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# Short- and long-term effects of temperature on partial nitrification in a sequencing batch reactor treating domestic wastewater

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ABSTRACT

Partial nitrification to nitrite has been frequently obtained at high temperatures, but has proved difficult to achieve at low temperatures when treating low strength domestic wastewater. In this study, the long-term effects of temperature on partial nitrification were investigated by operating a sequencing bath reactor with the use of aeration duration control. The specific ammonia oxidation rate decreased by 1.5 times with the temperature decreasing from 25 to 15 °C. However, low temperature did not deteriorate the stable partial nitrification performance. Nitrite accumulation ratio was always above 90%, even slightly higher (above 95%) at low temperatures. The nitrifying sludge accumulated with ammonia-oxidizing bacteria (AOB), but washout of nitrite-oxidizing bacteria (NOB) was used to determine the short-term effects of temperature on ammonia oxidation process. The ammonia oxidation rate depended more sensitively on lower temperatures; correspondingly the temperature coefficient $\theta$ was 1.172 from 5 to 20 °C, while $\theta$ was 1.062 from 20 to 35 °C. Moreover, the larger activation energy (111.5 kJ mol$^{-1}$) was found at lower temperatures of 5–20 °C, whereas the smaller value (42.0 kJ mol$^{-1}$) was observed at higher temperatures of 20–35 °C. These findings might be contributed to extend the applicability of the partial nitrification process in wastewater treatment plants operated under cold weather conditions. It is suggested that the selective enrichment of AOB as well as the washout of NOB be obtained by process control before making the biomass slowly adapt to low temperatures for achieving partial nitrification to nitrite at low temperatures.



## 1. Introduction

Nitrogen removal from wastewater is critical to prevent the toxic effect on aquatic life, oxygen depletion and eutrophication in receiving water. Compared with physical–chemical treatments, biological nitrogen removal (BNR), being more effective and relatively inexpensive, has been widely adopted in practice. Shortcut nitrogen removal via the nitrite pathway, as one of the cost-effective and sustainable BNR processes, has the potential of reducing the requirements for aeration consumption and carbon source. Compared with the traditional BNR process, this novel process results in saving up to 25% of the oxygen consumption in the nitrification stage, and 40% of the carbon source requirements in the denitrification stage [1,2].

The selective enrichment of ammonia-oxidizing bacteria (AOB) and the inhibition-limitation-washout of nitrite-oxidizing bacteria (NOB) are critical for achieving and maintaining of partial nitrification to nitrite [3,4]. In recent years, stable partial nitrification to nitrite has been achieved frequently in the systems treating wastewater containing a high-level ammonium, such as the anaerobic digester supernatant and landfill leachate [5,6]. In contrast to continuous systems, sequencing batch reactors (SBRs) have become quite common for obtaining high nitrite accumulation due to the flexibility of process control [3]. Some suggested factors to achieve stable partial nitrification in literature include low dissolved oxygen (DO) concentration, high temperature, appropriate sludge retention time (SRT), high substrate concentration, free ammonia (FA) and free nitrous acid (FNA) inhibition and aeration pattern [7–9]. However, some strategies might lead to negative effects, such as filamentous bulking caused by low DO. Moreover, the accumulation of nitrite under low DO condition can trigger the distinct emission of nitrous oxide ($N_2O$) via denitrification by AOB [10–12]. Significantly aeration duration control, whereby aeration is terminated as soon as ammonium oxidation is complete, has been developed, and it has been demonstrated that it is an

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effective and robust method to obtain stable nitrite accumulation [13–16].

In particular, temperature, as a key operation parameter, has distinct effects on the competition between AOB and NOB [17,18]. The activation energies of AOB and NOB as well as their sensitivities to temperature changes are distinctly different. Raising temperature can not only promote the growth rates of AOB, but also expand the differences of specific growth rates between AOB and NOB [6,19]. AOB would out-compete NOB at the relatively higher temperatures, due to higher specific growth rate for AOB. For example, Hellinga et al. reported that AOB had a higher maximal specific growth rate than NOB at 20 °C, while the specific growth rate of AOB was lower than that of NOB at 15 °C [6]. In general, the optimal temperature for expanding the differences of specific growth rates between AOB and NOB is recommended to be above 25 °C [6,7]. Therefore, most of the studies on partial nitrification to nitrite were achieved at relatively high water temperatures [20–24]. SHARON (Single reactor High activity Ammonia Removal Over Nitrite), as the most typical process of partial nitrification, is the first full-scale process in which high nitrite accumulation could be achieved [6,25,26]. Apart from high ammonium concentrations and short SRT, high temperature (35 °C) is a very critical control parameter to achieve the partial nitrification in SHARON process. However, such strict conditions limit its application since high temperature is not generally available for the common municipal or domestic wastewater.

Although many effective methods, such as immobilization and bioaugmentation, have been developed to protect the nitrifiers from low temperatures [27,28], the stable nitrite accumulation has proven difficult to achieve at low temperatures [15]. Several reports on achieving nitrite accumulation at low temperatures were especially in SBRs treating wastewater with high ammonium concentrations [28,29]. If the high nitrite accumulation and sufficient organism activity are possible at low temperatures, the shortcut nitrogen removal via the nitrite pathway might be expected to extend its applicability, particularly on treating domestic wastewater in the cold regions. In addition, most of the works focused on the short-term effects of temperature on the respiratory activities of the mixed culture of AOB and NOB [18,23]. The nitrifying bacterial community, especially the ratio of AOB was not mentioned when conducting the short-term effects of temperature on ammonia oxidation process.

Therefore, the objectives of this study are: (1) to investigate both the long-term and short-term temperature effects on ammonia oxidation process of an accumulated AOB culture within a relatively broad temperature range (long-term effects: from 12 to 25 °C; short-term effects: from 5 to 35 °C); (2) to evaluate the stability of partial nitrification performance within an ample temperature interval that covers the operating temperature range of most of the domestic wastewater treatment plants (WWTPs). It is expected to provide useful information for a clearer understanding of the behavior of AOB and to build the basis for extending the applicability of the partial nitrification process.

## 2. Materials and methods

### 2.1. A lab-scale SBR and operation conditions

Long-term experiments were performed in a lab-scale SBR with 10 L working volume. The pH and DO values were monitored during the reaction phase. Each cycle consisted of feeding (3 min), aerobic reaction, settling (30 min), decanting (30 min) and idling. The length of aeration reaction was not fixed and the aeration valve was switched off when detecting the characteristic point (ammonia valley) in pH profile [30]. The ammonia valley was detected by the real-time aeration duration control built before [13,15]. Five liters of clarified supernatant was withdrawn from the reactor at the end of settling phase and five liters fresh wastewater was pumped into the reactor during the filling phase. SRT was controlled at about 30 days by withdrawing the sludge from the reactor at the end of the aerobic reaction. The reactor operation consisted of three phases: phase I (days 1–30) was used for the start up of partial nitrification by aeration duration control; the maintaining of partial nitrification was investigated at room temperature (18–25 °C) during phase II (days 31–91); at the following phase III (days 92–166), the stability of partial nitrification was investigated at low temperature (12–17 °C). Temperature of the reactor was progressively decreased in phases II and III, which is similar with the methodology described in the previous study [13].

### 2.2. Wastewater and seed sludge characteristics

The seed sludge was collected from the secondary clarifier of a lab-scale pre-denitrification reactor in our lab [31]. The feed to the SBR was collected from an on-campus sewer line and the wastewater characteristics can be found in the previous study [4]. The average chemical oxygen demanding (COD) and ammonium concentrations in influent are 215 and 58.1 mg $L^{-1}$, respectively.

### 2.3. Analytical methods

The temperature, pH and DO were detected on line using WTW pH/DO meter (WTW Multi 340i, Germany). $NH_4^+$-N, $NO_2^-$-N, $NO_3^-$-N, mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were measured according to Standard Methods [32].

### 2.4. Fluorescence in situ hybridization (FISH)

Sample fixation and hybridization steps were carried out according to the method previously described by Amann et al. [33]. Table 1 lists the details of 16S rRNA-targeted oligonucleotide probes used in this study. FISH was carried out with $EUB_{mix}$ specific for members of the domain bacteria, Nstpa662 specific for *Nitrospira* and Nit3 specific for *Nitrobacter* [34,35]. Both Nso1225 and Nso190 were used to detect ammonia-oxidizing β-*Proteobacteria* [35,36]. The images of FISH samples were captured using an OLYMPUS-BX52 fluorescence microscope (Japan). Randomly selected 50 fields of each sludge sample were used to quantify by using Image-pro plus 6.0 Software®, where the relative abundance of the interested bacteria was determined as mean percentage of all bacteria [37].

### 2.5. Batch test

After the parent reactor (SBR described above) had been operated at low temperature for two months, batch tests were conducted to investigate the short-term effect of temperature on ammonia oxidation process. By FISH analysis, the nitrifying microorganisms for batch experiments were accumulated with AOB but with negligible NOB (more details later). At the beginning of each experiment, the sludge was prepared by harvesting the mixed liquor from the parent reactor. The collected mixed liquor was first concentrated in a centrifuge (3000 rpm × 5 min) and the supernatant was decanted to remove the residual inorganic nitrogen. The concentrated cells were re-suspended and washed twice with the effluent from the lab-scale SBR. The rinsed cells were transferred to a 250 mL Erlenmeyer flask with 100 mL of domestic wastewater. The Erlenmeyer flask was immerged in an electric-heated thermostatic water bath. The concentration of cells in the flask was in the range of 1500–2000 mg $L^{-1}$ as MLVSS. The initial ammonium concentration was about 40 mg N $L^{-1}$. The maximum specific substrate-utilization rates of AOB were determined

**Table 1**
FISH probes used, their target groups and hybridization conditions in this study.

| Probe name | %Formamide | Sequence (5′-3′) | Fluorochrome labeled | Target group |
|---|---|---|---|---|
| $EUB_{mix}$[a] | 55 (with Nso190)<br>35 (with Nso1225)<br>35 (with Ntspa662)<br>40 (with Nit3) | EUB338:GCTGCCTCCCGTAGGA GT<br>EUB338-II:GCAGCCACCCGTAGGTGT<br>EUB338-III:GCTGCCACCCGTAGGTGT | FITC | Eubacteria |
| Nso190 | 55 | CGATCCCCTGCTTTTCTCC | Cy3 | Many but not all ammonia-oxidizing β-*Proteobacteria* |
| Nso1225 | 35 | CGCCATTGTATTACGTGTGA | Cy3 | Almost all ammonia-oxidizing β-*Proteobacteria* |
| Ntspa662 | 35 | GGAATTCCGCGCTCCTCT | Cy3 | *Nitrospira* |
| Nit3 | 40 | CCTGTGCTCCATGCTCCG | Cy3 | *Nitrobacter* |

[a] $EUB_{mix}$ is an equimolar mixture of probes EUB338, EUB338-II and EUB338-III.

through linear regression of the measured ammonia profiles. The nitrite production rates were determined similarly from the measured nitrite profiles. The temperature levels were varied from 5 to 35 °C. Triplicate experiments were conducted at every temperature level. The average DO was about 3 mg $L^{-1}$ and pH was in the range of 7.4–7.8.

### 2.6. Temperature coefficient and activation energy

The effect of temperature on a constant rate relative to a standard temperature (293 K herein) can be expressed by the simplified Arrhenius equation [38]:

$$r_T = r_{293} \times \theta^{(T-293)} \quad (1)$$

where the $r_T$ is the reaction rate at the temperature $T$ (in K) and $\theta$ is the temperature coefficient.

The activation energy of a reaction can be determined graphically by taking the natural logarithm of Arrhenius equation, as shown in the following equation (2):

$$\ln r_T = \frac{-E_a}{RT} + \ln A \quad (2)$$

where $A$ is the frequency factor for the reaction, $R$ is the universal gas constant, $T$ is the temperature in K, and $E_a$ is the activation energy.

## 3. Results and discussion

### 3.1. Typical performance of partial nitrification SBR at room and low temperatures

During the start-up period (phase I), the average DO was above 2 mg $L^{-1}$ and the temperature was lower than 27 °C. Moreover, the mean of $NH_4^+$-N in the influent was lower than 60 mg $L^{-1}$, which results in FA concentrations often lower than 1.0 mg $L^{-1}$ under pH < 8 conditions. Therefore, these operation conditions were not favorable to expand the differences of specific growth rates between AOB and NOB. In fact, partial nitrification was achieved by selecting appropriate aeration duration. The application of real-time aeration duration control was in favor of implementation of nitrite accumulation. The aerobic phase was terminated as soon as ammonium oxidation was complete in order to prevent from converting nitrite into nitrate. The end point of ammonium oxidation could be reliably detected from the on-line pH. Commonly, there was an inflexion point (ammonia valley) in pH profile at the end of nitritation (from $NH_4^+$ to $NO_2^-$) [39]. The value of dpH/d$t$ was commonly used to detect the ammonia valley, which can be detected by judging the point of dpH/d$t$ from negative to positive. Long time operation with the use of aeration control was beneficial for the nitrite accumulation and the dominant growth of AOB over NOB. However, extended aeration encouraged the transition of partial nitrification to complete nitrification. More details about achieving partial nitrification to nitrite with the use of aeration duration control can be found in the pervious studies [13,15].

Typical variations of nitrogen species and the control parameters including DO, pH and dpH/d$t$ at room temperature (21.5 °C) are shown in Fig. 1. It was observed that $NH_4^+$-N decreased with elapsed time under aerobic condition, whereas there was a corresponding increase of $NO_2^-$-N concentration. The ammonia valley appearing in pH profile (the point of dpH/d$t$ from negative to positive) could indicate distinctly that the ammonium oxidation was complete, and then the aeration would be stopped. At the end of aeration phase, ammonia concentration was lower than 1 mg N $L^{-1}$ and nitrite concentration was about 27.8 mg N $L^{-1}$. The nitrate production rate (0.003 g N $g^{-1}$ MLSS $d^{-1}$) was distinctly lower than the nitrite production rate (0.051 g N $g^{-1}$ MLSS $d^{-1}$). At the end of aeration phase, nitrate concentration was only 2.6 mg $L^{-1}$.

Typical variations of nitrogen concentrations and control parameters at low temperature (13.5 °C) are shown in Fig. 2. At low temperatures the aeration duration control could still not only indicate the completion of ammonia oxidation process, but also achieve the high nitrite accumulation ratio. Compared to the results at room temperature (Fig. 1), the aeration duration would be prolonged due to lower rates of ammonia oxidation with the decrease in temperature. It was demonstrated that aeration duration control was quite robust and effective on detecting characteristic point despite of temperature variation. At the end point of ammonia oxidation, the ammonia valley in pH profile and the inflexion point in dpH/d$t$ were easily observed. In addition, the ammonia oxidation was also complete (ammonia concentration was lower than 1 mg N $L^{-1}$) and the transformation from nitrite to nitrate was avoided (nitrate concentration was almost negligible). The nitrate production rate (0.001 g N $g^{-1}$ MLSS $d^{-1}$) was also distinctly lower than the nitrite production rate (0.040 g N $g^{-1}$ MLSS $d^{-1}$). The differences of substrate oxidation rates between AOB and NOB were gradually expanded with increase in operation time.

### 3.2. The long-term effects of temperature on partial nitrification

The feasibility of achievement of the partial nitrification process at lower temperatures has been investigated. During the overall operation period, the aeration phase was always controlled long enough for effective ammonia oxidation, but was inadequate for nitrite to be further oxidized. Considering that a rapid change in the operational conditions could lead to a destabilization of the biological system, partial nitrification at low temperatures was achieved by progressively decreasing temperature. Fig. 3 shows the operational performance during the operation period (phase II and phase III), including ammonia removal efficiency ($NH_4$-N%), nitrite accumulation ratio ($NO_2$-N% = $NO_2^-$-N/$NO_x^-$-N, $NO_x^-$-N = $NO_2^-$-N + $NO_3^-$-N), nitrate conversion ratio ($NO_3$-N% = $NO_3^-$-N/$NO_x^-$-N) and temperature variations. Ammonium was totally depleted at both high and low temperatures, the average of ammonia removal efficiency was higher than 97%. The sludge in the reactor was gradually adapted to lower operating temperatures. Although low

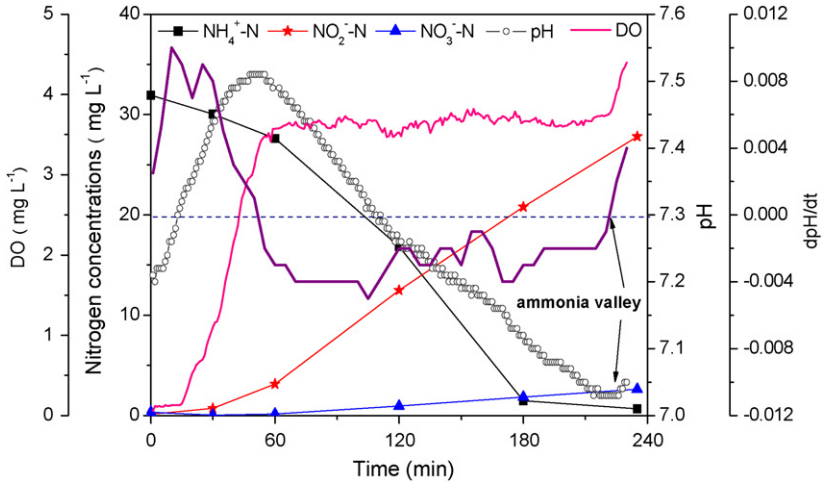


**Fig. 1.** Typical variations of nitrogen species and control parameters at temperature of 21.5 °C.

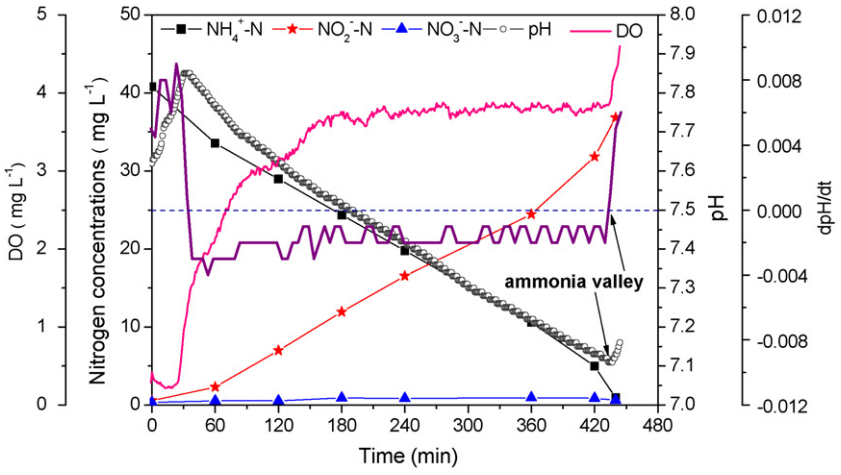


**Fig. 2.** Typical variations of nitrogen species and control parameters at temperature of 13.5 °C.

temperatures affected biochemistry reaction rates, the ammonium oxidation process was still capable of being completed. Significantly, low temperatures did not deteriorate the stable partial nitrification performance. The mean of nitrite accumulation ratio was 95% all along phase III, which was slightly higher than the ratio in phase II (90%). The reason for higher nitrite accumulation ratio at low temperatures was that the nitrifying microorganism community got optimized by aeration duration control for long-term operation (more details can be found in Section 3.3). According to the operation experiences, it is suggested that a feasible and advisable start-up strategy to achieve partial nitrification process at low temperatures could have two steps. The first one would be the selective production of the accumulated AOB by aeration duration control or other optimized conditions, such as high temperature or inhibitors. Then, the second step would be the slow adaptation of the biomass to low temperatures. In this study, the selective accumulation of AOB and the washout of NOB with the use of aeration control for long time operation was the critical point to maintain stable partial nitrification at low temperatures.

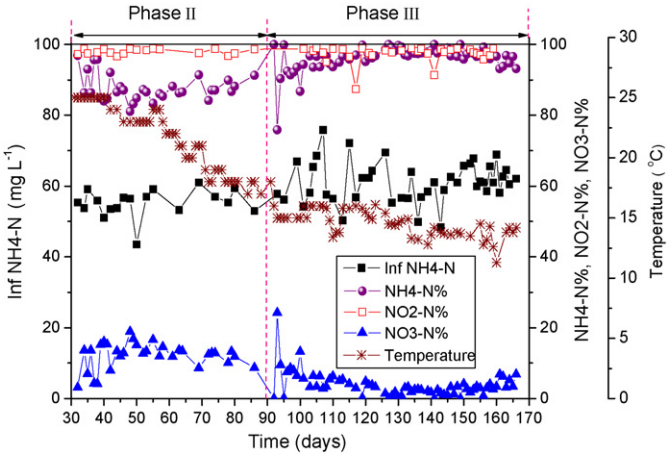


**Fig. 3.** SBR operation performance and temperature during phase II and phase III.

The temperature dependency profile obtained for the biomass in partial nitrification reactor (Fig. 4) was similar and consistent with the results obtained by other authors [15,24]. An exponential increase of the maximum specific ammonia oxidation rate was observed for temperatures up to 35 °C. When the temperature decreased from 25 to 15 °C, the specific ammonia oxidation rate decreased by 1.5 times from 0.064 to 0.043 g $NH_4^+$-N $g^{-1}$ MLSS $d^{-1}$. Moreover, the specific ammonia oxidation rate increased as temperature increased and was satisfactorily described ($R^2 = 0.96$) using the Arrhenius expression ($\theta = 1.051$). Knowles et al. [40] presented temperature coefficient $\theta$ of 1.103 for nitrification. Painter and Loveless [41] found temperature coefficient of 1.076. The temperature coefficient in this current study was slightly lower

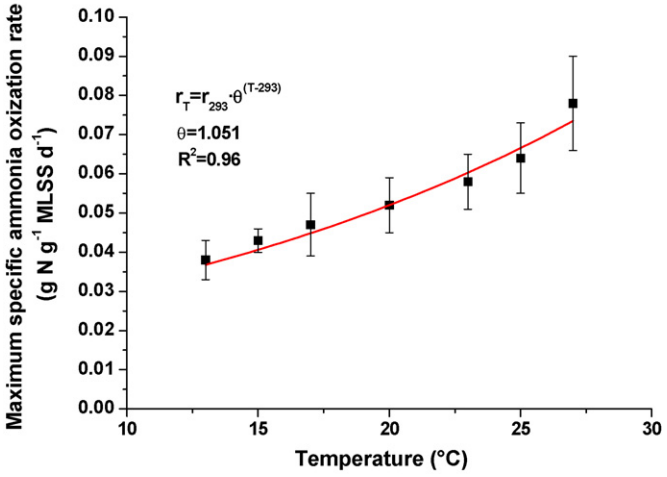


**Fig. 4.** Long-term effect of temperature on the specific ammonia oxidation rate (error bars indicate standard deviations of measurements).

than the reported value (1.12) in ASM2 [42], which indicated the lower dependence of the nitrification reaction rate on temperature. This lower dependence was mainly attributed to the fact that the microorganism was adapted to low temperature ammonia oxidation process by gradually lowering temperature strategy. Popel and Fischer [43] proposed that the effect of temperature on nitrification in activated sludge processes was often lower than expected from literature data, for the reason that reactor configuration, hydraulic residence time (HRT) and effluent concentration might have also played an important role in reducing the observed influence of temperature.

### 3.3. Sludge population structure at low temperature

The key point for the stable nitrite accumulation during long-term operation depends on achieving the optimization of nitrifying microorganism community, which implies AOB become the dominant nitrifying bacteria, while NOB are gradually washed out from the system. As noted previously, the low temperatures did not deteriorate stable partial nitrification, which was also attributed to the fact that the nitrifying microorganism community got optimized. The composition of the microbial population was characterized using FISH method. The quantification of the microbial population distribution indicated that, approximately, the size of AOB was 3–4% and the size of NOB was 2.5–4% (including *Nitrospira* of $2.4 \pm 0.4\%$ and *Nitrobacter* of $0.7 \pm 0.3\%$). After long-term application of aeration duration control, the AOB population size increased to 7–9%, but the NOB population size was almost negligible in the SBR. Since COD in influent was available for the growth of heterotrophic bacteria, the dominant organism was common heterotrophic bacteria in this system. Heterotrophs accounted for above 85% of total bacteria. The fraction of heterotrophs was estimated from the remaining relative area other than those of AOB and NOB. Compared to heterotrophic bacteria, the percentage of nitrifying bacteria was less than 15% accounting for the overall organisms. Although AOB was not the dominant bacteria, they were the dominant nitrifying bacteria compared to NOB in the system. Typical images of AOB and NOB under different phases are shown in Fig. 5.

Although FISH analysis is a semi-quantitative estimation and cannot give accurate population size [36], it is almost certain that AOB were more predominant than NOB in the reactor based on operational performance with such higher nitrite accumulation ratio. As mentioned above, the possible reason for the elimination or the reduction of the NOB population was associated with the long-term application of aeration duration control. On the other hand, since nitrate was still produced at the end of the experiment (Fig. 3), one can suspect whether NOB in smaller percentage still existed in the system and whether they could grow again. However, the immediately switch-off aeration after detecting the end point of ammonia oxidation was not enough for NOB completely oxidizing nitrite to nitrate in a cycle. Moreover, the AOB population size was distinctly dominant over the NOB population size in the reactor. In this case, fewer NOB could not attain enough or more growth opportunities, thus they could not out-compete the accumulated AOB. Therefore, the nitrite accumulation was able to be steadily maintained in the reactor, which kept operating at even low temperatures. This point has also been demonstrated in a pilot-scale SBR treating municipal wastewater for long time operation under low temperature [15]. Therefore, the approach of obtaining the selective enrichment of AOB as well as the washout of NOB by process control before making the biomass slowly adapt to low temperatures is feasible to achieve successfully stable nitrite production at low temperatures.

### 3.4. The short-term effects of temperature on partial nitrification

The short-term effects of temperature on the respiratory activities of AOB had been investigated in literature. However, mixed culture of nitrifying bacteria was used or the ratio of AOB was not mentioned when using the enriched culture of AOB. In this study, the short-term effects of temperature on ammonia oxidation process were estimated after the parent reactor had been operated at low temperatures for two months. The maximum ammonia oxidation rate of the nitrifying sludge accumulated with AOB (7–9% relative to the domain bacteria) was measured in batch tests in triplicate at temperatures between 5 and 35 °C. The ammonia oxidation rate and the nitrite production rate increased with temperature increase (Fig. 6). At temperatures between 5 and 10 °C, the ammonia oxidation rates were very slow. However, the main product of nitrification was still nitrite and almost no nitrate was produced with varying temperatures. The maximum ammonia utilization rate can be determined from the slope of ammonia removal as illustrated in Fig. 6a. In estimating the slopes, initial points that had been obtained at a relatively high substrate concentration were chosen for linear regression. The relationship of ammonia consumption and time was linearly fitted very well ($R^2 > 0.99$ in all cases), which indicated that the ammonia oxidation followed the zero-order reaction kinetics. The trend of ammonium oxidation rate with increasing temperature was comparable to the observations made by other studies. Groeneweg et al. [44] observed that the ammonium consumption kinetics increased by a factor of 3 as the temperature rose from 10 to 30 °C. Kim et al. [24] presented that the specific ammonia oxidation rate increased by 5.3 times with the increase in temperature from 10 to 30 °C. In addition, Wang and Yang [45] found the increase in ammonium oxidation rate by a factor of 4.5 when temperature increased from 12 to 30 °C using the enriched culture of AOB (but the ratio of AOB was not mentioned). As for the specific ammonia oxidation rate in this study, it increased by 24 times as the temperature increased from 5 to 35 °C and increased by 12 times as the temperature increased from 10 to 30 °C.

Moreover, the relationship between the maximum specific ammonia oxidation rate and temperature is illustrated in Fig. 7. The maximum specific ammonia oxidation rate from batch tests increased as temperature rose from 5 to 35 °C and was satisfactorily described ($R^2 = 0.929$) using the Arrhenius expression ($\theta = 1.065$). In fact, the temperature coefficient should be considered separately in different temperature intervals. The increasing slope of maximum specific ammonia oxidation rate as increasing temperature was higher from 5 to 20 °C, compared to temperature from 20 and 35 °C. The temperature effects on the maximum specific ammonia utilization rate were better predicted with the simplified

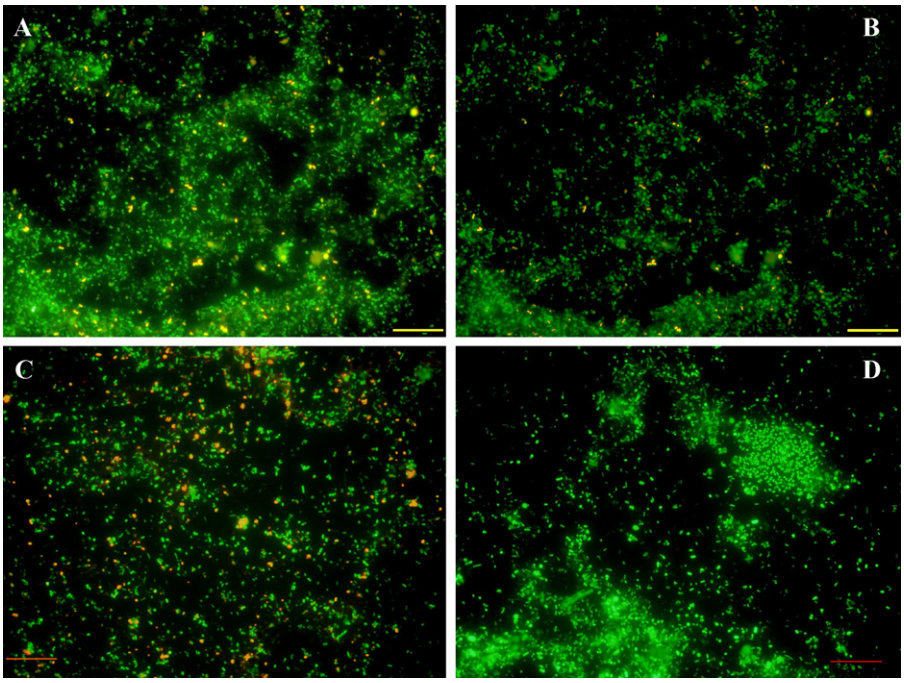


**Fig. 5.** FISH images for AOB and NOB during operation period. (A) AOB (with Cy3 labeled Nso190) in seed sludge; (B) NOB (with Cy3 labeled Ntspa662) in seed sludge; (C) AOB (with Cy3 labeled Nso190) at low temperature on day 150; and (D) NOB (with Cy3 labeled Ntspa662) at low temperature on day 150. Eubacteria are shown in green and AOB are shown in yellow in pictures A and C. NOB are shown in yellow in picture B. In picture D, the overall field taken by Eubacteria was shown in green and NOB were not detected. Bar = 20 μm. It was observed sometimes that the fluorescence intensities obtained with Nso1225 were weaker than those obtained with Nso190, the probe Nso190 was mainly used to for quantitative FISH. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Arrhenius equation: $\theta = 1.172$, $R^2 = 0.995$ under temperature from 5 to 20 °C and $\theta = 1.062$, $R^2 = 0.955$ under temperature from 20 to 35 °C. The higher temperature coefficient indicates that temperature has more distinct effect on the ammonia oxidation rate. Therefore, it can be concluded that the ammonia oxidation rate was influenced by temperature more sensitively at low temperatures than at high temperatures. Temperature dependency factors for the ammonia oxidation process have been published elsewhere and several are shown in Table 2. The temperature coefficient obtained in this work actually agreed with published data on nitrification with activated sludge as well as biofilm, which is normally quoted in the range 1.05–1.12 [38,42,46,47]. In ASM2, the temperature coefficient recommended is 1.12 under temperature in the range of 10–25 °C [42], which is not given separately in different temperature intervals. The quality of the fits using ASM models might become more accurate, if two different temperature coefficients are used to calibrate kinetics coefficients. In addition, temperature coefficient in the expression for the maximum effective specific growth rate should be wastewater specific. It is recommended that experiments be carried out to obtain values for a particular wastewater over the range of temperature likely to be encountered during operation period.

Furthermore, different temperature coefficients were observed when comparing the short-term and long-term effects of temperature on the biomass. As long-term effect, AOB was progressively adapted to low temperature and low temperature did not cause a distinct decrease of the activity of AOB. The predicted temperature coefficient was 1.051 in long-term effect of temperature. As short-term effect, the temperature coefficient was 1.065, which was a bit higher than that under long-term effect. Higher temperature coefficient might be associated that AOB cannot adopt the rapid changing of temperature. In addition, the difference between short-term effect and long-term effect of temperature on partial nitrification might caused by the shift of the AOB species in the biomass. Further investigation is required to demonstrate if the microbial community structure of AOB has shifted with the changing temperature.

The activation energy of ammonia oxidation process can be determined graphically by taking the natural logarithm of Arrhenius equation. An Arrhenius plot in Fig. 8 shows a break at 20 °C and two different activation energies, as indicated by the two different slopes. The activation energies of ammonia oxidation in the temperature ranges of 5–20 and 20–35 °C were determined from the slope of the two straight lines and were 111.5 and 42.0 kJ mol$^{-1}$, respectively. The larger value was found at lower temperatures, whereas the smaller value was observed at higher temperatures, as in the other studies [24,48]. Moreover, the energy of activation of ammonia oxidation at the lower temperature range (5–20 °C)

**Table 2**
Comparison of temperature coefficients ($\theta$) for AOB.

| $\theta$ | Culture | Conditions | Reactor type | Temperature range (°C) | Reference |
|---|---|---|---|---|---|
| 1.098 | Activated sludge | Domestic sewage | Batch test | 15–20 | [38] |
| 1.098 | Biofilm | Ammonia-limiting | Moving-bed | 10.2–23.3 | [47] |
| 1.058 | Biofilm | Oxygen-limiting | Moving-bed | 12.5–28.1 | [47] |
| 1.045 | Activated sludge | Tanning wastewater | Full-scale WWTP | 20–35 | [46] |
| 1.120 | Activated sludge | – | – | 10–25 | [42] |
| 1.051 | Partial nitrification sludge | Long-term effect | SBR | 13–25 | This study |
| 1.172 | Partial nitrification sludge | Short-term effect | Batch test | 5–20 | This study |
| 1.062 | Partial nitrification sludge | Short-term effect | Batch test | 20–35 | This study |

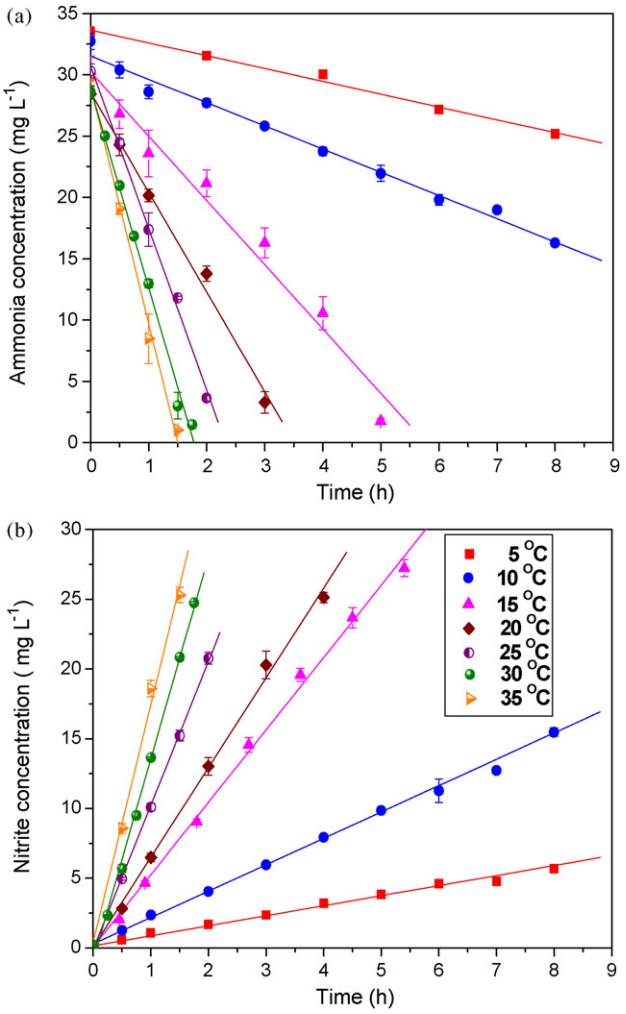


**Fig. 6.** The aerobic metabolism of ammonia oxidation organisms as function of temperature: (a) ammonium; (b) nitrite. Error bars indicate standard deviations of measurements.

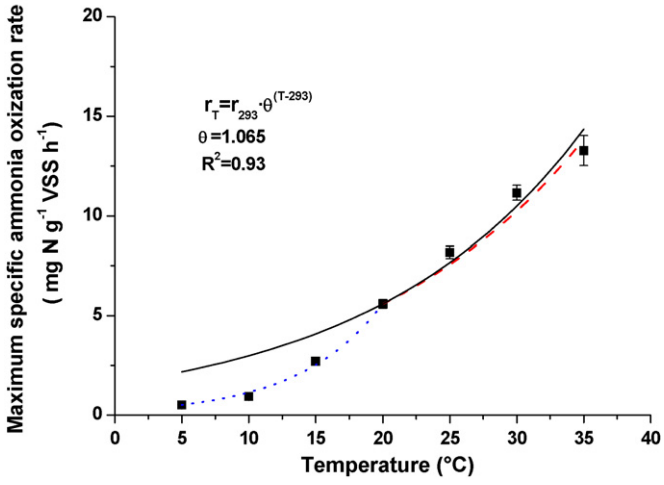


**Fig. 7.** Short-term effect of temperature on the maximum specific ammonia oxidation rate (error bars indicate standard deviations of measurements; black line is fit for temperature from 5 to 35 °C; blue short dash line is fit for temperature from 5 to 20 °C and red dash line is fit for temperature from 20 to 35 °C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

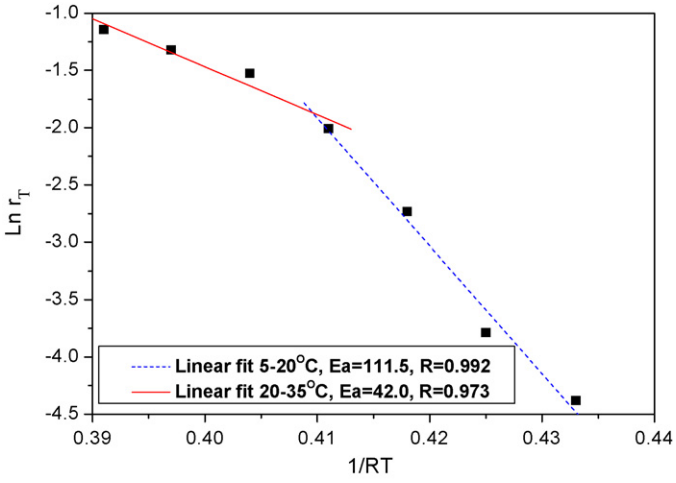


**Fig. 8.** Activation energy of ammonia oxidation reaction with partial nitrification sludge.

**Table 3**
Comparison of activation energies for AOB reported in literature and this study.

| Activation energy (kJ mol$^{-1}$) | Culture | Temperature range (°C) | Reference |
|---|---|---|---|
| 24.6 | Enriched culture | 12–34 | [49] |
| 29 | Activated sludge | 10–35 | [50] |
| 38.6 | Activated sludge | 20–30 | [24] |
| 42.0 | Partial nitrification sludge | 20–35 | This study |
| 49–54 | Activated sludge | 4–25 | [51] |
| 56 | immobilized cells | 20–35 | [48] |
| 62.3 | *Nitrosomonas europaea* | 11–25 | [52] |
| 66.9 | Enriched culture | 9–34 | [53] |
| 73 | Free *Nitrosomonas* cells | 10–35 | [48] |
| 86.4 | *Nitrosomonas europaea* | 2–30 | [54] |
| 87.1 | Activated sludge | 10–20 | [24] |
| 111.5 | Partial nitrification sludge | 5–20 | This study |
| 163 | Immobilized cells | 10–20 | [48] |
| 253 | Enriched culture | 4–12 | [49] |
| 317 | Activated sludge | 5–17 | [55] |

was greater by a factor of 2.5 compared to the higher temperature range (20–35 °C).

The comparison in details of activation energies for AOB reported in different studies is listed in Table 3. Based on the comparison, activation energies are relatively lower for pure culture or enriched AOB than values for mixed culture. The result of apparent energy activation under higher temperature range in this study was higher than the value observed by Kim et al. [24], but the activation energy under lower temperature range was lower than that reported by Weon et al. [49]. The main reason for different activation energy values might be the different nitrifying community in the seed sludge for batch tests, as well as microorganism cultivation history. The dominant nitrifying bacteria for batch test in this work was the accumulated AOB that had adapted to low temperature ammonia oxidation process, compared to mixed culture of AOB and NOB that had not encountered low temperatures in the study by Kim et al. [24]. Moreover similar with our observation, two studies reported two distinct activation energies in ammonia oxidation by AOB at two different temperature ranges. As suggested by Kim et al. [24], the different activation energies under lower temperatures and higher temperatures might be attributed to the turnover of rate-limiting step between the oxidation of $NH_3$ to $NH_2OH$ and the oxidation of $NH_2OH$ to $NO_2^-$. In addition, two different activation energies were also observed using immobilized cells of AOB, but the only value (73 kJ mol$^{-1}$) was obtained with free cells of AOB [48]. Therefore, Benyahia and Polomarkaki [48] suggested that dif-

ferent rate-limiting steps for nitrification using immobilized cells might apply according to the range of temperatures employed. In order to elucidate the mechanism and define the rate-limiting step at different temperature ranges, further investigation is required to study the temperature dependence of two key enzymes involved in ammonia oxidation reaction, including ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO).

## 4. Conclusions

Long- and short-term effects of temperature on partial nitrification were investigated and several conclusions are obtained as follows:

(1) By decreasing the temperature gradually, the partial nitrification in an SBR established by controlling aeration duration was successfully operated at low temperatures. Low temperature did not deteriorate stable partial nitrification performance and nitrite accumulation ratio was always above 90%, although the ammonia oxidation rate decreased as temperature decreased.
(2) The sludge with only AOB as the dominant nitrifying bacteria was used to investigate the short-term effects of temperature on ammonia oxidation process. The temperature coefficient was calculated separately, $\theta$ was 1.172 under temperature from 5 to 20 °C and was 1.062 under temperature from 20 to 35 °C, respectively.
(3) The larger activation energy (111.5 kJ mol$^{-1}$) was found in the range of lower temperatures (5–20 °C), whereas the smaller value (42.0 kJ mol$^{-1}$) was observed at higher temperatures of 20–35 °C. Two different activation energies indicated that the rate-limiting step of ammonia oxidation process was not identical at different temperature ranges.
(4) The selective enrichment of AOB and the washout of NOB by adopting aeration duration control was the critical point to achieve and maintain partial nitrification to nitrite in the reactor or WWTPs operated under low temperatures.

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