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Nutrients removal and nitrous oxide emission during simultaneous nitrification, denitrification, and phosphorus removal process: impact of temperature

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ABSTRACT

Simultaneous nitrification, denitrification, and phosphorus removal (SNDPR) process was achieved in a SBR system operated in anaerobic–aerobic (low dissolved oxygen (DO)) mode. The contaminant removal and nitrous oxide (N_2O) emission characteristics in the reactor at two different temperatures (25 and 10˚C) were investigated to evaluate the impacts of low temperature on SNDPR process. The results showed that TP and TN removal efficiencies of the reactor were 95.6 and 77%, respectively at 25˚C indicating that simultaneous nitrogen and phosphorus removal were successfully achieved in the reactor. However, the removal of TN and ammonium was weakened significantly by the low temperature. The low temperature has little impact on phosphorus removal, mainly due to the enrichment of PAOs at low temperature. The N_2O emission mainly occurred during the low DO aerobic period. N_2O emission amount at 10˚C was calculated as 648.8 μg N/g MLSS, which was about 43.3% higher than that at 25˚C, and 10.11% of nitrogen removed was converted to N_2O at low temperature, showing that low temperature stimulated the emission of N_2O during SNDPR process.

Keywords: Nitrous oxide emission; Nutrients removal; SNDPR; Temperature; PHA

1. Introduction

Simultaneous nitrification, denitrification, and phosphorus removal (SNDPR) process is a novel biological nutrients removal (BNR) system, which couples simultaneous nitrification–denitrification (SND) process and denitrifying phosphorus removal process in one reactor. Nitrogen and phosphorus could be removed simultaneously using the intracellular carbon source during SNDPR process, saving the COD requirement. In addition, it has a 20–30% lower cell yield. Therefore, compared with conventional BNR process, SNDPR is highly beneficial in terms of a lower COD demand, reduced aeration cost, and less sludge production [1].

It has been found that denitrification can be accomplished by the so-called denitrifying phosphorus accumulating organisms (DPAOs) in

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anaerobic–anoxic-enhanced biological phosphorus removal (EBPR) systems, allowing simultaneous nitrate–nitrite reduction and phosphorus uptake using the same COD [2]. In alternating anaerobic–aerobic mode with a low dissolved oxygen (DO), concentration, nitrification, denitrification, and phosphorus removal can be carried out simultaneously [1]. The SNDPR process has been achieved by many researchers using different reactors, such as SBR [3], MBR [4], oxidation ditch [5], and granular sludge systems [6].

However, it was reported that SNDPR process could produce a significant amount of nitrous oxide (N_2O) during nitrogen removal [7]. N_2O is considered as one critical greenhouse gas and has a global-warming potential approximately 300 times larger than CO_2 of equal mass. In addition, N_2O is a significant contributor to the destruction of ozone layer [8]. Over the last decade, the issue of N_2O emission has attracted more and more attentions of the governments and researchers [9–11]. It has proved that BNR process is an important source of N_2O, and can contribute to N_2O generation varying between 0 and 95% of nitrogen load [12].

N_2O can be produced via nitrification, denitrification, dissimilatory reduction of NO_3^- to NH_4^+, and chemo-denitrification processes during the microbial transformations of nitrogenous compounds [13]. During SNDPR, many processes could induce the production of N_2O. At the low DO condition, nitrifier denitrification and heterotrophic denitrification are considered as the dominate nitrogen removal process and both could produce much N_2O [14]. Moreover, during denitrifying phosphorus removal process, denitrification is carried out using intracellular poly-β-hydroxyalkanoates (PHA) as carbon source, which can stimulate the emission of N_2O [2]. The N_2O emission characteristics and mechanisms of SNDPR process would be more complex due to the coupling of low-DO SND and denitrifying phosphorus removal processes. In order to optimize the SNDPR process and make it environmental friendly, the N_2O emission characteristics must be investigated in depth.

N_2O emission during BNR is affected by many factors, such as oxygen level, COD/N ratio, carbon source, and nitrite concentration [9]. In addition, N_2O is mainly produced by the metabolism of micro-organisms during nitrogen transformation, which is influenced significantly by environmental temperature. It was reported that temperature was the vital factor influencing both nitrification and denitrification during SND process [15]. Moreover, the denitrifying phosphorus removal process could be completely inhibited at low temperature [16]. The enzymes related to nitrogen conversion could also be affected by temperature. Previous literature showed that the low temperature could affect the active sites of N_2O reductase, thereby causing a lower N_2O emission rate at low temperature [17]. Furthermore, the microbial community structure is also affected by temperature. It was reported that the competition between PAOs and glycogen-accumulating organisms (GAOs), which were two dominant heterotrophic microbes in EBPR system, was greatly affected by temperature and found that PAOs were dominant at low temperature [18]. When operated in winter, the simultaneous nitrogen and phosphorus removal of SNDPR process would be affected by the low environmental temperatures, and the nutrients removal pathway and removal characteristics may change, thereby influencing the emission of N_2O. However, to date, few literatures are available regarding the impact of low temperature on nutrients removal and N_2O emission characteristics during SNDPR process.

In this study, the SNDPR process was achieved in a lab-scale SBR reactor operated in alternating anaerobic–aerobic (low DO condition) mode, and then the reactor was operated at low temperatures (10˚C). The nutrients removal and N_2O emission characteristics during SNDPR process were investigated. Furthermore, the impacts of temperature on nutrients removal and N_2O emission during SNDPR process were also evaluated to further explore the N_2O emission mechanism during SNDPR process.

2. Materials and methods

2.1. Reactor setup and operation

Experiments were carried out in a lab-scale SBR which was made of a transparent, rigid plexiglas cylinder with an effective volume of 8 L. The schematic description of the experiment system was shown in Fig. 1. Biomass was enriched in the SBR seeding with sludge from a local municipal wastewater treatment plant (Xuzhou, China), and the concentration of mixed liquor suspended solids (MLSS) in SBR was maintained at approximately 3,200–3,500 mg/L.

The SBR was operated with a cycle time of 6 h, consisted of a 1.5-h anaerobic period and a 3.5-h aerobic period, followed by 45-min settling and 15-min decanting. Four liters of synthetic wastewater was pumped into the reactor in the first 5 min of the anaerobic period. An electric agitator with a rectangular paddle was used to keep sludge suspended during anaerobic period. During the aerobic period, air supply was regulated by using an on/off control system to keep the DO at between 0.35 and 0.65 mg/L. After

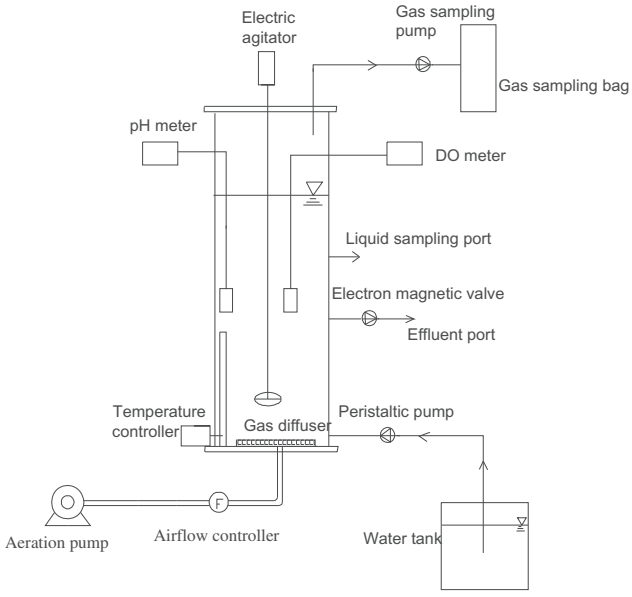

Fig. 1. Schematic description of the experiment system.

the settling period, 4 L of supernatant was removed, resulting in a hydraulic retention time of 12 h. Before settling, about 0.5 L mixed liquor was wasted to keep the solids retention time at approximately 16 d. The pH in the reactor was recorded, but not controlled, and fluctuated between 7.0 and 7.5.

In order to achieve SNDPR quickly, the reactor was operated at room temperature (25 ± 2℃) during the startup period. After SNDPR process was achieved in the reactor, the impacts of environmental temperature on nutrients removal and N_2O emission characteristics were explored. The SBR was operated at 25 ± 2℃ and 10 ± 2℃, respectively for two months. During each period, the removal efficiencies of nitrogen, phosphorous, and COD were evaluated every three days. In addition, the N_2O emission rate during one running cycle was measured twice per month by collecting the off-gases at intervals of 30 min. Meanwhile, the liquid and solid samples were taken to measure the water quality and intracellular polymers content.

2.2. Synthetic feed

For better investigation on N_2O emission characteristics, synthetic municipal wastewater was used in this study to eliminate the influence of water quality fluctuation. The wastewater contained, per liter: 512 mg CH_3COONa, 153 mg NH_4Cl, 200 mg $NaHCO_3$, 33 mg KH_2PO_4, 55 mg K_2HPO_4, 10 mg $MgSO_4{\cdot}7H_2O$, 10 mg $FeSO_4{\cdot}7H_2O$, 17 mg $CaCl_2$, and 1 mL nutrient solution. The composition of nutrient solution can be found in the previous study [19]. The complete influent contained 400 mg/L COD, 40 mg/L NH_4-N, and 15 mg/L TP.

2.3. Analytical methods

The analysis of COD, NH_4^+-N, NO_3^--N, NO_2^--N, TN, TP and MLSS were conducted in accordance with the standard methods [20]. TN was determined by a TOC/TN Analyzer (Multi N/C-3100, analytikjena, Germany). DO was measured with a DO meter (HQ30d53LDO™, Hach, USA). N_2O concentration was determined by the gas chromatography (SP-3410, China) with an electron capture detector and a Poropak Q column. The concentration of intracellular PHAs (including poly-b-hydroxybutyrate (PHB), polyhydroxyvalerate (PHV), and poly-3-hydroxy-2-methylvalerate (PH2MV)) was measured according to the method described by Oehmen et al. [21]. Glycogen was analyzed according to the method presented by Zeng et al. [22].

The SND efficiency was calculated according to the equation described by Zeng et al. [23]. The emission rate and quantity of N_2O were calculated using the equation described by Hu et al. [19]. N_2O-N conversion rate was calculated by Eq. (1):

$$N_2O\text{-N conversion rate} = N_2O\text{-N/TN removed} \times 100\% \quad (1)$$

All statistical tests were performed with the statistical program SPSS 19.0 (SPSS Inc., Chicago, USA). For all tests, differences were considered significant only if $p < 0.05$.

3. Results and discussion

3.1. Contaminant removal performance of SNDPR system

Table 1 shows the contaminant removal performance of the SNDPR reactor at each temperature. COD removal efficiency of SNDPR process was high (>90%) and there was no significant difference when running at two temperatures ($p = 0.138$). The removal of organic matter mainly relied on the metabolism of heterotrophic microbes. It was reported that low temperature could slow down the microbial activity, as well as chemical and enzymatic reactions, leading to the decrease of COD removal [24]. However, in this study, the impact of temperature on COD removal during SNDPR process was inconspicuous, and the COD removal efficiency was as high as 90.4% at low temperature. In fact, the previous literature has shown

Table 1
Mean influent and effluent contaminant concentrations with standard deviations (in brackets) and removal efficiencies at 25 and 10˚C during SNDP process. All the data are mean values of at least 15 experiments

	25˚C			10˚C		
Parameters	Influent (mg/L)	Effluent (mg/L)	Removal efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Removal efficiency (%)
COD	390.5 (29.2)	33.8 (3.7)	91.3	396.8 (16.1)	38.1 (1.1)	90.4
TP	15.8 (0.4)	0.7 (0.3)	95.6	14.6 (2.0)	0.9 (0.6)	93.8
TN	35.2 (4.2)	7.8 (3.5)	77.7	32.8 (1.0)	11.0 (3.5)	66.5
NH_4^+	33.8 (2.7)	0.2 (0.2)	99.3	38.6 (0.6)	6.2 (1.2)	84.0
NO_2^-	0.6 (0.4)	0.2 (0.2)	–	0.3 (0.2)	1.3 (0.9)	–
NO_3^-	3.6 (2.0)	5.2 (2.1)	–	4.2 (1.3)	7.9 (3.2)	–

that although there was positive correlation between temperature and organic matter removal in cold climate, the correlation coefficient was small [25]. It illustrated that the influence of low temperature on the removal of organic matter was negligible. In the present study, the organic matter used in the synthetic wastewater was only acetate, which was easily biodegradable. In addition, the anaerobic duration in this study was long enough for the heterotrophic denitrifiers and PAOs to absorb the carbon. Therefore, although the microbial activity would be lowered due to the low temperature, the COD removal efficiency at low temperature was still similar to that at high temperature.

The SNDPR reactor gained favorable TP and TN removal efficiency at 25˚C. The TP removal efficiency reached to 95.6% and the TN removal efficiency was also above 77%. The results indicated that the simultaneous nitrogen and phosphorus removal occurred in the reactor and the SNDPR process was successfully achieved at 25˚C. When the reactor ran at 10˚C, the removal efficiency of TP was still as high as 93.8%, which was only slightly lower than that at 25˚C. Although it was reported that DPAOs were sensitive to temperature changes in the range of 10–30˚C, and the metabolism could be completely inhibited at low temperature [16], the impact of low temperature on phosphorus removal observed in the present study was insignificant ($p = 0.065$). The reason may be that the low temperature favored the growth and enrichment of PAOs, which was also reported by Brdjanovic et al. [26]. In addition, the nitrification was inhibited and the activities of nitrifiers were lowered at low temperature, thereby the PAOs would be in favor of phosphorus uptake due to the less competition for DO. Helmer and Kunst also found that a drop in temperature from 20 to 10˚C had no significant impact on the efficiency of EBPR [27].

However, the removal of TN and ammonium was weakened significantly by the low temperature ($p < 0.05$). TN removal efficiency at 10˚C was only 66.5%, which was 14.4% lower than that at 25˚C. In addition, the removal efficiency of NH_4-N decreased from 99.3 to 84%. Meanwhile, the effluent nitrite and nitrate concentrations increased at low temperature. Nitrogen removal during SNDPR process was conducted via nitrification coupled with denitrification (include heterotrophic denitrification, nitrifier denitrification, denitrifying phosphorus removal, etc.). It can be seen that the decrease of TN removal efficiency at low temperature was mainly due to the incompletely oxidation of ammonium. Many literatures have shown that the nitrification would be weakened at low temperature due to the decrease of the activity of nitrifiers [25,28]. Furthermore, the effluent concentrations of nitrite and nitrate at 10˚C were higher than that at 25˚C, indicating that the simultaneous denitrification rate during the low DO aerobic period decreased by the low temperature.

3.2. Nitrogen, phosphorus and carbon transformation of SNDPR reactor

In order to further explore the characteristics of the contaminant removal in SNDPR reactor at different temperatures, the cyclic nitrogen, phosphorus, and carbon transformations were detected. Fig. 2 shows the nitrogen transformation in the reactor during one operation cycle at different temperatures. It can be seen that at 25˚C, the concentrations of TN and NH_4-N kept unchanged during anaerobic period (Fig. 2(a)). The TN removal mainly occurred during the low DO aerobic period and the removal rate was high in the first 150 min of aerobic period. During this period, NH_4-N concentration decreased fast for nitrification, and the ammonium oxidation rate was calculated as

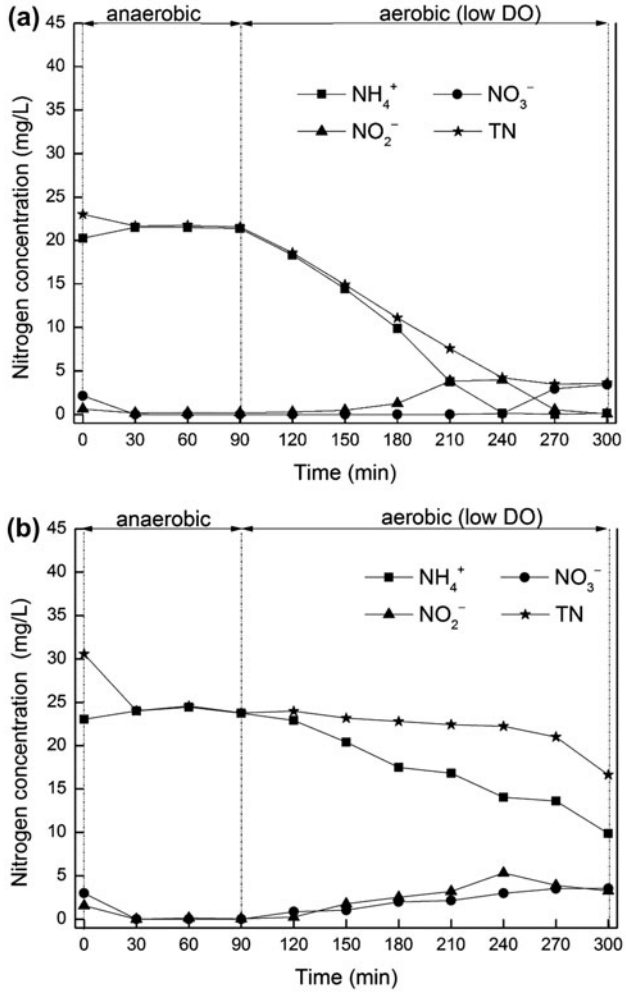

Fig. 2. Time profiles of nitrogen concentration during one cycle at different temperatures: (a) 25℃ and (b) 10℃. All data are mean values of at least 5 experiments.

8.5 mg N/L/h. At the same time, nearly no nitrate accumulated. The results indicated that simultaneous denitrification process during the low DO aerobic period was enhanced, and the SND process was successfully achieved, leading to high TN removal efficiency. Then during the last 30 min of aerobic period, the ammonium was almost completely removed. Although the nitrite concentration increased transitorily at 210 min, it decreased quickly to about zero, accompanied by the increase of nitrate concentration to about 4 mg/L. The SND efficiency of the SNDPR reactor at 25℃ was calculated as 83.7%.

The nitrogen transformation in SNDPR reactor at 10℃ was different to that at 25℃, especially during the low DO aerobic period (Fig. 2(b)). The TN removal also mainly occurred during the aerobic period. However, the removal rate was lower obviously than that at 25℃. NH_4-N was removed slowly during the aerobic period and the final concentration was about 9.9 mg/L. The ammonium oxidation rate was only 3.9 mg N/L/h, which was about 54% lower than that at 25℃. The results were in accordance with the previous literature that the nitrification rate doubled as temperature increased by 10℃ within the range of 5–30℃ [29]. The low temperature inhibited the activity of nitrifiers, leading to a low ammonium oxidation rate. Moreover, both nitrate and nitrite accumulated during the low DO aerobic period at 10℃. The concentration of NO_x-N increased at the beginning of aerobic period and the effluent concentration was about 6.8 mg/L. The SND efficiency of the reactor at 10℃ was only 53.0%, which was much lower than that at 25℃. The results revealed that the low temperature greatly inhibited the simultaneous nitrification and denitrification process during the low DO aerobic period, and the activities of nitrification and denitrification were decreased by the low temperature.

The transformation of COD and TP, as well as the contents of intracellular glycogen and PHA during one operation cycle of the SNDPR reactor were detected at different temperatures, and the results were shown in Fig. 3. Fig. 3(a) illustrated the transformation characteristics at 25℃. It can be seen that COD was almost removed completely in the first 30 min during the anaerobic period, which was accompanied by PHA synthesis, glycogen consumption and phosphorus release. COD was utilized by heterotrophic microorganisms, including denitrifiers, PAOs and GAOs. The influent carbon was consumed quickly for denitrification process occurring at the beginning of aerobic period (Fig. 2), and also for PHA synthesis by PAOs and GAOs. During the anaerobic period, PAOs could take up organic substrates and stored as PHA using the energy obtained partly from the glycogen utilization but mostly from the hydrolysis of the stored polyphosphate, resulting in the phosphorus release and glycogen consumption [30]. Then during the aerobic period, the PAOs oxidized the stored PHA to supply energy for the biomass growth, phosphorus uptake, and glycogen recovery, leading to the decrease of phosphorus and PHA concentration and the increase of glycogen content.

In this study, PHB was the dominant form of intracellular PHA, and a spot of PHV was found in the reactor. The results proved that both GAOs and PAOs exist in the SNDPR system, because the reason for the PHV production is the presence of GAOs [31]. Based on the different stoichiometry of PAOs and GAOs models, it can be estimated how much acetate is taken up by either PAOs or GAOs, and the activities of PAOs and GAOs can be evaluated according to the

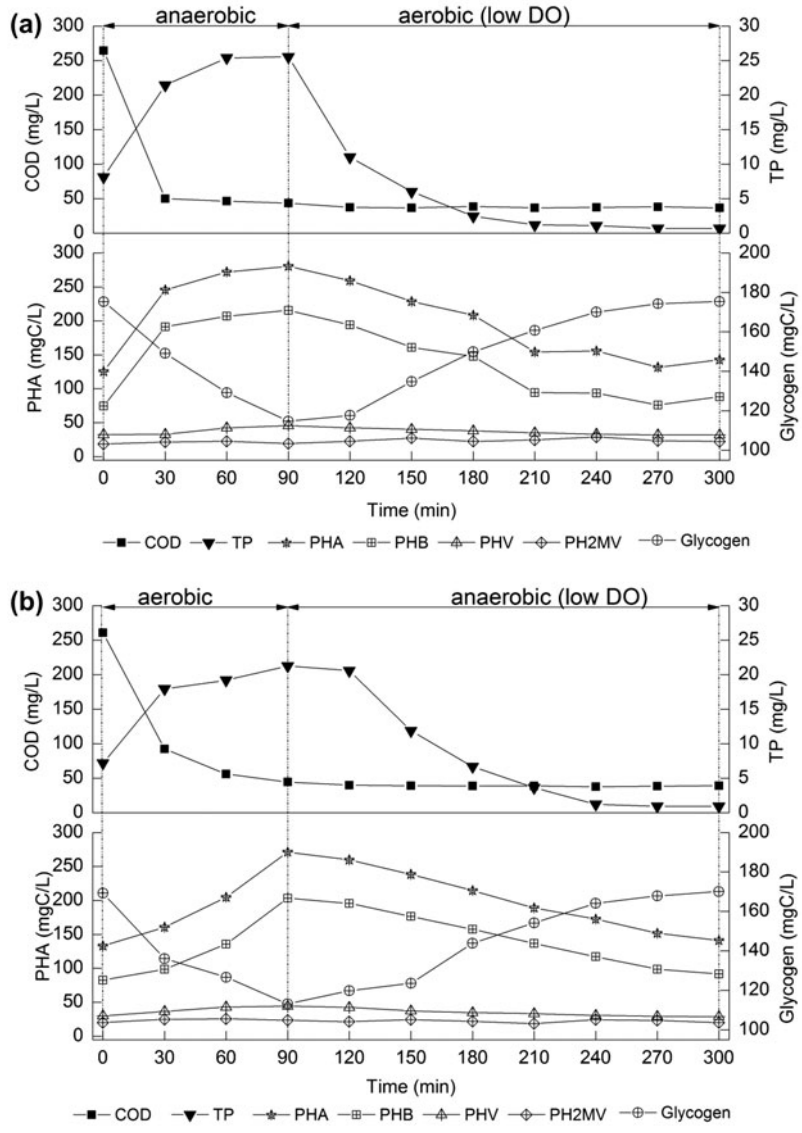

Fig. 3. Time profiles of COD, TP, PHA, and glycogen contents during one cycle at different temperatures: (a) 25℃ and (b) 10℃. All data are mean values of at least 5 experiments.

method developed by Zeng et al. [31]. It can be described as:

$$a = \frac{(1 + 2\alpha_{GAO})\mathrm{HAc} - \mathrm{Gly}}{0.5 + 2\alpha_{GAO}} \quad (2)$$

$$b = \frac{\mathrm{Gly} - 0.5\mathrm{HAc}}{0.5 + 2\alpha_{GAO}} \quad (3)$$

where a and b are the amounts of acetate taken by PAOs and GAOs, respectively, α_{GAO} represents the energy required to transport 1 C-mol acetate across the GAO cell membrane and is pH dependent. At pH 7.0, α_{GAO} is approximately 0.060 mol ATP/C-mol HAc.

Using the above equations, it was calculated that at 25℃, a = 4.99 mmol C/L (=69% of HAc), and b = 2.27 mmol C/L (=31% of HAc). The results indicated that PAOs instead of GAOs were the dominate biomass in the reactor. The previous literature demonstrated that PAOs and GAOs preferred acetate as carbon source, and accumulated PHB [32]. It was noteworthy that the amount of PHB utilized was more than the quantity of synthesized glycogen (C-mol based) during the aerobic period. It was quite likely that the PHB was utilized as carbon source to drive

denitrification during the low DO aerobic period (Fig. 2(a)).

The time profiles of phosphorus and carbon transformation during the SNDPR process at 10℃ were different to that at 25℃ (Fig. 3(b)). The COD concentration declined slowly during the anaerobic period and reached to the minimum value at 90 min. Although the effluent concentration remained unchanged, the removal rate of COD was lowered at the low temperature due to the decrease of microbial activity. TP concentration during anaerobic period increased to about 21.3 mg/L at 10℃, which was lower than that at 25℃ (25.5 mg/L). Furthermore, the uptake rate of phosphorus during the low DO aerobic period at 10℃ was also lowered. According to Eqs. (2) and (3), PAOs and GAOs took up 75 and 25% HAc, respectively. It can figure out that the PAOs were enriched at the low temperature. Therefore, the uptake of phosphorus was not influenced significantly at the low temperature, although the activity of DPAOs may be inhibited. At low temperature, simultaneous denitrification process during the low DO aerobic period was weakened (Table 1 and Fig. 2), and the activities of nitrifiers were also lowered. Although the nitrogen removal efficiency was inhibited by the low temperature, it would favor the phosphorus removal by PAOs because it would ease the competition for carbon and electron between PAOs and denitrifiers.

3.3. N_2O emission characteristics during SNDPR process

The N_2O emission rates during one operation cycle of the SNDPR reactor at different temperatures were shown in Fig. 4. Meanwhile, the N_2O emission amount per cycle and conversion rate were also calculated and shown in Table 2. It can be seen that the N_2O emission rate during anaerobic period was approximately zero, no matter at 25 or 10℃. The emission amount during anaerobic period was 3.59 and 3.13 μg N/g MLSS for 25 and 10℃, respectively, which accounted for about 0.8 and 0.5% of the whole N_2O emission. During the anaerobic period, the carbon source was sufficient, which was conducive to denitrification process without accumulation of N_2O. The temperature had no significant impact on N_2O emission during the anaerobic period ($p = 0.762$).

The N_2O emission of the SNDPR reactor mainly occurred during the low DO aerobic period. When running at 25℃, the N_2O emission rate of the SNDPR reactor increased rapidly at the beginning of aerobic period, and reached to the maximum value of 8.02 μg/g MLSS/min at about 210 min. Then, the emission rate decreased gradually to about 0.25 μg/g MLSS/min at the end. The average emission amount during the low DO aerobic period was calculated as 451.39 μg N/g MLSS, and about 5.76% of TN removed was converted to N_2O. During the SNDPR process, N_2O could be produced via different pathways, such as heterotrophic denitrification, nitrifier denitrification, denitrifying phosphorus removal, and so on [14]. At the low DO condition, nitrifier denitrification would be more favorable, leading to significant amounts of N_2O emission [33]. In addition, the heterotrophic denitrification would be inhibited due to the limited carbon source and the existence of oxygen, resulting in incompletely denitrification and accumulation of N_2O. Moreover, the denitrifying phosphorus removal was reported to be one important source of N_2O [34]. At the beginning of aerobic period, the denitrification could be carried out using PHA as carbon source, which would induce the competition of denitrifying enzymes for electron donor, resulting in the drastic increase of N_2O emission rate [9]. Then with the consumption of PHA, the rate of simultaneous denitrification and the denitrifying phosphorus removal decreased, accompanied by the reduction of N_2O emission rate.

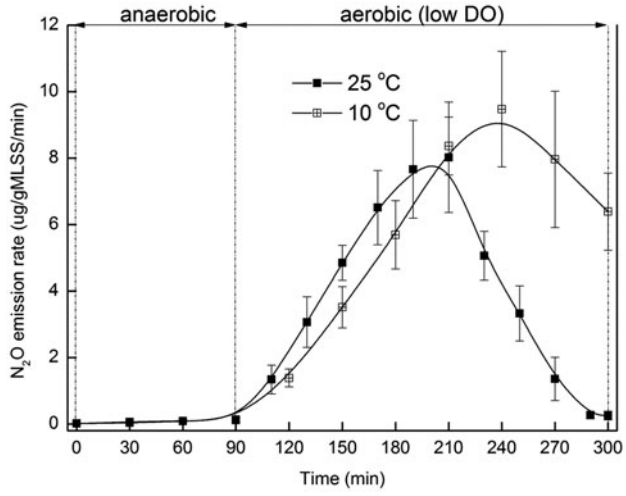

Fig. 4. Time profiles of N_2O emission rate during one operation cycle at different temperatures. All data are mean values of at least 5 experiments.

However, the time course of N_2O emission rate during the aerobic period at 10℃ was different to that at 25℃ (Fig. 4). At the beginning of aerobic period, the N_2O emission rate increased, which was similar to that of 25℃. Nevertheless, the increase rate was slightly lower. It may be caused by the low ammonium oxidation rate at the low temperature (Fig. 2(b)). The emission rate increased to the peak at about 240 min, and the maximum emission rate was about

Table 2
N_2O emission amount per cycle with standard deviations (in brackets) in the reactor at 25 and 10℃. All the data are mean values of at least 4 experiments

Temperature (℃)	N_2O-N emission during anaerobic period (μg N/g MLSS)	N_2O-N emission during aerobic period (μg N/g MLSS)	Total N_2O-N emission (μg N/g MLSS)	N_2O-N conversion rate (%)
25	3.59 (1.83)	451.39 (36.54)	454.98 (38.22)	5.67
10	3.13 (1.26)	648.80 (43.48)	651.93 (42.35)	10.11

9.47 μg/g MLSS/min, which was much higher than that at 25℃. Then the emission rate decreased gently and slowly to about 6.39 μg/g MLSS/min at 300 min. The average N_2O emission amount during the aerobic period at 10℃ was calculated as 648.80 μg N/g MLSS, which was 43.3% higher than that at 25℃. Additionally, the N_2O-N conversion rate at 10℃ was about 10.11%, and more TN removed was converted to N_2O at low temperature. The results showed that the low temperature stimulated the emission of N_2O. It could be explained from the following aspects. Firstly, the low temperature weakened the activities of nitrifiers and denitrifiers, and the nitrification and denitrification process conducted incompletely, leading to more N_2O emission [13]. Secondly, the accumulation of NO_2^- at the low temperature induced more N_2O emission. Nitrite was considered as an important influence factor for N_2O emission and it could stimulate the production of N_2O both during nitrification and denitrification processes [9]. Although nitrite concentration increased at 210 min at high temperature, it was removed quickly due to the high denitrification rate. However, the NO_2^- concentration increased as soon as the beginning of aeration at low temperature and maintained at relative high level during the whole aerobic period (Fig. 2). Therefore, the N_2O production at low temperature was much higher than that at high temperature.

4. Conclusions

The SNDPR reactor gained favorable TP and TN removal efficiency at 25℃ indicated that simultaneous nitrogen and phosphorus removal occurred in the reactor. However, the removal of TN and ammonium were weakened significantly by the low temperature, mainly due to the incompletely oxidation of ammonium. The low temperature greatly lowered the SND efficiency during the SNDPR process. The impact of temperature on phosphorus removal was insignificant, mainly due to the enrichment of PAOs at low temperature. The SNDPR process stimulated the emission of N_2O, and at low temperature the N_2O emission amount was much higher than that at high temperature.

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