Due to the potential benefits on energy/cost savings from partial nitrification, this study carried a series of laboratory tests to study the combined influence from water temperature, organic carbon and dissolved oxygen (DO) on partial nitrification. The purpose of this study was to obtain a general direction to operate activated sludge systems for partial nitrification under changing environment conditions and wastewater strengths. The time profiles of the mixed liquor NO$_2$-N and NO$_3$-N during a 11 h aeration phase were monitored in a 6 L bench scale sequencing batch reactor (SBR) under varying water temperatures (20°C to 30°C), organic carbon abundances (initial mixed liquor COD/N ratio ranged 2.8 to 14), and DO levels (0.4 to 4.0 mg L$^{-1}$). Results showed that organic carbon abundance should be firstly lowered to below a certain threshold level (14 in this study) in order to allow nitrification to occur. Then, water temperature should be kept at relatively higher levels (at least 30°C) and DO should be controlled at relatively lower levels (depending on aeration duration). However, the adverse effect on partial nitrification from low water temperatures can be compensated by a low DO, while the favoring effect on partial nitrification from high water temperatures can be reversed by a high DO and/or excess aeration. Although it was not the focus of this study, simultaneous nitrification and denitrification (SND) was also indicated viable when organic carbon abundance was kept at relatively higher levels but still below the inhibitory level for nitrification. Successive study is recommended to further quantify the interactions between these influential factors on partial nitrification and to improve and better understand the reliability and sensitivity of activated sludge systems in achieving partial nitrification under normally always changing reaction conditions. The overall conclusion is that partial nitrification is under normally always changing reaction conditions.

Key words: Best management practice, bioreactor operation, nitrogen removal, wastewater, water temperatures, organic carbon abundances, dissolved oxygen (DO).

INTRODUCTION

Nitrification (NH$_4^+$-N $\rightarrow$ NO$_2^-$-N $\rightarrow$ NO$_3^-$-N) followed by denitrification (NO$_3^-$-N $\rightarrow$ N$_2$) has been adopted as a standard practice by the wastewater industrial to achieve nitrogen removal, and nitrification and denitrification are often spatially separated as two independent phases and conducted in different reactors (Rittmann and McCarty, 2000; Robertson et al., 1988; VanNiel, 1991; Yoo et al., 1999). For engineering convenience, aeration for nitrification is also often designed without taking the oxygen credits from the following denitrification. However, 25% reduction in aeration requirements and 40% reduction in carbon requirements could be achieved theoretically if biological nitrification process can be regulated to stop at NO$_2^-$ (partial nitrification) and let following denitrification to be carried via NO$_2^-$ (Abeling and Seyfried, 1992; Fux et al., 2003; Turk and Mavinic, 1986; Yoo et al., 1999). As energy consumption is becoming a major issue for the modern human society, energy/cost saving from wastewater treatment naturally becomes the interest of...
Quite a few strategies have been tested in the past decade to favor partial nitrification (to enhance NO$_3^-$ production instead of NO$_2^-$). Some typical examples are the combination of Single Reactor for High Ammonia Removal Over Nitrite (SHARON) (Hellenga et al., 1998; Jetten et al., 1997) and Anaerobic Ammonium Oxidation (ANAMMOX) (Mulder et al., 1995; Schmidt et al., 2003), Completely Autotrophic Nitrogen Removal Over Nitrite (CANON) (Sliekers et al., 2002; Strous et al., 1997), and Oxygen-Limited Autotrophic Nitrification-Denitrification (OLAND) (Kuai and Verstraete, 1998). However, even with these reported successes, controlling nitrification to stop at NO$_2^-$ has been consistently considered as the most challenging step (Weissenbacher et al., 2007; Yoo et al., 1999). Furthermore, since demographical and geographical variations characterize an important feature of wastewater, the robustness of such systems is also frequently questioned for their practical applications (Fux et al., 2003; Mulder, 2003).

Temperature, dissolved oxygen (DO), and organic carbon abundance have been identified as influential factors for the activities of nitrifying bacteria. It has been observed that ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) have different tolerances for temperature changes (Jetten et al., 1997). AOB can sustain at temperatures higher than 28°C while NOB activities start to be inhibited (Jetten et al., 1997; Wu et al., 2007). AOB and NOB were also observed to have different oxygen sensitivities with AOB can sustain better than NOB at lower DO conditions (Balmelle et al., 1992; Peng et al., 2007; Wu et al., 2007). Regulating aeration on/off based on mixed liquor oxidation reduction potential (ORP) and pH levels has been tested at laboratory scales to prevent excess aeration so as to favor partial nitrification (Gao et al., 2009; Guo et al., 2009).

It has been observed that there exists a competition for ammonium nitrogen (NH$_4^+$-N) between nitrifying bacteria and heterotrophic bacteria within the microbial communities of activated sludge systems (Strauss and Lamberti, 2000; Verhagen and Laanbroek, 1991). A critical ratio of organic carbon to nitrogen (normally Total Kjeldahl Nitrogen plus NH$_4^+$-N) exists. Below this critical ratio, heterotrophic bacteria are not N-limited and nitrifying bacteria are able to compete successfully for NH$_4^+$-N so as to allow nitrification to occur (Strauss and Lamberti, 2000; Verhagen and Laanbroek, 1991). Thus, an environment of low organic carbon loading is considered more favorable for partial nitrification compared to the higher ones (Ahn, 2006; Khin and Annachhatre, 2004; Mosquera-Corral et al., 2005). Generally speaking, the different level activities of AOB and NOB under changing environment conditions are often the main cause of observing different NO$_2^-$ and NO$_3^-$ production levels (Cecen and Gonenc, 1994; Peng et al., 2007; Turk and Mavinic, 1986).

The purpose of this study was to obtain a general direction to operate activated sludge systems for partial nitrification under changing environment conditions and wastewater strengths. Due to the geographical and demographical nature of wastewater, there is essentially no such a system that can achieve the treatment requirement at only one unique preset working point. Therefore, the results of this study should not be interpreted as a universal or paramount panacea to guarantee the establishment of partial nitrification.

**MATERIALS AND METHODS**

**Experiment apparatus**

The experimental SBR was made of a polymethyl methacrylate cylinder of 30 cm height and 20 cm inside diameter. The reactor was put on a heating plate (Thermo Scientific, USA) to maintain a constant water temperature and was aerated for 11 h with its DO being maintained at 2 mg/L. The room temperature was also controlled during each test. A magnetic stirrer was also used to mix the liquor during the aeration phase. The air flow was adjusted by an inline air flow regulator to maintain the target DO level for each test. A DO meter was also used to monitor the DO concentration during each treatment stage. The DO and water temperature were measured with a DO meter (YSI® Model 52). Water pH was measured by a pH meter (Orion® Model 828).

**Sludge seeding, SBR startup and operation**

The sludge used for this study was obtained from the Harbin Municipal Wastewater Treatment Plant, the City of Harbin, Heilongjiang Province, China. The plant adopts an A/O process and its receiving wastewater contains roughly 220 mg L$^{-1}$ biological oxygen demand (BOD$_5$) and 50 mg L$^{-1}$ NH$_4^+$-N at an annual average basis. The sludge used in this study was taken from the stage “O” (oxic). The wastewater used in this study was synthesized. Ammonium chloride (NH$_4$Cl) was used as the nitrogen source and sodium acetate (NaAc) was used as the carbon source. Other ingredients (mg L$^{-1}$) were: CaCl$_2$, 10; KH$_2$PO$_4$, 22; MgSO$_4$·7H$_2$O, 50; NaHCO$_3$, 3.4 g l$^{-1}$ NH$_4^+$-N. Trace element solution was also supplied into the synthetic wastewater at 1 ml L$^{-1}$ with the following ingredients (g L$^{-1}$): EDTA, 57.1; ZnSO$_4$·7H$_2$O, 3.9; CaCl$_2$·2H$_2$O, 7.0; MnCl$_2$·4H$_2$O, 5.1; FeSO$_4$·7H$_2$O, 5.0; (NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O, 1.1; CuSO$_4$·5H$_2$O, 1.6; CoCl$_2$·6H$_2$O, 1.6. The final water pH was adjusted to approximately 7.0 by either NaOH or HCl.

The sludge seeding was started by first mixing 5-L fresh activated sludge with 1-L synthetic wastewater in the reactor. Then the reactor was aerated for 11 h with its DO being maintained at approximately 2.0 mg L$^{-1}$. After this, the mixed liquor was settled for 1 h and the 3-L supernatant was decanted. After the initial sludge seeding, the reactor was continuously operated twice a day with 3 L fresh wastewater flash filling, 11 h aeration, 1 h sedimentation, and 3 L supernatant decant (time negligible). The volumetric exchange ratio (VER) was kept at 50%. The reactor temperature was maintained at 25°C and the pH of the mixed liquor was only monitored but not controlled (ranged 7.0 to 7.8). Ammonium nitrogen (NH$_4^+$-N) in the feeding synthetic wastewater was gradually lowered from 150 to 50 mg L$^{-1}$ during the first 10 days, with the carbon source being also adjusted to keep the initial mixed liquor COD/N (COD from acetate and N from NH$_4$Cl) ratio at approximately 6.0. A 100 ml of sludge from the settled 3 L MLSS was discharged every day (every 2 SBR cycles) to maintain the MLSS between 2000 to 2500 mg/L, corresponding to a sludge residence time (SRT) of around 30 days.

The scenario testing was conducted after the SBR effluent was...
was followed for water chemical analysis. COD was measured by Standard methods for examination of water and wastewater (1998) followed for water chemical analysis. COD was measured by the closed reflux method, and NH₄-N was measured by the Nesslerization method. NO was measured by an ion chromatography (DIONEX® Model 100).

**RESULTS**

**Water temperature**

The time profiles of the mixed liquor NH₄-N, NO₂-N, NO₃-N, and COD during the 11 h aeration phase under the three tested water temperatures (20, 25, and 30°C) are illustrated in Figure 1. It was observed that as the water temperature was increased not only the highest achievable NO₃-N level was lowered in the reactor (from 19.8 mg L⁻¹ under 20°C to 8.9 mg L⁻¹ under 30°C), but also its first occurrence time was delayed (from 1 h under 20°C to 4.5 h under 30°C). Meanwhile, the highest achievable NO₂-N level was elevated in the reactor (from 3.2 mg L⁻¹ under 20°C to 18 mg L⁻¹ under 30°C) and its first occurrence time was brought earlier (from 1.5 hours under 20°C to 1.0 hour under 30°C). Also, a quicker NH₄-N removal, corresponding to an increased amount of NO₂-N production, was observed as the water temperature was increased. Nevertheless, the NO₂-N level was only observed higher than that of NO₃-N under the water temperature of 30°C.

The water temperature increase induced transition from NO₃-N favoring environment to NO₂-N favoring environment, and it confirmed the theory of temperature selection on AOB and NOB. It has been observed that by maintaining the water temperature between 30 and 40°C, NOB activities can be totally suppressed while AOB can still sustain (Jetten et al., 1997). A pronounced temperature selection on AOB over NOB was observed even between 20 to 28°C within an activated sludge system under a DO of 2.0 mg L⁻¹ (Wu et al., 2007).

Although water temperature increase favors NO₂-N producing, the different time profiles of NO₂-N and NO₃-N suggest the duration of aeration phase should also be adjusted to match the optimum timing for NO₂-N harvesting with limited presence of NO₃-N. Similarly, the control of aeration duration has been demonstrated as an important factor to "preserve" NO₂-N from being nitrified into NO₃-N (Blackburne et al., 2008; Fux et al., 2003; Gao et al., 2009; Guo et al., 2009; Peng et al., 2007; Yoo et al., 1999). Using the testing results under the 30°C for example (Figure 1C), setting the aeration phase at 4.5 h seems already adequate to harvest NO₂-N at its prime while NO₃-N was just about to show up in the SBR.

**Organic carbon abundance**

The time profiles of the mixed liquor NH₄-N, NO₂-N, NO₃-N, and COD during the 11 h aeration phase under the four tested initial mixed liquor COD/N ratios (2.8, 6.0, 9.8, and 14) are illustrated in Figure 2. One of the significant observations was that no sign of nitrification was observed when the initial mixed liquor COD/N ratio was kept at 14. Since there potentially exists a competition for NH₄-N between nitrifying bacteria and heterotrophic bacteria (Strauss and Lamberti, 2000; Verhagen and Laanbroek, 1991), the observed no nitrification might be caused by the stimulated heterotrophic bacteria activities that overwhelmingly competed for NH₄-N with nitrifying bacteria, and consequently suppressed the nitrification process (Knowles, 1982; Hanaki et al., 1990; Strauss and Lamberti, 2000; Verhagen and Laanbroek, 1991). Even as the initial mixed liquor COD/N ratio was increased from 2.8 to 9.8 under which nitrification process was able to be carried under the experimental conditions, NO₂-N and NO₃-N productions were found affected differently.

From the initial mixed liquor COD/N ratio 2.8 (Figure 2A) to 6.0 (Figure 2B), the highest achievable NO₂-N and NO₃-N levels were fairly unchanged, but the time required to reach their highest levels were brought earlier (from 11 h under COD/N ratio 2.8 to 4.5 h under COD/N ratio 6.0). From the initial mixed liquor COD/N ratio 6.0 (Figure 2B) to 9.8 (Figure 2C), however, NO₂-N was observed being nitrified into NO₃-N after reaching its highest level. A denitrification tendency was even observed on NO₃-N under the initial mixed liquor COD/N ratio of 9.8. A general observation is that the NO₃-N production benefited from the organic carbon abundance increase with elevated highest levels and earlier reaching of its highest level. Meanwhile, NO₂-N only benefited with an earlier reaching of its peak level, but with the risk of being nitrified into NO₃-N as the organic carbon abundance gets even higher.

The different “beneficial” effects received by NO₂-N and NO₃-N from the organic carbon abundance increase might be explained by Zhang et al. (2002) who observed that NOB, AOB, and heterotrophic bacteria changed within an activated sludge system in response to changing organic carbon (NaAc) loadings (0~200 mg
Figure 1. The time profiles of the mixed liquor NH$_4$-N, NO$_2$-N, NO$_3$-N, and COD during the 11 h aeration phase under the three tested water temperatures: 20° C (A), 25° C (B), and 30° C (C). (Initial mixed liquor COD/N was maintained at approximately 6.0, DO was maintained at approximately 2.0 mg/L, and pH was observed between 7.1 to 7.8. Error bars of standard deviation were not shown).

Figure 2. The time profiles of the mixed liquor NH$_4$-N, NO$_2$-N, NO$_3$-N, and COD during the 11 h aeration phase under the four tested initial mixed liquor COD/N ratios: 2.8 (A), 6.0 (B), and 9.8 (C), and 14 (D). (Water temperature was maintained at 30° C, DO was maintained at approximately 2.0 mg/L, and pH was observed between 7.1 to 7.8. Error bars of standard deviation were not shown).

TOC L$^{-1}$ under low DO (0.5~1.0 mg L$^{-1}$) conditions. Their experimental results showed that the organic carbon loading increase stimulated heterotrophic bacteria growth and wiped out over 99% of AOB and NOB. However, the adverse effect on NOB was more than 100 times than AOB suffered. Therefore, it is possible that the adverse
effect on NOB from organic carbon abundance already started under the initial mixed liquor COD/N ratio of 9.8, but was not strong enough to inhibit AOB yet. Nevertheless, this organic carbon level was already strong enough to sustain a denitrification process. Similar denitrification phenomenon was also observed in a previous study (Wang et al., 2010) that NO₂⁻-N formed under a DO of 2.0 mg L⁻¹ and a COD/N ratio lower than the nitrification inhibitory level was denitrified, potentially due to the still abundant presence of organic carbon. The observed denitrification potential suggested a simultaneous nitrification and denitrification (SND) possibility within this experimental SBR. As nitrification is shown here under both beneficial and adverse effects from organic carbons, it is actually indicating that operating a SND process is also tricky (Robertson et al., 1988).

It should also be noticed that the reason of observing higher NO₂⁻-N levels than that of NO₃⁻-N in these organic carbon abundance tests might be caused by the adopted 30°C water temperature that favors partial nitrification. Therefore, although lowering the carbon abundance seems to be the prerequisite for nitrification, a partial nitrification favoring environment should not be expected by adjusting only the organic carbon abundance. However, the different benefits obtained by NO₂⁻-N and NO₃⁻-N productions from organic carbon abundance can be exploited for an optimum timing (duration of aeration phase) for NO₂⁻-N harvesting. Using the experimental results for example, it seems optimum to control the aeration phase at 4.5 h under the test of initial mixed liquor COD/N ratio of 6.0 or at 6.0 h under the test of initial mixed liquor COD/N ratio of 9.8.

**DO**

The time profiles of the mixed liquor NH₄⁺-N, NO₂⁻-N, NO₃⁻-N, and COD during the 11 h aeration phase under the four tested DO levels (0.4, 1.2, 2.0, and 4.0 mg L⁻¹) are illustrated in Figure 3. It was observed that the DO increase not only elevated the highest achievable levels of NO₂⁻-N (from 9.1 mg L⁻¹ under DO 0.4 to 22.7 mg L⁻¹ under DO 4.0 mg L⁻¹) and NO₃⁻-N (from 4.7 mg L⁻¹ under DO 0.4 to 12.3 mg L⁻¹ under DO 4.0 mg L⁻¹), but also push them earlier to reach their highest levels. NH₄⁺-N depletion was also getting quicker as the DO was increased, corresponding to the elevated nitrification speed. However, the NO₂⁻-N production seemed benefited from the DO increase earlier than NO₃⁻-N. This phenomena can be explained by that NOB has a less affinity for oxygen than that of AOB as a result of their different oxygen saturation levels: 1.1 mg O₂ L⁻¹ for NOB and 0.3 mg O₂ L⁻¹ for AOB (Wu et al., 2007), with AOB can adapt to relatively lower DO levels, under which condition NOB would have already been inhibited (Wiesmann, 1994; Wu et al., 2007). Although an extremely low DO is detrimental to AOB and NOB both, this DO related biological stress will be relieved earlier on AOB than NOB as the DO being gradually increased (Peng et al., 2007; Wiesmann, 1994; Wu et al., 2007).

Again, possibly due to the 30°C water temperature, the NO₂⁻-N levels were found always higher than that of the NO₃⁻-N under all the four tested DO levels. Nevertheless, NO₂⁻-N was noticeably nitrified into NO₃⁻-N under the test of DO 4.0 mg L⁻¹ (Figure 3D), indicating a potential to get a NO₃⁻-N level higher than that of NO₂⁻-N if the aeration phase was extended to longer than 11 h or a higher than 4.0 mg L⁻¹ DO level was applied. Gao et al. (2009) also claimed that excess aeration after NO₂⁻-N was dominantly produced can still lead to nitrification of NO₂⁻-N. It should also be aware that partial nitrification can still be established under room temperatures lower than 30°C by controlling DO at relatively lower levels (Peng et al., 2007; Wang et al., 2010). When higher water temperature is not able to be obtained economically, limiting DO then might be a practical way to achieve partial nitrification (Peng et al., 2007). In this study, this observed earlier NO₂⁻-N production increase than that of NO₃⁻-N under the DO increase seemed will also lead to this conclusion. However, this study did not carry any further experiment to verify this possibility.

Similar to the test of DO and organic carbon abundance, it is also indicated that an appropriate aeration duration should be set to match the optimum timing for NO₂⁻-N harvesting under varying DO conditions. Therefore, combing all previous studies including this one, the general picture is shown here that temperature effect favoring between AOB and NOB can be undermined or boosted by aeration intensity and/or duration.

**CONCLUSION**

1) Although it is no doubt that partial nitrification will be more favored at higher water temperatures, the turning point for NO₂⁻-N production to surpass NO₃⁻-N production is still depending on the overall reaction conditions and the site specific wastewater constituents. The beneficial effect on partial nitrification from high water temperatures can be compromised by increased organic carbon abundance and/or a high DO level.

2) A too high organic carbon abundance is detrimental to the overall nitrification process even it is at a favoring temperature level for partial nitrification. However, when being presented at lower than the inhibitory level, the increase on organic carbon abundance can increase both NO₂⁻-N and NO₃⁻-N productions. NO₃⁻-N production benefits more at relatively higher organic carbon abundances while NO₂⁻-N benefits more at relatively lower organic carbon abundances.

3) DO control is interchangeable for temperature control to achieve partial nitrification. Low DO levels favor partial nitrification and can compensate the lost beneficial effect on partial nitrification caused by a lowered water
Figure 3. The time courses of mixed liquor NH$_4$-N, NO$_2$-N, NO$_3$-N, and COD during the 11 h aeration phase under the four tested DO levels: 0.4 mg/L (A), 1.2 mg/L (B), 2.0 mg/L (C), and 4.0 mg/L (D). (Water temperature was maintained at 30°C, initial mixed liquor COD/N was maintained at approximately 6.0, and pH was observed between 7.1 to 7.6. Error bars of standard deviation were not shown).

temperature, while high DO levels can undo the favoring effect on partial nitrification from relatively higher water temperatures.

4) NO$_2$-N and NO$_3$-N production profiles will change under varying reaction conditions. Consequently, the duration of aeration phase, as an integrated reactor operation parameter, must also be tuned accordingly to accommodate the optimum timing for NO$_2$-N harvesting.

ACKNOWLEDGEMENT

This work was financially supported by the National Natural Science Foundation of China (NSFC) (51008239) and the Fundamental Research Funds for the Central Universities (5082010) and the Open Project of State Key Laboratory of Urban Water Resource and Environment (HIT) (No.QA200810 and QAK201014).

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