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Rapidly startup of partial nitrification in sequencing batch reactor and microbiological analysis

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ABSTRACT

Both temperature and dissolved oxygen (DO) have impact on partial nitrification. Through experiment and analysis in this study, the collaborative effect of temperature and DO on partial nitrification can be expressed as (temperature increases 1°C)/(DO decreases 0.1 mg L^{-1}) = 4.28. However, increasing temperature for wastewater treatment plant is scarcely possible. With room temperature (19–22°C), 140 cycles experiments were conducted in sequencing batch reactor (SBR). Results showed that DO concentration of 0.2–0.8 mg L^{-1} is suitable for rapidly startup of partial nitrification. No visible difference between 0.2 and 0.5 mg L^{-1} and 0.5–0.8 mg L^{-1} on rapidly startup of partial nitrification was existent. Microbiological analysis showed that system mainly contained 10 groups, *β-Proteobacteria*, uncultured bacterium, and *Bacteroidetes* were three dominant populations whose percentage were 32.5, 28.75, and 17.5%, respectively. Compared to the percentage of nitrite-oxidizing bacteria (NOB), ammonium-oxidizing bacteria (AOB) had an obvious advantage, which demonstrated the enrichment of AOB and achievement of partial nitrification. A model related to nitrite-accumulated rate (ρ), AOB biomass (X_A), and NOB biomass (X_N) was established in this study. Combined microbiological analysis with model calculation, the proportion of X_N not related to nitrite oxidation can be draw during startup of partial nitrification.

Keywords: Partial nitrification; Rapidly startup; Temperature; Dissolved oxygen; Microbiological analysis; Model

1. Introduction

Over the last decade, nitrogen removal via nitrite instead of nitrate has been widely regarded as the optimal pathway for nitrification and de-nitrification process that can reduce oxygen demand and organic source demand due to nitrification by up to 25 and 40%, respectively [1,2]. Meanwhile, for anaerobic ammonia oxidation process, partial nitrification is crucial. The key of achieving partial nitrification is to restrain the conversion from nitrite to nitrate. Recently, numerous studies has focused on achieving partial nitrification [3–5], for which a number of feasible control measures and strategies has established,

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e.g. low dissolved oxygen (DO) concentration, high temperature, alkaline pH, inhibitors, and short sludge retention time. It is reported that low DO limits the growth of nitrite oxidizing bacteria (NOB) and, consequently, leading to nitrite accumulation, which is due to the oxygen saturation coefficients of ammonium and nitrite oxidation are known to be 0.3 and 1.1 mg L^{-1}, respectively [6]. When temperature is above 30˚C, ammonium oxidizing bacteria (AOB) grows significantly faster than NOB [7]. Free ammonia concentrations as low as 0.1 mg L^{-1} can promote NOB inhibition [8], and pH acts on the NH_4^+–NH_3 equilibrium: alkaline pH shifts the chemical equilibrium to free ammonia, inhibiting NOB [9]. The generation cycle of AOB is shorter than NOB [2], thus, partial nitrification can be achieved by controlling SRT between the generation cycle of AOB and NOB.

Although many scholars have pointed out the realization of nitrite accumulation with high temperature or low DO concentration, the reported favorable values in activated sludge are different. Some reference results are listed in Table 1. Obviously, otherness is objective existent as Table 1 described. In addition, the effect of temperature and DO on partial nitrification are correlative which has been demonstrated by experiment in our research group [10–12]. Therefore, it is vital to study on the collaborative effect of temperature and DO on partial nitrification. To the best of our knowledge, for the treatment of low strength, municipal-type wastewater in wastewater treatment plant, increasing temperature in aeration tank is scarcely possible. Fortunately, with the development of automatic control technology low DO concentration could be easily obtained. Automatic control technology mainly consist of control system, aeration system, and DO probe. Desired DO concentration range was set in advance for control system, actual DO concentration is transferred to control system through DO probe, then control system would send instructions to aeration system, if actual DO concentration less than low limit of desired DO concentration, aeration system would start; if actual DO concentration greater than upper limit of desired DO concentration, aeration system would stop.

Nitrite-accumulated rate (ρ) in the effluent during nitrification is the most intuitive and effective one of all indicators to evaluate the performance of partial nitrification. The value of ρ can be calculated using mathematical formula (1). NO_2^-, NO_3^- both refers the produced during ammonia oxidation process, excluding contained by influent.

$$\rho = \frac{[NO_2^-]}{[NO_2^-] + [NO_3^-]} \quad (1)$$

Viewed from microbiology, the change of nitrite-accumulated rate is caused by the variation of AOB and NOB. A model related to nitrite-accumulated rate (ρ), AOB biomass (X_A), and NOB biomass (X_N) has been proposed in this study. Research on rapidly startup of partial nitrification through DO control is the key of this study. In actual project, during the stage of startup, effluent must reach the national standard. Meanwhile, for SBR process, the rapidly startup is not only related to increased rate of nitrite accumulated rate per cycle but also the nitrification time of each cycle. The total reaction time needed for startup has used to evaluate the effect of different DO concentrations on rapidly startup of partial nitrification.

2. Materials and methods

2.1. Experimental setup

A sequencing batch reactor (Fig. 1) with working volume of 5.2 L was operated in Beijing University of Technology, China. The system mainly contains timing stirrer, DO probe, pH probe, water quality analyzer, water bath with constant temperature, heater, aerator, and reactor made of plexiglass. The reactor is cube structure with 18 × 12 × 24 cm.

Table 1
Some reported favorable values in activated sludge for partial nitrification

Temperature (˚)		DO (mg L^{-1})	
Favorable value	Provenience	Favorable value	Provenience
≥30	[13]	≤0.5	[15]
≥28	[14]	≤0.5	[17]
≥25	[15]	≤1.0	[18]
11–15 and 31–33	[16]	≤1.4	[19]

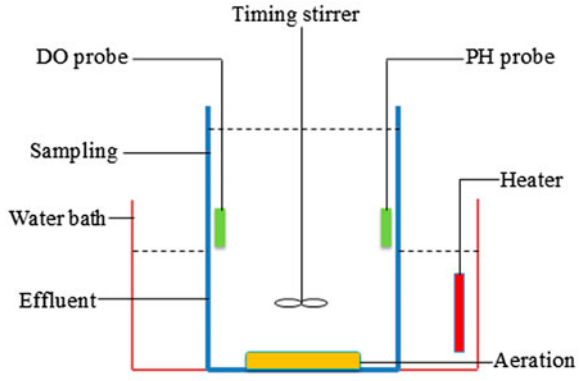

Fig. 1. Schematic diagram of experimental setup.

2.2. Wastewater and inoculated sludge

An artificial wastewater consisted of 70 mg L^{-1} (NH_4^+-N) NH_4Cl as the main substrate, 500 mg L^{-1} ($CaCO_3$) $NaHCO_3$ as the alkalinity source, 3 mg L^{-1} (P) KH_2PO_4 as the source of phosphorus and pH 7.8–7.9. Ingredient of trace elements is the same as the research of Yin et al. [20]. Inoculated sludge was taken from the aeration tank of Gaobeidian WWTP in Beijing, China, which has a good nitrification performance, and its f (MLVSS/MLSS) and sludge volume index (SVI) were 0.75 and 90, respectively. During the experimental process, MLSS of the wastewater was kept about 3,500 mg L^{-1}.

2.3. Experiment for studying collaborative effect of temperature and DO on partial nitrification

As Table 1 shown, each three different levels of temperature and DO were chosen to conduct this experiment, which was comprehensive and contained 9 set of tests (T_1O_1, T_1O_2, T_1O_3, T_2O_1, T_2O_2, T_2O_3, T_3O_1, T_3O_2, T_3O_3). T_1, T_2, T_3 represent 31, 25, and 19°C, respectively, O_1, O_2, O_3 represent 0.5, 1.4, and 2.3 mg L^{-1}, respectively.

Each test includes 20 biological treatment cycles: in the first cycle, temperature and DO were controlled on request, samples were taken at intervals of certain time, and then, the concentrations of NH_4^+, NO_2^-, and NO_3^- of each sample were measured. Nitrification time could be known from ammonium curve, noted t_1. Nitrite accumulated rate at this time was calculated, noted ρ_1; from the second cycle to the 19th cycle, aeration time was t_1; in the 20th cycle, experiment was carried on as same as the first cycle, nitrification time, and nitrite-accumulated rate were noted t_2 and ρ_2, respectively. In addition, the range of pH in each cycle was about 7.9–7.2.

2.4. Startup of partial nitrification in SBR

Experiment of startup of partial nitrification was conducted with room temperature (19–22°C), DO concentration was in sequence controlled at 0.2–0.5, 0.5–0.8, 0.8–1.1, and 1.1–1.4 mg L^{-1} to achieve the startup of partial nitrification. Total time was used to compare their effect on rapidly startup of partial nitrification with mathematical formula (2). Mathematical formula (2) is based on two hypotheses: the increased rate of nitrite-accumulated rate is constant with invariable external environment such as temperature, DO and so on; the relationship between nitrification time and nitrite accumulated rate is linear.

$$t_z = \frac{90}{v} \times \frac{t_3 + t_4}{2} \tag{2}$$

where t_z is total reaction time, unit is min; v is increased rate of nitrite accumulated rate, unit is % per cycle; t_3 and t_4 are nitrification times when nitrite accumulated rate are 0 and 90%, unit was min.

2.5. Analytical procedures

All samples were analyzed after filtration with 0.45-μm filter paper. NH_4^+, NO_2^-, NO_3^-, MLSS, MLVSS, and SVI were measured according to Standard Methods [21], MLSS and MLVSS were measured using weighing method; SVI was calculated by SV/MLSS, and SV was measured using precipitation method; concentrations of NH_4^+ and NO_2^- were measured using colorimetric methods, while NO_3^- was analyzed using ultraviolet spectrophotometric method. The temperature and pH were detected on line using WTW level 2 pH meters (WTW Company, Germany). DO were controlled by rotor flow meter and continuously monitored by WTW, pH/oxi340i meter with DO probes (WTW Company, Germany).

2.6. Bacterial community analysis

For the phylogenetic analysis, a sample was obtained after cycle 130 which represented the achievement of startup of partial nitrification. DNA was extracted from the sample using the Ultra Clean™ Soil DNA kit (MoBio Laboratories, Solana Beach, CA), which includes bead beating and a spin-column purification steps [22], and then DNA was stored at −20°C until further processed.

The amplification of the 16S rRNA gene of total bacteria was performed using the universal primer set 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r

(5′-TACGGYTACCTTGTTACGACTT-3′). The mixture solutions (50 μl) for PCR amplification of total bacteria consisted of 5 μl 10 × PCR buffer, 1 μl dNTP (2.5 mmol L^{-1}), 1 μl 27f (20 μmol L^{-1}), 1 μl 1492r (20 μmol L^{-1}), 0.5 μl Taq DNA polymerase, 0.5 μl template DNA and 41 μl PCR-grade sterile water. The PCR program for the total bacterial 16S rRNA gene measurement was 1.5 min at 95℃; 5 cycles of 0.5 min at 95℃, 0.5 min at 60℃, 2 min at 72℃; 5cycles of 0.5 min at 95℃, 0.5 min at 55℃, 2 min at 72 ℃; 15cycles of 0.5 min at 95℃, 0.5 min at 50℃, 2 min at 72℃; 10 min at 60℃.

The PCR products were purified with the purification kit (Sangon, China) and cloned using pMD18-T plasmid vector system (TaKaRa, Japan). Eighty clones were randomly selected for sequencing. All sequences obtained were compared with the reference microorganisms available in GenBank by BLAST tool. The GenBank accession numbers in this study are KP411846-KP411869.

3. Results and discussion

3.1. Collaborative effect of temperature and DO on partial nitrification

Nitrite-accumulated rate (ρ) was used to evaluate the performance of partial nitrification and the variation about it of each test is described in Fig. 2 in which ρ_1, ρ_2 represents nitrite accumulated rate before and after each test, respectively, $\Delta\rho$ represents the changed value.

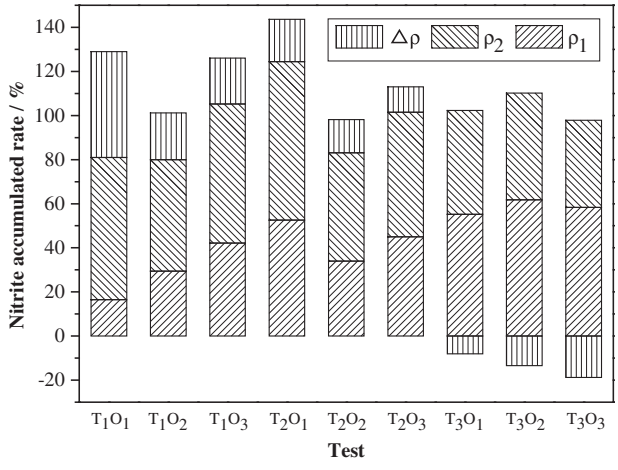

Fig. 2. Variation of nitrite-accumulated rate during each test.
Notes: ρ_1—nitrite accumulated rate before each test; ρ_2—nitrite accumulated rate after each test; $\Delta\rho = \rho_2 - \rho_1$.

Much information can be obtained. The previous six tests present positive performance and the last three tests present negative performance, which indicate that temperature become the limiting factor when it declines to 19℃, even though DO concentration (0.5 mg L^{-1}) is the positive factor. When temperature (T_1, T_2, T_3) is fixed, the positive effect of DO on nitrite accumulated rate reduces along with increased DO concentration (O_1, O_2, O_3). When DO (O_1, O_2, O_3) is fixed, the effect of temperature on nitrite-accumulated rate changes from positive to negative along with temperature (T_1, T_2, T_3) declines. It can be draw a simple conclusion that temperature plays a major role in partial nitrification of this study, meanwhile the positive role of DO also can be confirmed, although it is not as significant as that of temperature.

The changed value of nitrite-accumulated rate ($\Delta\rho$) is obtained from Fig. 2. For comparing the effect of temperature and DO on partial nitrification, using K_T represent the average effect of three DO levels (O_1, O_2, O_3) on nitrite accumulated rate, and K_T equals the average value of $\Delta\rho$ of three DO levels under the fixed temperature. K_O represent the average effect of three temperature levels (T_1, T_2, T_3) on nitrite accumulated rate, and K_O equals the average value of $\Delta\rho$ of three temperature levels under the fixed DO concentration. So the effect of temperature on partial nitrification can be seen through variation of K_T which ignores the effect of DO. Similarly, the effect of DO on partial nitrification can be seen through variation of K_O which ignores the effect of temperature. Fig. 3(a) and (b) describe the variation of K_T and K_O, respectively.

According to the results of linear fit in Fig. 3(a) and (b), the collaborative effect of temperature and DO on partial nitrification can be expressed as (temperature increases 1℃)/(DO decreases 0.1 mg L^{-1}) = 4.28. In this study, the intervals of temperature and DO are 6℃ and 0.9 mg L^{-1}. Then, 6℃/0.9 mg L^{-1} = 2.85 which indicate that temperature is the main factor compared to DO in this study.

3.2. Rapidly startup of partial nitrification in SBR

The variation of nitrite accumulated rate and nitrification time during 140 run cycles are shown as Fig. 4.

Experiment includes five stages of A, B, C, D and E. Stage A represents the recovery of activity. Stage B, C and D belong to the startup of partial nitrification. Stage E represents the operation of partial nitrification. Their corresponding DO concentrations were controlled at 1.5–2.0, 0.2–0.5, 0.5–0.8, 0.8–1.1, and 1.1–1.4 mg L^{-1}, respectively. During stage A, nitrification time decreases significantly after 10 cycles

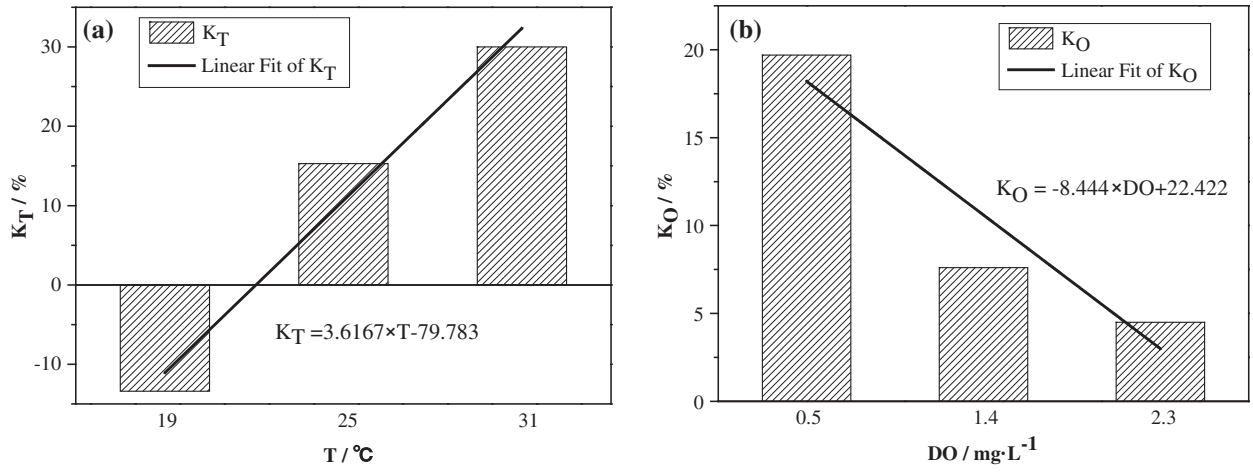

Fig. 3. Variation diagrams of K_T and K_O. (a) Linear fitting chart of K_T and (b) linear fitting chart of K_O.

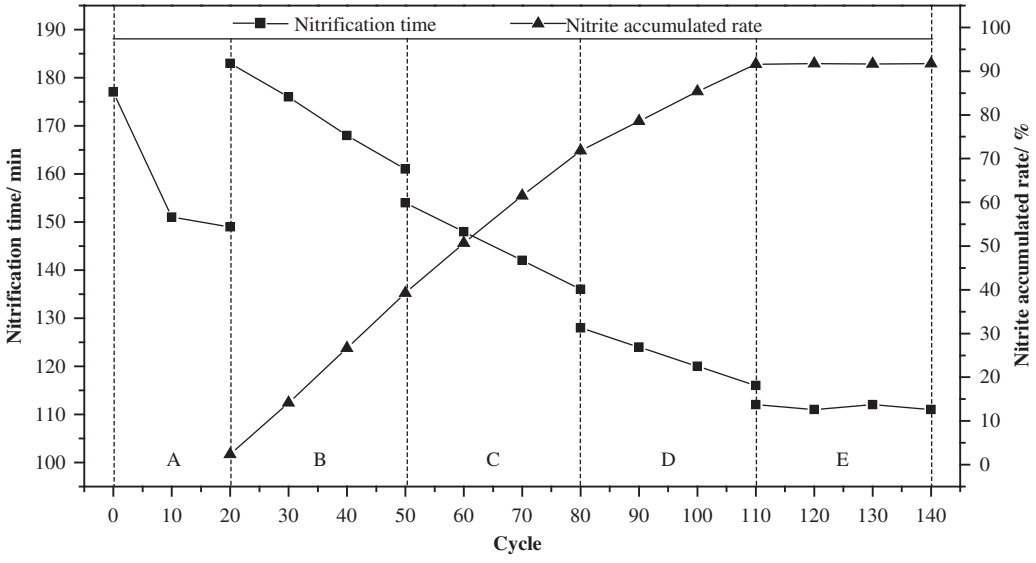

Fig. 4. Results of experiments about startup of partial nitrification.

running, and then nitrification time is almost unchanged from cycle 10 to cycle 20, which indicates the activity of microorganisms has recovered to normal level.

The results show that the startup and operation of partial nitrification can be achieved through increasing DO concentration gradually. Stage B, C, and D are all positive for startup of partial nitrification. During the stage E, throughout 30 cycles, nitrite accumulated rate and nitrification time are almost unchanged, which is needed for steady operation.

As Fig. 4 shown, the hypotheses of mathematical formula (2) can be confirmed from stage B, C, and D. For comparing the effectiveness of rapidly startup, calculation results of total time are stage B (11,917 min), stage C (12,350 min), and stage D (19,509 min). It is clearly that total times of stage B and C are close, and much less than that of stage D which indicates that DO concentration of 0.2–0.8 mg L^{-1} is suitable for rapidly startup of partial nitrification. It is interesting that B (0.2–0.5 mg L^{-1}) and C (0.5–0.8 mg L^{-1}) have similar impact on rapidly startup of partial nitrification. When DO concentration is 0.5 mg L^{-1}, the growth rate of AOB is about 60% of the normal level [23]. In this study, the increment about MLVSS of stage B and C were 170 and 230 mg L^{-1}, respectively. Obviously, sludge yield under stage B was lower than stage C which indicates doubling time under stage B was higher. Compared to C

(0.5–0.8 mg L^{-1}), even the competitive advantage of AOB under B (0.2–0.5 mg L^{-1}) is more significant, but doubling time of AOB increases, that is why no visible difference between B (0.2–0.5 mg L^{-1}) and C (0.5–0.8 mg L^{-1}) on rapidly startup is existent.

3.3. Characteristic of bacterial community

Sludge sample was taken from cycle 130 which represents the achievement of startup of partial nitrification. 16S rDNA gene cloning results of total bacteria are described in Table 2.

From Table 2, system mainly contains 10 groups. The *β-Proteobacteria*, uncultured bacterium and *Bacteroidetes* are dominant populations with 32.5, 28.75, and 17.5% percentage, respectively. The percentage of *Acidobacteria* is 7.5%. While, the percentage of *Nitrospirae*, *Spirochaetes*, *Verrucomicrobia*, *α-Proteobacteria*, *δ-Proteobacteria*, and *Chloroflexi* are relative small, which are 3.75, 3.75, 2.5, 1.25, 1.25, and 1.25%, respectively. The above-mentioned results are in accord with research of Wagner that *β-Proteobacteria* and *Bacteroidetes* are always the dominant population in wastewater treatment system [24]. Additionally, a portion of bacteria in the system is uncultured bacterium. It demonstrates the shortage of traditional microbiology analysis determination technique which mainly contains pure isolation, culture and so on. Meanwhile, the superiority of molecular biological technique is confirmed with which uncultured bacterium can be detected and the original microbial information in the sample will be obtained effectively and quickly.

The *β-Proteobacteria* in this system contains *Nitrosomonas* and *Denitratisoma*, and the percentage of *Nitrosomonas* (22.5%) is apparently higher than that of *Denitratisoma* (10%). It indicates that autotrophic bacteria become dominant in *β-Proteobacteria* which is different from previous reports [12,25]. Thus, a reasonable explanation may be lack of organic carbon source in artificial wastewater. Autotrophic bacteria in this system are *Nitrosomonas* and *Nitrospira*. Its percentage was 26.25% which is higher than that of conventional wastewater treatment process. However, it is interesting that the percentage of autotrophic bacteria is also lower than heterotrophic bacteria in total phylogenetic group. It may be explained as the generation cycles of AOB and NOB are about 8–36 and 12–59 h, respectively, which are obviously higher than that of

Table 2
16S rRNA cloning results of total bacteria

Clone	Percentage of clone (%)	Accession number	The most similar bacteria	Similarity (%)	Phylogenetic group	Percentage of group (%)
1	1.25	DQ186614	*Micavibrio* sp.	88	*α-Proteobacteria*	1.25
2	15	AJ224941	*Nitrosomonas* sp.	99	*β-Proteobacteria*	32.5
3	2.5	KF810114	*Denitratisoma*	98		
4	7.5	KF810117	*Denitratisoma*	99		
5	5	AF287297	*Nitrosococcus*	99		
6	2.5	AB079053	*Nitrosomonas* sp.	99		
7	1.25	AJ233908	*Melittangium*	93	*δ-Proteobacteria*	1.25
8	2.5	AB539999	*Bacteroidetes*	95	*Bacteroidetes*	17.5
9	6.25	GQ274116	Uncultured	86		
10	6.25	NR_108511	*Chryseolinea*	92		
11	2.5	FJ263933	*Chitinophagaceae*	95		
12	3.75	AY234727	Bacterium	92	*Acidobacteria*	7.5
13	1.25	GU187027	*Acidobacteria*	94		
14	2.5	GU187039	*Acidobacteria*	97		
15	3.75	NR_074700	*Candidatus*	99	*Nitrospira*	3.75
16	3.75	NR_119299	*Leptonema illini*	99	*Spirochaetes*	3.75
17	2.5	GU129926	*Spartobacteria*	88	*Verrucomicrobia*	2.5
18	1.25	NR_109544	*Ornatilinea*	90	*Chloroflexi*	1.25
19	10	JX040363	Uncultured	99	Uncultured	28.75
20	1.25	AB286378	Uncultured	99	bacterium	
21	3.75	GU454914	Uncultured	94		
22	5	KC539798	Bacterium	99		
23	5	HQ158632	Uncultured	99		
24	3.75	KC253303	Uncultured	98		

heterotrophic bacteria. Thus, heterotrophic bacteria can utilize internal carbon source in sludge for rapid growth. A relative reasonable explanation for entirely different percentages of heterotrophic bacteria in total phylogenetic group and phylum *β-Proteobacteria* may be that when organic carbon is limited, *Denitratisoma* has not advantage for competing internal carbon source in sludge with other heterotrophic bacteria.

Nitrosomonas, *Nitrospira,* and *Denitratisoma* are involved which are all related to nitrogen removal. Their percentages are 22.5, 3.75, and 10%, respectively. Relatively common AOB includes *Nitrosomonas* and *Nitrosospira*, but only *Nitrosomonas* is detected in the reactor. Pure culture study [26] showed that growth rate of *Nitrosomonas* is faster. For example, the maximum specific growth rate (μ_{max}) of *N. europaea* can reach to 0.088 h^{-1}, but the μ_{max} of *Nitrosospira* is 0.033–0.035 h^{-1}. It may explain that *Nitrosomonas* is the preponderant AOB. *Nitrospira* is an important NOB, and only it belonged to NOB has been detected. Studies [27,28] have shown that functional NOB is *Nitrospira* not *Nitrobacter*. *Denitratisoma* belongs to denitrifying bacteria. However, this study did not set denitrification process and no organic carbon was added. It may because the reactor is cube structure where anoxic area might exist. Additionally, organic carbon might derive from sludge or endogenous carbon. Compared to the percentage of NOB (*Nitrospira*), AOB (*Nitrosomonas*) has an obvious advantage, which demonstrates the enrichment of AOB and achievement of partial nitrification.

3.4. Model related to nitrite-accumulated rate (ρ), AOB biomass (X_A), and NOB biomass (X_N)

Based on the Monod equation, the relationship between the specific growth rate of AOB and concentrations of NH_4^+ and DO is:

$$\mu_{AOB} = \mu_A \frac{S_{NH}}{K_{A,NH} + S_{NH}} \cdot \frac{S_O}{K_{A,O} + S_O} \tag{3}$$

The relationship between the specific growth rate of NOB and concentrations of NO_2^- and DO is:

$$\mu_{NOB} = \mu_N \frac{S_{NO}}{K_{N,NO} + S_{NO}} \cdot \frac{S_O}{K_{N,O} + S_O} \tag{4}$$

Then the growth rate of AOB and NOB are:

$$V_{AOB} = \mu_{AOB} \cdot X_A = \mu_A \frac{S_{NH}}{K_{A,NH} + S_{NH}} \cdot \frac{S_O}{K_{A,O} + S_O} \cdot X_A \tag{5}$$

$$V_{NOB} = \mu_{NOB} \cdot X_N = \mu_N \frac{S_{NO}}{K_{N,NO} + S_{NO}} \cdot \frac{S_O}{K_{N,O} + S_O} \cdot X_N \tag{6}$$

According to the deduction process of GAO [29], the relationship between microbial growth and NH_4^+ consumption can be expressed as:

$$\frac{dS_{NH}}{dt} = -\frac{V_{AOB}}{Y_A} = -\frac{1}{Y_A} \cdot \mu_A \frac{S_{NH}}{K_{A,NH} + S_{NH}} \cdot \frac{S_O}{K_{A,O} + S_O} \cdot X_A \tag{7}$$

Similarly, the relationship between microbial growth and NO_2^- consumption can be expressed as:

$$\frac{dS_{NO}}{dt} = -\frac{V_{NOB}}{Y_N} = -\frac{1}{Y_N} \cdot \mu_N \frac{S_{NO}}{K_{N,NO} + S_{NO}} \cdot \frac{S_O}{K_{N,O} + S_O} \cdot X_N \tag{8}$$

In Eqs. (7) and (8), Y_A, μ_A, $K_{A,NH}$, $K_{A,O}$, Y_N, μ_N, $K_{N,NO}$, and $K_{N,O}$ are constant coefficients under certain operational conditions. When DO concentration (S_O) is controlled at a constant level, the term $(S_O)/(K_{A,O} + S_O)$ and $(S_O)/(K_{N,O} + S_O)$ can be regarded as constant. $K_{A,NH}$ and $K_{N,NO}$ are 1.1, 0.5 mg L^{-1} [6]. In this study, concentration of NH_4^+ in nitrification effluent was about 5–10 mg L^{-1}, so $S_{NH}/(K_{A,NH} + S_{NH}) \approx 1$, similarly, during the startup of partial nitrification nitrite accumulated, and the nitrite accumulation rate increased, $S_{NO} >> K_{N,NO}$, so $S_{NO}/(K_{N,NO} + S_{NO}) \approx 1$. For each one cycle of SBR, X_A and X_N also can be considered constant in this cycle. Then, dS_{NH}/dt and dS_{NO}/dt are constants in each one cycle of SBR in this study.

Nitrite accumulated rate (ρ) in nitrification effluent has a direct relation with dS_{NH}/dt and dS_{NO}/dt in each one cycle of SBR, due to dS_{NH}/dt and dS_{NO}/dt are constants, so relation between ρ, dS_{NH}/dt and dS_{NO}/dt can be expressed as follows:

$$\frac{dS_{NH}/dt}{dS_{NO}/dt} = \frac{1}{1-\rho} \tag{9}$$

Substitute Eqs. (7)–(9), then we obtain:

$$\frac{\frac{1}{Y_A} \cdot \mu_A \cdot \frac{S_O}{K_{A,O} + S_O} \cdot X_A}{\frac{1}{Y_N} \cdot \mu_N \cdot \frac{S_O}{K_{N,O} + S_O} \cdot X_N} = \frac{1}{1-\rho} \tag{10}$$

Simplify Eq. (10), then we obtain:

$$\frac{X_A}{X_N} = \frac{1}{1-\rho} \cdot \frac{\frac{\mu_N}{Y_N} \cdot \frac{S_O}{K_{N,O} + S_O}}{\frac{\mu_A}{Y_A} \cdot \frac{S_O}{K_{A,O} + S_O}} \tag{11}$$

Using Eq. (11), the value of X_A/X_N in cycle 130 was calculated, according to the research of Wiesmann [6], Y_A, $K_{A,O}$, Y_N, and $K_{N,O}$ were 0.2, 0.3, 0.057, and 1.1, respectively. In addition, μ_A and μ_N were 2.0 and 2.4 based on the calculation results of Fang [30]. DO concentration of cycle 130 was controlled at 1.1–1.4 mg L^{-1}, and S_O was 1.25, and ρ was about 0.9. The calculation value of X_A/X_N in cycle 130 was 27.8. However, the result of microbiological analysis was $X_A/X_N = 22.5\%/3.75\% = 6$. Huge disparity was existent between calculated value and measured value. Reasons may be both of X_A and X_N used in model are activated and all AOB and NOB can degrade NH_4^+ and NO_2^-, respectively. Actually, X_A and X_N determined by microbiological analysis also include degenerative or died. Substantially, the startup of partial nitrification is a process to restrain NOB and wash out it. So we can assume that X_A determined by microbiological analysis were all activated for degrading NH_4^+. Then, during the startup of partial nitrification, with the microbiological analysis and model calculation, we can draw the proportion of X_N not related to nitrite oxidation. In this study, 3.75% of total bacteria were determined as NOB with molecular biological technique. When combined with model calculation, we can find that about 80% of X_N was not related to nitrite oxidation. Along with the long-term operation, they would be washed out.

4. Conclusions

The collaborative effect of temperature and DO on partial nitrification was also demonstrated in this study which could be expressed as (temperature increased 1℃)/(DO decreased 0.1 mg L^{-1}) = 4.28. With room temperature (19–22℃), DO concentration of 0.2–0.8 mg L^{-1} is suitable for rapidly startup of partial nitrification, it was interesting that there was not visible difference between 0.2–0.5 mg L^{-1} and 0.5–0.8 mg L^{-1} on rapidly startup of partial nitrification. Microbiological analysis demonstrated the enrichment of AOB and achievement of partial nitrification. The proportion of NOB biomass not related to nitrite oxidation can be obtained with the model in this study which is related to nitrite accumulated rate, AOB biomass and NOB biomass.

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Nomenclature

DO	—	dissolved oxygen
AOB	—	ammonium oxidizing bacteria
NOB	—	nitrite oxidizing bacteria
ρ	—	nitrite accumulated rate
X_A	—	AOB biomass
X_N	—	NOB biomass
μ_{AOB}	—	specific growth rate of AOB
μ_A	—	maximum specific growth rate of AOB
S_{NH}	—	ammonium concentration
$K_{A,NH}$	—	half-maximum rate concentration for S_{NH} for AOB
S_O	—	DO concentration
$K_{A,O}$	—	half-maximum rate concentration for DO for AOB
μ_{NOB}	—	specific growth rate of NOB
μ_A	—	maximum specific growth rate of NOB
S_{NO}	—	nitrite concentration
$K_{N,NO}$	—	half-maximum rate concentration for S_{NO} for NOB
$K_{N,O}$	—	half-maximum rate concentration for DO for NOB
V_{AOB}	—	growth rate of AOB
V_{NOB}	—	growth rate of NOB
Y_A	—	yield coefficient for AOB
Y_N	—	yield coefficient for NOB

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