_2^-$	3.10	3.17	0.075 ± 0.011	0.18 ± 0.03
	$NO_2^- \rightarrow NO_3^-$	1.06 ± 0.02	1.06	0.073 ± 0.021	0.06 ± 0.02
20-day	$NH_4^+ \rightarrow NO_3^-$	4.09 ± 0.05	4.21	N/A	N/A
	$NH_4^+ \rightarrow NO_2^-$	3.09	3.16	0.078 ± 0.010	0.19 ± 0.03
	$NO_2^- \rightarrow NO_3^-$	1.05 ± 0.04	1.05	0.075 ± 0.033	0.06 ± 0.04
WWTP	$NH_4^+ \rightarrow NO_3^-$	4.07 ± 0.08	4.20	N/A	N/A
	$NH_4^+ \rightarrow NO_2^-$	3.08	3.15	0.081 ± 0.018	0.20 ± 0.04
	$NO_2^- \rightarrow NO_3^-$	1.05 ± 0.03	1.05	0.079 ± 0.025	0.06 ± 0.02
Reference	$NH_4^+ \rightarrow NO_3^-$	4.25[a], 4.33[b]			0.10-0.15[a]
	$NH_4^+ \rightarrow NO_2^-$	3.22[b]			0.14 ± 0.02[c] 0.169[d] 0.05 to 0.29[e,f] 0.34[g]
	$NO_2^- \rightarrow NO_3^-$	1.11[b]			0.072 ± 0.01[c] 0.07[d] 0.02 to 0.084[e,f]

(a, Metcalf & Eddy, 2003; b, Werzernak and Gannon, 1967; c, Blackburne et al., 2007; d, Ahn et al., 2008; e, Alleman, 1984; f, Beccari et al., 1979; g, Furumai and Rittmann, 1994).

Table 3 – Average biomass ammonia oxidation rate (AOR) and nitrite oxidation rate (NOR) estimated in batch respirometric tests and batch kinetics tests (Mean ± stdev, n =3).

Process	Based on OUR		Batch kinetics tests
	OUR_{max} (mg-O_2/L-h)	AOR or NOR (mg-N/h-L)	AOR or NOR (mg-N/h-L)
$NH_4^+ \rightarrow NO_2^-$	15.99 ± 0.45	4.95 ± 0.14	5.05 ± 0.17
$NO_2^- \rightarrow NO_3^-$	4.56 ± 0.25	4.40 ± 0.24	4.29 ± 0.06

II: Combined Effects of Solids Retention Time and Dissolved Oxygen on Nitrification, Nitrifying Bacterial Communities, and Aeration Needs in the Activated Sludge Process

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Abstract

The combined effects of solids retention time (SRT) and dissolved oxygen (DO) on nitrification, nitrifying bacterial communities, and aeration needs in activated sludge were studied. When the DO was high, ammonia-oxidizing bacteria (AOB) had both higher specific growth rate and higher endogenous decay coefficient than nitrite-oxidizing bacteria (NOB) did. Under long-term low DO conditions (DO ≤ 0.5 mg/L), the endogenous decay of both AOB and NOB was slowed down. As a result, the nitrifier biomass concentration increased, canceling some of the adverse effect of low DO on nitrification. Finally, complete nitrification was almost achieved in the 10, 20, and 40-day SRT reactors with a DO of 0.37, 0.25, and 0.16 mg/L, respectively. Under long-term low DO conditions, NOB became a better oxygen competitor than AOB since their oxygen affinity increased significantly. *Nitrosomonas europaea/eutropha*–like AOB were dominant with all tested SRTs and DO levels. *Nitrobacter*- and *Nitrospira*-like NOB played the main role in nitrite oxidation in the 5 and 40-day SRT reactors, respectively.

In all reactors, *Nitrospira* increased considerably when the DO was reduced to ≤ 0.5 mg/L. Compared to a baseline condition (SRT = 10 days and DO = 2 mg/L), aeration need was reduced by about 20% under these two conditions (SRT = 10 days and DO = 0.37 mg/L; SRT = 40 days and DO = 0.16 mg/L). In the reactor with a 20-day SRT, it was found that, when the DO was around 0.25 mg/L, the aeration need fluctuated significantly and the boom of filamentous bacteria decreased the oxygen transfer efficiency considerably.

Keywords

Activated sludge, nitrification, dissolved oxygen, solids retention time, nitrifying bacterial community

1. Introduction

With increasing concerns for energy crisis and climate change associated with the fossil fuel consumption, energy conservation is becoming an emerging topic in wastewater treatment. In the aeration tank of activated sludge process, the aeration need mainly depends on the actual oxygen demand and oxygen transfer efficiency. The actual oxygen demand is determined by the amount of pollutants oxidized and biomass produced. The oxygen transfer efficiency, however, is associated with aeration devices, operational DO, sludge property, temperature, and MLSS concentration (Metcalf & Eddy, 2003). Therefore, two directions can reduce aeration need: improve the oxygen transfer efficiency or reduce the actual oxygen demand. Theoretically, if the aeration tank is running with a DO of 0.5 mg/L instead of 2 mg/L, the oxygen transfer efficiency would be enhanced by about 16% (Metcalf & Eddy, 2003).

The majority of biochemical reactions for biological wastewater treatment occur in the aeration tank: heterotrophic bacteria remove BOD and nitrifying bacteria oxidize ammonia. Both BOD degradation and nitrification use oxygen as the electron acceptors. Generally, nitrifying bacteria are less competitive to utilize DO than heterotrophic bacteria (Metcalf & Eddy, 2003). As a result, the critical operational DO in aeration tank is usually determined by the nitrification needs. When the DO was below 2 mg/L, the growth of nitrifying bacteria can be inhibited (Metcalf & Eddy, 2003). Therefore, though the oxygen transfer efficiency increases under a low DO condition, incomplete nitrification may occur. However, the nitrification performance under a low DO condition can be enhanced by extending solids retention time (SRT) (Stenstrom, 1980). On the other hand, a longer SRT results in a lower biomass production, meaning a higher oxygen demand for biomass decay. However, under a low DO condition, the sludge endogenous decay can be inhibited, thereby increasing the sludge production (Abbassi et al., 1999). As a result, there may be no significant difference for the actual oxygen demand under two conditions: a short SRT with a high DO versus a long SRT with a low DO. Finally, the actual aeration need to achieve the same treatment under a long SRT and low DO condition may be reduced since the oxygen transfer efficiency can be improved by a low DO.

Nitrification is completed by two steps: ammonia oxidized into nitrite by ammonia-oxidizing bacteria (AOB) and then to nitrate by nitrite-oxidizing bacteria (NOB) (Metcalf & Eddy, 2003). Generally, the growth of NOB is slower than AOB (Blackburne et al., 2008). In many cases, partial nitrification occurred under low DO conditions (Okabe et al., 1999; Sliekers et al., 2005; Blackburne et al., 2008; Park et al., 2010; Li et al., 2011) and then NOB was thought to be more sensitive to a low DO than AOB. However, in these studies, the effect of SRT on partial nitrification with a low DO was not evaluated. It is also possible that NOB is a better competitor than AOB under low DO conditions when SRT was long.

The short-term effect of low DO on nitrification kinetics was well studied and a Monod based expression was usually used to describe its impact (Stankewich, 1972; Park et al., 2004; Weon et al., 2004; Park and Noguera, 2006; Kaelin et al., 2009). In the short-term DO impact tests, the process of endogenous decay did not play a main role; therefore, the effect of low DO on nitrifier endogenous decay was ignored (Park et al., 2004; Weon et al., 2004; Park and Noguera, 2006). Under long-term low DO conditions, however, the process of endogenous decay plays an important role in the nitrifier biomass concentration. Thus it is important to know the effect of low DO on the endogenous decay of nitrifiers. In the existing models, the low DO was thought to have no inhibition on nitrifier decay (Stenstrom, 1980; Metcalf and Eddy, 2003) or have the same inhibition on nitrifier growth and decay (Henze et al., 2000; Manser et al., 2005). However, these hypotheses have not been tested.

In activated sludge, *Nitrosomonas*-like AOB and *Nitrobacter*-like NOB were considered the most popular nitrifiers (Metcalf & Eddy, 2003). However, recent findings indicated that *Nitrosospira*-like AOB and *Nitrospira*-like NOB might dominate the activated sludge systems as well (Dytczak et al., 2008; Li et al., 2007; Limpiyakorn et al., 2005; Sonthiphand and Limpiyakorn, 2011). The kinetics performance between *Nitrosomonas* and *Nitrosospira* and between *Nitrobacter* and *Nitrosospira* was significantly different. *Nitrobacter* was 10 times more active than *Nitrospira* (Kim and

Kim, 2006). Therefore, *Nitrosomonas*-like AOB especially *Nitrosomonas europaea/eutropha* lineage and *Nitrobacter*-like NOB were suggested to be "*r*" strategists, having high specific growth rates but low substrate affinity, while *Nitrosospira*-like AOB and *Nitrospira*-like NOB were thought to be "*K*" strategists, having low specific growth rates but high substrate affinity (Dytczak et al., 2008; Schramm et al., 1999; Kim and Kim, 2006). In addition, the sublineages in the group of AOB and NOB may have significantly different oxygen affinity (Park and Noguera, 2006). Beside AOB, ammonia-oxidizing archaea (AOA) were found to be present in some wastewater treatment processes as well (Park et al., 2006; Zhang et al., 2009; Limpiyakorn et al., 2011), but no nitrite-oxidizing archaea was found (You et al., 2009).

Due to the great diversity of nitrifiers, the nitrifying bacteria communities in activated sludge may shift with the variations in SRT, DO, substrate concentration, and temperature (Park and Noguera, 2004; Kim and Kim, 2006; Li et al., 2008; Yu et al., 2010). In previous studies, these factors were evaluated individually and it was difficult to determine which factor would play the main role. In addition, it is possible that the nitrifiers with a high oxygen affinity will be selected under long-term low DO conditions, thereby increasing nitrification performance.

The objective of this research was to examine the combined effects of SRT and DO concentration on nitrification, nitrifying bacterial communities, oxygen demand, and aeration need in the activated sludge process. Models describing the combined effects of SRT and DO concentration on nitrification will be validated and calibrated using experimental data. Then the effect of long-term low DO on the growth and decay of AOB and NOB will be determined.

2. Materials and methods

2.1 Reactor design and operation

Three same sized bench scale complete-mix reactors (31.5 L) were set up as shown in Fig. 1 and were fed continuously with synthetic wastewater to achieve a hydraulic retention time (HRT) approximately 12 hours. Influent chemical oxygen demand (COD) and ammonia nitrogen concentrations were 180 mg/L and 48 ± 2 mg/L, respectively. The COD was provided with glucose, while ammonia was provided with ammonium carbonate. In addition, trace elements were provided in the influent, with concentrations of Mn^{2+} = 0.2 mg/L (from $MnCl_2 \cdot 4H_2O$), Mo^{5+} = 0.12 mg/L (from $MoCl_5$), Co^{2+} = 0.001 mg/L (from $CoCl_2$), Zn^{2+} = 0.05 mg/L (from $ZnCl_2$), and Fe^{2+} = 0.005 mg/L (from $FeSO_4 \cdot 7H_2O$). Tap water with soluble Ca and Mg greater than 20 mg/L was used as the solvent. The pH in the aeration tank ranged from 7.0-7.5, controlled by a buffer containing K_2HPO_4 and Na_2CO_3. Operational temperature was approximately 20 ˚C. The seed activated sludge was collected from an oxidation ditch in the Rolla Southeast Wastewater Treatment Plant.

Nitrification performance with various SRTs and DO levels, as presented in Table 1, was determined in these bench scale reactors. In the experiment without a DO limitation (DO > 4.0 mg/L), the three reactors were operated with 5, 10, and 20-day SRT, respectively. Preliminary findings indicated that the effluent ammonia and nitrite concentrations in the 5-day SRT reactor were generally higher than 1 and 5 mg-N/L, respectively, suggesting that a 5-day SRT was too short to achieve complete nitrification. Therefore, low DO impact experiment was not continued. After finishing the 5-day SRT experiment, the same reactor was tested with a SRT of 40 days.

During the experiment, the operational parameters, including DO, pH, oxidation reduction potential (ORP), temperature, inflow rate, aeration intensity, and mixed liquor suspended solids (MLSS) in the reactors were monitored regularly. The concentrations of effluent ammonia, nitrite, and nitrate were measured normally 3 times a week. The effluent biochemical oxygen demand (BOD) was measured when the system stabilized under each condition.

After running for at least 2 SRTs at each DO level, the maximum biomass ammonia oxidation rate (AOR) and nitrite oxidation rate (NOR) were measured using a respirometric approach, e.g., through the oxygen uptake rate (OUR) measurement. A microscope (Olympus-CKX41, Center Valley, PA) was used to observe the sludge floc. The effluent quality, MLSS concentration, nitrification rate, and microscope observation were used to judge the stability of the reactors. After the system was stabilized under each condition (at least 2 SRTs), a conventional batch kinetic test was carried out to determine the maximum biomass ammonia and nitrite oxidation rates directly. In addition, a sludge sample (2 mL) was collected and centrifuged at 13,000 rpm for 2 minutes to separate water and solids. The sludge pellet was persevered under -80˚C for DNA extraction.

2.2 Batch respirometric tests

The batch respirometric tests were conducted to determine the biomass maximum AOR and NOR, which could be used to indicate the abundance of active AOB and NOB. A pulse-flow PF-8000 aerobic/anaerobic respirometer (Respirometer System and Application, Fayetteville, Arkansas, USA) was used to conduct the batch respirometric tests and its scheme was shown in a previous study (Liu and Wang, 2012). To each batch reactor, 500 mL of sludge sample were added. After approximately 5 hours, the endogenous respiration rate in the reactor became constant. Five mL of ammonium bicarbonate stock solution (NH_4HCO_3, NH_4^+-N = 3052 ± 45 mg/L) or sodium nitrite stock solution ($NaNO_2$, NO_2^--N = 3150 ± 14 mg/L) were added into each reactor. The pH in all reactors was controlled at approximately 7.5 using a buffer solution containing KH_2PO_4 and $NaHCO_3$. All reactors were intensively mixed with a magnetic mixer at 1,450 rpm to achieve a DO level greater than 4 mg/L. The OUR of each reactor was recorded every 6 minutes. After all of the added substrate was consumed, the respiration rate decreased to the same level as it was before the substrate addition, and the batch test was terminated.

In the batch respirometric tests, biomass ammonia oxidation and nitrite oxidation rates could be calculated based on OUR. The detailed calculation protocol was shown in a previous study (Liu and Wang, 2012). To confirm the ammonia and nitrite oxidation rates determined through respirometric tests, conventional batch kinetic tests were conducted under the same conditions. In the conventional batch kinetic test, the concentration of ammonia or nitrite, instead of OUR, as a function of time were measured.

2.3 Batch DO impact tests

After finishing the long-term performance tests in the 40-day SRT reactor with a DO around 4 mg/L and 0.2 mg/L, batch DO impact tests were conducted to determine the effect of DO concentration on ammonia and nitrite oxidation rates. In the batch DO impact tests, the inflow was discontinued and ammonium or nitrite stock solution was added into the reactor to increase the initial ammonia or nitrite concentration to about 30- N/L. The ammonia or nitrite oxidation rate under different DO levels, around 4, 2.5, 1.5, 0.75, 0.5, and 0.25 mg/L, were measured. The pH was always controlled around 7.5 by a buffer containing K_2HPO_4 and $NaHCO_3$. The experimental temperature was around 20 ˚C.

2.4 DNA extraction

Ultraclean® soil isolation kits (MoBio Laboratories, Solana Beach, CA) were used to isolate the DNA from the stored sludge pellets by following the manufacturer's protocol. DNA samples were stored at - 20 ˚C for subsequent assays. We confirmed the intact DNA on 1% agarose gel electrophoresis.

2.5 Terminal restriction fragment length polymorphism (T-RFLP) analysis

T-RFLP was applied to analyze the nitrifying bacterial communities in all sludge samples based on 16S rRNA gene for AOB and NOB (Regan et al., 2000). The detailed protocol was described in a previous study (Siripong and Rittmann, 2007). An initially low concentration of DNA from nitrifiers could result in a low yield of PCR products and then poor T-RFLP electropherograms. The DNA from AOB and NOB 16S rRNA gene was amplified by nested PCR, using universal amplification to increase initial DNA template concentration, followed by the specific amplification of AOB and NOB 16S rRNA genes. The primers used in the universal amplification and specific amplification are presented in Table 2 and the PCR programs are shown in Table 3. After specific amplifications, the PCR products were purified and digested with MspI restriction endouclease at 37 ˚C for 3 hours (Promega, Madison, WI). QIAquick purification kits (Qiagen Inc., Valencia,CA) were used for the PCR products purification. Digested PCR products were analyzed by an Applied Biosystem 3130 genetic analyzer (Applied Biosystems, Foster city, CA). The peak results were analyzed using Peak Scanner software version 1.0 (Applied Biosystems, Foster city, CA) and the fragment sizes were compared to the expected standard fragment sizes (Siripong and Rittmann, 2007) shown in Table 4.

2.6 Real-time PCR analysis

To investigate the changes in the nitrifying bacteria populations according to the variations in SRT and DO concentration, real-time PCR assays were conducted on all DNA samples.

Three independent real-time PCR assays were conducted to quantify total 16S rRNA of AOB, *Nitrobacter*-like NOB, and *Nitrospira*-like NOB in each DNA sample. Each reaction tube was separately loaded with 2 uL of template DNA, 6 pmol of the forward and reverse primers, 10 uL power SYBR green master mix (No 4367659, Invitrogen, NY, USA), and PCR grade water for a final volume of 20 uL. Primers with their sequences used for each assay are shown in Table 2. For AOB 16S rRNA, two forward primers (CTO 189A/B and CTO 189C) were used as described previously by Hermansson and Lindgren (2001). Real-time PCR program for each assay are shown in Table 3. All real-time PCR assays were performance in triplicate per sample and included standards and control reactions without DNA template. The specificity of each PCR assay was confirmed using melting curve analysis and agarose gel electrophoresis. Standards were made using serially diluted plasmid DNA with 10^2 – 10^8 copies/μL. The preparation of plasmid DNA followed the protocol made by Dr. Zhiqiang Hu’s research group in University of Missouri. All primers and probes are produced by Integrated DNA Technologies, Inc. (Coralville, Iowa, USA).

Recent literature indicated that ammonia-oxidizing archaea (AOA) might be present in the activated sludge processes (Park et al., 2006; Zhang et al., 2009;

Limpiyakorn et al., 2011). Therefore, it was possible that the AOA were present in our reactors as well. The primers, Arch-amoAF and Arch-amoAR (Francis et al., 2005), were used to amplify AOA amoA gene in our DNA samples, but no DNA bands at 635 bp were found on 1.5% agarose gel electrophoresis, indicating that AOA probably were not present in our reactors.

2.7 Models development

When there is no DO limitation, the effluent BOD concentration from a steady-state complete-mix reactor is a function of SRT (Metcalf and Eddy, 2003). Following the same procedure in the model development for BOD, the model to calculate the effluent ammonia concentration in the steady-state can be developed and also is expressed as a function of SRT.

$$S_{NH} = \frac{K_{s,NH}\lfloor 1 + K_{d0,AOB} SRT \rfloor}{SRT\left(\mu_{0,AOB} - K_{d0,AOB}\right) - 1} \tag{1}$$

Similarly, the effluent nitrite concentration in the steady-state can also be expressed as a function of SRT.

$$S_{NO} = \frac{K_{s,NO}\lfloor 1 + K_{d0,NOB} SRT \rfloor}{SRT\left(\mu_{0,NOB} - K_{d0,NOB}\right) - 1} \tag{2}$$

Based on the material mass balance, the AOB biomass concentration in the steady-state can be expressed as a function of SRT, ammonia removal and hydraulic retention time.

$$X_{AOB} = \frac{SRT \times Q}{V}\left[\frac{Y_{AOB}(S_{0,NH} - S_{NH})}{1 + K_{d0,AOB} SRT}\right] \tag{3}$$

In a sludge sample, it is difficult to determine the net active AOB biomass concentration, but the maximum ammonia oxidation rate (AOR_m) can be detected easily, which can be used to indicate the sludge ammonia oxidation capacity.

$$AOR_m = X_{AOB} k_{NH} = \frac{SRT \times Q}{V} \left[\frac{Y_{AOB}(S_{0,NH} - S_{NH})}{1 + K_{d0,AOB} SRT} \right] k_{NH} \tag{4}$$

Because

$$\mu_{0,AOB} = Y_{AOB} k_{NH} \tag{5}$$

$$AOR_m = \frac{SRT \times Q}{V} \left[\frac{\mu_{0,AOB}(S_{0,NH} - S_{NH})}{1 + K_{d0,AOB} SRT} \right] \tag{6}$$

Similarly, the maximum nitrite oxidation rate (NOR_m) can be used to indicate the nitrite oxidation capacity of the sludge.

$$NOR_m = \frac{SRT \times Q}{V} \left[\frac{\mu_{0,NOB}(S_{0,NO} - S_{NO})}{1 + K_{d0,NOB} SRT} \right] \tag{7}$$

Generally, when the DO is lower than 4 mg/L in activated sludge, especially lower than 2 mg/L, the growth rate of AOB and NOB may be limited. On the other hand, the low DO may reduce the endogenous decay rate of AOB and NOB. Assuming the low DO has inhibition on both nitrifier growth rate and endogenous decay rate, the parameters of maximum specific growth rate, μ, and endogenous decay coefficient , K_d, in Equations (1-7) need to be corrected. Monod based DO impact expression is widely used to indicate the effect of low DO on nitrifier growth and decay (Metcalf and Eddy, 2003; Henze et al., 2000). Therefore, Equations (8) and (9) can be used to express the effect of low DO on AOB and NOB growth and Equations (10) and (11) can be used to indicate the effect of low DO on AOB and NOB endogenous decay. The DO is assumed to have no impact on the values of Y and K_S (Henze et al., 2000; Metcalf and Eddy, 2003).

$$\mu_{AOB} = \mu_{0,AOB} \frac{DO}{K_{DO-g,AOB} + DO} \tag{8}$$

$$\mu_{NOB} = \mu_{0,NOB} \frac{DO}{K_{DO-g,NOB} + DO} \tag{9}$$

$$K_{d,AOB} = K_{d0,AOB} \frac{DO}{K_{DO-d,AOB} + DO} \tag{10}$$

$$K_{d,NOB} = K_{d0,NOB} \frac{DO}{K_{DO-d,NOB} + DO} \tag{11}$$

In the literature, DO is assumed to have the same impact on nitrifier growth and endogenous decay. In this case,

$$K_{DO-g,AOB} = K_{DO-d,AOB} \quad \text{and} \quad K_{DO-g,NOB} = K_{DO-d,NOB} \tag{12}$$

However, this assumption has not been validated.

Using Equations (8) and (9) to correct the effect of low DO on AOB and NOB growth and Equations (10) and (11) to correct the effect of low DO on AOB and NOB endogenous decay, Equations (1), (2), (6) and (7) need to be changed into Equations (13), (14), (15), and (16), respectively.

$$S_{NH} = \frac{K_{s,NH}\left[1 + K_{d,AOB} SRT\right]}{SRT\left(\mu_{AOB} - K_{d,AOB}\right) - 1} \tag{13}$$

$$S_{NO} = \frac{K_{s,NO}\left[1 + K_{d,NOB} SRT\right]}{SRT\left(\mu_{NOB} - K_{d,NOB}\right) - 1} \tag{14}$$

$$AOR_m = \frac{SRT \times Q}{V}\left[\frac{\mu_{AOB}(S_{0,NH} - S_{NH})}{1 + K_{d,AOB} SRT}\right] \tag{15}$$

$$NOR_m = \frac{SRT \times Q}{V}\left[\frac{\mu_{NOB}(S_{0,NO} - S_{NO})}{1 + K_{d,NOB}SRT}\right] \tag{16}$$

Equations (13) and (14) can be used to predict the combined effect of SRT and DO on the effluent ammonia and nitrite concentrations, respectively. Equations (15) and (16) can be used to predict the maximum ammonia and nitrite oxidation rates for the biomass cultivated with various SRTs and DO concentrations.

If the effluent ammonia and nitrite concentrations are fixed, then the minimum operational SRT is a function of DO concentration. Rearrange Equations (13), and (14), and Equations (17) and (18) are obtained:

$$SRT = \frac{K_{S,NH} + S_{NH}}{S_{NH}\mu_{0,AOB}\frac{DO}{K_{DO-g,AOB} + DO} - (K_{S,NH} + S_{NH})K_{d,AOB}\frac{DO}{K_{DO-d,AOB} + DO}} \tag{17}$$

$$SRT = \frac{K_{S,NO} + S_{NO}}{S_{NO}\mu_{0,NOB}\frac{DO}{K_{DO-g,NOB} + DO} - (K_{S,NO} + S_{NO})K_{d,NOB}\frac{DO}{K_{DO-d,NOB} + DO}} \tag{18}$$

where, X_{AOB} and X_{NOB} are the net active AOB and NOB biomass concentrations, respectively; k_{NH} and k_{NO} are the specific maximum ammonia and nitrite oxidation rates, respectively; Y_{AOB} and Y_{AOB} are the synthetic yield coefficients for AOB and NOB, respectively; $K_{s,NH}$ and $K_{s,NO}$ are the half-velocity substrate constants for ammonia and nitrite oxidation, respectively; $K_{d0,AOB}$ and $K_{d0,NOB}$ are the endogenous decay coefficients for AOB and NOB without DO limitation, respectively; $K_{d,AOB}$ and $K_{d,NOB}$ are the endogenous decay coefficients for AOB and NOB with DO limitation, respectively; $\mu_{0,AOB}$ and $\mu_{0,NOB}$ are the maximum specific growth rates for AOB and

NOB without DO limitation, respectively; μ_{AOB} and μ_{NOB} are the maximum specific growth rates for AOB and NOB with DO limitation, respectively; S_{NH} and S_{NO} are the effluent ammonia and nitrite concentrations, respectively; $(S_{0,NH} - S_{NH})$ and $(S_{0,NO} - S_{NO})$ are the ammonia and nitrite oxidized, respectively; Q is the inflow rate; V is the effective volume of aeration tank; $K_{DO-g,AOB}$, $K_{DO-d,AOB}$, $K_{DO-g,NOB}$, $K_{DO-d,NOB}$ are the half-saturation oxygen constants for AOB growth, AOB decay, NOB growth, and NOB decay, respectively; AOR_m and NOR_m are the maximum ammonia oxidation rate and the maximum nitrite oxidation rate, respectively. .

2.8 Oxygen demand calculation

In activated sludge, the actual oxygen demand is determined by the BOD biodegradation, nitrification, and biomass production. The oxygen demand for BOD biodegradation can be calculated using Equation (19).

$$R_{BOD} = Q(S_{0,BOD} - S_{BOD}) \tag{19}$$

The oxygen demand for nitrification can be determined by Equation (20).

$$R_N = R_1 \times Q \times S_{NO_3^-} + R_2 \times Q \times S_{NO_{2^-}} \tag{20}$$

The reduction of oxygen demand due to biomass production can be calculated by Equation (21).

$$R_X = -1.42 P_{Bio} \tag{21}$$

Under low DO conditions, simultaneous nitrification and denitrification may occur and some BOD will be consumed by denitrification, thereby reducing the oxygen demand for BOD biodegradation. One g-N of nitrate is equivalent to 2.86 g oxygen

(Metcalf and Eddy, 2003). So the reduction of oxygen demand due to denitrification could be calculated by Equation (22):

$$R_D = -2.86QS_{NO_{3-reduced}} \tag{22}$$

Then the total oxygen demand can be calculated by Equation (23):

$$R_T = R_{BOD} + R_N + R_S + R_D \tag{23}$$

where, R_{BOD} and R_N are the oxygen demand for complete BOD biodegradation and nitrification, respectively; R_X and R_D are the oxygen demand reduction due to biomass production and denitrification, respectively; R_T is the total oxygen demand; $S_{0,BOD}$ and S_{BOD} are the influent and effluent BOD_u concentrations, respectively; $S_{NO_3^-}$ and $S_{NO_2^-}$ are the concentrations of ammonia and nitrite oxidized, respectively; P_{bio} is the biomass production; Q is the inflow rate; R_1 and R_2 are the oxygen used for generating 1 unit of nitrate or nitrite from nitrification, respectively, and the values of R_1 and R_2 were determined in a previous study, which were 4.23 and 3.17 g-O_2/g-N, respectively. In Equation (22), $S_{NO_{3-reduced}}$ is the concentration of reduced nitrate, equal to the difference of effluent total nitrogen (ammonia + nitrite + nitrate) with an unlimited DO and limited DO under the same SRT.

2.9 Chemical analysis

The mixed liquor suspended solid concentration (MLSS), the mixed liquor volatile suspended solid concentration (MLVSS), and BOD_5 were determined following standard methods SM 2540 D, SM 2540 E, and SM 5210 B (Clesceri et al., 1998). An Orion model 370 pH meter with a PerpHecT pH electrode (Orion 9206BN) was used to measure the pH. A YSI 58 DO meter with a YSI 5239 probe was used to measure DO. A

HACH test kit TNT 832 was used to measure ammonia-N concentration when it was higher than 2 mg-N/L and a TNT 830 was used when it was lower than 2 mg-N/L. A HACH test kit TNT 840 was used to measure the nitrite-N concentration when it was higher than 0.6 mg-N/L and a TNT 839 was used when it was lower than 0.6 mg-N/L. The nitrate-N concentration was measured by a HACH test kit TNT 835.

3. Results and discussion

3.1. Effluent quality and sludge production

3.1.1. Effluent quality

The effluent ammonia, nitrite, and nitrate concentrations, under different SRTs and DO concentrations, are given Fig. 2. At the 5-day SRT [Fig. 2(a)], the effluent ammonia concentration gradually decreased in the first 50 days and was approximately 2 mg-N/L in the second 50 days. The effluent nitrite concentration performed an increasing trend in the first 50 days and ranged from 3 to 15 mg-N/L in the second 50 days. In the 5-day SRT reactor, the average effluent ammonia and nitrite concentrations during the second 50 days were 2.6 ± 2.3 and 9.2 ± 3.4 mg-N/L (Table 5), respectively. The nitrite accumulated, indicating that the nitrite oxidation rate was smaller than the ammonia oxidation rate at this particular SRT. With the same operational temperature and SRT, similar effluent ammonia concentration (3.8 ± 2.2 mg-N/L) was detected in Duan et al.’s membrane bioreactor (2009). In their study, the effluent nitrite concentration was not reported. Therefore, ammonia oxidation was almost completed under the 5 days’ SRT, while it was too short to complete nitrite oxidation.

In the 10-day SRT reactor, with a DO of approximately 4 mg/L [Fig. 2(b)], the effluent ammonia and nitrite concentrations decreased to less than 1 mg-N/L after about

20 days and 50 days running, respectively. This confirmed that the growth of NOB was slower than AOB. Finally, the stabilized effluent ammonia and nitrite concentrations were 0.04 ± 0.01 and 0.15 ± 0.05 mg-N/L, respectively, suggesting that complete nitrification was achieved. When the DO was reduced to approximately 2 mg/L, no significant change in either the effluent ammonia or the nitrite concentrations occurred. On the second day when the DO was reduced from 2 to 1 mg/L, the effluent ammonia concentration increased to about 1.5 mg-N/L and the effluent nitrite increased to around 8 mg-N/L, indicating that nitrite oxidation was more sensitive to low DO in this situation. On the third day, however, the effluent ammonia and the nitrite concentrations decreased to about 0.1 and 1.0 mg-N/L, respectively. After approximately 40 more days, the effluent nitrite decreased gradually from 1.0 to 0.3 mg-N/L. Finally, the stabilized effluent ammonia and nitrite concentrations at a DO of approximately 1 mg/L were approximately 0.04 and 0.29 mg-N/L (Table 5), respectively.

On the second day (after the DO was reduced from 1.0 to 0.37 mg/L), both the effluent ammonia and nitrite concentrations increased to approximately 1 and 9 mg-N/L, respectively, indicating that incomplete nitrification occurred. The accumulation of nitrite also suggested that, in this situation, the nitrite oxidation rate was smaller than the ammonia oxidation rate. In the first two weeks, after the DO was reduced to 0.37 mg/L, the effluent ammonia concentration increased to approximately 10 mg-N/L. In this period, however, the effluent nitrite concentration was gradually decreasing, probably due to less nitrite was produced. After approximately 2 months (with a DO near 0.37 mg/L), surprisingly, both the effluent nitrite and the ammonia concentrations decreased to lower than 1 mg-N/L, indicating that complete nitrification was achieved in the 10-day SRT reactor with this low DO concentration. Finally, both the effluent ammonia and the nitrite concentrations were stabilized at 0.85 ± 0.73 and 0.46 ± 0.17 mg-N/L, respectively (Table 5).

After the DO was reduced from 0.37 to 0.19 mg/L, the effluent ammonia concentration increased to approximately 8 mg-N/L immediately. The effluent nitrite, however, did not accumulate. After approximately 50 days of operating with a DO near 0.19 mg/L, the effluent ammonia concentration increased to approximately 37 mg-N/L. As a result, the effluent nitrate concentration decreased to nearly 3.5 mg-N/L. Strangely, the effluent nitrite concentration was always lower than 1 mg-N/L. This suggested that the nitrite oxidation rate was greater than the ammonia oxidation rate in this situation. This phenomenon was in contrast to previous reports. Previous studies indicated that NOB was more sensitive to low DO concentrations (Laanbroek & Gerards, 1993; Laanbroek et al., 1994; Metcalf & Eddy, 2003; Sliekers et al., 2005); and, when the DO was limited, the effluent nitrite would accumulate (Metcalf & Eddy, 2003; Sliekers et al., 2005; Blackbune et al., 2008). In this study, however, when the effluent ammonia accumulated considerably, with a DO near 0.19 mg/L, the effluent nitrite was generally lower than 0.2 mg-N/L.

The nitrification performance in the 20-day SRT reactor was examined with 5 different DO concentrations [Fig. 2(c)]. The reduction of DO from 4 to 2 and to 1 mg/L did not lead to a significant change in the effluent ammonia, nitrite, or nitrate concentrations. When the DO was near 0.38 mg/L, both the effluent ammonia and the effluent nitrite accumulated occasionally in the first two weeks, likely due to the fluctuation of operational DO concentration. The DO was reduced to approximately 0.15

mg/L after operating with a DO near 0.38 mg/L for roughly 2 months. The effluent ammonia accumulated after nearly one week of operation at a DO around 0.15 mg/L and the DO concentration was improved to about 0.25 mg/L. Finally, the effluent ammonia and nitrite concentrations were approximately 2 and 0.1 mg-N/L, with a DO near 0.25 mg/L, respectively.

At the 40-day SRT, the nitrification performance was examined at 4 different DO levels [Fig. 2(d)]. With an unlimited DO (DO ≥ 4.0 mg/L), complete nitrification was achieved very quickly. Within the first 3 weeks (after the reduction of DO from 4 to 0.4 mg/L), the effluent ammonia concentration increased obviously. Unlike in the reactor with 10-day SRT, no significant increase in the effluent nitrite concentration occurred. Complete nitrification was achieved three weeks after the DO was reduced., The effluent ammonia concentration increased again when the DO was reduced continuously from 0.43 to 0.21 mg/L. Nevertheless, the effluent nitrite did not accumulate. This finding was also in contrast to previous reports that the nitrite would accumulate when the DO was limited (Metcalf & Eddy, 2003; Sliekers et al., 2005; Blackbune et al., 2008). After approximately 4 weeks running, with a DO of 0.21 mg/L, the effluent ammonia concentration decreased, and complete nitrification was almost achieved. To find out the minimum safe operational DO with this particular SRT, the DO decreased again to nearly 0.16 mg/L. After the reduction of DO to 0.16 mg/L, the effluent ammonia concentration increased slightly, finally stabilizing at approximately 2 mg-N/L. As in the reactor with 10 or 20-day SRT, no nitrite accumulated with this low DO and its average concentration was lower than 0.1 mg-N/L (Table 5).

The DO was an important control parameter for activated sludge processes with nitrification. When the DO was low, nitrification could be inhibited and incomplete nitrification could occur. The reports regarding the effect of DO on nitrification, however, varied widely throughout the literatures. Downing and Scragg (1958) reported that a DO of at least 0.3 mg/L was needed for nitrification to occur; nitrification ceased entirely when the DO was below 0.2 mg/L (Downing et al., 1964). Wild et al. (1971) found that the nitrification rates in activated sludge were unaffected by a DO concentration above 1.0 mg/L. However, Nagel and Haworth (1969) reported that the nitrification rate in activated sludge doubled when the DO concentration increased from 1.0 to 3.0 mg/L. In a membrane bioreactor, the effluent ammonia concentration was greater than 20 mg-N/L when the DO was between 0.15 and 0.55 mg/L (Hocaoglu et al., 2011). Both Hanaki et al. (1989) and Bellucci et al. (2011), however, found that complete nitrification could be achieved when the DO was near 0.5 mg/L. With an even lower DO level (0.24 mg/L), nitrification was still completed in a chemostat reactor (Park et al., 2004). In the benchmark textbook, a DO of 2 mg/L was recommended for activated sludge processes with nitrification (Metcalf & Eddy, 2003). In our 5-day SRT reactor, an average 95% of the ammonia was removed with an unlimited DO concentration, while the effluent nitrite accumulated considerably. In our 10-day SRT reactor, complete nitrification was achieved after operating with a DO of 0.37 mg/L for nearly 2 months. Only about 10% of ammonia was nitrified when the DO was reduced to 0.19 mg/L in the 10-day SRT reactor. The reactors, however, with 20 and 40-day SRT could achieve complete nitrification with a DO of 0.25 and 0.16 mg/L, respectively. These findings indicated that a reactor with a longer SRT could achieve complete nitrification with a lower DO. A higher concentration of nitrifying bacteria was expected to be present with a longer SRT. A higher nitrifier concentration could compensate the adverse effect of low DO on nitrification rate. Therefore, conflicting reports of DO's impact on nitrification likely resulted from the non-uniformity of operational SRT.

Nitrification was completed in two steps. Generally, the nitrite oxidation (the second step of nitrification) was suggested to be more sensitive to low DO concentrations than ammonia oxidation (the first step of nitrification) (Laanbroek & Gerards, 1993; Laanbroek et al., 1994; Metcalf & Eddy, 2003; Sliekers et al., 2005). In the 10-day SRT reactor [Fig. 2(b)], the effluent nitrite concentration increased significantly when the DO was reduced to near 1 mg/L, while ammonia accumulation was not found. In addition, when the DO was reduced to 0.37 mg/L in the 10-day SRT reactor, both the effluent ammonia and the nitrite concentrations increased significantly in the first week. However, the effluent nitrite concentration was much greater than the effluent ammonia, indicating that the nitrite oxidation rate was smaller than the ammonia oxidation rate in this situation. These observations were in agreement with previous reports that the nitrite oxidation was more sensitive to low DO concentrations. When the DO was reduced to 0.43 mg/L in the 40-day SRT reactor, however, the effluent ammonia accumulated; the effluent nitrite did not. In all reactors with a DO of approximately 0.2 mg/L, the effluent ammonia concentration was much higher than the effluent nitrite. These observations suggested that ammonia oxidation was more sensitive to limited DO than nitrite oxidation.

Under low DO conditions, the simultaneous nitrification and denitrification might occur in the activated sludge process (Metcalf & Eddy, 2003; Guo et al., 2009; Hocaoglu et al., 2011). The nitrite was an intermediate production in the denitrification and might be the preferred electron acceptor over nitrate (Wang et al., 2008; Guo et al., 2009). Therefore, the low concentration of effluent nitrite could have been the result of denitrification. However, as shown in Figs. 2(b), 2(c), and 2(d), the sum of effluent ammonia, nitrate, and nitrite under low DO conditions was about 2 mg-N/L less than that under unlimited DO conditions, indicating that denitrification was insignificant. Another possibility is that NOB had increased their oxygen affinity under long-term low DO conditions, which made them a better competitor for oxygen than AOB.

In the reactors with 10, 20, and 40-day SRT, complete nitrification was almost achieved, with a minimum DO level of 0.37, 0.25, and 0.16 mg/L, respectively. Please note that, after the reduction of the DO, e.g. from 1 mg/L to 0.37 mg/L in the 10-day SRT reactor, from 4 to 0.43 mg/L then to 0.21 and to 0.16 mg/L in 40-day SRT reactor, incomplete nitrification occurred obviously in the initial period. Surprisingly, complete nitrification was achieved again after operating a long time with the same low DO concentrations. In these periods, no changes were made to any of the operational conditions, including ammonia loading, temperature, pH, and SRT. We can offer two possible explanations for the recovery of complete nitrification under long-term low DO conditions. The oxygen affinity of nitrifying bacteria could have increased under long-term low DO conditions (Kowalchuk et al., 1998; Park & Noguera, 2006). Both the AOB and the NOB biomass concentrations increased, which could have reduced the adverse impact of low DO on the nitrification rate.

3.1.2. MLSS concentration

The MLSS concentrations, under different SRTs and DO levels, are given in Fig. 3. As expected, a higher SRT resulted in a higher MLSS concentration. The MLSS concentration for the 5-day SRT was approximately 0.6 g/L [see Fig. 3(a)]. At the 10-day SRT, the MLSS concentration changed little when the DO ranged from 4 to 1 mg/L and it was about 1.0 g/L. After operating for 50 days with a DO of 0.37 mg/L, the MLSS concentration in the 10-day SRT reactor increased to approximately 1.25 g/L. Similarly, the average MLSS concentration in the 40-day SRT reactor increased from 2.48 g/L to 3.46 g/L when the DO was reduced from 4 to 0.43 mg/L (Table 5). At the 20-day SRT, however, the MLSS concentration began to increase, with a DO of 2 mg/L, and fluctuated significantly when the DO was lower than 0.5 mg/L.

The sludge production in the steady-state, with different DO levels and SRTs, is illustrated in Fig. 3(e). A higher SRT with an unilimited DO level resulted in a lower sludge production rate. When the DO was unlimited, the sludge production in the 5, 10, and 20-day SRT reactors were 1.91, 1.64, and 1.38 greater, respectively, than that in the 40-day SRT reactor. When the DO was near 0.4 mg/L, the sludge production in the 10-day and 40-day SRT reactors increased by 25% and 40%, respectively. At the 20-day SRT, the sludge production increased by 23% and 15%, at a DO near 1 and 0.25 mg/L, respectively. Abbassi et al. (1999) obtained similar observations, indicating that that the low DO could enhance sludge production. The activated sludge was, primarily, composed of heterotrophic bacteria (Metcalf & Eddy, 2003). The effluent BOD concentrations were similar under all of the tested DO levels (Table 5). Thus, the increase in sludge production likely resulted from the inhibition of heterotrophic biomass endogenous decay by the low DO. The endogenous decay rate of heterotrophic biomass could be reduced under low DO conditions, (Siegrist et al., 1999; Henze et al., 2000). The biomass loss due to the decay would then decrease. Therefore, when the same amount of heterotrophic biomass was synthesized, the sludge production (observed yield) increased under low DO conditions. A higher sludge production could lead to a lower oxygen demand in the aeration tank (Metcalf and Eddy, 2003) and also benefit the wastewater treatment plants that use biomass for biogas production.

As shown Fig. 3(e), when the DO was reduced from approximately 0.4 to 0.2 mg/L in both the 10-day and the 40-day SRT reactors, the sludge production decreased slightly. Additional mechanisms, such as the shift of heterotrophic bacteria communities, must be involved. Activated sludge contained a diversity of heterotrophic bacteria (Metcalf & Eddy, 2003). Some of them might have a higher synthetic yield coefficient or lower decay rate (Lavallée et al., 2002). If the sludge was dominated by the heterotrophic bacteria with a higher yield coefficient or lower decay rate, the sludge production would increase. Therefore, the fluctuating MLSS concentration in the 20-day SRT, with a DO less than 0.5 mg/L, was likely due to the shift of heterotrophic bacterial communities.

3.1.3 Biomass nitrification rate

In activated sludge, only a small portion of MLSS was composed of nitrifying bacteria (Randall et al., 1992). Thus, the change in the MLSS concentration (shown in Fig. 3) can’t indicate a variation in the nitrifier biomass concentration. The net concentration of active nitrifying biomass in an activated sludge sample, however, is difficult to quantify. In contrast, both the ammonia and nitrite oxidation rates for a given sludge sample can be easily detected, which can be used to indicate the concentrations of active AOB and NOB, respectively. The maximum AOR and NOR for the sludge, cultivated with different SRTs and DO levels, were measured using both the respirometric approach and the conventional batch kinetics test. The results are shown in Fig. 4.

A longer SRT can result in a higher nitrifying biomass concentration, thus improving nitrification performance. As expected, the sludge with a longer SRT had a much higher AOR and NOR, as shown in Figs. 4(a) and 4(b). As previously discussed, the low DO has an adverse effect on AOB and NOB growth; therefore, it seems that less nitrifying bacteria will be enriched in the process with a lower DO concentration. On the contrary, when the DO was reduced to ≤ 0.5 mg/L, both the AOR and the NOR increased considerably in all of the reactors [Figs. 4(a) and 4(b)]. No significant change occurred in either the AOR or the NOR when the DO was reduced from 4 to 1 mg/L. When the DO was reduced to approximately 0.2 mg/L, both the AOR and the NOR continued to increase for the 40-day SRT sludge and dropped dramatically for the 10-day SRT sludge. Please note that, when the DO was lower than 0.3 mg/L, complete nitrification was nearly achieved in the 20 and 40-day SRT reactors, but only about 10% of ammonia was removed in the 10-day SRT reactor. Both less AOB biomass and nitrite were yielded when less ammonia was oxidized. Finally, the biomass concentrations of both AOB and NOB in the 10-day SRT reactors dropped, resulting in the reduction of biomass maximum AOR and NOR.

The effluent quality data (Fig. 2) indicated that complete nitrification was recovered after about 2 months, with a DO of 0.37 mg/L, in the 10-day SRT reactor. The recovery of complete nitrification probably resulted from an increase in both AOB and NOB concentrations under low DO conditions. Both Hanaki et al. (1989) and Bellucci et al. (2011) also found that the observed yield rate for AOB increased significantly in a low

DO system (DO ≤ 0.5 mg/L), while no significant increase was detected for NOB. The detailed mechanisms, however, were not revealed by Hanaki et al. (1989) and Bellucci et al. (2011).

Bellucci et al. (2011) believed an increase in the observed yield rate of AOB, under low DO conditions, might be a result of the mixotrophic metabolism of AOB. Some AOB in the low DO systems utilized organic compounds as a carbon source, which could decrease the energy need to convert CO_2 to cellular carbon in autotrophic metabolism system. As a result, the energy requirement for AOB cell synthesis was reduced, thereby increasing the cell yield for AOB. Bellucci et al. (2011), however, did not prove their hypothesis. Indeed, *Nitrosomonas europaea*–like AOB were able to utilize some organic as the carbon source, e.g., fructose, pyruvate, and acetate (Lee et al., 1999; Hommes et al., 2003; Schmidt, 2009). In our experiments, glucose was the sole organic carbon source in the influent. Hommes et al.'s (2003), however, reported that, in the chemolithoheterotrophic growth of *Nitrosomonas europaea* (ammonia as an energy source, organic as a carbon source, and oxygen as an oxidant), glucose could not be utilized as the organic carbon source, suggesting that the chemolithoheterotrophic growth of *Nitrosomonas europaea* probably was not present in our reactors. In contrast to Bellucci et al.' view, Hommes et al. (2003) also found that *Nitrosomonas europaea* using either fructose or pyruvate as the carbon source had a lower growth rate or yield rate than using CO_2. In this case, even if *Nitrosomonas europaea* had utilized other organics (e.g., metabolism byproducts) as the carbon source in our systems under low DO conditions, a higher observed yield rate for AOB could not be observed. *Nitrosomonas europaea* could also perform chemoorganotrophic growth (organic as the energy source and nitrite as the electron acceptor) under anoxic condition (Abeliovich & Vonshak, 1992; Beaumont et al., 2002). The chemoorganotrophic growth of *Nitrosomonas europaea*, however, could not help remove ammonia. As previously discussed, either the nitrite or the nitrate removal due to denitrification in our systems was insignificant. Therefore, probably there is no chemoorganotrophic growth in our systems. Consequently, we do not think the explanation for a higher observed yield rate of AOB under low DO conditions given by Bellucci et al. (2011) was the major the mechanism in our system.

Nitrosomonas europaea–like AOB could perform anaerobic ammonia oxidation as well using nitrite as an oxidant (Abeliovich & Vonshak, 1992). In an anaerobic batch test, however, the ammonia concentration in the sludge, cultivated with a low DO, did not show a decrease in the presence of nitrite (data not shown). This finding indicating that anaerobic ammonia oxidizing bacteria were not present in our system.

According to the discussion in last paragraphs, additional mechanisms must account for the increase in the observed yield of both AOB and NOB biomass under long-term low DO conditions. The observed yield of nitrifying biomass under the same SRT was not only determined by the synthetics yield but also the endogenous decay. The synthetic yield rate of nitrifiers was independent of the DO concentration (Metcalf & Eddy, 2003). When the same amount of ammonia or nitrite was oxidized with a high and low DO, the synthetic yield of AOB or NOB would be the same. If the low DO had inhibition on both AOB and NOB endogenous decay, then both AOB and NOB biomass loss due to decay would be reduced. Therefore, the observed yield rate of both AOB and NOB biomass increased if complete nitrification was still achieved. In the 10, 20, and 40-day SRT reactors with a low DO of 0.37, 0.25, and 0.16 mg/L, respectively, complete nitrification was almost achieved, suggesting that there was no significant change in the synthetic yield of AOB and NOB. Thus, an increase in the observed yield of both AOB and NOB biomass was likely due to the inhibition of low DO on the decay of both AOB and NOB.

This explanation, however, requires the prior assumption that the elevation of both AOR and NOR for the sludge cultivated with low DO concentrations was the result of an increase in both the AOB and NOB biomass concentrations. The elevation of AOR and NOR was also likely due to a nitrifying bacterial community shift rather than an increase in both the AOB and NOB biomass concentrations. For example, a shift from "r" strategist AOB to a "K" strategist AOB could increase AOR significantly with the same AOB biomass concentration. Therefore, to fully support our explanation, further studies were needed to prove that more AOB and NOB were enriched under low DO conditions.

3.2. Modeling the combined effects of SRT and DO on nitrification

3.2.1. Modeling nitrification performance without DO limitation

Fig. 5(a) illustrates the average effluent ammonia and nitrite concentrations in the steady-state. Overall, a higher SRT resulted in lower ammonia and nitrite cocnentrations. At the 5-day SRT, the average effluent ammonia and nitrite cocnentrations were approximately 2.5 and 9.0 mg-N/L, respectively, indicating that ammonia oxidation was nearly completed and the 5 day SRT was too short to complete nitrite oxidation. When the SRT was ≥ 10 days, the effluent ammonia and nitrite cocnentrations were much lower than 1 mg-N/L, suggesting that both ammonia and nitrite oxidation were completeted. When the SRT was ≤ 20 days, the effluent nitrite concentration in the steady-state was always higher than the effluent ammonia, while it was lower than the effluent ammonia at the 40-day SRT. Fig. 5(b) illustrates the average AOR_m and NOR_m for the sludge cultured with various SRTs when the influent ammonia was 48 mg-N/L. A longer SRT resulted in both higher ammonia and nitrite oxidation rates. AOR_m was greater than NOR_m when the SRT was ≤ 20 days, while they were very close under the 40-day SRT.

Fig. 5 indicates that a longer SRT improved nitrification signifcantly, especifically for nitrite oxidation. When the SRT was ≤ 20 days, ammonia oxidation seemed to have an advantege over nitrite oxidation. At the 40-day SRT, however, nirtite oxidation seemed more competitive. This discrepancy likely resulted from the kinetics difference between AOB and NOB. Unfortunately, the current modeling generally looked at nitrifcaition as a single step, and the separate kinetics information for both AOB and NOB was very limited. To determine the kinetic parameters for AOB and NOB in activated sludge, Equations (6) and (7) were first used to fit the effect of SRT on biomass AOR_m and NOR_m, respectively. Both the endogenous decay cofficients (K_d) and the maximum specific growth rates for AOB and NOB (μ) were determined in this data fitting. The maximum specific ammonia utilization rate (k_{NH}) can be calculated using Equation (5), [based on μ_{AOB} and the AOB yield coefficients (Y_{AOB}) from our previous study (Liu & Wang, 2012)]. The AOB biomass concentration (X_{AOB}) can be caulcuated with Equation (4) using AOR_m and k_{NH}. Similarly, both the maximum specific nitrite oxidation rate (k_{NO}) and the NOB biomass concentration (X_{NOB}) can be caulcuated with Equations similar to (5) and (4), respectively.

Euqations (1) and (2) were used to fit the effect of SRT on the effluent ammonia and the nitrite concentrations, respectively. The values of endogenous decay coeffcients and the maximum specific growth rates were used in this datat fitting. Thus, the half-velocity constants for ammmona oxidation and nitrite oxidation (K_S) were determined through curve fitting. Note that complete nitrification was not achieved at the 5-day SRT; therefore, those data points were not used in the data fitting for quality in Fig. 5. Table 6 illustrates the kinetic costants for both AOB and NOB.

As shown Fig. 5, the modeling curves are in agreement with the experimental data, indicating that the complete nitrification performance under various SRTs can be simulated with a set of constants shown in Table 6. Overall, the estimated kinetic parameters for both AOB and NOB were significantly different. As determined in our previous study, NOB had a much lower yield coefficient than AOB did (Liu and Wang, 2012), suggesting that the NOB biomass concentration might be lower than AOB in the same process. NOB, however, had a much higher k value than AOB did, consistent with Weon et al.'s finding (2004). As a result, the nitrite could usually be removed as soon as it was generated. Though NOB were more active on substrate oxidation, the maximum specific growth rate of NOB was still lower than the growth rate of AOB. The estimated value of μ for AOB and NOB were 0.24 and 0.18 d^{-1}, respectively. They both were lower than typical values, but still in the reported range.

The K_d values for AOB and NOB were very low (as presented in Table 6). Therefore, the performance of both AOB and NOB under a short SRT is primarily determined by each maximum specific growth rate. Because AOB grew faster than NOB, which made AOB advantageous over NOB under a short SRT. As a result, when the SRT was ≤ 20 days, the effluent ammonia concentration in the steady-state was always lower than effluent nitrite concentration [Fig. 5(a)]. Similarly, AOR_m was higher than NOR_m [Fig. 5(b)]. On the contrary, the endogenous decay could play an important role under a long SRT. AOB had a greater value of K_d than NOB had, indicating that more AOB biomass would be decayed than NOB did under the same SRT. As a result, when the SRT was 40 days, the effluent nitrite concentration was lower than effluent ammonia concentration and the values of AOR_m and NOR_m became close. It is possible that the NOR_m will be greater than AOR_m under an even longer SRT. The calculated minimum SRTs ($SRT_{\min} = 1/(\mu - K_d)$) for AOB and NOB were 5.7 and 7.7 days, respectively. Therefore, the 5-day SRT was close to the minimum required SRT for AOB, but much shorter than that for NOB. As a result, for the 5-day SRT reactor, ammonia oxidation was almost completed, while a high effluent nitrite concentration was observed (Fig. 5(a)). The 10-day SRT was longer than the minimum SRTs for AOB and NOB; therefore, complete nitrification could be achieved in the 10-day SRT reactor.

As shown in Table 6, the K_S values for both AOB and NOB determined in this study were much smaller than the reported values, indicating that both AOB and NOB in our systems had a high substrate affinity. The bacteria with a higher substrate affinity could survive better under the low substrate conditions. In our systems, with a SRT $\geq$ 10 days, both the effluent ammonia and the effluent nitrite concentrations in the steady-state were very low, suggesting that the estimated K_S values were reasonable. In reality, the accurate value of K_S for a specific group of bacteria was very difficult to determine; it might vary significantly with the changes of experimental conditions. The discrepancy between our estimates and other reported values might be because different experimental method and conditions were used.

3.2.2. Modeling nitrification performance with DO limitation

The effluent ammonia and nitrite concentrations in the steady-state, with different DO levels, are given in Figs. 6(a) and 6(c). When the DO was ≥ 1 mg/L, complete nitrification was achieved in 10, 20, and 40-day SRT reactors and the DO had no significant impact on either effluent ammonia or nitrite concentrations. When the DO was reduced to 0.37 mg/L, the steady-state effluent ammonia and nitrite concentrations in the 10-day SRT reactor increased to approximately 0.85 and 0.46 mg-N/L, respectively. In the 40-day SRT reactor, however, the reduction of DO from 4 to 0.4 mg/L did not increase the steady-state concentrations of either effluent ammonia or nitrite. When the DO was reduced to approximately 0.2 mg/L, the steady-state effluent ammonia concentrations in the 10, 20, and 40-day SRT reactors increased to nearly 37, 2.2, and 2.0 mg-N/L, respectively, but the effluent nitrite concentrations in the three reactors were still lower than 0.2 mg-N/L. This finding indicated that NOB was a better competitor than AOB was under the long-term low DO conditions.

To determine the effect of DO on the growth and decay of AOB and NOB, Equations (13), (14), (15), and (16) were used to predict the combined effects of SRT and DO on effluent ammonia concentration, effluent nitrite concentration, biomass maximum AOR, and biomass maximum NOR, respectively. The best-fit of models to the experimental data are presented in Fig. 6., The parameters of half-saturation constant for oxygen (K_{DO}) for the endogenous decay of AOB and NOB were identified through the data fitting for AOR and NOR. The optimal values of K_{DO} for the growth of both AOB and NOB were determined in the data fitting for effluent quality. The estimated K_{DO} values are listed in Table 6.

As shown in Fig. 6, some experimental data points were not matched well, especially in the data fitting for effluent ammonia and nitrite concentrations. All the modeling curves, however, indicated the trend very well. Under low DO conditions, complete nitrification was just barely achieved and a slight variation in the operational DO or inflow rate could lead to in a large fluctuation in the effluent quality. On the other hand, different species of nitrifiers were included in the group of either AOB or NOB and their kinetic performance might be significantly different (Schramm et al., 1999; Kim & Kim, 2006; Dytczak et al., 2008). The community for either AOB or NOB in the sludge might change with either SRT or DO concentration. As a result, the kinetic parameters for the entire group of either AOB or NOB might be changed as well. Moreover, the sludge floc size could impact the kinetic parameters as well, especially for K_S and K_{DO} (Beccari et al., 1992). Therefore, all those made it difficult to achieve good fitting quality using a set of constant kinetics parameters. Nevertheless, the models with estimated parameters could indicate the trend and the major mechanisms very well.

With the same DO concentrations, a longer SRT would result in lower concentrations of effluent ammonia and nitrite as shown in Figs. 6(a) and 6(c). There was a turning point for the effect of DO on effluent ammonia and nitrite concentrations under the same SRT. When the DO was higher than the turning point, both the effluent ammonia and the nitrite concentrations increased slightly with a decrease in the DO concentration and complete nitrification was achieved. When the DO was either close to or lower than the turning point, however, both effluent ammonia and nitrite concentrations increased dramatically with a decrease in the DO concentration, resulting in incomplete nitrification. This turning point could be used as the minimum operational

DO for the activated sludge process with nitrification. Additionally, SRT had a great impact on the turning point and a longer SRT would result in a smaller DO turning point. By comparing the curves in Figs. 6(a) and 6(c), one can observe that, under the same SRT, the turning point DO for effluent nitrite was lower than that for effluent ammonia, indicating that under long-term low DO conditions the minimum operational DO would be controlled by ammonia oxidation. When the operational DO was controlled at this turning point, complete nitrification could be achieved with the minimum aeration input possible.

As illustrated in Figs. 6 (b) and 6(d), the modeling curve could indicate the combined effect of SRT and DO on biomass AOR and NOR well. Definitely, under the same DO level, a longer SRT resulted in higher values of both AOR and NOR. When the DO was ≥ 1 mg/L, both AOR and the NOR increased slightly with an increase in the DO concentration. When the DO was ≤ 1 mg/L, both the AOR and the NOR increased significantly with a decrease in the DO the concentration. Similarly, there was also a turning point for the effect of DO on AOR and NOR. When the DO was lower than the turning point, AOR or NOR began to decrease quickly. The turning points in Figs. 6 (a) and 6(b) or Figs. 6(c) and 6(d) were almost the same values. When the DO was lower than the turning point, incomplete nitrification could occur, and then less nitrifiers would be yielded. As a result, the biomass nitrification rate would decrease quickly.

The K_{DO} values for AOB growth and decay were 0.39 and 0.52 mg/L, respectively, indicating that the low DO had a similar inhibition on AOB growth and decay (see table 6). Previously, the low DO was thought to have either no inhibition on AOB decay (Stenstrom, 1980; Metcalf and Eddy, 2003) or the same inhibition on the growth and decay (Henze et al., 2000; Manser et al., 2005). Previous studies generally focused on the effect of low DO on AOB growth. Only one value of K_{DO} was reported for the endogenous decay of AOB (Munz et al., 2011). As presented in Table 6, the estimated K_{DO} value for AOB growth was in agreement with previous study. The estimated K_{DO} value for AOB decay, however, was much smaller than that determined by Munz et al. (2011). They determined the K_{DO} for AOB decay under starvation conditions through batch tests.

The estimated K_{DO} value for NOB decay was 0.69 mg/L, indicating that the low DO had great inhibition on NOB biomass decay. No reference K_{DO} values were found for NOB decay. Manser et al. (2006), however, found that NOB had a much smaller decay coefficient under anoxic conditions than they did under aerobic conditions, which supported that the DO played an important role in the biomass decay of NOB. The reference values of K_{DO} for NOB growth was between 0.34 and 1.5 mg/L (see table 6), generally higher than that for AOB growth, which supported the claim that NOB was more sensitive to low DO than AOB (Sliekers et al., 2005; Blackburne et al., 2008). In our study, however, the estimated K_{DO} value for NOB growth was approximately 0.08 mg-O_2/L, much lower than both the reference values as well as those for AOB growth and NOB decay. The low K_{DO} value for NOB growth was reasonable because the lowest experimental DO concentrations did not significantly impact the steady-state effluent nitrite concentrations. The estimated K_{DO} value for NOB growth in this study, however, might be inaccurate as it was much lower than the minimum experimental DO concentrations.

3.2.3. Short–term effect of DO on nitrification rate

Park and Noguera (2006) reported that a strain of *Nitrosomonas europaea*-like AOB had a much higher oxygen affinity (K_{DO} = 0.24 mg/L) than a stain of *Nitrosomonas oligotropha*-like AOB (K_{DO} = 1.22 mg/L). This finding indicated that the sublineages in the group of AOB might have significantly different oxygen affinity. It is likely that the nitrifiers with a high oxygen affinity can be selected in the systems with a long-term low DO concentration. The value of K_{DO} for the entire group of both AOB and NOB can decrease; the nitrification performance under low DO conditions will increase. The batch DO impact tests were conducted on the 40-day SRT sludge, cultivated with a high DO (DO = 4 mg/L) and a low DO (0.16 mg/L), to determine whether or not there was an increase in the oxygen affinity for the nitrifying bacteria cultivated with a long-term low DO concentration. These results are given in Fig. 7. The Monod-based DO impact expression was used to fit the experimental data, and the parameter of K_{DO} was obtained.

Though the low DO had significant inhibition on AOR in both sludge, it was likely that the low DO caused a little less inhibition in the sludge cultivated with a low DO (see Fig. 7). When the DO was reduced from 4 to approximately 0.2 mg/L (in the batch DO impact tests), the AOR decreased by 84% and 75% in the sludge cultivated with a high DO and a low DO, respectively. The K_{DO} values for ammonia oxidation were 0.71 and 0.39 mg/L in the sludge cultivated with a high DO and a low DO, respectively (Table 7). These results indicated that the oxygen affinity for the entire group of AOB increased slightly.

When the DO was reduced from 4 to approximately 0.2 mg/L in the batch test (see Fig. 7), the NOR decreased by about 72% and 10% in the sludge cultivated with a high DO and a low DO, respectively. This finding indicates that the low DO performed much less inhibition on NOR in the sludge cultivated with a DO of 0.16 mg/L. The K_{DO} values for nitrite oxidation were 0.43 and 0.04 mg/L in the sludge cultivated with a high DO and a low DO, respectively, as shown in Table 7. These results indicate that, unlike AOB, the group of NOB increased their oxygen affinity significantly under long-term, low DO conditions.

The K_{DO} values for the sludge cultivated under long-term low DO conditions, determined in the batch DO impact tests, were in agreement with those in Table 6. Under long-term low DO conditions, the oxygen affinity of AOB increased insignificantly, while NOB increased their oxygen affinity considerably, which made NOB the better competitor for oxygen than AOB. Consequently, no nitrite accumulated and the effluent nitrite concentration was always lower than the effluent ammonia under long-term low DO conditions. Therefore, two major contributions were found for the recovery of complete nitrification under low DO conditions: (1) an elevated nitrifying bacteria concentration resulting from the inhibition of endogenous decay by low DO concentrations and (2) an increased oxygen affinity of nitrifiers. The first one occurred on both AOB and NOB, while the second one occurred primarily on NOB.

3.2.4. Correlation between SRT and DO

As previously discussed, ammonia oxidation was almost completed in the 5-, 10-, 20-, and 40-day SRT reactors with a DO of 4.5, 0.37, 0.25, and 0.16 mg/L, respectively. Complete nitrite oxidation could not be achieved in the 5-day SRT reactor. No nitrite accumulated in the 10, 20, and 40-day SRT reactors, even under low DO conditions (DO ≤ 0.5 mg/L). This finding indicates that SRT had a great impact on the operational DO required to achieve complete nitrification. Equations (17) and (18) were developed to describe the correlations between SRT and DO for ammonia oxidation and nitrite oxidation in a complete mix reactor, respectively. When both the effluent ammonia and the nitrite concentrations were set at 1 mg-N/L, the correlations between SRT and DO for ammonia oxidation and nitrite oxidation are plotted using the parameters in Table 6. These plots are given in Fig. 8.

The model curve for AOB matched the experimental data points well (Fig. 8). The reactor with a longer SRT could achieve complete nitrification with a lower DO concentration. The correlation between SRT and DO for ammonia oxidation was different than what it was for nitrite oxidation. NOB needed a longer SRT than AOB to achieve complete oxidation, mainly due to NOB growing more slowly than AOB. When the SRT was higher than 10 days, however, AOB required a higher DO than NOB to achieve complete oxidation, mainly due to the low DO having less inhibition on the growth of NOB than AOB.

3.3. Nitrifying bacterial communities

3.3.1. T-RFLP

The nitrifying bacterial communities in the sludge cultivated with different SRTs and DO concentrations were determined using T-RFLP specifically designed for the identification of AOB and NOB with signature terminal fragment (TF) lengths (Regan et al., 2002). Fig. 9 shows the electropherograms of AOB.

As exhibited in Fig. 9, all the samples showed a dominant peak at 161 bp, a signature peak of Group 1 *Nitrosomonas europaea/eutropha* lineage and Group 4 *Nitrosomonas marina* lineage (Table 4). In our study, the influent to the reactors was freshwater, so that the marine AOB species in *Nitrosomonas marina* lineage were not relevant. Thus *Nitrosomonas europaea*/*eutropha* were the dominant AOB in all the samples. In addition to the major peak at 161 bp, a small peak 272 bp was also detected in all the samples, which represented the potential presence of Group 1, 2, 3, or 5. We also detected a small peak at 101 bp in some samples, which represented the presence of *Nitrosospira*-like AOB. In all samples prepared with an unlimited DO, the peak at 101 bp was not present or extremely small, indicating that *Nitrosospira*-like AOB may not present in these samples. However, in the 10 day-SRT sludge with a DO lower than 1 mg/L and in the 40-day SRT sludge with a DO of 0.2 mg/L, a clear peak at 101 bp was detected. This difference suggested that the low DO might have promoted the growth of *Nitrosospira*-like AOB.

Nitrosomonas-like AOB, especially *Nitrosomonas europaea/eutropha* lineage, were suggested to be "r" strategists (growing fast and having a low substrate affinity), while *Nitrosospira*-like AOB was thought to be "K" strategists (growing slowly and having a high substrate affinity) (Schramm et al., 1999; Kim and Kim, 2006; Dytczak et al., 2008). Theoretically, a long SRT and a low ammonia concentration would promote the dominance of *Nitrosospira*-like AOB, while a low SRT and a high substrate concentration would favor the competition of *Nitrosomonas*-like AOB. Under the 10, 20, and 40-day SRTs without DO limitation, though the steady-state effluent ammonia concentrations were much lower than 0.1 mg-N/L, *Nitrosomonas*-like AOB were still dominant. In Yu et al.'s study (2010), in the reactors with SRTs ≤ 30-day, *Nitrosomonas*-like AOB were dominant, while considerable *Nitrosospira*-like AOB was present under the infinite SRT. This indicated that the SRT, not substrate concentration, was the major factor for the competition between *Nitrosomonas* and *Nitrosospira* in activated sludge.

The DO was another important impact factor for AOB community (Gieseke et al., 2001; Park and Noguera, 2004; Park and Noguera, 2007; Li et al., 2007), while the reports on DO impact was controversial. Under low DO conditions, Li et al. (2007) found that *Nitrosospira* outcompeted *Nitrosomonas*, but in other studies (Gieseke et al., 2001; de Bie et al., 2001; Park and Noguera, 2004), *Nitrosomonas* were found to be the dominant AOB. Gieseke et al. (2001) reported that *Nitrosomonas oligotropha*-like AOB had a high affinity for oxygen. But a strain of *Nitrosomonas* oligotropha-like AOB (NL7), isolated in a reactor with a long-term low DO (0.12 – 0.24 mg/L), had a low oxygen affinity (K_{DO} = 1.22 mg/L) (Park and Noguera, 2007). On the contrary, a strain of *Nitrosomonas europaea*-like AOB (ML1), isolated from the same reactor had a high oxygen affinity (K_{DO} = 0.24 mg/L) (Park and Noguera, 2007). In our study, *Nitrosomonas europaea/eutropha*-like AOB were the prevalent AOB in the samples cultivated with a low DO concentration (≤ 0.5 mg/L).

The electropherograms of *Nitrobacter*-like NOB and *Nitrospira*-like NOB are shown in Fig. 10. All *Nitrobacter* specific T-RFLP profiles (Fig. 10(a)) represented a prominent peak at 136 bp, which indicated that *Nitrobacter*-like NOB were present in all sludge samples. There were some unexpected peaks, which could be the result of an incomplete digestion, uncharacterized *Nitrobacter* species, or imperfect matcher primers (Spirong and Rittmann, 2007). The *Nitrospira* specific T-RFLP profiles (Fig. 10(b)) showed a dominant peak at 272 bp in all samples and a high peak at 261 bp in most samples, which indicated the presence of *Nitrospira*-like NOB. The peak at 261bp occurred in samples with SRTs higher 10 day and DO levels higher than 1 mg/L. However, when the DO was ≤ 0.5 mg/L, this peak (261 bp) disappeared in the 10-day SRT sludge and its intensity decreased significantly in 20 and 40-day SRT sludge. This suggested that some sublineages in the group of *Nitrospira* could not survive well under low DO conditions. However, T-RFLP assays using 16S rRNA gene could not differentiate the sublineages in the group of *Nitrospira*.

3.3.2. Real-time PCR

As discussed previously, the sludge cultivated with a low DO concentration had higher AOR and NOR (Fig. 4), probably due to more nitrifiers were enriched. To confirm that, the 16S rRNA gene copies for AOB, *Nitrobacter*-like NOB, and *Nitrospira*-like NOB in the sludge cultivated with different SRTs and DO concentrations were quantified using real-time PCR assays. The results are shown in Fig. 11.

Overall, the number of AOB under all conditions was in the range of 9.5×10^{10} to 3.5×10^{11} copies/L, which was in the same order of magnitude as those determined in a Kim et al.' study (2011), but approximately 1 - 2 order of magnitude larger than those detected by Limpiyakorn et al. (2005) and Sonthiphand and Limpiyakorn, 2011. As presented in Fig. 11, when the DO was ≥ 4 mg/L, AOB ranged from 9.5×10^{10} to 1.8×10^{11} copies/L and more AOB was found in 40-day SRT sludge. When the DO was reduced to 0.5 mg/L, or less, the number of AOB was in the range of 2.0×10^{11} - 3.5×10^{11} copies/L, suggesting that that the population size of AOB was almost doubled.

Fig. 11 indicates that *Nitrobacter* and *Nitrospira* were coexisting with all tested SRTs and DO concentrations, consistent with the results from T-RFLP assays. When the DO was ≥ 4 mg/L, the percentage of *Nitrobacter* within the total NOB (*Nitrobacter* +

Nitrospira) was approximately 55%, 20%, 10%, and 5% in the reactors with 5, 10, 20, and 40-day SRT, respectively, strongly suggesting that a longer SRT would benefit the competition of Nitrospira. When the DO was reduced to ≤ 0.5 mg/L, the number of *Nitrobacter* increased only in the 10-day SRT reactor, while no significant change was found in the reactors with 20 and 40-day SRTs. Unlike *Nitrobacter*, the concentration of *Nitrospira* increased considerably in all reactors after the reduction of DO to ≤ 0.5 mg/L. As a result, under low DO conditions the percentage of *Nitrobacter* was reduced to approximately 1% and 0.7% in the 20 and 40-day SRT reactors, respectively, but it remained almost the same in the 10-day SRT reactor. Considering that *Nitrobacter* were about 10 times more active than *Nitrospira* (Kim and Kim, 2006; Blackburne et al., 2007), the actual role played by *Nitrobacter* in nitrite oxidation was supposed to be greater than their relative population percentage.

According to the results from Fig. 11, the DO had great impact on the population size of nitrifiers. Generally, more AOB and NOB (*Nitrobacter* + *Nitrospira*) were detected in the sludge cultivated with a DO ≤ 0.5 mg/L, which suggests that the increase in the maximum nitrification capacity for the sludge cultivated under low DO conditions (Fig. 4) was mainly due to more nitrifiers were enriched. Moreover, a conclusion that a low DO would inhibit nitrifier endogenous decay, drawn previously, was validated. If the endogenous decay of nitrifying bacteria was inhibited by a low DO concentration, the nitrifying bacteria concentration would increase under long-term low DO conditions. As a result, the sludge nitrification capability would increase and the adverse effect of low DO on nitrification rate in the sludge would be reduced.

In activated sludge, generally only genera *Nitrobacter* and *Nitrospira* were found in the group of NOB (Limpiyakorn et al., 2005; Li et al., 2007; Dytczak et al., 2008; Sonthiphand and Limpiyakorn, 2011). Results shown in Fig. 11 indicate that the SRT played an important role in the competition between *Nitrobacter* and *Nitrospira* under the unlimited DO conditions. In addition to SRT, the nitrite concentration was another important factor for the competition between *Nitrobacter* and *Nitrospira* (Okabe et al., 1999; Kim and Kim, 2006; Nogueira and Melo, 2006). *Nitrobacter* and *Nitrospira* were hypothesized to be r-strategists (growing fast and having a low substrate affinity) and K-strategists (growing slowly and having a high substrate affinity), respectively (Schramm et al., 1999; Kim and Kim, 2006; Nogueira and Melo, 2006). In our 5-day SRT reactor, the effluent nitrite concentration was high (generally > 5 mg/L), which would benefit the competition of r-strategists. However, in the reactors with a SRT ≥ 10 days, the effluent nitrite concentration in the steady-state was lower than 0.2 mg-N/L, which would favor the competition of K-strategists. Fig. 11 indicated that when the DO was unlimited, *Nitrobacter* were the superior competitor under the 5-day SRT, while *Nitrospira* were the better one under a long SRT. Therefore, the hypothesis was also supported in our study. Both SRT and nitrite concentration would impact the competition between *Nitrobacter* and *Nitrospira*. Even under the 10-day SRT without a DO limitation, the number of *Nitrospira* was greater than *Nitrobacter*, so it seemed that nitrite concentration played a more important role than SRT. Because nitrite concentration in the complete-mix process was determined by operational SRT when the DO was unlimited, the SRT was still the critical factor determining the NOB community.

Previous reports indicated that NOB was more sensitive to a low DO concentration than AOB and as a result, the effluent nitrite could accumulate if the DO was limited (Lannnbrok et al., 1994; Sliekers et al., 2005; Blackburne et al., 2008). However, under long-term low DO conditions in this study, it was found that NOB was a better competitor than AOB mainly due to NOB increased their oxygen affinity significantly. Real-time PCR assays indicated that, when the DO was ≤ 0.5 mg/L, the number of *Nitrospira* increased considerably in all reactors, but *Nitrobacter* increased significantly only in the 10-day SRT reactor and had no considerable change in the 20 and 40-day SRT reactors. The increase in the number of *Nitrobacter* in the 10-day SRT reactor at a DO of 0.37 mg/L was probably due to the elevated nitrite concentration (from 0.15 to 0.45 mg-N/L). When the DO was ≤ 0.5 mg/L in the 20 and 40-day SRT reactors, the effluent nitrite concentrations in the steady-state were still lower than 0.2 mg-N/L. Therefore, the increase in the number of *Nitrospira* in the 20 and 40-day SRT reactors suggested that *Nitrospira* were a better oxygen competitor than *Nitrobacter*. In this case, the increase in the oxygen affinity of NOB under long-term low DO conditions was due to more *Nitrospira* were enriched. But Blackburne et al.'s (2007) study indicated that the pure cultures of *Nitrobacter* and *Nitrospira* had a similar oxygen affinity (K_{DO} = 0.54 mg/L). In this case, it was hard to explain for the increase in the oxygen affinity of NOB under long-term low DO conditions. *Nitrospira* consisted of at least four distinct sublineages (Daims et al., 2001). Possibly, the sublineages in *Nitrospira* which had a higher oxygen affinity were enriched under long-term low DO conditions. The T-RFLP assay for *Nitrospira* seemed support this possibility. As shown in Fig. 10(b), the intensity for the peak at 261s decreased significantly in sludge samples cultivated under low DO conditions, indicating that the community of *Nitrospira* had shitted significantly. Another possibility was that, the same type of *Nitropisra* was enriched under long-term low DO conditions, but their oxygen affinity increased (Kowalchuk et al., 1998).

3.4. Oxygen demand and aeration need

3.4.1. Oxygen demand

Using Equations (19) – (23), the average oxygen demand in the steady-state under each condition was calculated and the results are shown in Table 8.

Overall, the oxygen demand for BOD biodegradation under different SRTs and DO levels was very similar since the effluent BOD concentrations were close. When the DO was ≥ 2 mg/L, the total oxygen demand under the same SRT was almost the same, while a higher SRT definitely resulted in more oxygen demand. As presented in Table 8, when the DO was ≥ 2 mg/L, the total oxygen demand for 40-day reactor was about 13.2 % more than that in 10-day SRT. Though the 5-day SRT reactor had the lowest oxygen demand, it could not achieve complete nitrification. Under a longer SRT, less sludge was produced (Fig. 3(e)) and then more oxygen would be needed for sludge decay.

Under the 10 - 40 day SRTs, when the DO was reduced to 0.5 mg/L, or less, the oxygen demand was reduced by about 7% - 10%. The reduction of oxygen demand under low DO conditions was mainly due to a higher sludge production and partly due to the occurrence of simultaneous nitrification and denitrification. As discussed previously, even when the DO was around 0.2 mg/L in our systems, only about 2 mg-N/L of nitrate was denitrified. So the reduction of oxygen demand due to denitrification was not significant. As exhibited in Table 8, the lowest total oxygen demand occurred in the 10-day SRT reactor with a DO of 0.19 mg/L, mainly resulting from incomplete nitrification.

When the DO was about 0.43 mg/L at the 40-day SRT, complete nitrification was achieved and about 2 mg-N/L of nitrate was denitrified. In addition, the sludge production in the steady-state increased by about 40% compared to a DO around 4 mg/L. Finally, the total oxygen demand in the 40-day SRT reactor with a DO of 0.43 mg/L was reduced to the similar level in the 10-day SRT reactor with a DO $\geq$ 2 mg/L. However, a low DO would benefit oxygen transfer and then the aeration need in the 40-day SRT reactor with a DO of 0.43 mg/L was expected to be lower than that in the 10-day SRT reactor with a DO around 2 mg/L. Beyond our expectation, the 10-day SRT reactor could achieve complete nitrification with a low DO of 0.37 mg/L. Consequently, the condition (10-day SRT with a DO of 0.37 mg/L) resulted in the lowest oxygen demand.

3.4.2. Aeration need

In the aeration tank, aeration need is not only determined by the actual oxygen demand but also oxygen transfer efficiency. The DO can impact oxygen transfer efficiency significantly in the aeration tank and a lower DO will benefit oxygen transfer. In addition to DO, MLSS concentration, microbial community, and activated sludge morphological properties may impact oxygen transfer as well. Aeration needs in all reactors under various DO concentrations are shown in Fig. 12.

As shown in Figs. 12(b) and 12(c), when the DO was reduced from about 4 to 2 mg/L in the 10 and 20-day SRT reactors, the aeration need was reduced by half. Table 8 indicated that, after the reduction of DO from 4 to 2 mg/L, there was no significant change in the total oxygen demand. This indicated that the saving of aeration with a DO around 2 mg/L were mainly due to the improvement of oxygen transfer efficiency.

However, after the DO was reduced from 2 to 1, 0.5, or even lower, the saving of aeration was not so significant.

The average aeration needs and the calculated oxygen utilization efficiency (the ratio of oxygen utilized to the oxygen supplied) under each condition are shown in Fig. 13. At the 10 and 20-day SRTs, when the DO was reduced from 4 to 2 mg/L, the required aeration was reduced by about 44%. When the DO was reduced from 2 to 1 mg/L, almost no aeration was saved at the 20 day SRT, while the aeration need in the 10-day SRT reactor was reduced by about 12%. A continuous reduction of DO from 1 to about 0.4 mg/L at the 10-day SRT reduced the aeration need by about 7%.

For current wastewater treatment plants, the operational DO and SRT generally were around 2 mg/L and 10 days, respectively. If set the aeration need at this condition as a baseline, about 19% and 20% of aeration could be saved under the conditions of SRT = 10 days with DO = 0.37 mg/L and SRT = 40 days with DO = 0.16 mg/L, respectively. As discussed previously, the actual oxygen demand was reduced by about 7 – 10% under low DO conditions. Therefore, the reduction of actual oxygen demand had partly contributed to the saving of aeration under low DO conditions. As shown in Fig. 13(b), under baseline condition (SRT = 10 days and DO = 2 mg/L) during our experiment, the oxygen utilization efficiency was about 2.0% and it increased to 2.2 % and 2.6% under the conditions of SRT = 10 days with DO = 0.37 mg/L and SRT = 40 days with DO = 0.16 mg/L, respectively. This indicated that, after the reduction of DO from 2 to about 0. 5 mg/L, or less, the oxygen transfer efficiency was also improved. Therefore, both the reduction of actual oxygen demand and the improvement of oxygen transfer efficiency had contributed to the saving of aeration needs under long-term low DO conditions.

Moreover, the oxygen transfer efficiency was inversely proportional to airflow rate (U.S. EPA 1989). Therefore, in addition to the low DO, the reduction of aeration intensity had also contributed to the improvement of the oxygen utilization efficiency under low DO conditions (Fig. 13(b)). The highest oxygen utilization efficiency was obtained under a condition of SRT=10 days and DO = 0.19 mg/L. Under this condition, incomplete nitrification occurred and the required aeration was much lower than the others (Fig. 13(a)). Possibly, the high oxygen utilization efficiency under this condition was mainly improved by the low airflow rate.

Krampe and Krauth (2003) and Germain et al (2007) reported that the oxygen transfer efficiency was inversely proportional to MLSS concentration. As discussed previously, a longer SRT resulted in a higher MLSS concentration and as a result, the oxygen transfer efficiency at a higher SRT was supposed to be lower. As shown in Fig. 13 (b), however, at the same DO concentrations the 40-day SRT reactor had similar or a little higher oxygen utilization efficiency, indicating that the higher MLSS concentration at the 40-day SRT did not inhibit oxygen transfer. This observation was in the agreement with other reports (U.S. EPA 1989; Rosso et al., 2005a; Rosso et al., 2005b), which showed that the oxygen transfer efficiency was directly proportional to SRT.

Strangely, the situation in the 20-day SRT reactor was totally different. When the DO was reduced from 2 to below 0.5 mg/L, though oxygen demand was reduced, almost no aeration was saved. This indicated that the oxygen transfer efficiency was not improved under low DO conditions. As shown in Fig. 12 (c), when the DO was maintained around 0.25 mg/L, the required aeration fluctuated dramatically. Therefore, other mechanisms must be involved.

3.4.3. Effect of bulking sludge on oxygen transfer efficiency

As discussed previously, to maintain the DO around 0.25 mg/L in the 20-day SRT reactor (Fig. 12(c)), the required airflow rate fluctuated dramatically. The average DO concentration, required airflow, actual oxygen demand, oxygen utilization efficiency, and sludge settling ability during different period in the 20-day SRT were summarized in Table 9. As shown in Table 9, in the period of 322nd to 431st day, the average operational DO was in the range of 0.17 to 0.36 mg/L and there was no significant change in the actual oxygen demand. However, the required aeration fluctuated greatly. In the periods of 322 to 333, 398 to 410, and 435 to 431, the required aeration ranged from 4.2 to 5.6 scfh with oxygen utilization efficiency in the range of 2.2% to 1.6%. However, the required aeration in the periods of 343 – 355 and 422 – 431 was only about 3.2 scfh, with oxygen utilization efficiency approximately 2.9%. Please note that, the sludge in the periods of 322 to 333, 398 to 410, and 435 to 431 had almost no settling in 30 minutes, but the sludge in the periods of 343 – 355 and 422 – 431 had obvious settling. So it was speculated that the fluctuation in the aeration need was caused by the shift of microbial communities and sludge morphological properties.

The microscope images for the sludge taken in the periods (Table 9) are shown in Fig. 14. Obviously, filamentous bacteria thrived in the periods of 322 – 333, 398 – 410, and 431 – 435 when the system required a high aeration. However, in the periods of 343 – 355 and 422 – 431, good settling sludge floc was formed and almost no or very few filamentous bacteria were found. Therefore, the low oxygen transfer efficiency in the periods of 322 – 333, 398 – 410, and 431 – 435 was probably caused by the boom of filamentous bacteria. The boom of filamentous bacteria could increase the viscosity of mixed liquor (Meng et al., 2007). With the increase in the viscosity, the film resistance would be enlarged and finally, the oxygen transfer efficiency decreased (Garcia-Ochoa and Gomez, 2009). Moreover, the flow regime would impact oxygen transfer as well (Quyang and Yang, 2007; Rosso et al., 2010). Thus the effect of filamentous bacteria on oxygen transfer could also be achieved by changing the flow regime in the aeration tank.

Though the filamentous bacteria thrived severely in the both periods of 322 – 333 and 398 – 410, the oxygen utilization efficiency in the first period was much lower than that in the second one. Comparing the floc images taken on the days of 326 and 404, it was likely that different type of filamentous bacteria were present. In the sludge on the 326th day, the filamentous bacteria were like the type of *Microthrix parvicella*, while those on 404th day were more like Type 021N filamentous bacteria. Type 021N filamentous bacteria have a longer filament and possibly they can increase the viscosity of the mixed liquor more than *Microthrix parvicella* did.

4. Conclusion

Nitrification performance and nitrifying bacterial communities in the activated sludge reactors with different SRTs and DO levels were studied. In addition, the oxygen demand and aeration needs under each condition were compared.

When the DO was unlimited (DO ≥ 4 mg/L), AOB had an advantage over NOB with a SRT ≤ 20 days mainly because AOB had a higher specific growth rate. On the contrary, NOB had an advantage over AOB at the 40-day SRT mainly due to NOB had a smaller endogenous decay coefficient than AOB did. When the DO was ≤ 0.5 mg/L, the endogenous decay of AOB and NOB was inhibited and then the biomass concentrations of AOB and NOB increased. As a result, the adverse effect of low DO on nitrification was reduced. Finally, complete nitrification was almost achieved in the 10, 20, and 40-day SRT reactors with a low DO of 0.37, 0.25, and 0.16 mg/L, respectively. On the other hand, under long-term low DO conditions, the oxygen affinity of NOB increased considerably, while it increased very slightly for AOB. This made NOB became the better oxygen competitor than AOB under low DO conditions. As a result, no nitrite accumulated and the effluent nitrite concentration was lower than the effluent ammonia under long-term low DO conditions.

Under all tested SRTs and DO levels, *Nitrosomonas europaea/eutropha* were the dominant AOB. *Nitrobacter*–like NOB and *Nitrospira*-like NOB were coexisting under all conditions. But *Nitrobacter* played the main role in nitrite oxidation in the 5-day SRT reactor, while *Nitrospira*-like NOB played the main role at the 40-day SRT. In all reactors, when the DO was ≤ 0.5 mg/L, the number of *Nitrospira* increased significantly.

Compared to a baseline condition (SRT = 10 days and DO = 2 mg/L), about 20% of aeration was saved under these two conditions (SRT = 10 days and DO = 0.37 mg/L) and (SRT = 40 days and DO = 0.16 mg/L). In the reactor with a 20-day SRT, it was found that, when the DO was around 0.25 mg/L, the aeration need fluctuated significantly and the boom of filamentous bacteria decreased the oxygen transfer efficiency dramatically.

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REFERENCES

Abbassi, B., Dullstein, S., Räbiger, N., 1999. Minimization of excess sludge production by increase of oxygen concentration in activated sludge flocs; experimental and theoretical approach. Water Research 34(1): 139-146.

Abeliovich, A., Vonshak, A., 1992. Anaerobic metabolism of *Nitrosomonas europaea*. Archives of Microbiology 158(4): 267-270.

Amann, R. I., Binder, R. J., Olson, S., Chisholm, S. W., Devereux, R., Stahl, D. A., 1990. Combination of 16S ribosomal RNA targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Applied and Environmental Microbiology 56 (6): 1919–1925.

Beaumont, H. J. E., Hommes, N. G., Sayavedra-Soto, L. A., Arp, D. J., Arciero, D. M., Hooper, A. B., Westerhoff, H. V., Spanning, R. J. M. van., 2002. Nitrite reductase of *Nitrosomonas europaea* is not essential for production of gaseous nitrogen oxides and confers tolerance to nitrite. Journal of Bacteriology 184(9): 2557-2560.

Beccari, M., Di Pinto, A.C., Ramadori, R., Tomei, M. C., 1992. Effects of dissolved oxygen and diffusion resistances on nitrification kinetics. Water Research 26(8): 1099-1104.

Bellucci, M., Ofiteru, I. D., Graham, D. W., Head, I. M., Curtis, T. P., 2011. Low-dissolved-oxygen nitrifying systems exploit ammonia-oxidizing bacteria with unusually high yields. Applied and Environmental Microbiology 77(21): 7787-7796.

Blackburne R., Yuan Z., Keller J., 2008. Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. Biodegradation 19(2): 303-312.

Blackburne, R., Vadivelu, V. M., Yuan, Z., Keller, J., 2007. Kinetic characterisation of an enriched *Nitrospira* culture with comparison to *Nitrobacter*. Waster Research 41(14): 3033-3042.

Clesceri, L. S.; Greenberg, A.E.; Eaton, A. D. (1998) Standard methods for examination of water and wastewater, 20th edition. American Public Health Association, Washington, DC.

Daims, H., Nielsen, J. L., Nielsen, P. H., Schleifer, K. H., Wagner, M., 2001. In situ characterization of Nitrospira-like nitrite-oxidizing bacteria active in wastewater treatment plants. Applied Environmental Microbiology 67(11): 5273-5284.

de Bie, M. J. M., Speksnijder, A.G. C. L., Kowalchuk, G. A., Schuurman, T., Zwart, G., Stephen, J. R., Diekmann, O. E., Laanbroek, H. J., 2001. Shifts in the dominant populations of ammonia-oxidizing β-subclass Proteobacteria along the eutrophic Schelde estuary. Aquatic Microbial Ecology 23: 225–236.

Dionisi, H. M., Layton, A. C., Harms, G., Gregory, I. R., Robinson, K. G., Sayler, G. S., 2002. Quantification of *Nitrosomonas oligotropha*-like ammonia-oxidizing bacteria and Nitrospira spp. from full scale wastewater treatment plants by competitive PCR. Applied and Environmental Microbiology 68(1): 245-253.

Downing, A. L., Painter, H. A., Knowles, G., 1964. Nitrification in the activated sludge process. Journal of the Institute of Sewage Purification 64(2): 130-158.

Downing, A. L., Scagg, L. J., 1958. The effect of synthetic detergents on the rate of aeration in diffused air activated sludge plants. Water Waste Treatment Journal 7: 102

Duan, L., Moreno-Andrade, I., Huang, C. L., Xia, S., Hermanowicz, S. W., 2009. Effects of short solids retention time on microbial community in a membrane bioreactor. Bioresource Technology 100(14): 3489-3496.

Dytczak, M., Londry, K. L., Oleszkiewicz, J. A., 2008. Activated sludge operational regime has significant impact on the type of nitrifying community and its nitrification rates. Water Research 42(8-9): 2320-2328.

Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E., Oakley, B. B., 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. PNAS 102(41): 14683-14688.

Garcia-Ochoa, F., Gomez, E., 2009. Bioreactor scale-up and oxygen transfer rate in microbial processes: an overview. Biotechnology Advances 27(2): 153-176.

Germain, E., Nelles, F., Drews, A., Pearce, P., Kraume, M., Reid, E., Judd, S. J., Stephenson, T., 2007. Biomass effects on oxygen transfer in membrane bioreactors. Water Research 41(5): 1038-1044.

Gieseke, A., Purkhold, U., Wagner, M., Amann, R., Schramm, A., 2001. Community structure and activity dynamics of nitrifying bacteria in a phosphate-removing biofilm. Applied and Environmental Microbiology 67(3): 1351-1362.

Graham, D. W., Knapp, C. W., Van Vleck, E. S., Bloor, K., Lane, T., Graham, C. E., 2007. Experimental demonstration of chaotic instability in biological nitrification. The ISME Journal 1:385-394.

Guo, J., Peng, Y., Wang, S., Zheng, Y., Huang, H., 2009. Long-term effect of dissolved oxygen on partial nitrification performance and microbial community structure. Bioresource Technology 100(11): 2796-2802.

Hanaki, K., Wantawin,C., Ohgaki,S., 1989. Nitrification at low levels of dissolved oxygen with and without organic loading in a suspended-growth reactor. Water Research 24(3): 297-302.

Henze M., Gujer W., Mino T., Loosdrecht M. van. 2000. Activated sludge models ASM1, ASM2, ASM2d and ASM3. IWA publishing, Alliance House London UK.

Hermansson, A., Lindgren, P.E., 2001. Quantification of ammonia oxidizing bacteria in arable soil by real time PCR. Applied and Environmental Microbiology 67(2): 972-976.

Hocaoglu, S. M., Insel, G., Cokgor, E. U., Orhon, D., 2011. Effect of low dissolved oxygen on simultaneous nitrification and denitrification in a membrane bioreactor treating black water. Bioresource Technology 102(6): 4333-4340.

Hocaoglu, S. M., Insel, G., Cokgor, E. U., Orhon, D., 2011. Effect of sludge age on simultaneous nitrification and denitrification in membrane bioreactor. Bioresource Technology 102(12): 6665-6672.

Hommes, N. G., Sayavedra-Soto, A. L., Arp, D. J., 2003. Chemolithoorganotrophic Growth of *Nitrosomonas europaea* on Fructose. Journal of Bacteriology 185(23): 6809-6814.

Kaelin, D., Manser, R., Rieger, L., Eugster, J., Rottermann, K., Siegrist, H., 2009. Extension of ASM3 for two-step nitrification and denitrification and its calibration and validation with batch tests and pilot scale data. Water Research 43(6): 1680-1692.

Kane, M. D., Poulsen, L. K., Stahl, D. A., 1993. Monitoring the enrichment and isolation of sulfate-reducing bacteria by using oligonucleotide hybridization probes designed from environmentally derived 16s ribosomal RNA sequences. Applied and Environmental Microbiology 59 (3): 682–686.

Kim D. J., Kim S. H., 2006. Effect of nitrite concentration on the distribution and competition of nitrite-oxidzing bacteria in nitratation reactor systems and their kinetics characteristics. Water Research 40(5): 887-894.

Kim Y. M., Lee, D. S., Park, C., Park, D., Park, J. M., 2011. Effects of free cyanide on microbial communities and biological carbon and nitrogen removal performance in the industrial activated sludge process. Water Research 45(3):1267-1279.

Kowalchuk, G. A., Bodelier, P. L. E., Heilig, G. H. J., Stephen, J. R., Laanbroek, H. J., 1998. Community analysis of ammonia-oxidising bacteria, in relation to oxygen availability in soils and root-oxygenated sediments, using PCR, DGGE and oligonucleotide probe hybridization. FEMS Microbiology Ecology 27(4): 339-350.

Krampe, J., Krauth, K., 2003. Oxygen transfer into activated sludge with high MLSS concentrations. Water Science and Technology 47(11): 297-303.

Laanbroek H. J., Bodelier P. L. E., Gerards S., 1994. Oxygen consumption kinetics of *Nitrosomonas europaea* and *Nitrobacter hamburgensis* grown in mixed continuous cultures at different oxygen concentrations. Archives of Microbiology 161(2): 156-162.

Laanbroek, H. L.; Gerards, S., 1993. Competition for limiting amounts of oxygen between *Nitrosomonas europaea* and *Nitrobacter winogradskyi* grown in mixed continuous cultures. Archives of Microbiology 159(5): 453-459.

Lavallée, B., Lessard, P., Besser, C., 2002. Decay rate variability of active heterotrophic biomass. Water Science and Technology 46(1-2): 423-430.

Lee, N., Nielsen, P. H., Andreasen, K. H., Juretschko, S., Nielsen, J. L., Schleifer, K. H., Wagner, M., 1999. Combination of fluorescent in situ hybridization and microautoradiography — a new tool for structure-function analyses in microbial ecology. Applied Environmental Microbiology 65(3): 1289-1297.

Li, B., Irvin, S., Baker, K., 2007. The variation of nitrifying bacterial population sizes in a sequencing batch reactor (SBR) treating low, mid, high concentrated synthetic wastewater. Journal of Environmental Engineering and Science 6: 651-663.

Limpiyakorn, T., Shinohara, Y., Kurisu, F., Yagi, O., 2005. Communities of ammonia-oxidizing bacteria in activated sludge of various sewage treatment plants in Tokyo. FEMS Microbiology Ecology, 54(2): 205-217.

Lin, C., Stahl, D. A., 1995. Comparative analyses reveal a highly conserved endoglucanase in the cellulolytic genus Fibrobacter. Journal of Bacteriology 177(9): 2543–2549.

Liu, G., Wang, J., 2012. Probing the stoichiometry of the autotrophic nitrification process using the respirometric approach, Water Research 46(18): 5954-5962.

Manser, R., Gujer, W., Siegrist, H., 2005. Consequences of mass transfer effects on the inetics of nitrifiers. Water Research 39(19): 4633-4642.

Manser, R., Gujer, W., Siegrist, H., 2006. Decay processes of nitrifying bacteria in biological wastewater treatment systems. Water Research 40(12): 2416-2426.

Meng, F., Shi, B., Yang, F., Zhang, H., 2007. Effect of hydraulic retention time on membrane fouling and biomass characteristics in submerged membrane bioreactors. Bioprocess and Biosystems Engineering 30(5): 359-367.

Metcalf and Eddy Inc. (2003) Wastewater Engineering: Treatment and Reuse, 4th ed. McGraw-Hill, New York.

Mobarry, B. K., Wagner, M., Urbain, V., Rittmann, B. E., Stahl, D. A., 1996. Phylogenetic probes for analyzing abundance and spatial organization of nitrifying bacteria. Applied and Environmental Microbiology 62(6): 2156–2162.

Munz, G., Lubello, C., Oleszkiewicz, J. A., 2011. Modeling the decay of ammonium oxidizing bacteria. Water Research 45(2):557-564.

Nagel, C. A., Haworth, J. G., 1969. Operational factors affecting nitrification in the activated sludge process. In 42nd Annual Conference WPCF, Dallas, Tex.

Nogueira, R., Melo, L. F., 2006. Competition between Nitrospira spp. and Nitrobacter spp. in nitrite-oxidizing bioreactors. Biotechnology Bioengineering 95(1):169-175.

Okabe, S., Satoh, H., Watanabe, Y., 1999. In Situ Analysis of Nitrifying Biofilms as Determined by In Situ Hybridization and the Use of Microelectrodes. Applied and Environmental Microbiology 65(7): 3182-3191.

Ouyang, A, Yang, S. T., 2007. Effects of mixing intensity on cell seeding and proliferation in three-dimensional fibrous matrices. Biotechnology Bioengineering 96(2): 371-80.

Park, S., Bae, W., Rittmann, B. E., 2010. Operational boundaries for nitrite accumulation in nitrification based on minimum/maximum substrate concentrations that include effects of oxygen limitation, pH, and free ammonia and free nitrous acid inhibition. Environmental Science Technology 44(1):335-42.

Park, H. D., Noguera, D. R., 2007. Characterization of two ammonia-oxidizing bacteria isolated from reactors operated with low dissolved oxygen concentrations. Journal of Applied Microbiology 102(5): 1401-1417.

Park, H. D., Wells, G. F., Bae, H., Criddle, C. S., Francis, C. A., 2006. Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. Applied and Environmental Microbiology 72(8): 5643-5647.

Park, H. P., Noguera, D.R., 2004. Evaluating of the effect of dissolved oxygen on ammonia-oxdizing bacterial communities in activated sludge. Water Research 38(14-15): 3275-3286.

Randall, C. W., Barnard, J. L., Setensel, H. D., 1992. Design and retrofit of wastewater treatment plants for biological nutrients removal. Technomic Publishing Company, Inc. Lancaster, Pennsylvania.

Regan, J. M., Harrington, G. W., Noguera, D. R., 2002. Ammonia- and nitrite-oxidizing bacterial communities in a pilot-scale chloraminated drinking water distribution system. Applied and Environmental Microbiology 68 (1): 73–81.

Rittmann, B. E., McCarty, P., 2001. Environmental biotechnology: principles and applications. Boston: NcGraw-Hill.

Rosso, D., Iranpour R., Stenstrom, M. K., 2005a. Fifteen years of offgas transfer efficiency measurements on fine-pore aerators: key role of sludge age and normalized air flux. Water Environmental Research 77(3): 266-73.

Rosso, D., Larson, L. E., Stenstrom, M. K., 2010. Aeration of large municipal wastewater treatment plants: state of art. Pier final project report prepared for California Energy Commission.

Rosso, D., Stenstrom, M. K., 2005b. Comparative economic analysis of the impacts of mean cell retention time and denitrification on aeration systems. Water Research 39(16): 3773-80.

Schmidt, I., 2009. Chemoorganoheterotrophic Growth of *Nitrosomonas europaea* and *Nitrosomonas eutropha*. Current Microbiology 59(2): 130-138.

Schramm, A., de Beer, D., van den Heuvel, J. C., Ottengraf S., Amann, R., 1999. Microscale distribution of populations and activities of *Nitrosospira* and *Nitrospira* spp. along a macroscale gradient in a nitrifying bioreactor: quantification by in situ hybridization and the use of microsensors. Applied and Environmental Microbiology 65(8): 3690–3696.

Siegrist, H., Brunner, I., Koch, G., Phan, L. C., Le, V. C., 1999. Reduction of biomass decay rate under anoxic and anaerobic conditions. Water Science and Technology 39(1): 129-137.

Siripong S., Rittmann B. E., 2007. Diversity study of nitrifying bacteria in full-scale municipal wastewater treatment plants. Water Research 41(5): 1110-1120.

Sliekers, A. O., Haaijer, S. C., Stafsnes, M. H., Kuenen, J. G., Jetten, M. S., 2005. Competition and coexistence of aerobic ammonium- and nitrite-oxidizing bacteria at low oxygen concentrations. Applied Microbiology and Biotechnology 68(6): 808-817.

Sonthiphand, P., Limpiyakorn T., 2011. Change in ammonia-oxidizing microorganisms in enriched nitrifying activated sludge. Applied Microbial Biotechnology, 89(3): 843-853.

Stankewich, M. J. J., 1972. Biological nitrification with the high purity oxygenation process. In 27th annual purdue industrial waste conference. West Lafayette IN: Purdue University. Pp. 1-23.

Stenstrom, M. K., 1980. The effect of dissolved oxygen concentration on nitrification. Water Research 14(6): 643-649.

Stenstrom, M. K., Song, S. S., 1991. Effects of oxygen transport limitation on nitrification in the activated sludge process. Journal Water Pollution Control Federation 63(3): 208-219.

Wagner, M., Rath, G., Amann, R., Koops, H. P., Schleifer, K. H., 1995. In situ identification of ammonia-oxidizing bacteria. Systematic Applied Microbiology 18 (2): 251–264.

Wang, J., Peng, Y., Wang, S., Gao, Y., 2008. Nitrogen removal by simultaneous nitrification and denitrification via nitrite in a sequencing hubrid biological reactor. Chinese Joural of Chemical Engineering 16(5): 778-784.

Weon, S.Y., Lee, S. I., Koopman, B., 2004. Effect of temperature and dissolved oxygen on biological nitrification at high ammonia concentrations. Environmental Technology 25(11): 1211-1219.

Wild, H. E., Sawyer, C. N., Mcmahon, T. D., 1971. Facters affecting nitrifictaion kinetics. Journal Water Pollution Control Federation 43(9): 1845-1854.

You J., Das, A., Dolan, E. M., Hu, Z., 2009. Ammonia-oxidizing archaea involved in nitrogen removal. Water Research 43(7): 1801-1809.

Yu T., Qi R., Li D., Zhang Y., Yang M., 2010. Nitrifier characteristics in submerged membrane bioreactor under different sludge retention time. Water Research 44(9): 2823-2830.

Zhang, T., Jin, T., Yan Q., Shao, M., Wells G., Criddle C., Fang, H. H. P., 2009. Occurrence of ammonia-oxidizing Archaea in activated sludges of a laboratory scale reactor and two wastewater treatment plants. Journal of Applied Microbiology 107(3): 970-977.

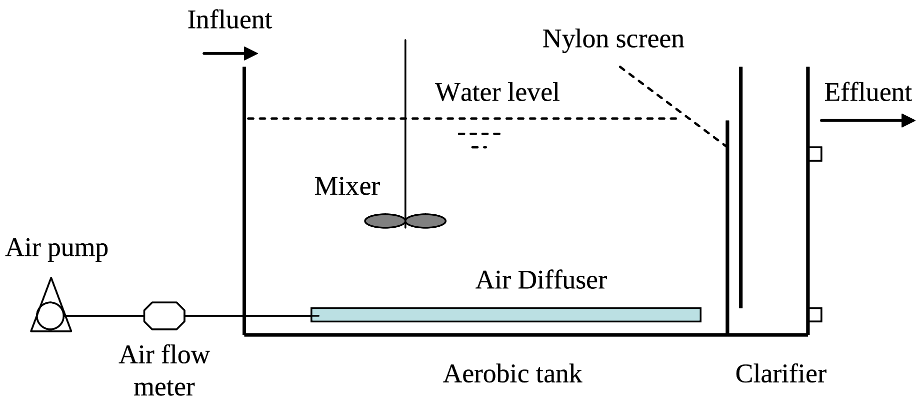
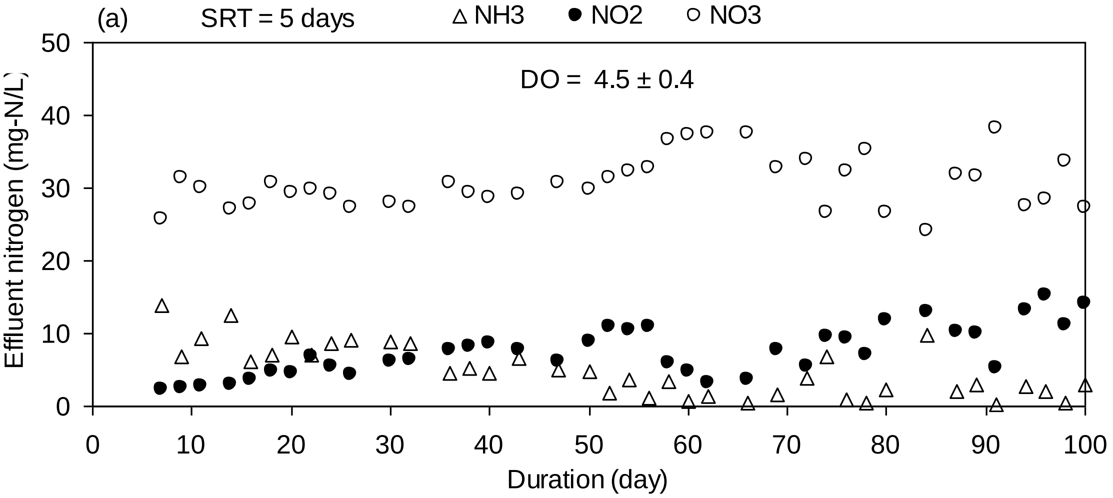
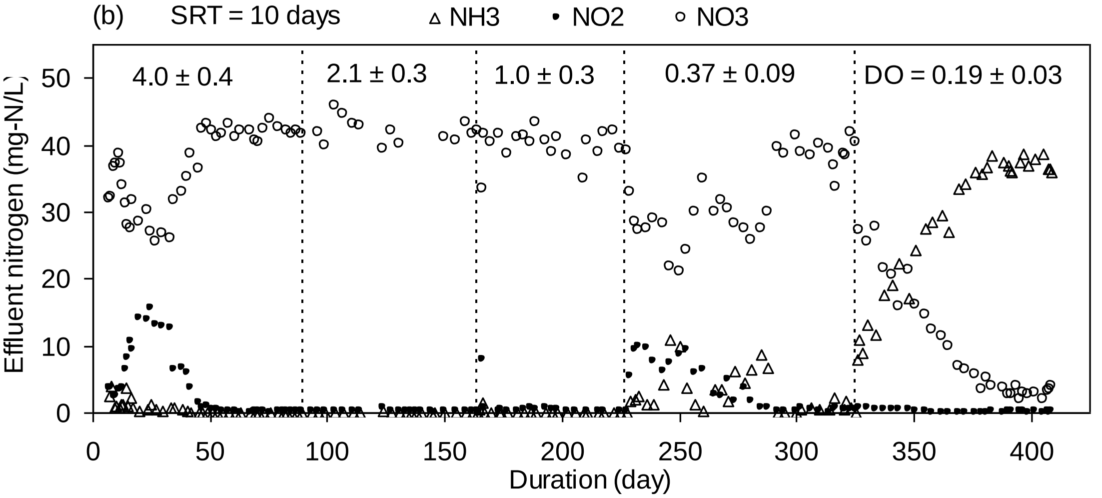
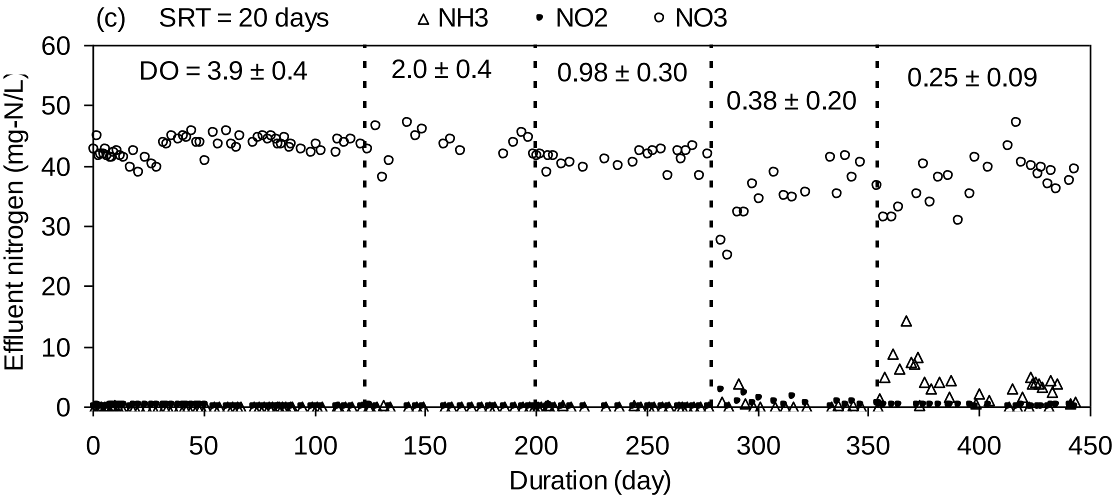


Fig. 1 – The schematic of a bench scale reactor


(a)
SRT = 5 days
△ NH3
● NO2
○ NO3
DO = 4.5 ± 0.4
Effluent nitrogen (mg-N/L)
0
10
20
30
40
50
0
10
20
30
40
50
60
70
80
90
100
Duration (day)


(b)
SRT = 10 days
△ NH3
• NO2
○ NO3
4.0 ± 0.4
2.1 ± 0.3
1.0 ± 0.3
0.37 ± 0.09
DO = 0.19 ± 0.03
Effluent nitrogen (mg-N/L)
0
10
20
30
40
50
0
50
100
150
200
250
300
350
400
Duration (day)


(c)
SRT = 20 days
△ NH3
• NO2
○ NO3
DO = 3.9 ± 0.4
2.0 ± 0.4
0.98 ± 0.30
0.38 ± 0.20
0.25 ± 0.09
Effluent nitrogen (mg-N/L)
0
10
20
30
40
50
60
0
50
100
150
200
250
300
350
400
450
Duration (day)

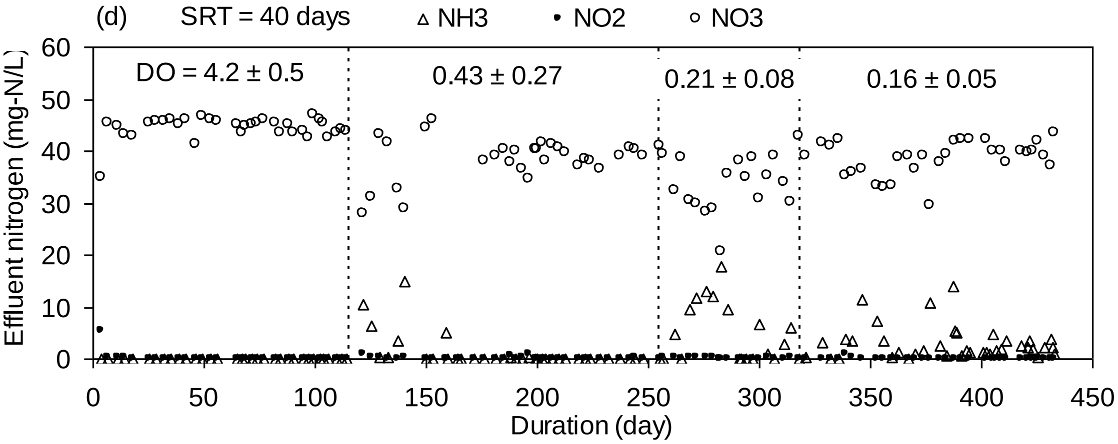
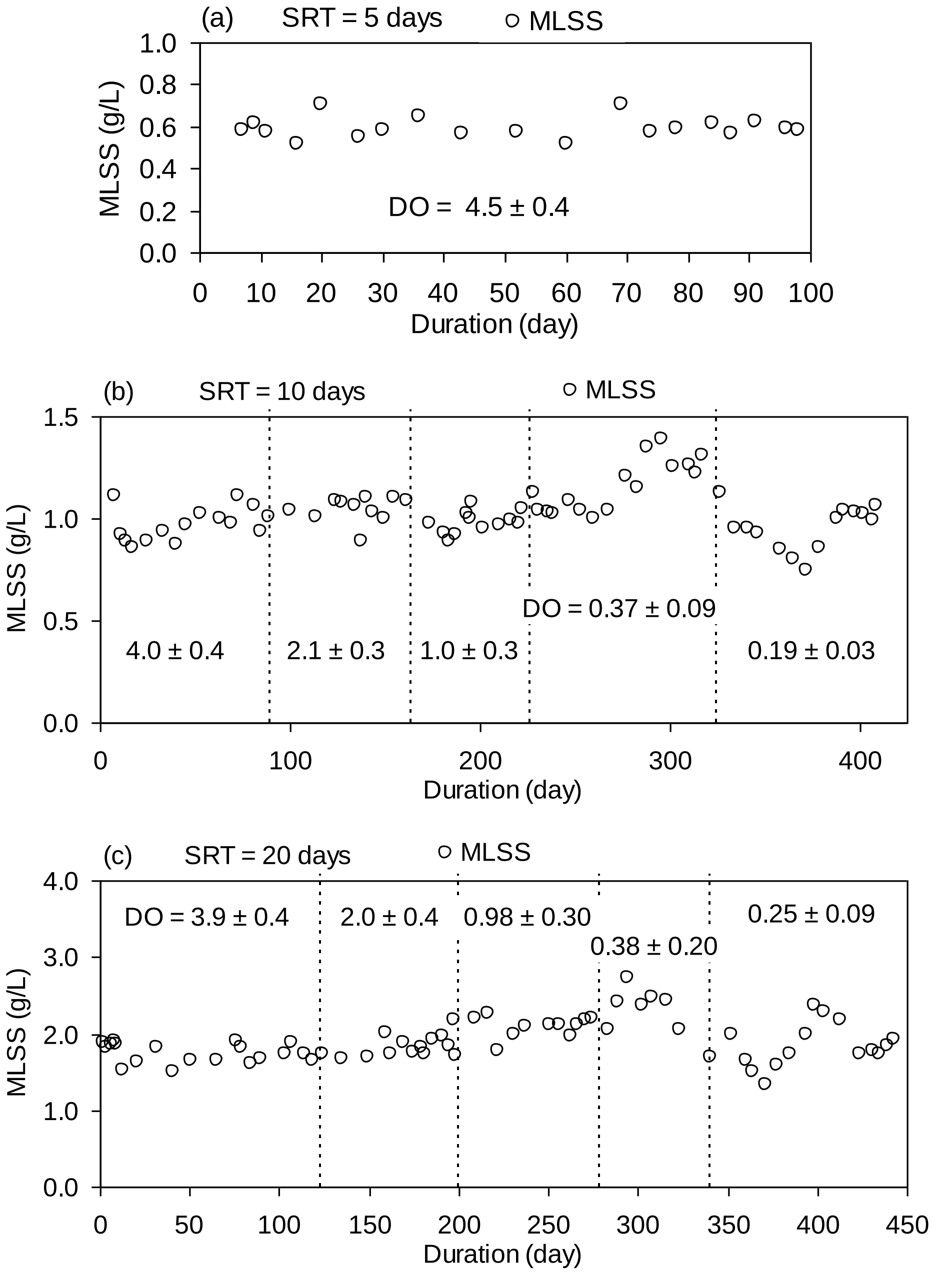


Fig. 2 – The effluent ammonia, nitrite, and nitrate concentrations with different dissolved oxygen (DO, mg/L) concentrations in the reactor with (a) 5, (b) 10, (c) 20 and (d) 40 days' solids retention time (SRT)


(a)
SRT = 5 days
○ MLSS
MLSS (g/L)
1.0
0.8
0.6
0.4
0.2
0.0
DO = 4.5 ± 0.4
0
10
20
30
40
50
60
70
80
90
100
Duration (day)
(b)
SRT = 10 days
○ MLSS
MLSS (g/L)
1.5
1.0
0.5
0.0
4.0 ± 0.4
2.1 ± 0.3
1.0 ± 0.3
DO = 0.37 ± 0.09
0.19 ± 0.03
0
100
200
300
400
Duration (day)
(c)
SRT = 20 days
○ MLSS
MLSS (g/L)
4.0
3.0
2.0
1.0
0.0
DO = 3.9 ± 0.4
2.0 ± 0.4
0.98 ± 0.30
0.38 ± 0.20
0.25 ± 0.09
0
50
100
150
200
250
300
350
400
450
Duration (day)

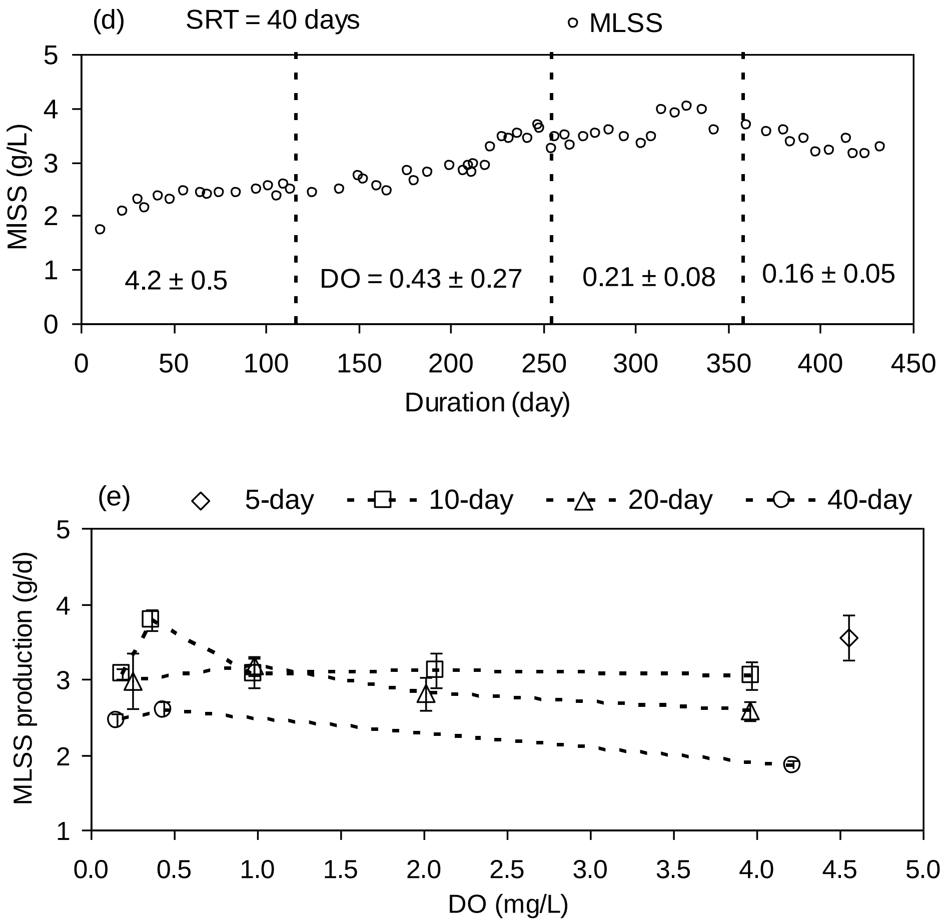


Fig. 3 – MLSS concentration under different dissolved oxygen (DO, mg/L) concentrations in the reactors with (a) 5, (b) 10, (c) 20 and (d) 40 days solids retention time (SRT); (e) sludge production in the steady-state under different DO concentrations and SRTs (Mean ± stdev).

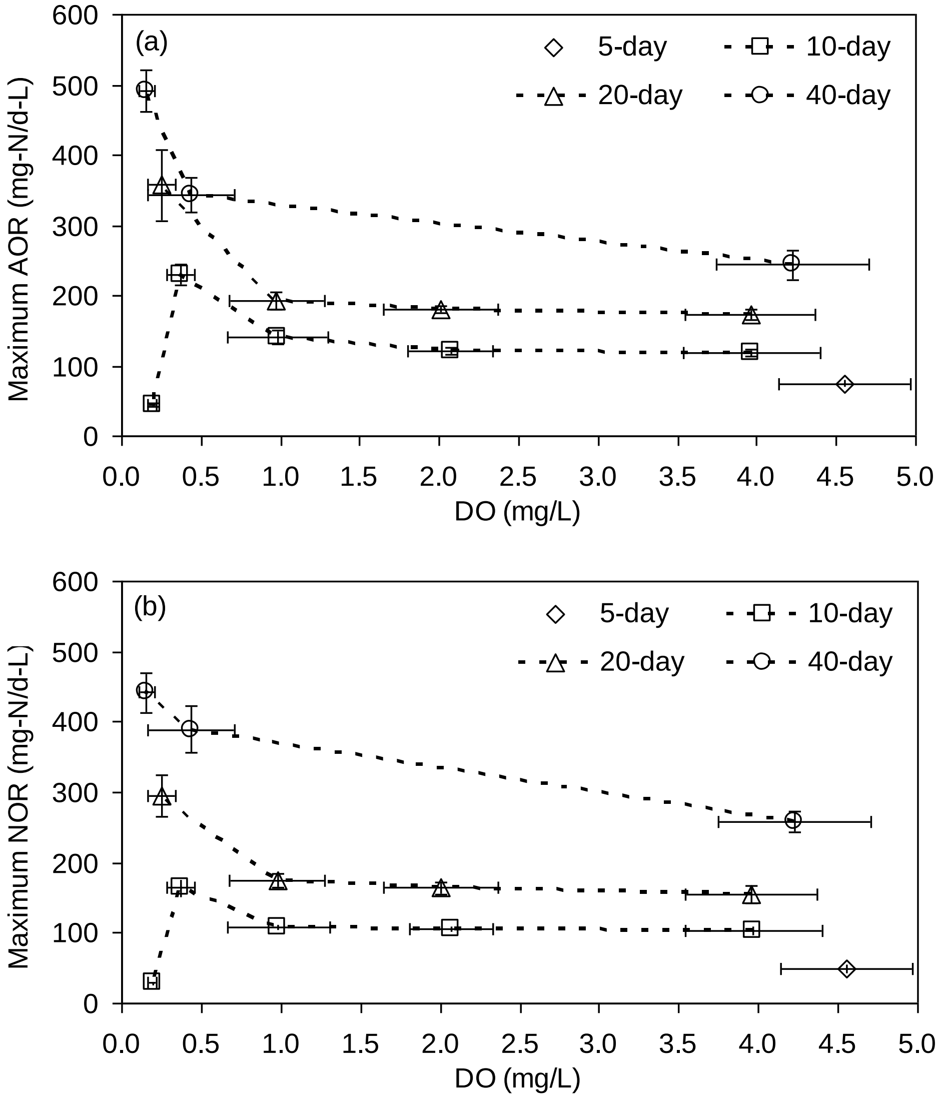


Fig. 4 – (a) Maximum ammonia oxidation rate (AOR) and (b) maximum nitrite oxidation rate (NOR) for the sludge cultivated with different dissolved oxygen (DO) concentrations and solids retention times (SRTs). (Mean ± stdev)

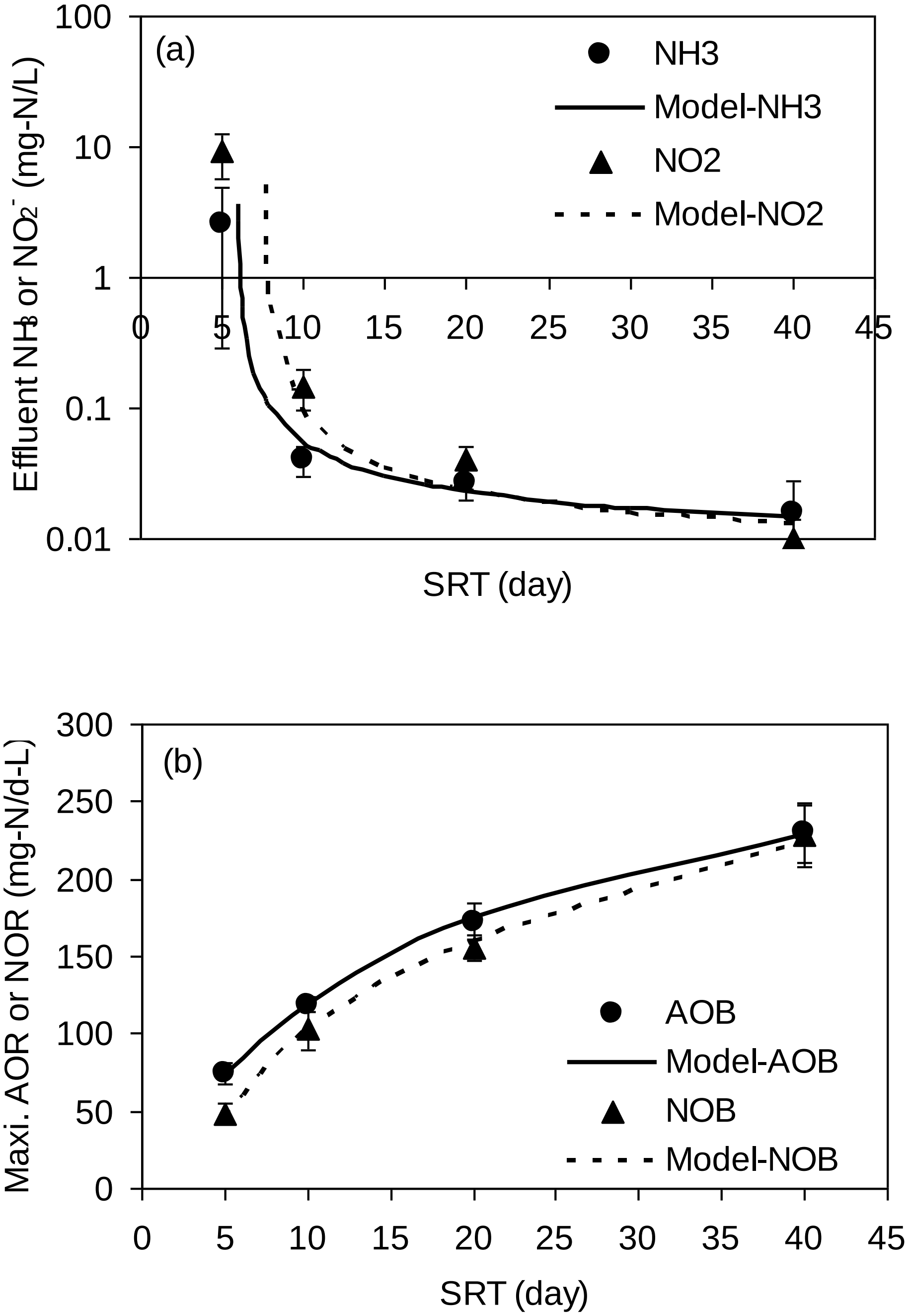
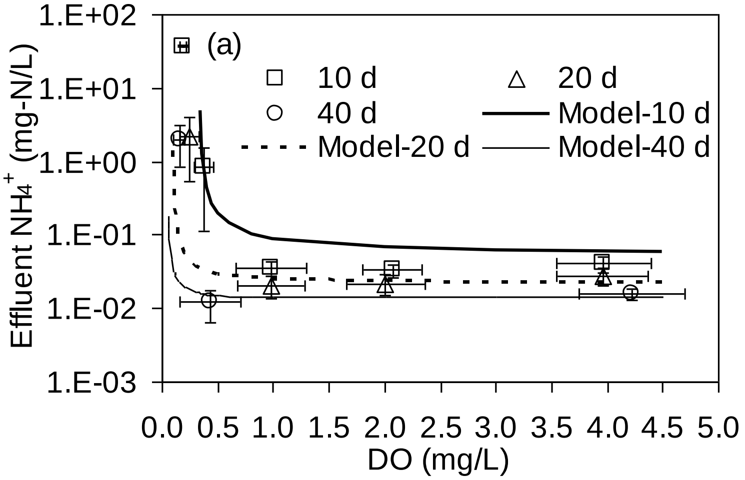
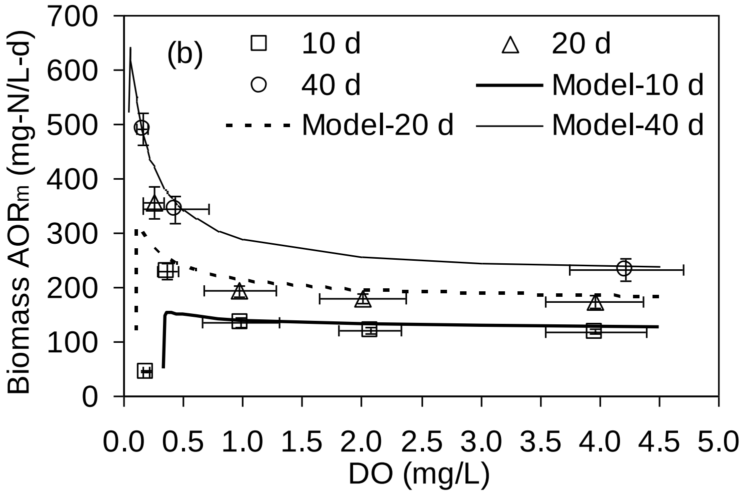
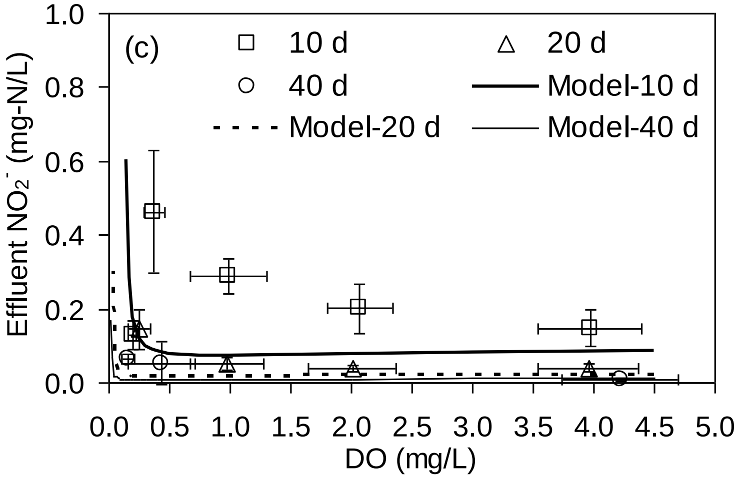


Fig. 5 – The effect of solids retention time (SRT) on (a) effluent ammonia and nitrite concentrations and (b) biomass maximum ammonia oxidation rate (AOR) and nitrite oxidation rate (NOR) under the unlimited DO conditions (Mean ± stdev). Lines are model fits using the parameters from Table 6.


(a)
10 d
20 d
40 d
Model-10 d
Model-20 d
Model-40 d
Effluent NH_4^+ (mg-N/L)
1.E+02
1.E+01
1.E+00
1.E-01
1.E-02
1.E-03
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
DO (mg/L)


(b)
10 d
20 d
40 d
Model-10 d
Model-20 d
Model-40 d
Biomass AOR_m (mg-N/L-d)
700
600
500
400
300
200
100
0
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
DO (mg/L)


(c)
10 d
20 d
40 d
Model-10 d
Model-20 d
Model-40 d
Effluent NO_2^- (mg-N/L)
1.0
0.8
0.6
0.4
0.2
0.0
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
DO (mg/L)

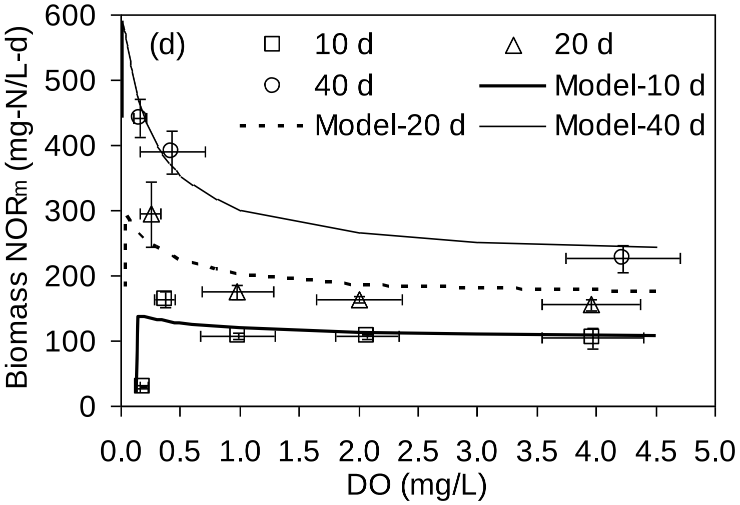


Fig. 6 – Combined effect of solids retention time (SRT) and dissolved oxygen (DO) concentration on (a) effluent ammonia, (b) biomass maximum ammonia oxidation rate (AOR), (b) effluent nitrite, and (d) biomass maximum nitrite oxidation rate (NOR). (Mean ± stdev). Lines are model fits using the parameters from Table 6.

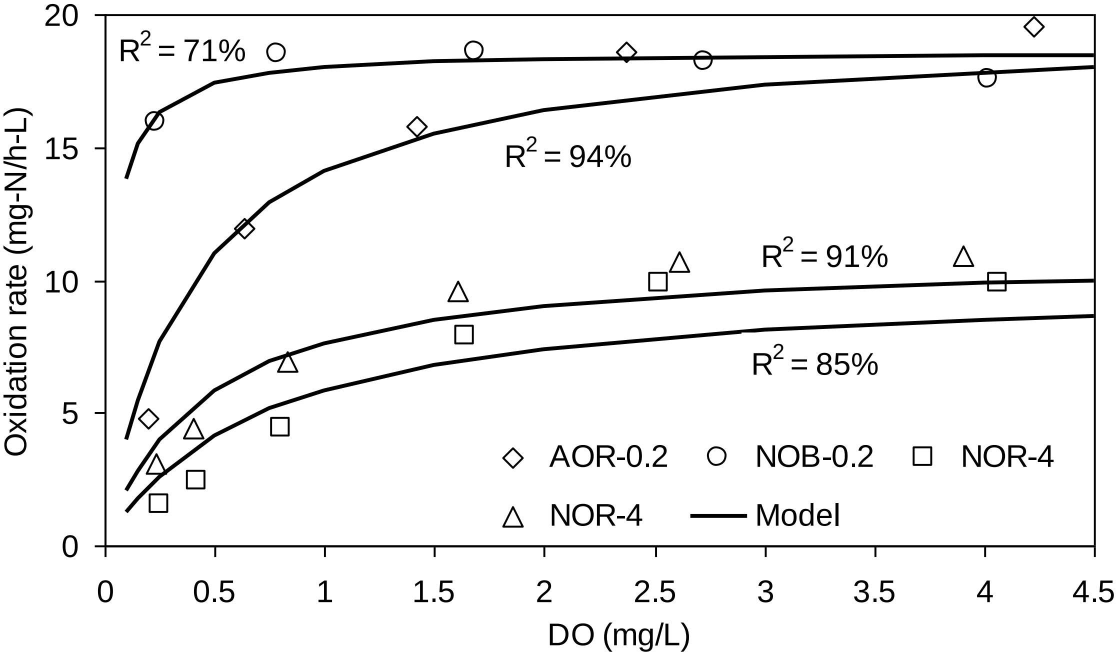


Fig. 7 – Effect of dissolved oxygen (DO) concentration on ammonia oxidation rate (AOR) and nitrite oxidation rate (NOR) in the 40-day SRT sludge cultivated with a high dissolved oxygen (DO, 4 mg/L) and a low DO (0.17 mg/L). Lines are model fits using the parameters from Table 7.

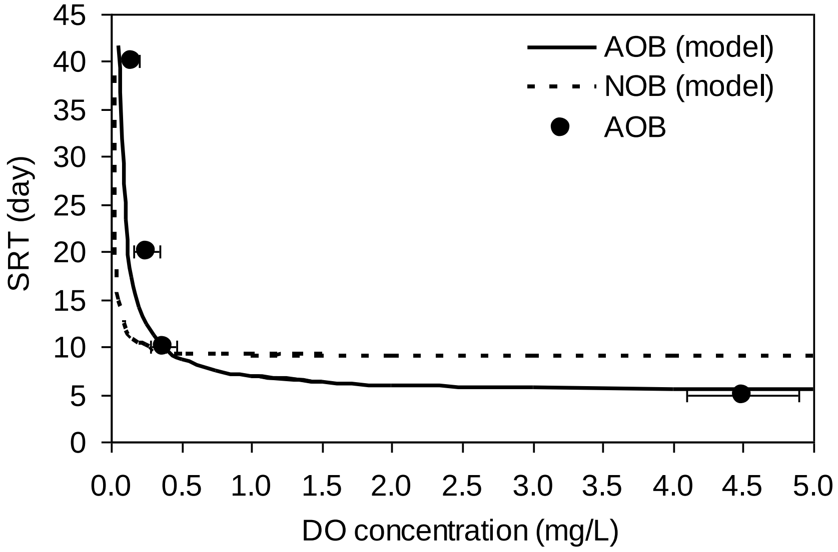


Fig. 8 – Effect of dissolved oxygen (DO) concentration on solids retention time (SRT) required to achieve effluent ammonia and nitrite concentrations of 1 mg-N/L at 20 ˚C in a complete-mix reactor based on kinetics coefficients in Table 6

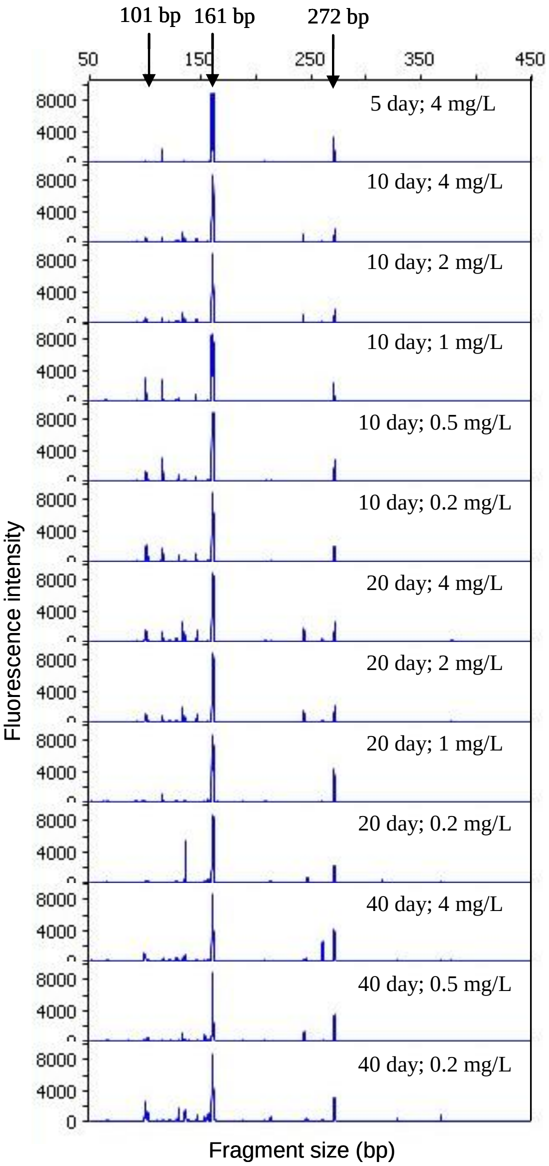


Fig. 9 – T-RFLP profiles of ammonia oxidizing bacteria (AOB) in the sludge cultivated with different solids retention times (SRTs, day) and dissolved oxygen concentrations (DO, mg/L)

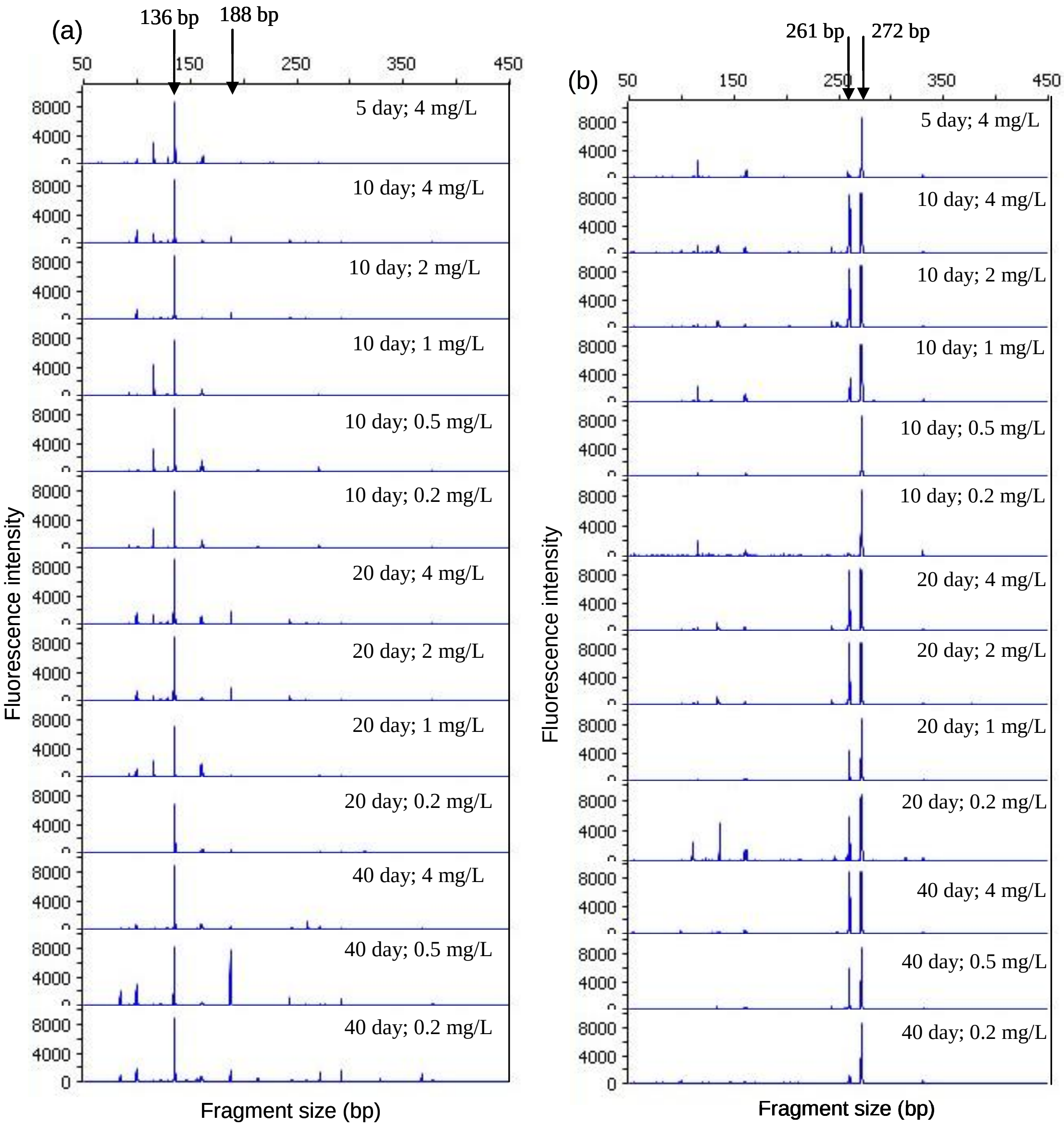


Fig. 10 – T-RFLP profiles of (a) Nitrobacter-like and (b) Nitrospira-like nitrite oxidizing bactiera (NOB) in the sludge cultivated with different solids retention times (SRTs, day) and dissolved oxygen concentrations (DO, mg/L)

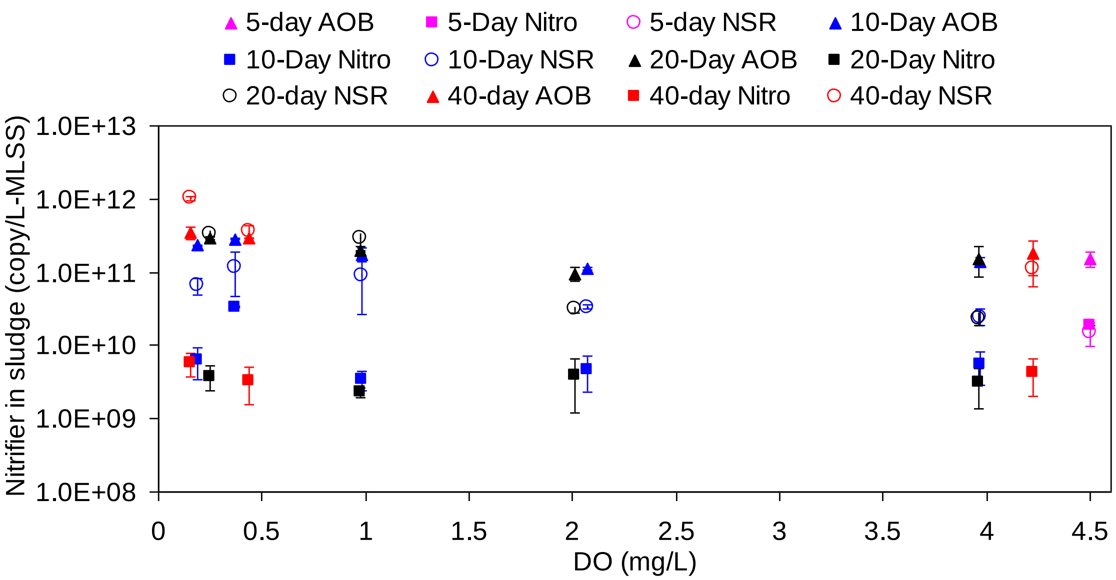
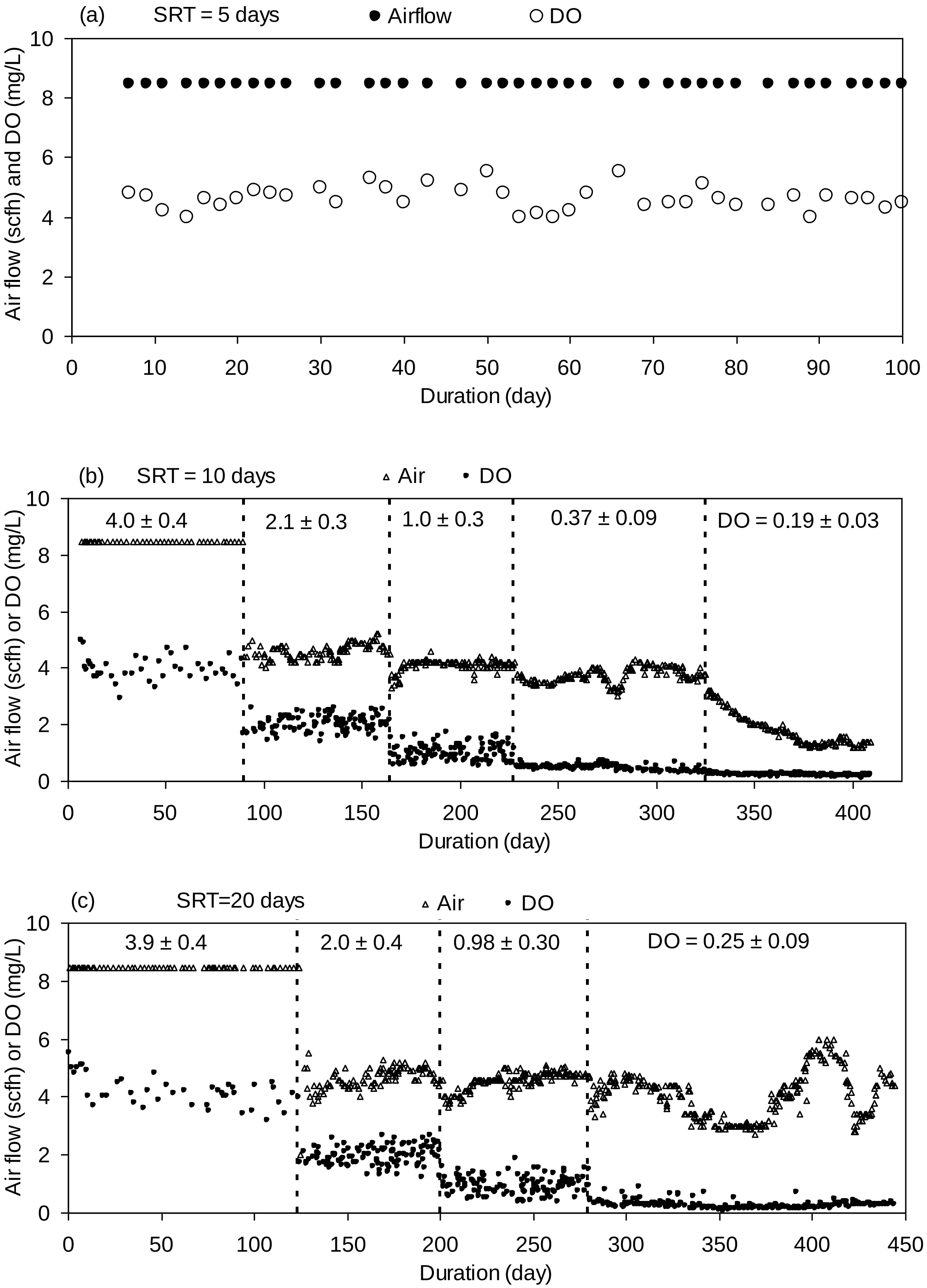


Fig. 11 – Copies per liter of 16S rRNA gene for ammonia oxidizing bacteria (AOB), *Nitrobacter*-lke (Nitro) nitrite oxidizing bacteria (NOB) and *Nitrospira*-like (NSR) NOB in activated sludge cultivated with different solids retention times (SRTs) and dissolved oxygen (DO) concentrations (Mean ± stdev).


(a)
SRT = 5 days
● Airflow
○ DO
Air flow (scfh) and DO (mg/L)
0
2
4
6
8
10
0
10
20
30
40
50
60
70
80
90
100
Duration (day)
(b)
SRT = 10 days
▵ Air
• DO
4.0 ± 0.4
2.1 ± 0.3
1.0 ± 0.3
0.37 ± 0.09
DO = 0.19 ± 0.03
Air flow (scfh) or DO (mg/L)
0
2
4
6
8
10
0
50
100
150
200
250
300
350
400
Duration (day)
(c)
SRT=20 days
▵ Air
• DO
3.9 ± 0.4
2.0 ± 0.4
0.98 ± 0.30
DO = 0.25 ± 0.09
Air flow (scfh) or DO (mg/L)
0
2
4
6
8
10
0
50
100
150
200
250
300
350
400
450
Duration (day)

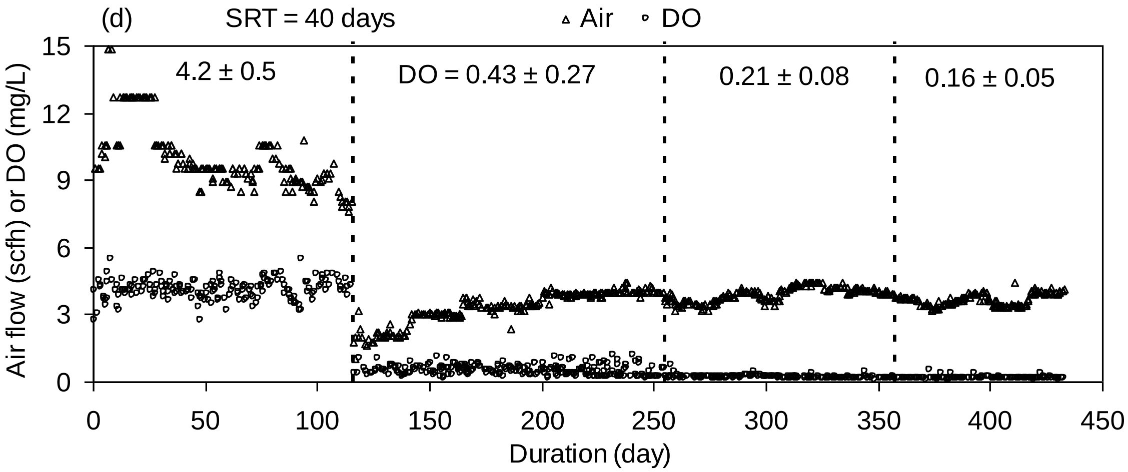


Fig. 12 – Aeration need with different dissolved oxygen (DO) concentrations in the reactors with (a) 5, (b) 10, (c) 20, and (d) 40 days SRT

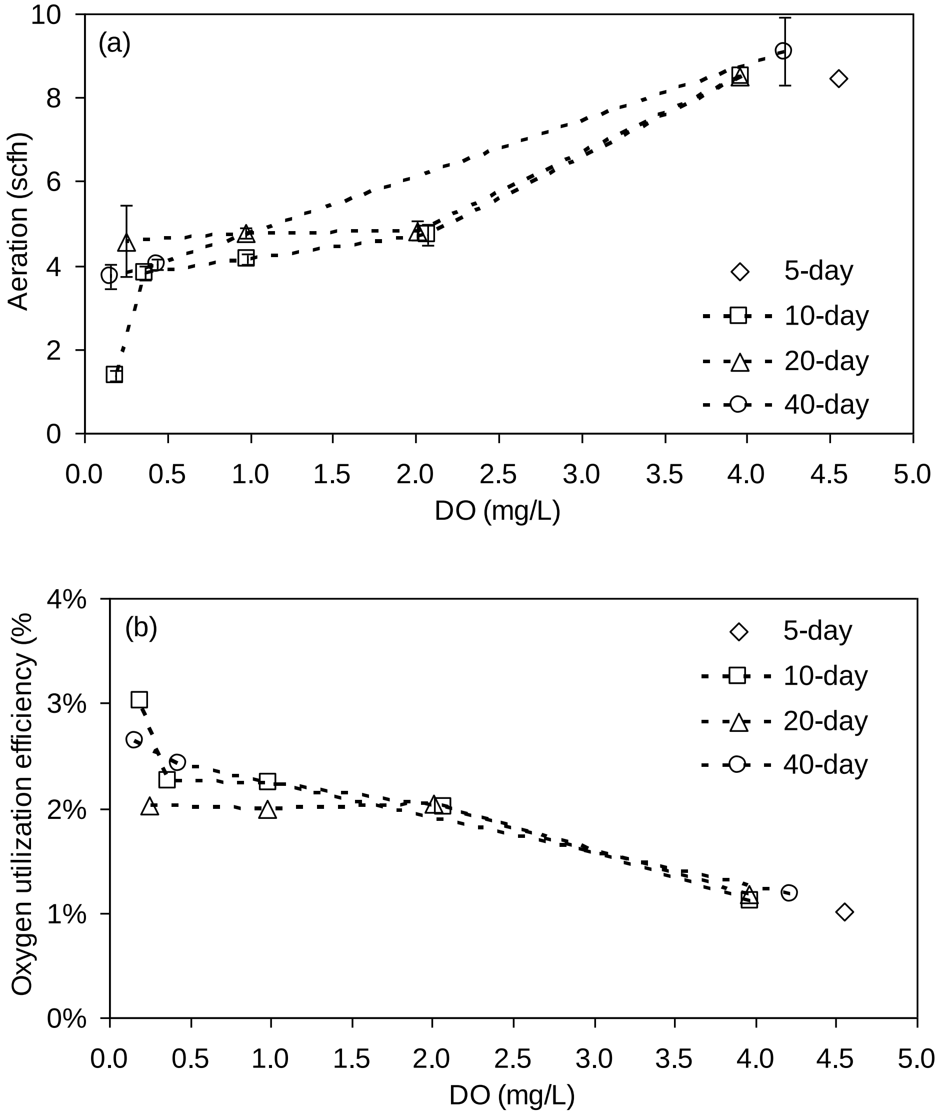


Fig. 13 – (a) Average aeration needs and (b) oxygen utilization efficiency in the steady-state in the reactors with different solids retention times (SRTs) and dissolved oxygen (DO) concentrations.

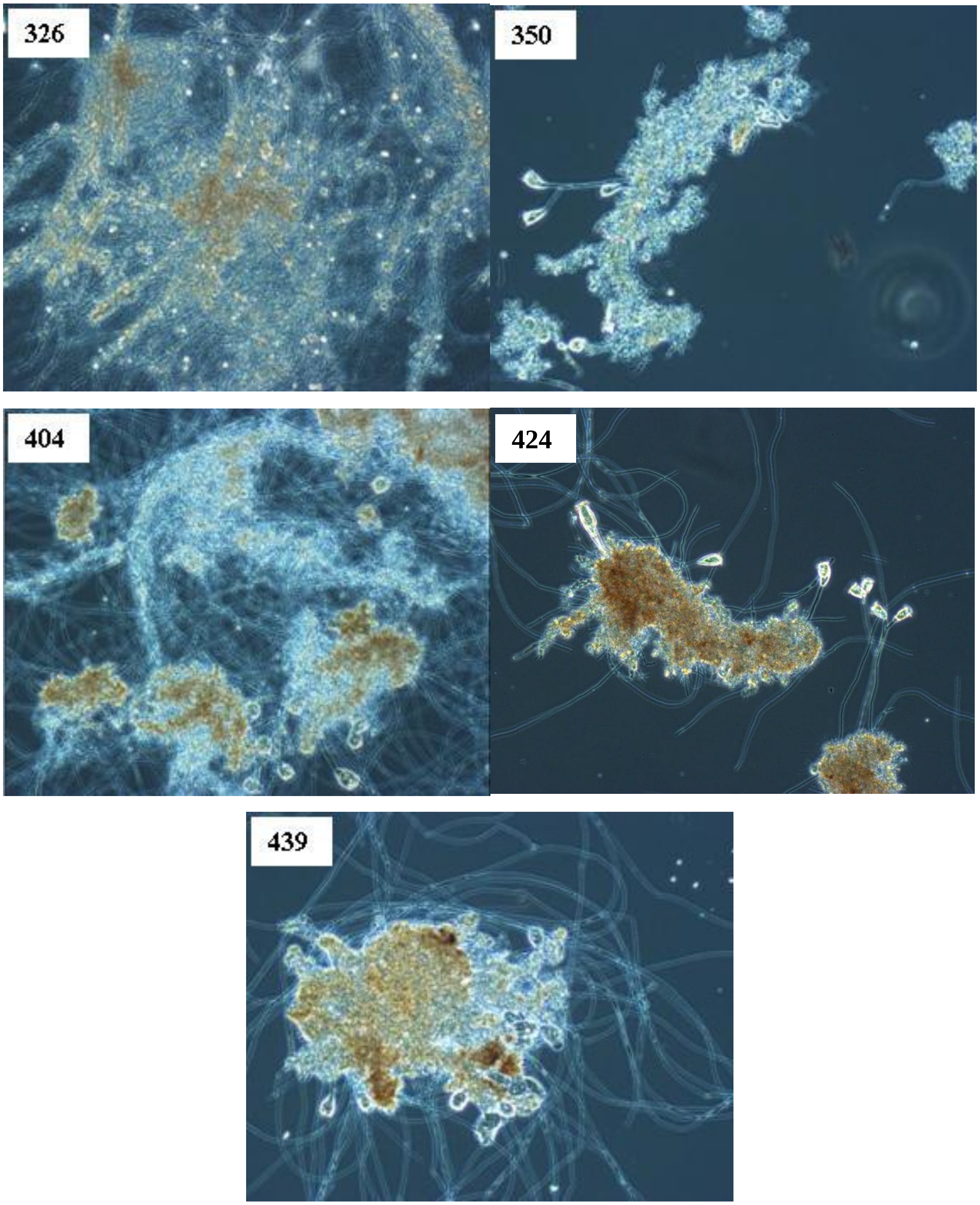


Fig. 14 – Typical microscope images for the sludge in the 20-day SRT reactor in the corresponding periods shown in Table 9 (the number in above images are the day when it they were taken)

Table 1 – Operational dissolved oxygen (DO) concentration and solids retention time (SRT)

SRT (day)	Unlimited DO	Limited DO			
	≥ 4.0 mg/L	2.0 mg/L	1.0 mg/L	0.5 mg/L	0.2 mg/L
5	×	N/A	N/A	N/A	N/A
10	×	×	×	×	×
20	×	×	×	N/A	×
40	×	N/A	N/A	×	×

Table 2 – Primers and probes used in the assays of T-RFLP and real-time PCR

Target	Primer/probe	Sequence	Reference
For T-RFLP			
Bacteria 16S rRNA	11f	5′-GTTTGATCCTGGCTCAG-3′	Kane et al., 1993
	1492r	5′-TACCTTGTTACGACTT-3′	Lin and Stahl, 1995
Bacteria 16S rRNA	Eub 338f	5′-(6-FAM)-ACTCCTACGGGAGGCAGC-3′	Amann et al., 1990
B-proteobacteria AOB 16S rRNA	Nso 1225r	5′-CGCCATTGTATTACGTGTGA-3′	Mobarry et al., 1996
Nitrobacter 16S rRNA	NIT3r	5'-CCTGTGCTCCATGCTCCG-3'	Wagner et al., 1995
Nitrospira 16S rRNA	Ntspa685r	5′-CGGGAATTCCGCGCTC-3′	Regan et al., 2002
For real-time PCR			
AOB 16S rRNA	CTO 189fA/B	5′-GGAGRAAAGCAGGGGATCG-3′	Hermansson and Lindgren, 2001
	CTO 189fC	5′-GGAGGAAAGTAGGGGATCG-3′	Hermansson and Lindgren, 2001
	RT1r	5′-CGTCCTCTCAGACCARCTACTG-3′	Hermansson and Lindgren, 2001
Nitrobacter 16S rRNA	Nitro 1198f	5′-ACCCCTAGCAAATCTCAAAAAACCG-3′	Graham et al., 2007
	Nitro 1423r	5′-CTTCACCCCAGTCGCTGACC-3′	Graham et al., 2007
Nitrospira 16S rRNA	NSR 1113f	5′-CCTGCTTTCAGTTGCTACCG-3′	Dionisi et al., 2002
	NSR 1264r	5′-GTTTGCAGCGCTTTGTACCG-3′	Dionisi et al., 2002
For the amplification of AOA amoA gene			
AOA *amoA* gene	Arch-amoAF	5'-STAATGGTCTGGCTTAGACG-3'	Francis et al., 2005
	Arch-amoAR	5'-GCGGCCATCCATCTGTATGT-3'	Francis et al., 2005

Table 3 – PCR programs used in the assays of T-RFLP and real-time PCR

Target	Primers/probe	PCR program	Reference
T-RFLP			
Universal PCR	11f 1492r	5 min at 95˚C; 30 cycles of 30 s at 95˚C, 30 s at 56˚C, and 45 s at 72 ˚C; and a final elongation for 10 min at 72 ˚C	Revised based on Siripong and Rittmann, 2007
AOB 16S rRNA	Nso 1225r Eub 338f	5 min at 95˚C; 30 cycles of 90 s at 95˚C, 30 s at 60 ˚C, and 90 s at 72 ˚C; and a final elongation for 10 min at 72 ˚C	
Nitrobacter 16S rRNA	NIT3r Eub 338f		
Nitrospira 16S rRNA	Ntspa685r Eub 338f		
Real-time PCR			
AOB 16S rRNA	CTO 189A/B CTO 189C RT1r	2 min at 50 ˚C, 10 min at 95 ˚C; 40 cycles of 30 s at 95 ˚C, 60 s at 60 ˚C, and 30 s at 72 ˚C	Revised based on Kim et al., 2011
Nitrobacter 16S rRNA	Nitro 1198f Nitro 1423r	2 min at 50 ˚C, 10 min at 95 ˚C; 40 cycles of 20 s at 95 ˚C, 60 s at 58 ˚C, and 40 s at 72 ˚C	
Nitrospira 16S rRNA	NSR 1113f NSR 1264r	2 min at 50 ˚C, 10 min at 95 ˚C; 40 cycles of 30 s at 95˚C, 60 s at 60 ˚C, and 30 s at 72 ˚C	
Amplification for AOA amoA gene			
AOA amoA gene	Arch-amoAF Arch-amoAR	5 min at 95 ˚C; 30 cycles of 40 s at 94˚C, 60 s at 53 ˚C, and 60 s at 72 ˚C, and 15 min at 72 ˚C	Francis et al., 2005

Table 4 – Expected terminal fragments (TF) size and their corresponding ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) groups based on terminal restriction fragment length polymorphism (T-RFLP) of 16S rRNA (Siripong et al., 2007)

Nitrifiers	Geneus	Lineage	TF size (bp)
AOB	*Nitrosomonas*	*Europaea/eutropha* lineage	164-166, 276
		Oligotropha lineage	276
		Cryotolerans lineage	276
		Marina lineage	166
		Communis lineage	276
	Nitrosospira		105-107
NOB	*Nitrobacter*		141, 196
	Nitrospira		134, 194, 265-267, 277, 333

Table 5 – Average effluent quality and mix liquor suspended solids (MLSS) concentration in the steady-state in the reactors with various solids retention times (SRTs) and dissolved oxygen (DO) concentrations (Mean ± stdev).

SRT (day)	Duration day	DO (mg/L)	NH_4^+ (mg-N/L)	NO_2^- (mg-N/L)	NO_3^- (mg-N/L)	BOD_5 (mg/L)	MLSS (g/L)
5	50 - 100	4.55	2.56 ± 2.27	9.15 ± 3.40	32.0 ± 4.1	4.3 ± 1.4	0.59 ± 0.05
10	61 - 89	3.97	0.04 ± 0.01	0.15 ± 0.05	41.9 ± 0.9	2.2 ± 0.6	1.02 ± 0.06
	133 - 164	2.07	0.033 ± 0.007	0.20 ± 0.07	41.8 ± 1.1	1.8 ± 0.5	1.04 ± 0.08
	208 - 228	0.98	0.036 ± 0.008	0.29 ± 0.05	39.5 ± 2.4	1.6 ± 0.8	1.02 ± 0.07
	305 - 325	0.37	0.85 ± 0.73	0.46 ± 0.17	38.7 ± 2.4	1.8 ± 0.3	1.26 ± 0.05
	383 - 409	0.19	37.2 ± 1.0	0.13 ± 0.04	3.1 ± 0.7	2.0 ± 0.7	1.02 ± 0.03
20	84 - 124	3.96	0.027 ± 0.007	0.040 ± 0.011	43.2 ± 0.8	1.6 ± 0.3	1.72 ± 0.09
	175 - 201	2.01	0.022 ± 0.007	0.039 ± 0.01	43.1 ± 1.7	1.5 ± 0.6	1.87 ± 0.15
	251 - 280	0.98	0.021 ± 0.007	0.052 ± 0.015	41.5 ± 1.7	1.3 ± 0.6	2.12 ± 0.08
	401 - 444	0.25	2.2 ± 1.7	0.15 ± 0.05	38.9 ± 3.6	1.1 ± 0.9	1.98 ± 0.24
40	77 - 115	4.22	0.016 ± 0.003	0.010 ± 0.004	44.4 ± 1.4	1.4 ± 1.7	2.48 ± 0.10
	222 - 255	0.43	0.012 ± 0.006	0.05 ± 0.06	39.2 ± 1.5	1.5 ± 0.9	3.46 ± 0.14
	391 - 433	0.16	2.0 ± 1.2	0.06 ± 0.01	40.5 ± 1.9	1.9 ± 0.9	3.26 ± 0.12

Table 6 – Stoichiometry and kinetics parameters for AOB and NOB (pH = 7.25 and T = 20 ˚C) (Mean ± stdev).

Parameters	AOB		NOB	
	This study	Reference	This study	Reference
Synthesis yield coefficient (g-VSS/g-N)	0.18[a]		0.06[a]	
Maxi. specific growth rate (d^{-1})	0.24 ± 0.01	0.66 – 0.77[a] 2.04[b] 0.8-1.1[c] 0.2-0.9[d]	0.18 ± 0.01	0.5-1.0[c]
Maxi. specific substrate utilization rate (g-N/g-VSS-d)	1.3	3.1[e]	3.0	13[e]
Half-velocity constant for substrate (mg-N/L)	0.023 ± 0.003	0.48-1.62[a] 0.5-1.0[d] 1.5[e]; 0.14[f]	0.020 ± 0.001	0.28[f] 2.7[e]
Endogenous decay coefficient (d^{-1})	0.066 ± 0.003	0.044-0.083[a] 0.05-0.15[d] 0.4[b]; 0.15[e,g] 0.05[h]	0.045 ± 0.006	0.15[e, g] 0.05[h]
Minimum SRT (d)	5.7	1.1[b]	7.7	
Half-saturation constant for oxygen, growth (mg-O_2/L)	0.29	0.5[f] 0.8[h] 0.5-1.0[i] 0.27-1.61[j] 0.43[k] 0.25-0.3[l]	0.08	0.68[f] 0.8[h] 0.5-1.5[i] 0.87-1.10[k] 0.34-1.84[l]
Half-saturation constant for oxygen, decay (mg-O_2/L)	0.48	1.6[c]	0.69	

a, Liu and Wang, 2012; b Park and Noguera, 2007; c, Munz et al., 2011; d, Kaelin et al., 2009; e, Metcalf and Eddy, 2003; f, Rittmann and McCarty, 2001; g, Manser et al., 2005; h, Manser et al., 2006; i, Henze et al., 2002; j, Weon et al., 2004; k, Stankewich et al., 1972; l, Stenstrom, 1980.

Table 7 – Estimated half-saturation constants for oxygen (K_{DO}) in the batch dissolved oxygen (DO) impact tests on ammonia and nitrite oxidation for the 40-day solids retention time (SRT) sludge cultivated with a high and a low dissolved oxygen (DO, mg/L) concentrations (Mean ± stdev).

DO	AOB	NOB
0.2	0.39 ± 0.08	0.04 ± 0.01
4.0	0.71 ± 0.21	0.43 ± 0.09

Table 8 – Oxygen demand in the steady-state under different solids retention times (SRTs) and dissolved oxygen (DO) concentrations.

SRT (day)	DO (mg/L)	Oxygen demand or oxygen demand reduction due to ($g-O_2/d$)				Total oxygen demand ($g-O_2/d$)
		BOD biodegradation	Denitrification	Nitrification	Biomass production	
5	4.55	11.10	0.00	10.4	-5.05	16.38
10	3.97	11.20	0.00	11.18	-4.33	18.06
	2.07	11.23	-0.01	11.18	-4.43	17.96
	0.98	11.24	-0.40	11.16	-4.36	17.64
	0.37	11.23	-0.37	10.91	-5.38	16.38
	0.19	11.21	-0.31	1.30	-4.36	7.84
20	3.96	11.24	0.01	11.51	-3.66	19.10
	2.01	11.25	0.00	11.51	-3.99	18.78
	0.98	11.26	-0.30	11.52	-4.50	17.97
	0.25	11.27	-0.34	10.94	-4.22	17.65
40	4.22	11.24	0.00	11.84	-2.64	20.44
	0.43	11.28	-0.94	11.84	-3.68	18.50
	0.16	11.28	-0.35	11.31	-3.47	18.77

Table 9 – Dissolved oxygen (DO), aeration intensity, actual oxygen demand, oxygen utilization efficiency, and sludge settling ability in different periods in the reactor with 20-day solids retention time (SRT)

Period	DO (mg/L)	Aeration (scfh)	Actual oxygen demand (g-O_2/d)	Oxygen utilization efficiency	SVI (mL/g-MLSS)
322 - 333	0.36 ± 0.19	4.2 ± 0.2	17.6	2.17%	Almost no settling
343 - 355	0.17 ± 0.02	3.1 ± 0.2	17.8	2.97%	205
398 - 410	0.21 ± 0.05	5.6 ± 0.2	17.3	1.61%	Almost no settling
422 - 431	0.30 ± 0.04	3.3 ± 0.2	18.0	2.85%	533
431 - 435	0.30 ± 0.03	4.6 ± 0.2	17.7	2.00%	Almost no settling

III: A potential approach to optimize aeration system operation based on effluent ammonia and nitrite

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Abstract

Correlations between the effluent quality parameters, such as effluent ammonia, nitrite and chemical oxygen demand (COD), and the key operational parameters of the wastewater treatment process, such as solids retention time (SRT), dissolved oxygen (DO), ammonia and organic loadings, and temperature, were investigated. At 10-day and 20-day SRTs, either effluent ammonia or effluent nitrite increased before other quality parameters responded to an insufficient DO condition. However, when the SRT was 40 days, effluent ammonia increased before the nitrite and COD under insufficient DO conditions. Under various influent loading and temperature conditions, the effluent quality, as indicated by the ammonia or nitrite concentration, only correlated with the operational DO. Therefore, effluent ammonia and nitrite can be used as key parameters to control the aeration system, and achieve the required effluent quality at a minimum aeration intensity. With this dynamic, quality-based control strategy the operation of the aeration system is optimized.

Key words

Wastewater treatment, aeration, optimization, ammonia, nitrite, quality-based control

1. Introduction

In activated sludge processes, the dissolved oxygen (DO) level is a critical operational parameter since it directly relates to both effluent quality and operation cost. Conventionally, the aeration tank should maintain a DO level of 2 mg/L to provide microorganisms with a certain activity (Metcalf & Eddy, 2003; Ma et al., 2006). However, in many treatment plants, especially those with long SRTs, the biodegradation can be completed at a DO level of less than 2 mg/L. In this case, maintaining a constant DO of 2 mg/L is unnecessary. On the other hand, insufficient DO could adversely impact biochemical oxygen demand (BOD) degradation and nitrification. Because the energy used for aeration contributes to the majority of the energy consumption of a treatment plant (McCarty et al., 2011), the main goal of aeration control is to minimize aeration while maintaining the required effluent quality. To meet this goal, an optimal operational DO should be used as the control parameter. However, given the large variations in wastewater flow, strength, and temperature, a constant DO level that can result in the required treatment without over aeration does not exist. In addition, due to the large variations in inflow quality and quantity, it is very difficult to maintain a constant DO at all times (Phillips and Fan, 2005). Maintaining a dynamic minimum DO level that can achieve the required effluent quality is the key to optimizing the aeration system for energy conservation.

Advanced control strategies, such as model-based predictive control or fuzzy control, are currently being investigated to minimize energy use for wastewater treatment (Manesis et al., 1998; Ferrer et al., 1998; Galluzzo et al., 2001; Ma et al., 2006; Holenda et al., 2008). Based on these model-based control strategies, an optimal set-point DO or air flow is determined and tracked through a number of equations and multiple variables, e.g., flow rate, temperature, influent ammonia, and sludge concentration. However, the kinetic parameters used in the equations are very difficult to determine and may vary with time (Cox 2004), which make control extremely difficult. Moreover, model-based control strategies strongly rely on the performance of many sensors, but the maintenance and calibration of these sensors are not simple.

Ammonia removal has become one of the most important goals for municipal wastewater treatment. In activated sludge processes, both BOD degradation and nitrification need sufficient DO. Nitrifying bacteria are believed to be less competitive in low DO than heterotrophic bacteria (Grady and Lim, 1980; Metcalf & Eddy, 2003). Therefore, if the DO in an aeration tank is not sufficient, effluent ammonia and/or nitrite may provide a faster feedback than effluent BOD by increasing their concentrations. Fig. 1 presents the general idea of using effluent ammonia and/or nitrite to control aeration intensity. Lower and upper threshold values need to be set. When the effluent ammonia or nitrite is greater than the upper threshold value, aeration intensity needs to be increased to improve nitrification. When the effluent ammonia, nitrite, or both of them, are below the lower threshold value, the aeration intensity needs to be decreased to save energy.

In addition to low DO, other conditions, such as a short solids retention time (SRT), low temperature, and peak ammonia and organic loadings may lead to incomplete nitrification, even when DO is sufficient (Hall and Murphy, 1985; Figueroa and Silverstein, 1992; Metcalf & Eddy, 2003; Liu et al., 2012). In these cases, the controller based on effluent ammonia or nitrite may give a false order to increase unnecessary aeration. Therefore, before the application of the proposed aeration control strategy, the following hypotheses have to be validated: (a) effluent ammonia or nitrite will give the first feedback to insufficient DO; (b) under certain conditions, insufficient DO is the only cause of incomplete nitrification; and (c) when DO is excessive, effluent ammonia and nitrite will be lower than a certain level. If only the first hypothesis is valid, effluent ammonia or nitrite can be used as indicators for possible insufficient aeration condition but can not be used for aeration control because the increased ammonia or nitrite could be caused by other operational conditions such as a peak inflow loading, a short SRT, a low temperature, etc. When the first two hypotheses are valid, effluent ammonia or nitrite can be used as key indicators for an insufficient aeration condition for aeration control. Only when all three hypotheses are valid, can effluent ammonia or nitrite show insufficient and excessive aeration conditions, and be used to control the aeration system to achieve the required effluent quality with minimal energy input.

2. Materials and methods

2.1 Experimental setup and SRT effect

Three bench-scale, complete-mix reactors with the same effective aeration volume of 31.5 L were set up. To determine the effect of SRT on nitrification without DO limitation (DO > 4 mg/L), the reactors were operated at SRTs of 5, 10, and 20 days, respectively. After finishing the test on the 5-day SRT, the SRT in that reactor was increased to 40 days. All reactors were fed with the same synthetic wastewater containing approximately 180 mg/L of chemical oxygen demand (COD) and 48 mg/L of ammonia-nitrogen (ammonia-N), provided by glucose and ammonium carbonate, respectively. In addition, trace elements were provided in the influent, with concentrations of Mn^{2+} = 0.2 mg/L (from $MnCl_2 \cdot 4H_2O$), Mo^{5+} = 0.12 mg/L (from $MoCl_5$), Co^{2+} = 0.001 mg/L (from $CoCl_2$), Zn^{2+} = 0.05 mg/L (from $ZnCl_2$), and Fe^{2+} = 0.005 mg/L (from $FeSO_4 \cdot 7H_2O$). Tap water with soluble Ca and Mg greater than 20 mg/L was used as the solvent. The hydraulic retention time (HRT) was approximately 12 hours and the temperature was 20.5 ± 1 ˚C for all reactors. The pH in the aeration tank ranged from 7.0 to 7.5, and was controlled by a buffer containing K_2HPO_4 and $NaHCO_3$. The SRT was controlled by daily sludge wasting from the aeration tank, and all sludge in the final clarifier was returned. The seed activated sludge was collected from an oxidation ditch in the Rolla Southeast Wastewater Treatment Plant. Effluent BOD, ammonia, nitrate, and nitrite, and the sludge production at steady-state were monitored regularly.

Preliminary findings indicated that complete nitrification could not be achieved at the 5-day SRT under DO > 4 mg/L, so no further tests were conducted on this SRT.

2.2 DO effect

After all reactors were stabilized, the DO levels were reduced to various ranges for 8 hours to determine the response of the reactor. All other operational conditions, such as SRT, influent substrate concentration, HRT, and temperature were maintained the same as before. The effluent COD, ammonia, nitrate, and nitrite were measured every 1 or 2 hours after the reduction of DO.

2.3 Shock load effect

After finishing experiment on the effect of DO, all reactors were changed back to an unlimited DO condition (DO > 4 mg/L). After stabilization, effects of ammonia and organic shock loads on reactor performance, especially nitrification, were tested. To test the effect of an ammonia shock load on the reactor performance, the influent ammonia concentration was increased to several times the previous concentration, while no change was made in the influent COD concentration. After increasing the influent ammonia concentration, the concentrations of effluent ammonia, nitrate, nitrite, and COD were measured at different time intervals. To test the effect of an organic shock load on the reactor performance, the influent COD concentration was increased to several times the previous concentration, while no changes in the influent ammonia concentration were made. In the organic shock load test, the concentrations of effluent COD, ammonia, nitrate, and nitrite were measured once each day. In both shock load tests, the DO concentration was maintained above 4 mg/L and the pH was maintained at a range of 7.0 - 7.5 for all reactors.

2.4 Field tests

A pilot-scale compete-mix activated sludge reactor was set up at the Southeast Wastewater Treatment Plant in Rolla, Missouri. The reactor was fed with raw municipal wastewater pumped from a location between the fine screen and the grit chamber. The effective volume of the aeration tank was 19.8 m^3. To simulate the loading variation, the pilot-scale reactor was fed at different inflow rates (from 38 to 114 m^3/d), resulting in HRTs in the range of 4.2 to 12.6 h. This field test was conducted during the period from June 2010 to February 2011, with the reactor temperature ranging from 8 to 27 ˚C. The SRT was controlled and maintained at approximately 30 - 40 days before November 2010. Starting from the middle of November, the SRT was increased to 60 – 80 days to compensate for the effect of low temperature on nitrification. During the experiment, constant aeration was provided. As a result of the variations in the inflow rate, strength, and temperature, the DO in the aerobic tank varied from 0.1 mg/L to 7 mg/L. Parameters were monitored approximately three times per week. These included mixed liquor suspended solids concentration (MLSS), mixed liquor volatile suspended solids concentration (MLVSS), pH, temperature and DO in the aeration tank, the inflow rate, the influent COD, suspended solids (SS) and total nitrogen (TN), and the effluent COD, ammonia, nitrite, and nitrate. A composite sample, consisting 96 grab samples per day collected using a GLS sampler (Teledyne ISCO, USA), was used for influent quality analysis. A grab sample at the final clarifier was used for effluent quality analysis.

2.5 Chemical analysis

MLSS or SS and MLVSS were determined followed standard methods 2540 D and 2540 E, respectively (Clesceri et al., 1998). A microscope (Olympus CKX41) was used to exam the microorganism community in the reactor. A YSI DO meter (YSI Model-58) with a YSI probe (YSI 08 C) measured the reactor DO and temperature; the DO probe was calibrated each time before measurement. An Orion model 370 pH meter with a PerpHecT pH electrode (Orion 9206BN) was used to measure the pH; the pH electrode was calibrated before each use. Chemical reagents used for COD, TN, ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), and nitrite nitrogen (NO_2^--N) analysis were purchased from HACH company (Loveland, Colorado). The 5-day BOD (BOD_5) was measured following the standard method 5210 B (Clesceri et al., 1998).

3. Results

3.1 Effect of SRT on nitrification without DO limitation

SRT is a critical parameter for nitrification, and incomplete nitrification will occur if SRT is too short, even under high DO conditions (Metcalf & Eddy, 2003). If incomplete nitrification is caused by a short SRT, rather than a low DO, the treatment will never achieve nitrification and it will be impossible to use effluent ammonia or nitrite to control aeration.

Table 1 shows a steady state effluent quality under different SRTs when the aeration tank had a DO of greater than 4 mg/L (unlimited DO). All reactors had low effluent BOD concentrations, indicating that the degradation of organic pollutants had been completed for all reactors. Table 1 also shows that reactors with 10-, 20- and 40-day

SRTs had effluent ammonia and nitrite concentrations below 0.5 mg-N/L, indicating that complete nitrification was achieved in these reactors. However, the 5-day SRT reactor had effluent ammonia-N and nitrite-N concentrations that were much greater than 1 mg-N/L, suggesting that the 5-day SRT was too short to achieve complete nitrification. Because a high nitrite concentration had accumulated within the 5-day SRT reactor, the reaction rate for second-step nitrification was lower than that for the first-step nitrification at this particular SRT. Therefore, in order to achieve complete nitrification, the SRT should be at least 10 days at room temperature, even when the DO concentration is greater than 4 mg/L.

3.2 DO effect

As discussed previously, in order to determine if the effluent ammonia and/or nitrite can be used as key parameters for aeration control, hypothesis (a) has to be validated, e.g. effluent ammonia or nitrite are more sensitive than other parameters to indicate the process performance and, therefore can give the first feedback on an insufficient aeration condition. Under different SRTs, bacterial communities are expected to be different (Metcalf & Eddy, 2003; Yu et al., 2010). As a result, reactors with different SRTs are expected to respond differently to an insufficient DO condition. Fig. 2 shows the effect of reduced DO on the performance of reactors with 10-, 20- and 40-day SRTs.

As shown in Fig. 2, reactors with different SRTs had significantly different responses to a reduced DO. For the 10-day SRT reactor, the effluent nitrite concentration noticeably increased when the DO was reduced to 1.5 mg/L, while a significant increase in effluent ammonia occurred when the DO was reduced to 0.8 mg/L, or less. For the 20-day SRT reactor, effluent nitrite noticeably increased when the DO was reduced to around 0.8 mg/L, but effluent ammonia increased considerably when the DO was less than 0.5 mg/L. For the 40-day SRT reactor, a noticeable increase in effluent nitrite concentration was not observed, although a significant increase in effluent ammonia only occurred when the DO was around 0.23 mg/L.

As expected, effluent COD did not increase significantly where incomplete nitrification occurred in the reactors with reduced DO conditions, confirming that nitrification was more sensitive to reduced DO. Therefore, ammonia or nitrite could give the first feedback of an insufficient DO condition. However, the responses of effluent ammonia and nitrite to insufficient DO, under different SRTs, were totally different. For 10-day and 20-day SRT reactors, noticeable increases in effluent nitrite occurred at DO levels higher than that for effluent ammonia. This was in agreement with the presence that nitrite oxidation was more sensitive to low DO, thereby resulting in nitrite accumulation (Laanbroek and Gerards, 1993; Laanbroek et al., 1994; Blackburne et al., 2008). Under these SRTs, effluent nitrite could report a insufficient DO condition better than effluent ammonia could. However, the concentration of nitrite was determined by the ammonia oxidation rate and the nitrite oxidation rate, and a low DO might have the opposite effect on its accumulation. When DO was further reduced, the increase in effluent ammonia was much more significant than that of effluent nitrite, due to the inhibition of the first-step nitrification, which resulted in less nitrite production. Therefore, under the 10-day and 20-day SRTs, the combination of ammonia and nitrite could better indicate insufficient DO than any individual parameter. At the 40-day SRT, the effluent nitrite had no significant increase with any DO condition, while effluent ammonia had a significant increase when the DO was reduced to 0.23 mg/L. Therefore, at this particular SRT, the effluent ammonia would give the first feedback before the effluent nitrite and COD when the DO was insufficient. In summary, either effluent ammonia or nitrite would give the first feedback as to insufficient DO under all SRT conditions. Thus hypothesis (a) has been validated. However, the idea of using effluent ammonia and nitrite to indicate insufficient DO can not be fully supported, even when hypothesis (a) was validated. If incomplete nitrification is caused by other conditions, such as peak ammonia loading, peak organic loading, short SRT, and low temperature, the increases in effluent ammonia and nitrite do not indicate insufficient DO.

Fig. 2 also shows that a reactor with a longer SRT could achieve complete nitrification under a very low DO condition. To achieve complete nitrification and avoid nitrite accumulation, the DO in reactors with 10-day and 20-day SRTs needs to be higher than 1.5 mg/L and 0.8 mg/L, respectively. However, for the 40-day SRT reactor, complete nitrification could be achieved at a DO of approximately 0.5 mg/L. Wastewater treatment plants operate with various SRTs and, in most cases, the operational SRT for a complete nitrification plant is much longer than the normal design value of 15 – 20 days. If aeration is controlled based on a set point DO of 2 mg/L, the excessive aeration will be provided, resulting in a significant waste of energy.

3.3 Effect of ammonia shock load

The influent load to a municipal wastewater treatment plant may vary considerably. It is possible that incomplete nitrification could occur at peak ammonia loading, even if the DO is unlimited. In this situation, ammonia/nitrite-based aeration control could give a false signal to demand more intensive aeration. In a wastewater treatment plant, the typical ratios of daily peak mass loading to average mass loading for BOD and total Kjeldahl nitrogen (TKN) are less than 4 and 3, respectively (Metcalf & Eddy, 2003); the shock loads last for 1 - 2 hours. Biomass concentration and bacterial communities under different SRTs are supposed to be different. As a result, reactors with different SRTs may have a different tolerance for an identical shock load. Fig. 3 shows the responses of reactors with 10-, 20- and 40- day SRTs under unlimited DO conditions to ammonia shock loads.

At the 10-day SRT, effluent nitrite concentration had noticeably increased after 2 hours while ammonia mass loading increased to 1.5 times. The effluent ammonia concentration had only showed an increase after 2 hours when ammonia loading was raised to 3 times. For the 20-day SRT, noticeable increases in ammonia and nitrite concentrations had only occurred after 2 hours when influent ammonia loading had increased to 6 and 3 times of the original value, respectively. In the 40-day SRT reactor, 6 times' ammonia loading only led to a slight effluent nitrite accumulation. In the ammonia shock load tests, no significant change was observed in effluent COD concentration (data not shown).

According to the results depicted in Fig. 3, the reactor with a higher SRT definitely had greater capability for handling on ammonia shock load. A longer SRT would result in a higher nitrifying biomass concentration, which might to be the main reason for the stability (Metcalf & Eddy, 2003).

Note that the results in Fig. 3 were obtained when elevated loadings were applied continuously. In all municipal wastewater treatment plants, the peak loads occurred twice per day, and each peak load lasts for only 1 – 2 hours, followed by a load that is smaller than the average (Metcalf & Eddy, 2003). Therefore, the shock load could be practically diluted and equalized within the aeration tank without causing an adverse operational issue. As indicated in Fig. 3, within these short periods, the 3-time peak loading did not result in a major effect on the reactors with 20-day and 40-day SRTs. It only slightly reduced the performance of the 10-day SRT reactor. However, in the field, influent loading is normally reduced to a level below average as soon as the peak loading period has passed. This provides an opportunity for the rector to recover. Moreover, the operational SRT of most treatment plants is significantly longer than 10 days. Therefore, reasonable ammonia shock loads would not lead to incomplete nitrification with unlimited DO when the SRT was higher than 10 days.

3.4 Effect of organic shock load

It was suspected that nitrification would be inhibited by high BOD concentration, especially in fixed-film processes (Figueroa and Silverstein, 1992; Downing and Nerenberg, 2008). Nitrification might be inhibited by high BOD concentration in the activated sludge processes as well (Sharma and Gupta, 2004). As a result, incomplete nitrification could occur at the peak BOD loading condition, even when DO was sufficient. To test the hypothesis, an organic shock load was applied to all reactors. As shown in Fig. 4(a), influent COD concentration increased from 180 mg/L to 360 mg/L on one day, then further increased to 720 mg/L on another day, and then increased even further, to 1080 mg/L, on the third day. During the increase in influent COD concentration, the influent ammonia concentration remained constant. The effluent BOD, ammonia, nitrate, and nitrite concentrations were monitored following the organic loading increase. As shown in Fig. 4(a), when the COD concentration increased to 1080 mg/L, an increase in effluent BOD concentration only occurred in the reactor with a 10-day SRT. As shown in Figs. 4(b) and 4(c), no significant change was found in the effluent ammonia and nitrite under various organic shock loads, indicating that an organic shock load had no impact on nitrification if the DO was sufficient. As shown in Fig. 4(c), the effluent nitrate concentration decreased notably with the increase in organic loading. This may have been caused by the assimilation of ammonia to biomass cells. As the organic loading increased, more heterotrophic biomass would have formed, and then more ammonia was used for biomass synthesis. As a result, less nitrate was generated.

For fixed-film processes, when the effluent BOD was lower than 20 mg/L, good nitrification was achieved. However, a higher BOD concentration inhibited nitrification (Figueroa and Silverstein, 1992). This inhibition was probably caused by the less competition for oxygen and space of nitrifying bacteria (Figueroa and Silverstein, 1992; Li et al., 2002). As shown in Fig. 4(a), at the peak organic loading, the effluent BOD for all reactors was still lower than 20 mg/L, indicating that, when DO was sufficient, activated sludge processes had great capacity to handle organic shock loads. In addition, compared to the fixed-film processes, activated sludge processes provided better oxygen transfer within the mixed liquor (Metcalf & Eddy, 2003). As a result, the effect of organic shock load on nitrification was not significant.

3.5 Pilot-scale validation

In activated sludge processes, effluent ammonia or nitrite concentration was mainly impacted by SRT, DO, temperature, and ammonia shock load. From the bench-scale experiment, it was found that, at the 10-day and 20-day SRTs, effluent nitrite initially gave the first feedback on insufficient DO before the effluent ammonia and COD did, and, when DO was further reduced, the effluent ammonia gave the first feedback before the effluent nitrite and COD. At the 40-day SRT, the effluent ammonia gave the first feedback on insufficient DO before the nitrite and COD did. In addition, with all test SRTs, about 1 to 2 hours of peak loading did not lead to noticeable incomplete nitrification. Therefore, the combination of ammonia and nitrite has great potential to be used as the control parameter for aeration system operation when the SRT is larger than 10 days. However, the effect of low temperature was not tested in the bench-scale reactors. It is still possible that incomplete nitrification could occur at a low temperature, even when the DO is adequate. However, the adverse effect of low temperature on nitrification may be overcome by extending the SRT. A field pilot-scale experiment was conducted to validate the findings obtained from lab bench-scale experiments and to test the hypothesis that complete nitrification can still be achieved with a low temperature when the SRT is long enough.

3.5.1 Reactor performance

Fig. S1 (Supplementary data) shows the changes in temperature, DO, MLSS, influent COD and TN loads, effluent COD, effluent ammonia, and effluent nitrite in the field pilot-scale reactor. The lowest temperature occurred during the period from January to February when it was approximately 8 ˚C. The highest temperature of approximately 27 ˚C occurred in July and August. The pilot-scale reactor was fed with different flow rates over the experimental period. As a result, the influent organic and TN loads varied significantly and in the ranges of 3.5 – 59.3 kg-COD/d and 0.38 to 3.84 kg-N/d, respectively. During the experiment, constant aeration was provided. As a result of changes in the inflow rate, strength, and temperature, the DO varied from 0.1 to 8.7 mg/L. Before the middle of November, the SRT was 30 – 40 days and the MLSS ranged from 1.8 to 6.3 g/L (with an average value of 3.9 ± 1.07 g/L). To overcome the effect of low temperature on nitrification in winter, the SRT was increased to 60 - 80 days, starting from the middle of November, and the average MLSS concentration was increased to 6.5 ± 1.28 g/L.

As presented in Fig. S1, there was no significant change in the effluent COD concentration under various loading, temperature, and DO conditions. The average effluent COD was 26 ± 10.8 mg/L. The effluent nitrite concentration during the experiment varied slightly, from 0.02 to 1.8 mg-N/L. However, the fluctuation of effluent ammonia was significant, from 0.03 to 18.0 mg-N/L. The highest effluent ammonia occurred during the time period when the DO was very low, rather than when there were fluctuation of other conditions, such as low temperature and high loadings. Even though the temperature in the reactor was generally lower than 10 ˚C in December, complete nitrification was achieved. Therefore, DO was a more sensitive operational parameter than the other parameters considered (such as temperature and influent loading) as long as the SRT was appropriate.

3.5.2 Discussion on pilot-scale experiment

Fig. 5 shows the correlation between the effluent ammonia and nitrite and the operational DO. There was a clear trend that a high effluent ammonia concentration occurred when the DO was low, indicating that the DO was the predominant factor for effluent ammonia concentration. The nitrite concentration also followed the same trend, but this observation was not conclusive due to the low nitrite concentration range. In most cases, when the DO was lower than 1.5 mg/L, or even 0.5 mg/L, the effluent ammonia concentration was still lower than 1 mg-N/L. When aeration is controlled, based on a DO set-point, some energy would be wasted in providing unnecessary aeration if complete nitrification could be achieved at a DO lower than that set-point. As shown in Fig. 5, when the DO was higher than 2 mg/L, the effluent ammonia and nitrite were always lower than 0.5 mg-N/L, indicating that 0.5 mg-N/L could be used as the lower threshold value for aeration control. Once both effluent ammonia and nitrite were lower than 0.5 mg-N/L, aeration could be decreased. For this particular treatment system, the nitrite concentration was always lower than the ammonia concentration due to the extremely long SRT; therefore, nitrite control was unnecessary. However, for systems that have lower SRTs, the nitrite concentration could respond to a low DO first; therefore, both ammonia and nitrite would be needed to control the aeration system to an ensure appropriate effluent quality.

As shown in Fig. S1 and Fig. 5, only in the first several days was a high effluent nitrite (higher than 1 mg-N/L) detected. However, no nitrite accumulation was detected after July, even when the DO was lower than 0.5 mg/L. In addition, no nitrite had accumulated with a low temperature, as exhibited in Fig. S1. The results from the field pilot-scale experiment fully support the conclusion that nitrite would not accumulate with low DO, when the SRT was long enough. Fig. S1 clearly indicates that there was no significant difference in the effluent COD with various DO concentrations and, when the SRT was long enough, a low temperature did not significantly impact the effluent COD.

4. Discussion

As discussed previously, to support the concept that effluent ammonia, effluent nitrite, or a combination could be used as key control parameters for aeration system in activated sludge processes, three hypotheses needed to be validated: (a) effluent ammonia or nitrite would give the first feedback with an insufficient DO level; (b) insufficient DO is the only cause for incomplete nitrification; and (c) when the DO is excessive, effluent ammonia or nitrite will be lower than a certain level. From the lab bench-scale experiment, it was found that complete nitrification could not be achieved at the 5-day SRT. Therefore, to apply the proposed control strategy, the SRT must be longer than 5 days. At the 10-day and 20-day SRTs, the effluent nitrite accumulated before the effluent ammonia did when the DO was inadequate, while there was no COD accumulation with all test DO concentrations. It was considered likely that effluent nitrite could indicate insufficient DO better than effluent ammonia, within these SRTs. However, further reduction of the DO resulted in more accumulated ammonia than nitrite, due to inhibition of the first step of nitrification. Consequently, a combination of ammonia and nitrite would indicate an insufficient DO level more effectively than any individual parameter considered. At the 40-day SRT, effluent ammonia would give the first feedback of insufficient DO before effluent nitrite and COD did; no noticeable nitrite and COD accumulations were found in any of the tested DO levels. As a result, with a 40-day SRT, effluent ammonia can be used as the only indicator for insufficient DO, and the nitrite indicator can be disabled. With all tested SRTs and DO levels, there was no significant COD concentration change, even when incomplete nitrification occurred. Thus, hypothesis (a) was validated. However, the idea of using effluent ammonia and nitrite to indicate insufficient DO is not fully supported, even though hypothesis (a) has been validated. Concentrations of effluent ammonia or nitrite increase when the DO is not sufficient, but the increase in concentrations of effluent ammonia or nitrite can not certainly indicate insufficient DO since incomplete nitrification may occur under other conditions, including peak ammonia loading, peak organic loading, and a low temperature.

As shown in Fig. 3, when reactors with 10-day and 20-day SRTs had continuous peak ammonia loading, the nitrite accumulated, especially in the 10-day SRT reactor. With a 40-day SRT, nitrite accumulation only occurred at 6 times’ the ammonia loading, when applied continuously. However, for municipal wastewater treatment plants, peak loading generally occurred twice per day, and each peak loading lasted only about 1 – 2 hours, followed by a low loading period (Metcalf & Eddy, 2003). Within these short periods, the peak loading did not result in a noticeable increase in the effluent ammonia and nitrite concentrations. Therefore, ammonia-nitrite based control would not be interrupted by peak ammonia loading, although the adjustment of aeration intensity would be made automatically. Please note that peak ammonia loading and organic loading may overlap during certain time period for a treatment plant. However, as shown in Fig. 4, the peak organic loading did not impact the nitrification process; therefore, the effect of peak organic loading can be ignored. Based on the field experiment, low temperature (8 ˚C) did not lead to incomplete nitrification with a long SRT. Consequently, an insufficient DO was the only cause for incomplete nitrification when the SRT is long enough, thereby supporting hypothesis (b). With validation of hypotheses (a) and (b), the idea of using effluent ammonia and nitrite to indicate insufficient DO is fully supported, as long as the SRT is longer than 10 days. It should be noted that a long SRT would definitely help to reduce the risk of ammonia-nitrite based aeration control with continuous peak ammonia loading.

To fully support an ammonia-nitrite based aeration control approach, we have to validate that effluent ammonia and nitrite can report excessive aeration as well. The bench-scale experiment and pilot-scale field testing determined that, when the DO was higher than 2 mg/L, the effluent ammonia and nitrite were lower than 0.5 mg-N/L. Therefore, low effluent ammonia and nitrite could indicate excessive aeration, and a concentration of 0.5 mg-N/L could be used as the lower threshold value for aeration control. When both effluent ammonia and nitrite concentrations are lower than 0.5 mg-

N/L, the aeration intensity would decrease. Nevertheless, the upper threshold value of ammonia and nitrite need to be determined based on the discharge permitted. If complete nitrification is required, the value of 2 mg-N/L for effluent ammonia and nitrite could be set as the upper threshold value. Therefore, hypotheses (a), (b), and (c) are validated and a combination of effluent ammonia and nitrite can be used as an integrated control parameter for aeration intensity.

Currently, a DO set-point control has been widely used. Although it is relatively simple, it does not indicate effluent quality (Phillips and Fan, 2005; Metcalf & Eddy, 2003). In our bench-scale experiments, complete nitrification was obtained in the 40-day SRT reactor with a DO of around 0.5 mg/L. As shown in Fig. 5, in most cases the effluent ammonia and nitrite concentrations were lower than 0.5 mg-N/L at DO levels that were lower than 2 mg/L, indicating that the treatment system could be operated with a DO lower than 2 mg/L without reducing effluent quality. A lower DO concentration benefited oxygen transfer and resulted in significant energy savings, especially in the summer. For example, when the saturation DO was 7 mg/L in the summer, by reducing the operational DO from 2 mg/L to 0.5 mg/L the DO deficit in oxygen transfer increased from 5 mg/L to 6.5 mg/L, resulting in a 30% increase in oxygen transfer efficiency. The key for ammonia-nitrite based aeration control is that it can provide the exact amount of aeration based on required effluent quality, thereby preventing of excessive aeration. In the model-based predictive control or fuzzy control, complicated models or programs were used to track the level of DO control, with typical inputs of inflow rate, influent and effluent ammonia concentrations, temperature, biomass concentration, and defaulted kinetics parameters (Manesis et al., 1998; Ferrer et al., 1998; Galluzzo et al., 2001; Ma et al., 2006; Holenda et al., 2008). However, non-linear variations in influent loading, temperature, and biomass kinetics make model-based predictive control and fuzzy control strategies very complicated and too fragile to succeed. Using the simple ammonia- nitrite based control strategy (i.e., quality-based feed-back control approach), aeration is provided to just meet the required effluent quality, thereby optimizing the operation of the aeration system.

Although effluent ammonia and nitrite can be used to indicate effluent quality and as key control parameters for aeration system to optimize the treatment process, there are some practical difficulties in their use currently. Lack of sensitivity and a slow response are the key issues with current on-line ammonia sensing technology. When using ammonia gas sensing technology, a separate reactor with an automatic pH adjustment system needs to be constructed to convert ammonium into ammonia gas (Instrumentation Testing Association, 2001). A probe senses the ammonia gas concentration to give a reading. This entire process lasts several minutes, and the probe needs frequent calibration, either manually or automatically. When using ammonium ion selective electrode, interference from the potassium ion needs to be continuously corrected, which may easily result in measurement and/or calibration errors (Nico2000 Ltd. Landon, UK). On-line nitrite monitoring, however, seemed easier and a spectral in-situ UV sensor was successfully applied in the wastewater treatment processes (Rieger et al., 2008). Nevertheless, a combination of ammonia and nitrite indicates that the overall performance of the treatment plants are affected by DO levels, influent loading, temperature, etc., and can be used to control the aeration system to achieve the required treatment quality with minimum energy input.

5. Conclusions

The effluent ammonia, nitrite, and COD in the activated sludge process, resulting from various SRTs, DO levels, peak ammonia and COD loadings, and temperature, were studied using complete-mix reactors. When the DO was not sufficient at a 20-day SRT, or less, either the effluent nitrite or ammonia would increase before any other wastewater quality parameters did. However, at the 40-day SRT, effluent ammonia increased first in response to an insufficient DO condition. Reasonable ammonia and organic shock loads did not lead to incomplete nitrification, and the effect of low temperature on nitrification was overcome by extending the SRT. Therefore, when the SRT was more than 10 days, a combination of effluent ammonia and nitrite could effectively report insufficient or excessive DO. This integrated parameter can be used to control an aeration system, to assure the required effluent quality with the minimum aeration intensity. When complete nitrification is required, the lower and upper threshold values of effluent ammonia and nitrite concentrations can be set at 0.5 and 2.0 mg-N/L, respectively. When both effluent ammonia and nitrite concentrations are below 0.5 mg-N/L, the aeration should be decreased. When either of these parameters is greater than 2 mg-N/L, the aeration should be increased.

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References

Blackburne, R.; Yuan, Z.; Keller, J. (2008) Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. Biodegradation, 19 (2), 303–312.

Clesceri, L. S.; Greenberg, A.E.; Eaton, A. D. (1998) Standard methods for examination of water and wastewater, 20th edition. American Public Health Association, Washington, DC.

Cox, C. D. (2004) Statistical distribution of uncertainty and variability in activated sludge model parameters. Water Environ. Res., 76(7), 2672-2685.

Downing, L. S.; Nerenberg, R. (2008) Effect of bulk liquid BOD concentration on activity and microbial community structure of a nitrifying, membrane-aerated biofilm. Appl. Microbiol. Biotechnol., 81(1), 153-162.

Ferrer, J.; Rodrigo, M. A.; Seco, A. (1998) Energy saving in the aeration process by fuzzy logic control. Water Sci. Technol. 38 (3), 209-217.

Figueroa, L. A.; Silverstein, J. (1992) The effect of particulate organic matter on biofilm nitrification. Water Environ. Res., 64(5), 728 – 733.

Galluzzo, M.; Ducato, R.; Bartolozzi, V.; Picciotto, A. (2001) Expert control of DO in the aerobic reactor of an activated sludge process. Comput. Chem. Eng. 25(4-6), 619-625.

Grady, C.P.L., Jr.; Lim, H. C. (1980) Biological wastewater treatment. Marcel Dekker, New York, N.Y.

Hall, E. R.; Murphy, K. L. (1985) Sludge age and substrate effects on nitrification kinetics. Res. J. Water Pollut. C., 57(5), 413-418.

Holenda, B.; Domokos, E.; Rédey, Á.; Fazakas, J. (2008) Dissolved oxygen control of the activated sludge wastewater treatment process using model predictive control. Comput. Chem. Eng., 32(6), 1270–1278.

Instrumentation testing association. (2001) Online ammonia analyzers for water and wastewater treatment applications: performance evaluation report. Henderson, NV, USA.

Laanbroek, H. L.; Gerards, S. (1993) Competition for limiting amounts of oxygen between *Nitrosomonas europaea* and *Nitrobacter winogradskyi* grown in mixed continuous cultures. Arch. Microbiol., 159(5), 453-459.

Laanbroek, H. L.; Bodelier PLE.; Gerards, S. (1994) Oxygen consumption kinetics of Nitrosomonas europaea and Nitrobacter hamburgensis grown in mixed continuous cultures at different oxygen concentrations. Arch. Microbiol., 161(2): 156-162.

Li, J.; Luan, Z.; Zhu, B.; Gong, Z.; Peng, D. (2002) Effects of colloidal organic matter on nitrification and composition of extracellular polymeric substances in biofilms. J. Chem. Tech. Biot., 77(12),1333–1339.

Ma, Y.; Peng, Y. Z.; Wang, X. L.; Wang, S. Y. (2006) Intelligent control aeration and external carbon addition for improving nitrogen removal. Environ. Modell. Softw., 21(6), 821-828.

Manesis, S.A.; Sapidis, D.J.; King, R.E. (1998) Intelligent control of wastewater treatment plants. Artif. Intell. Eng. 12 (3), 275-281.

McCarty, P.L.; Bae, J.; Kim, J. (2011) Domestic wastewater treatment as a net energy producer – can this be achieved? Environ. Sci. Tech., 45(17), 7100-7106.

Metcalf and Eddy Inc. (2003) Wastewater Engineering: Treatment and Reuse, 4th ed. McGraw-Hill, New York.

Nico2000 Ltd. Landon, UK. Technical specifications for the ammonium ion-selective electrode. http://www.nico2000.net/analytical/ammonium.htm#lowk

Phillips, D. L.; Fan, M. M. (2005) Aeration control using continuous dissolved oxygen monitoring in an activated sludge wastewater treatment process. Proceedings of the Water Environment Federation, 1617-1629.

Rieger, L.; Langergraber, G.; Kaelin, D.; Siegrist, H.; Vanrolleghem, P.A. (2008) Long-term evaluation of a spectral sensor for nitrite and nitrate. Water Sci. Tech., 57(10), 1563-1569.

Sharma, R.; Gupta SK. (2004) Influence of chemical oxygen demand/total Kjeldahl nitrogen ratio and sludge age on nitrification of nitrogenous wastewater. Water Environ. Res., 76(2), 155-161.

Yu,T.; Qi, R.; Li, D.; Zhang, Y.; Yang M. (2010) Nitrifier characteristics in submerged membrane bioreactors under different sludge retention times. Water Res., 44(9), 2823-2830.

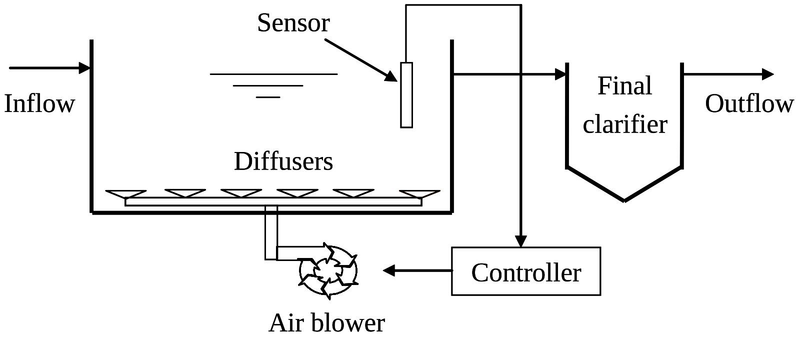

Fig. 1 – Scheme of the proposed aeration control strategy based on effluent ammonia and/or nitrite.

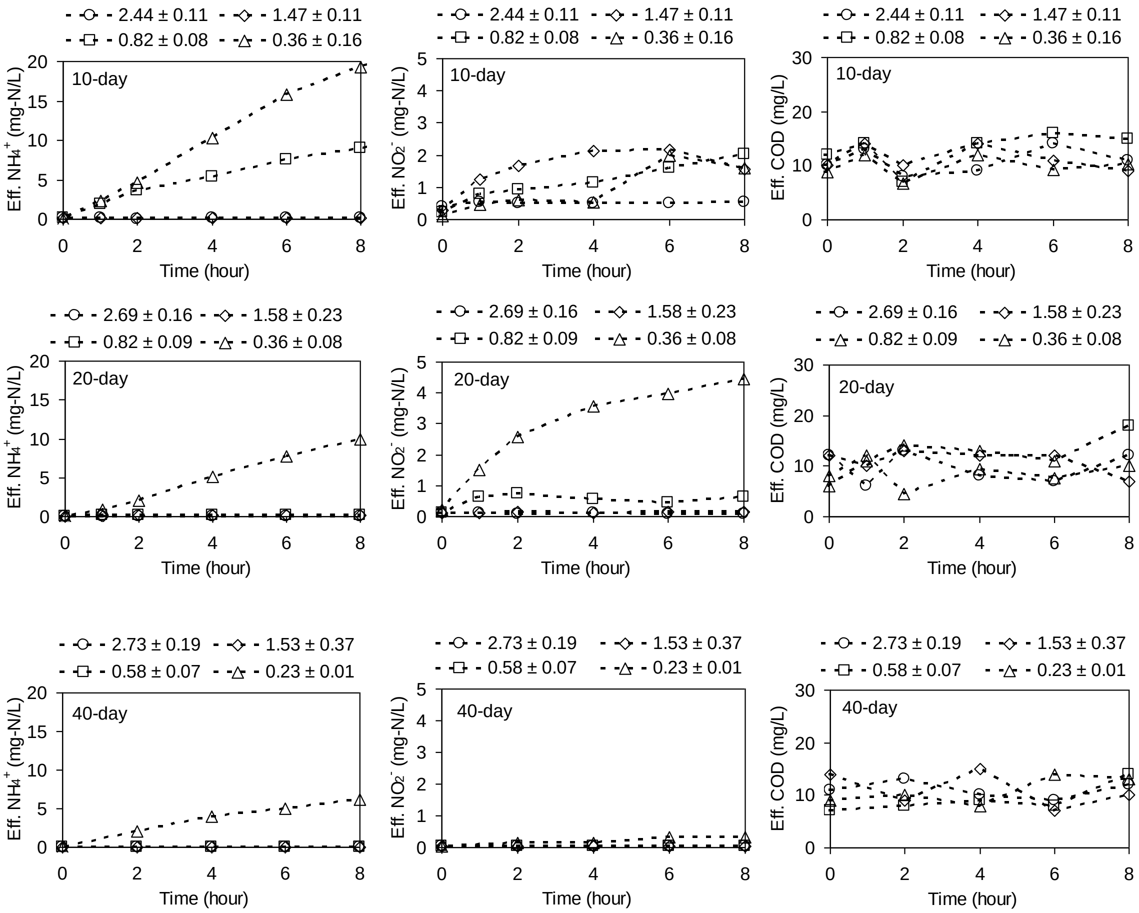

Fig. 2 – Effect of reduced DO on effluent COD, ammonia and nitrite for reactors with 10-, 20- and 40-day SRTs (pH = 7.0-7.5, Temperature =20.5 ± 1 ˚C).

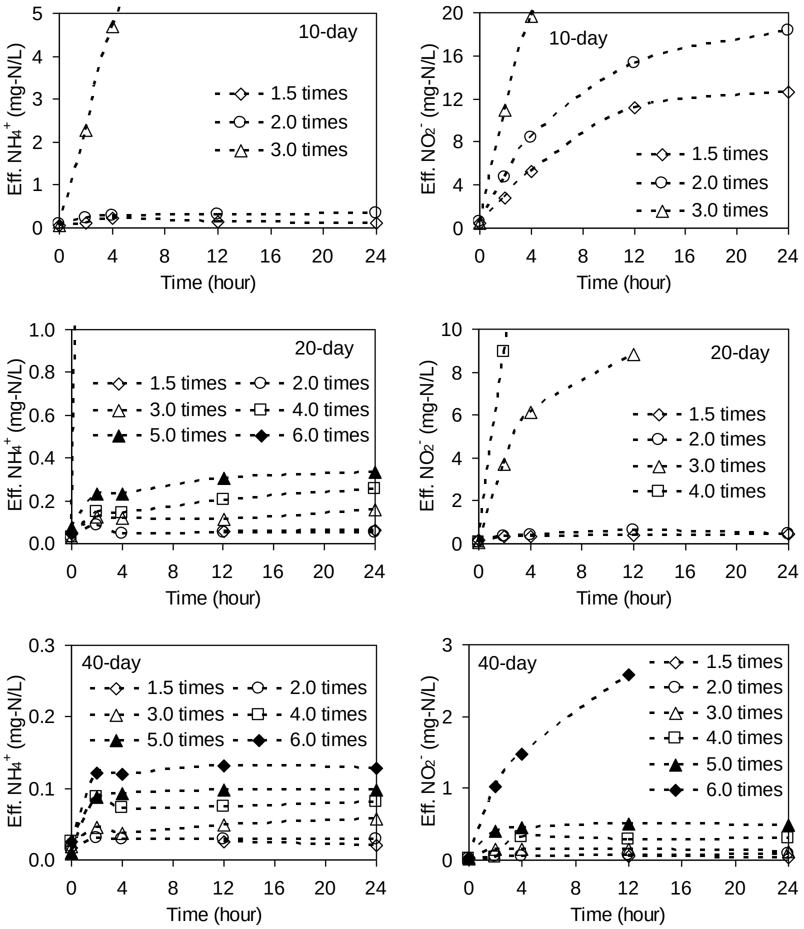

Fig. 3 – Effect of ammonia shock load on effluent ammonia and nitrite for reactors with 10-, 20- and 40-day SRTs (DO > 4 mg/L, pH = 7.0-7.5, Temperature =20.5 ± 1 ˚C).

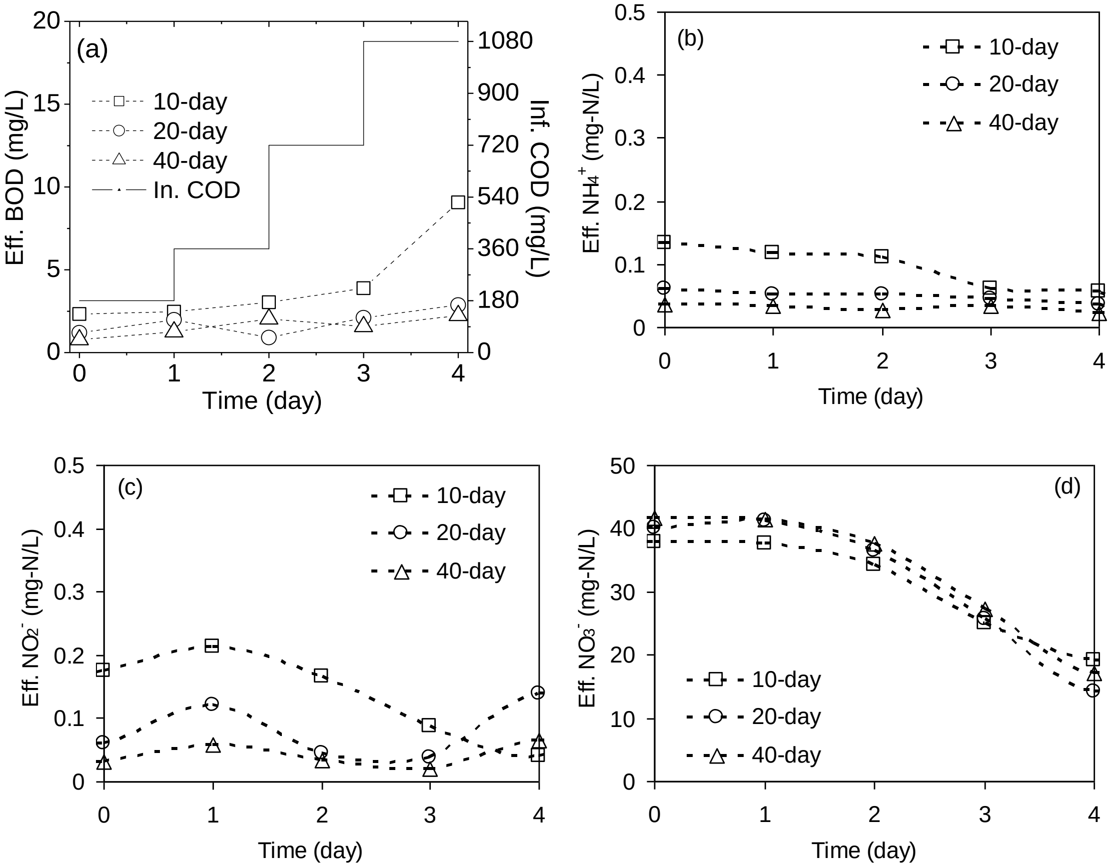

Fig. 4 – Effect of organic shock load on (a) effluent BOD, (b) ammonia, (c) nitrite and (d) nitrate in reactors with 10-, 20- and 40-day SRTs (DO > 4 mg/L, pH = 7.0-7.5, Temperature =20.5 ± 1 ˚C).

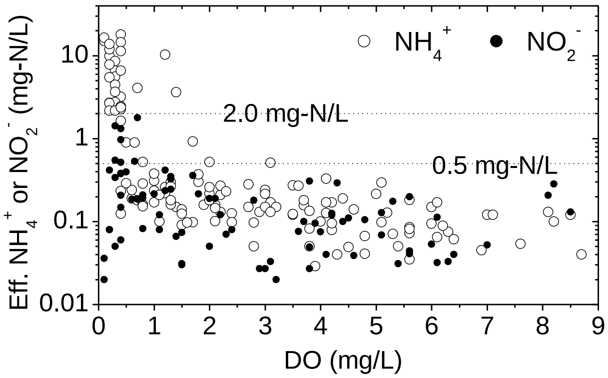

Fig. 5 – Correlations of effluent ammonia and nitrite with DO.

Table 1 – Steady-state effluent quality with different SRTs (Mean ± stdev, n ≥ 15) (DO > 4 mg/L, pH = 7.0-7.5, Temperature = 20.5 ± 1 ˚C).

SRT (day)	NH_4^+-N (mg-N/L)	NO_2^--N (mg-N/L)	NO_3^--N (mg-N/L)	BOD_5 (mg/L)
5	2.6 ± 2.3	9.2 ± 3.4	32.0 ± 4.1	4.3 ± 1.4
10	0.04 ± 0.01	0.15 ± 0.05	41.9 ± 0.9	2.2 ± 0.6
20	0.03 ± 0.007	0.04 ± 0.01	43.2 ± 0.8	1.6 ± 0.3
40	0.02 ± 0.003	0.01 ± 0.004	44.4 ± 1.4	1.7 ± 0.5

Supplementary data

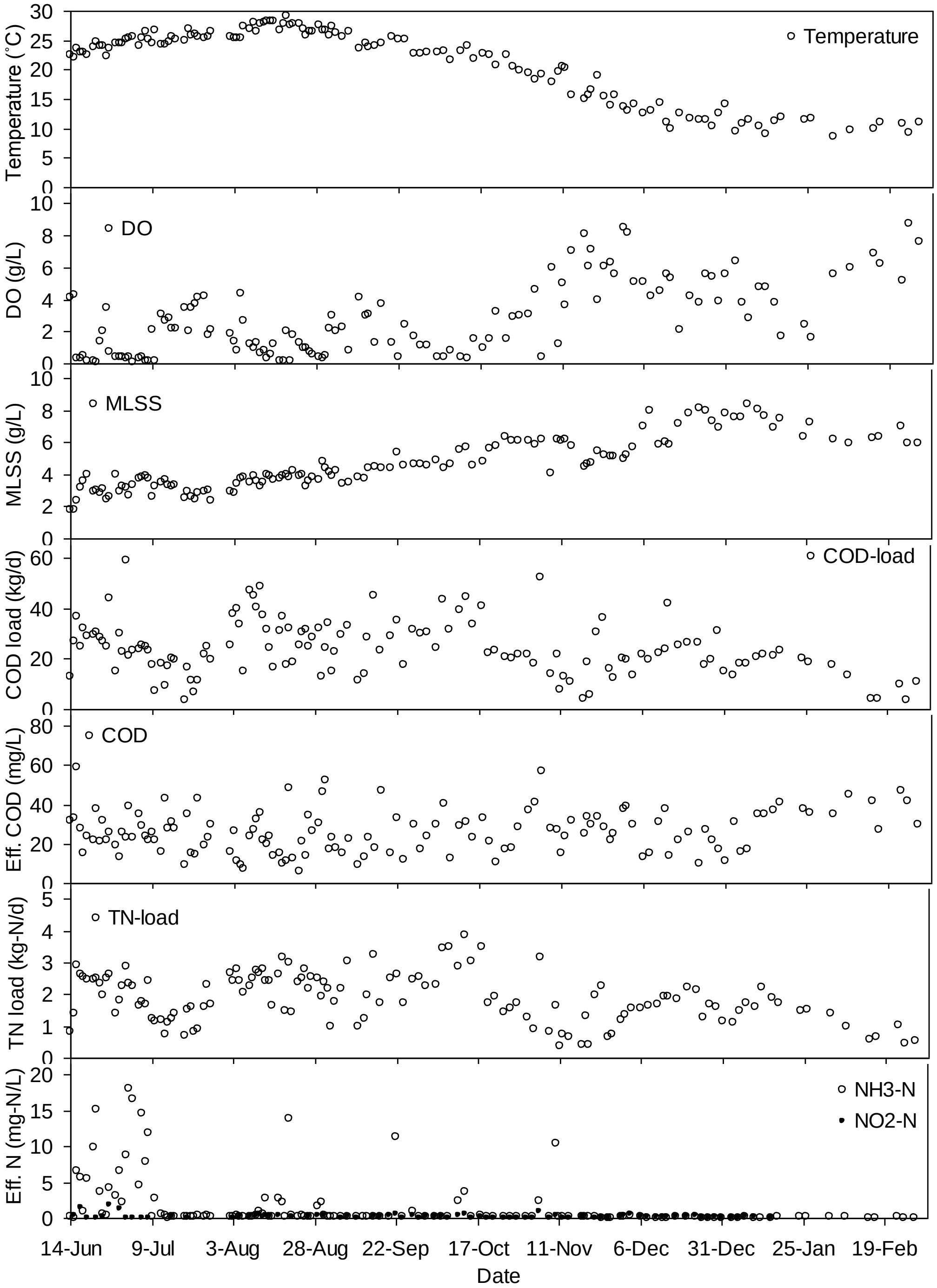

Fig. S1 – Temperature, DO concentration, MLSS concentration, influent COD and TN loadings, and effluent COD, ammonia, and nitrite concentrations in the reactor during field experiment.

SECTION

4. CONCLUSIONS

The primary results of this work are reported in three manuscripts for publication in peer-reviewed journals. Conclusions from this work have been reported in each paper, respectively, and are also presented below.

Objective 1: To probe the stoichiometry of the autotrophic nitrification process using a respirometric approach.

This objective is met and the results are shown in paper I. The results can be concluded as following:

(1) More accurate stoichiometric links between biomass yield, ammonia and nitrite oxidized, ammonia assimilated, and oxygen uptake for each step of the nitrification process were developed.

(2) The specific oxygen uptake was 4.23 mg-O_2/mg-N oxidized for complete nitrification, with 3.17 mg-O_2/mg-N oxidized for ammonia oxidation and 1.06 mg-O2/mg-N oxidized for nitrite oxidation.

(3) The fractions of electrons transferred into cell synthesis were approximately 7.5% for ammonia oxidation and 7.3% for nitrite oxidation.

(4) Biomass yield coefficients for ammonia oxidizers and nitrite oxidizers were approximately 0.18 and 0.06 g-VSS/g-N oxidized, respectively.

Objective 2: To quantify the combined effect of SRT and DO on nitrification performance: effluent ammonia and nitrite concentrations and AOB and NOB biomass concentrations (represented by biomass maximum ammonia and nitrite oxidation rates) This objective is met and the results are shown in paper II. The results can be concluded as following:

(1) Complete nitrification can not be achieved in the 5-day SRT reactor even when the DO is unlimited. In the 10, 20, and 40-day SRT reactors, complete nitrification was almost achieved with a low DO of 0.37, 0.25, and 0.16 mg/L, respectively.

(2) When the DO was unlimited, ammonia-oxidizing bacteria (AOB) had a higher specific growth rate and endogenous decay coefficient than the nitrite-oxidizing bacteria (NOB). As a result, AOB had an advantage over NOB under a short SRT, while NOB had an advantage under a long SRT.

(3) Under low DO conditions, the endogenous decay of AOB and NOB was inhibited and then their biomass concentration increased, thereby reducing the adverse effect of low DO on nitrification.

(4) Under long-term low DO conditions, NOB became a better competitor than AOB since the oxygen affinity of NOB increased significantly.

Objective 3: To compare the oxygen demand and aeration needs under different SRT and DO levels

This objective is met and the results are shown in paper II. The results can be concluded as following:

(1) Compared to a baseline condition (SRT = 10 days and DO = 2 mg/L), aeration need was reduced by about 20% under these two conditions (SRT = 10 days and DO = 0.37 mg/L) and (SRT = 40 days and DO = 0.16 mg/L).

(2) The reduction of aeration was mainly due to a higher sludge production and higher oxygen transfer efficiency under low DO conditions.

(3) In the reactor with 20-day SRT, it was found that the boom of filamentous bacteria reduced the oxygen transfer efficiency dramatically.

Objective 4: To elucidate the effect of DO and SRT on nitrifying bacteria communities

(1) *Nitrosomonas europaea/eutropha* – like AOB were dominant with all tested SRTs and DO levels.

(2) *Nitrobacter*-like NOB played the main role in nitrite oxidation in the 5-day SRT reactor, while *Nitrospira*-like NOB played the main role in the 40-day SRT reactor.

(3) When the DO was reduced to ≤ 0.5 mg/L, the number of *Nitrospira* increased considerably in all reactors, while *Nitrobacter* had no change in the 20 and 40-day SRT reactors.

Objective 5: To evaluate a potential aeration control strategy of using effluent ammonia or nitrite as the only parameter for aeration control

(1) This integrated parameter (combination of effluent ammonia and nitrite) has great potential to be used for aeration system control, to assure the required effluent quality with the minimum aeration intensity.

(2) When complete nitrification is required, the lower and upper threshold values of effluent ammonia and nitrite concentrations can be set at 0.5 and 2.0 mg-N/L, respectively. When both effluent ammonia and nitrite concentrations are below 0.5 mg-N/L, the aeration should be decreased. When either of these parameters is greater than 2 mg-N/L, the aeration should be increased.

5. SIGNIFICANCE AND IMPACT

In the field of biological wastewater treatment, the roles of SRT and DO concentration in nitrification have been studied separately in great detail. To the best of our knowledge, however, this is the first time to study the combined effects of SRT and DO concentration on nitrification, nitrifying bacterial communities, and aeration needs in the activated sludge process.

Previously, to achieve complete nitrification in the activated sludge process, the DO concentration was recommended to maintain at about 2 mg/L. However, in this study, we find nitrification can be completed with a DO around 0.2 mg/L. A low operational DO can reduce the aeration need significantly and help nutrients removal in the advanced activated sludge processes.

In this research, all the kinetic parameters for ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) are determined and then the performance of AOB and NOB in the activated sludge process under different SRTs and DO levels can be better understood. Previous studies generally focused on the effect of low DO concentration on the growth of nitrifying bacteria and few studies were conducted on the effect of low DO concentration on nitrifying bacteria endogenous decay. In this research, it is found that the process of nitrifier endogenous decay also plays a very important role. Under low DO conditions, the decay of nitrifying bacteria will be slowed down and then the nitrifier biomass concentration will increase, thereby reducing the adverse effect of low DO on nitrification. In previous reports, NOB were thought to be more sensitive to the low DO concentrations. In this research, under long-term low DO conditions, NOB can increase their oxygen affinity significantly and finally, NOB become the better competitor for oxygen than AOB.

Finally, a potential aeration control strategy is developed in this research. It is much simpler than the DO-set point or model-based control strategies. Theoretically, with this control strategy, the process can meet treatment requirements with a minimum aeration input.

In summary, this research provides a deeper understanding of nitrification performance under low DO conditions, which will lead to great advancement for the operation of wastewater treatment plant and novel technology development for significant energy saving and environmental sustainability during wastewater treatment.

6. FUTURE WORK

In this work, NOB increased their oxygen affinity significantly under long-term low DO conditions. To better understand the mechanisms, it is interesting to do a deeper analysis on the change of NOB community. Possibly, the sublineages with a high oxygen affinity in the group of NOB are selected under long-term low DO conditions.

In this work, the boom of filamentous bacteria in the 20-day SRT reactor inhibited the oxygen transfer considerably. Therefore, it is interesting to identify the filamentous bacteria which have inhibited the oxygen transfer.

Under low DO conditions, aeration can be reduced, while a low DO concentration may favor the growth of filamentous bacteria, which will inhibit the oxygen transfer. Possibly, a low operational DO will not lead to aeration saving. Therefore, it is necessary to study the control of sludge bulking problems under low DO conditions.

In this work, it is found that the combination of effluent ammonia and nitrite has great potential to be used as the only aeration control parameter in activated sludge process. But this control approach is not practiced in this study. It is necessary to set up this approach and evaluate its performance in a pilot-scale wastewater treatment process.

BIBLIOGRAPHY

Abbassi, B., Dullstein, S., Räbiger, N. Minimization of excess sludge production by increase of oxygen concentration in activated sludge flocs; experimental and theoretical approach. Water Research 1999, 34(1), 139-146.

Bellucci M., Ofiteru, I. D., Graham, D. W., Head, I. M., Curtis, T. P. Low-dissolved-oxygen nitrifying systems exploit ammonia-oxidizing bacteria with unusually high yields. Applied and Environmental Microbiology 2011, 77(21), 7787-7796.

Blackburne R., Yuan Z., Keller J. Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. Biodegradation 2008, 19(2), 303-312.

Cox, C. D. Statistical distribution of uncertainty and variability in activated sludge model parameters. Water Environmental Research 2004, 76(7), 2672-2685.

Daims, H., Nielsen, P. H., Nielsen, J., Juretschko, S., Wagner, M. Novel nitrospira-like bacteria as dominant nitrite-oxidizer in bioflims from wastewater treatment plants: Diversity and in situ physiology. Water Science and Technology 2000, 41, 85-90.

de Bie, M. J. M., Speksnijder, A.G. C. L., Kowalchuk, G. A., Schuurman, T., Zwart, G., Stephen, J. R., Diekmann, O. E., Laanbroek, H. J. Shifts in the dominant populations of ammonia-oxidizing β-subclass Proteobacteria along the eutrophic Schelde estuary. Aquatic Microbial Ecology 2001, 23, 225–236.

Downing, A. L., Painter, H. A., Knowles, G. Nitrification in the activated sludge process. Journal of the Institute of Sewage Purification 1964, 64(2), 130-158.

Downing, A. L., Scagg, L. J. The effect of synthetic detergents on the rate of aeration in diffused air activated sludge plants. Water Waste Treatment Journal 1958, 7, 102.

Dytczak, M., Londry, K. L., Oleszkiewicz, J. A. Activated sludge operational regime has significant impact on the type of nitrifying community and its nitrification rates. Water Research 2008, 42(8-9), 2320-2328.

Ferrer, J.; Rodrigo, M. A.; Seco, A. Energy saving in the aeration process by fuzzy logic control. Water Science and Technology 1998, 38 (3), 209-217.

Galluzzo, M.; Ducato, R.; Bartolozzi, V.; Picciotto, A. Expert control of DO in the aerobic reactor of an activated sludge process. Computer & Chemical Engineering 2001, 25(4-6), 619-625.

Gieseke, A., Purkhold, U., Wagner, M., Amann, R., Schramm, A. Community structure and activity dynamics of nitrifying bacteria in a phosphate-removing biofilm. Applied Environmental Microbiology 2001, 67(3): 1351-1362.

Grady, C.P.L., Jr.; Lim, H. C. Biological wastewater treatment. Marcel Dekker, New York, N.Y., 1980.

Hanaki, K., Wantawin,C., Ohgaki,S. Nitrification at low levels of dissolved oxygen with and without organic loading in a suspended-growth reactor. Water Research 1989, 24(3), 297-302.

Henze M., Gujer W., Mino T., Loosdrecht M. van. Activated sludge models ASM1, ASM2, ASM2d and ASM3. IWA publishing, Alliance House London UK., 2000.

Hocaoglu, S. M., Insel, G., Cokgor, E. U., Orhon, D. Effect of sludge age on simultaneous nitrification and denitrification in membrane bioreactor. Bioresource Technology 2011, 102(12), 6665-6672.

Holenda, B.; Domokos, E.; Rédey, Á.; Fazakas, J. Dissolved oxygen control of the activated sludge wastewater treatment process using model predictive control. Computer & Chemical Engineering 2008, 32(6), 1270–1278.

Kaelin, D., Manser, R., Rieger, L., Eugster, J., Rottermann, K., Siegrist, H. Extension of ASM3 for two-step nitrification and denitrification and its calibration and validation with batch tests and pilot scale data. Water Research 2009, 43(6), 1680-1692.

Kim D. J., Kim S. H. Effect of nitrite concentration on the distribution and competition of nitrite-oxidzing bacteria in nitratation reactor systems and their kinetics characteristics. Water Research 2006, 40, 887-894.

Koops HP., Pommerening-Röser, A. Distribution and ecophysiology of nitrifying bacteria emphasizing culture species. FEMS Microbiology Ecology 2001, 37, 1-9.

Laanbroek H. J., Bodelier P. L. E., Gerards S. Oxygen consumption kinetics of *Nitrosomonas europaea* and *Nitrobacter hamburgensis* grown in mixed continuous cultures at different oxygen concentrations. Archives of Microbiology 1994, 161(2), 156-162.

Laanbroek, H. L.; Gerards, S. Competition for limiting amounts of oxygen between *Nitrosomonas europaea* and *Nitrobacter winogradskyi* grown in mixed continuous cultures. Archives of Microbiology 1993, 159(5), 453-459.

Li B., Irvin S., Baker K. The variation of nitrifying bacterial population sizes in a sequencing batch reactor (SBR) treating low, mid, high concentrated synthetic wastewater. Journal of Environmental Engineering and Science 2007, 6, 651-663.

Lim J., Do H., Shin S. G., Hwang S. Primer and probe sets for group-specific quantification of the genera Nitrosomonas and Nitrosospira using real-time PCR. Biotechnology and Bioengineering 2007, 99, 1374-1382.

Limpiyakorn T., Shinohara Y., Kurisu F., Yagi O. Communities of ammonia-oxidizing bacteria in activated sludge of various sewage treatment plants in Tokyo. FEMS Microbiology Ecology 2005, 54(2), 205-217.

Ma, Y.; Peng, Y. Z.; Wang, X. L.; Wang, S. Y. Intelligent control aeration and external carbon addition for improving nitrogen removal. Environmental Modelling and Software 2006, 21(6), 821-828.

Manesis, S.A.; Sapidis, D.J.; King, R.E. Intelligent control of wastewater treatment plants. Artificial Intelligence in Engineering 1998, 12 (3), 275-281.

Manser, R., Gujer, W., Siegrist, H. Consequences of mass transfer effects on the inetics of nitrifiers. Water Research 2005, 39(19), 4633-4642.

McCarty, P.L.; Bae, J.; Kim, J. Domestic wastewater treatment as a net energy producer – can this be achieved? Environmental Science and Technology 2011, 45(17), 7100-7106.

Metcalf and Eddy Inc. Wastewater Engineering: Treatment and Reuse, 4th ed. McGraw-Hill, New York, 2003.

Nagel, C. A., Haworth, J. G. Operational factors affecting nitrification in the activated sludge process. In 42nd Annual Conference WPCF, Dallas, Tex, 1969.

Park S., Bae W., Rittmann B. E. Operational boundaries for nitrite accumulation in nitrification based on minimum/maximum substrate concentrations that include effects of oxygen limitation, pH, and free ammonia and free nitrous acid inhibition. Environmental Science Technology 2010, 44(1), 335-42.

Park, H. D., Wells, G. F., Bae, H., Criddle, C. S., Francis, C. A. Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. Applied and Environmental Microbiology 2006, 72(8), 5643-5647.

Park, H. P., Noguera, D.R. Evaluating of the effect of dissolved oxygen on ammonia-oxidizing bacterial communities in activated sludge. Water Research 2004, 38(14-15), 3275-3286.

Phillips, D. L.; Fan, M. M. Aeration control using continuous dissolved oxygen monitoring in an activated sludge wastewater treatment process. Proceedings of the Water Environment Federation 2005, 1617-1629.

Schramm A., de Beer, D., van den Heuvel, J. C., Ottengraf S., Amann, R. Microscale distribution of populations and activities of *Nitrosospira* and *Nitrospira* spp. along a macroscale gradient in a nitrifying bioreactor: quantification by in situ hybridization and the use of microsensors. Applied Environmental Microbiology 1999, 65(8), 3690–3696.

Siripong S., Rittmann B. E. Diversity study of nitrifying bacteria in full-scale municipal wastewater treatment plants. Water Research 2007, 41(5), 1110-1120.

Sliekers, A. O., Haaijer, S. C., Stafsnes, M. H., Kuenen, J. G., Jetten, M. S. Competition and coexistence of aerobic ammonium- and nitrite-oxidizing bacteria at low oxygen concentrations. Applied Microbiology Biotechnology 2005, 68(6), 808-817.

Sonthiphand, P., Limpiyakorn T. Change in ammonia-oxidizing microorganisms in enriched nitrifying activated sludge. Applied Microbial Biotechnology 2011, 89(3), 843-853.

Stankewich, M. J. J. Biological nitrification with the high purity oxygenation process. In 27th annual purdue industrial waste conference. West Lafayette IN: Purdue University, 1972, Pp. 1-23.

Stenstrom, M. K. The effect of dissolved oxygen concentration on nitrification. Water Research 1980, 14(6), 643-649.

Teske A., Alm E., Regan J. M., Toze S., Rittmann B. E., Stahl D. A. Evolutionary relationship among ammonia-oxidizing and nitrite oxidizing bacteria. Journal of Bacteriology 1994, 176, 6623-6630.

Weon, S.Y., Lee, S. I., Koopman, B. Effect of temperature and dissolved oxygen on biological nitrification at high ammonia concentrations. Environmental Technology 2004, 25(11), 1211-1219.

Wild, H. E., Sawyer, C. N., Mcmahon, T. D. Facters affecting nitrifictaion kinetics. Journal Water Pollution Control Federation 1971, 43(9), 1845-1854.

You J., Das, A., Dolan, E. M., Hu, Z. Ammonia-oxidizing archaea involved in nitrogen removal. Water Research 2009, 43(7), 1801-1809.

Yu T., Qi R., Li D., Zhang Y., Yang M. Nitrifier characteristics in submerged membrane bioreactor under different sludge retention time. Water Research 2010, 44(9), 2823-2830.

Zhang, T., Jin, T., Yan Q., Shao, M., Wells G., Criddle C., Fang, H. H. P. Occurrence of ammonia-oxidizing Archaea in activated sludges of a laboratory scale reactor and two wastewater treatment plants. Journal of Applied Microbiology 2009, 107(3), 970-977.

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