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Simultaneous nitritation and denitritation of domestic wastewater without addition of external carbon sources at limited aeration and normal temperatures

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ABSTRACT

Nitrogen removal via nitrite and simultaneous nitrification-denitrification (SND) are two processes for nitrogen removal from wastewater. The combination of these two processes, i.e., simultaneous nitritation-denitritation or SND via nitrite, is highly beneficial for the domestic wastewater treatment in terms of lower carbon requirements, reduced oxygen demand and less biomass production. A lab-scale sequencing batch reactor (SBR) treating domestic wastewater with low C/N ratios was operated to investigate simultaneous nitritation-denitritation without addition of external carbon sources under limiting aeration and normal temperatures (19 ± 1°C). The results showed that at a longer sludge retention time (SRT) of 50–66 d and an average dissolved oxygen (DO) concentration of 0.65 mg/L, nitritation was successfully achieved with nitrite accumulation rate over 95%. Fluorescence in-situ hybridization (FISH) analysis proved that ammonia oxidizing bacteria (AOB) became dominant nitrifying bacteria. Furthermore, denitritation occurred during the above aerobic period. The average total nitrogen (TN) removal through simultaneous nitritation-denitritation was maintained at 52% with a maximum of 63.1%. Low DO concentration under limited aeration is the key factor to achieve simultaneous nitritation-denitritation. Under long-term operation with low DO concentrations, the altering of nitrifying communities, establishment of anoxic micro-environment for denitrifiers growth and the characteristics of COD and NH_4^+-N biodegradation promoted the occurrence of simultaneous nitritation-denitritation.

Keywords: Simultaneous nitritation and denitritation; Domestic wastewater; Limited aeration; Low DO concentration; Normal temperature

1. Introduction

Conventional biological nitrogen removal is accomplished by a two-stage treatment, i.e. separated aerobic nitrification and anoxic denitrification [1]. Recently, several innovative technologies for nitrogen removal have been developed, among which, nitrogen removal via nitrite and simultaneous nitrification-denitrification (SND) was reported to be technically feasible and economically favorable [2,3]. Nitrogen removal via nitrite implies partial nitrification of NH_4^+ to nitrite (nitritation) and subsequently direct reduction of nitrite to N_2 gas (denitritation) [4,5], whereas SND defines that nitrification and denitrification can

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occur concurrently in one reactor under aerobic conditions with low dissolved oxygen (DO) [6,7]. The combination of these two processes, i.e. simultaneous nitritation-denitritation or SND via nitrite pathway, is highly beneficial for the domestic wastewater treatment with low C/N ratios in terms of lower carbon requirements, reduced oxygen demand and less biomass production [8–10]. The key points of achieving simultaneous nitritation-denitritation are: (1) oxidizing ammonia to nitrite instead of nitrate, i.e. establishment of nitritation; (2) nitritation and denitritation occurring concurrently in one reactor under aerobic conditions.

Regarding the establishment of nitritation, many studies have been showing the main factors affecting nitritation, such as higher temperature [11], short sludge retention time (SRT) [12], low DO concentration [13,14] and higher free ammonia (FA) concentration [15,16]. Previous researches suggested that the temperatures of 28–38°C are favorable for nitrogen removal via nitrite due to the specific growth rate of ammonia oxidizing bacteria (AOB) higher than that of nitrite oxidizing bacteria (NOB) [17,18]. SHARON process created by Delft University of Technology is a successful full-scale application of nitritation-denitritation at high temperatures of 30–35°C and SRT of 1.5 d [12]. However, the temperature of real domestic wastewater (usually at 10–25°C), especially in winter, cannot reach the optimal temperature of 30°C for nitrogen removal via nitrite. In the temperature range of 10–20°C, nitrite build-up can hardly be maintained due to the specific growth rate of NOB higher than that of AOB [17]. Therefore, relatively low wastewater temperature is the major obstacle for achievement and full-scale application of nitrogen removal via nitrite. Since both temperature and FA concentration of domestic wastewater cannot reach the optimal values for nitrogen removal via nitrite, DO concentration became the major regulating method due to the stronger DO affinity of AOB than NOB at low DO concentrations [19]. Very limited research has been undertaken on nitritation start-up for the treatment of real domestic wastewater from seed sludge with complete nitrification-denitrification at normal temperatures.

However, high nitrite accumulation from nitritation should not be negligible due to its harm to the operation of biological wastewater treatment. This problem can be effectively solved by simultaneous nitritation-denitritation without apparent nitrite toxicity effects for the microorganisms in the reactor. The mechanism of SND has been investigated based on spatially heterogeneous aerobic and anaerobic niches, and distinct metabolic pathway of the microbial populations involved [10]. It is clear so far that DO has been recognized as one of the key factors in SND as well as low DO concentration favors nitrogen removal via nitrite [20,21], and thus, the operation with low DO concentration has the potential of achieving SND via nitrite. Presently, most studies focus on SND phenomena in biofilm systems and granular sludge processes treating synthetic or industrial wastewater [22–25], which easily favors the establishment of anoxic microenvironments for SND. Furthermore, in order to improve SND efficiency (>80%), most studies added external carbon sources to promote denitrification [8,10]. Contrastively, very few studies are related to SND via nitrite in activated sludge process, particularly for the treatment of real domestic wastewater without addition of external carbon sources at normal temperatures, because it is hard to establish anoxic microenvironments for SND in activated sludge flocs, and normal temperature is unfavorable for nitritation.

This study aimed to (1) start up nitritation from seed sludge with complete nitrification-denitrification at normal temperature of 19 ± 1°C, i.e., establish the effective methods to make AOB become dominant nitrifying bacteria and NOB inhibitive, (2) develop the appropriate methods to promote and stabilize SND via nitrite for the treatment of real domestic wastewater, and (3) determine the mechanism of SND via nitrite, particularly with regard to the effect of low DO concentration.

2. Materials and methods

2.1. Experimental set-up and operation

A lab-scale sequencing batch reactor (SBR, working volume 11 L) with a bubble air diffuser, mechanical stirring and on-line measurement of DO and pH was used during the experiments (Fig. 1). The reactor was controlled at 19 ± 1°C using temperature controller and electric heater. The airflow meter controlled the aeration rate to achieve the desired DO concentration. An appropriate amount of settled sludge was directly discharged from the reactor at the end of sludge-settling period to maintain the mixed liquor suspended solid (MLSS) concentration at 3,000–3,500 mg/L and sludge retention time (SRT) at about 50–66 days.

The SBR was operated for 148 days including four successive phases (Table 1). The average DO concentration was below 1 mg/L throughout the experiments. The aerobic duration of each cycle in phases I, II and III was fixed at 3, 4 and 5 h, respectively, and the aerobic duration of each cycle in phase IV was real-time controlled. Typically cyclic studies on the 85th, 125th, 139th day in phase IV with real-time control and TN removal via nitrite were performed. Furthermore, wasted sludge from the reactor during day 65 to day 123 was collected, and then operated at normal DO

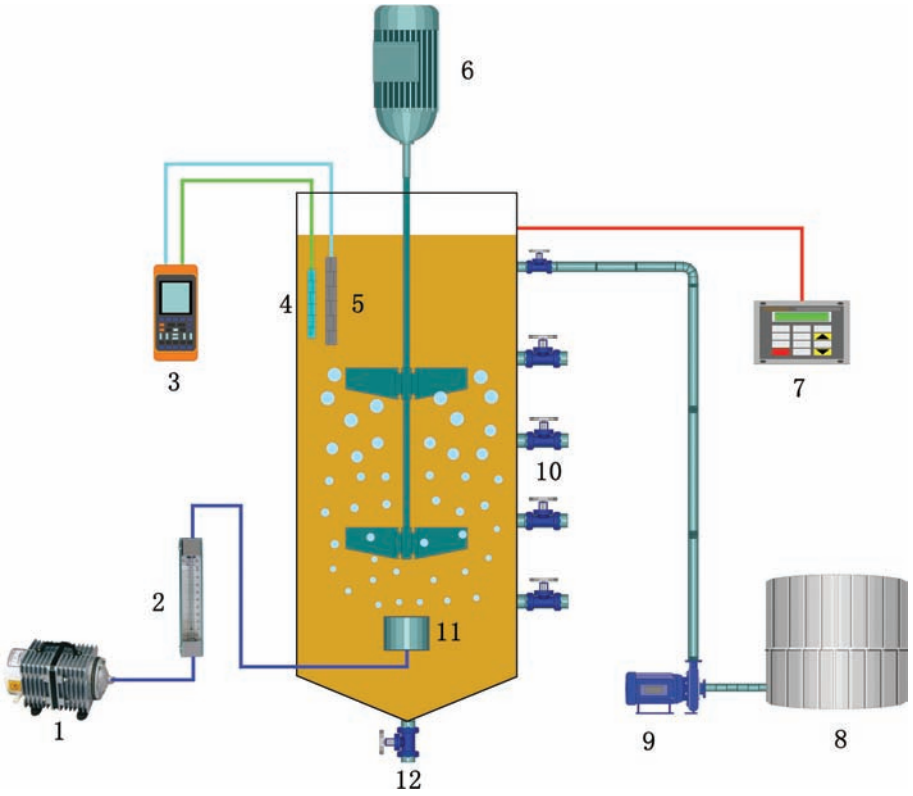


Fig. 1. Lab-scale SBR reactor. 1 air pump; 2 airflow meter; 3 DO and pH meter; 4 pH sensor; 5 DO sensor; 6 stirrer; 7 temperature controller; 8 influent tank; 9 feeding pump; 10 sampling valves; 11 air diffuser; and 12 waste sludge.

level of 2 mg/L for one month to achieve a stable performance, which aimed to compare with the operation at low DO levels.

Each cycle of SBR consisted of 5 min feeding and aeration, followed by 30 min settling and 5 min decanting. For each cycle, only the length of the aerobic period was either preset or real-time controlled by on-line measurement of DO and pH.

2.2. Wastewater and seed sludge

Real domestic wastewater with low C/N (COD/TN) ratio of 2:1 was used as influent. The composition of the domestic wastewater is shown in Table 2.

The inoculated sludge with complete nitrification-denitrification was taken from the recycling sludge of Gaobeidian WWTP (wastewater treatment plant) in Beijing. Fluorescence in-situ hybridization (FISH) analysis showed that nitrifying communities in seed sludge included both AOB and NOB.

2.3. Analytical methods

Ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), total nitrogen (TN), COD and MLSS were measured according to the APHA standard methods [26]. DO and pH were

Table 1
SBR operation for SND via nitrite at normal temperatures

Phase	Aeration rate ($L \cdot h^{-1}$)	DO ($mg \cdot L^{-1}$)		Aerobic duration (h)	Operational days (cycles)
		DO range	Average DO		
I	40	0.04–0.75	0.46	Preset 3 h	18 d (54 cycles)
II	40	0.04–0.88	0.52	Preset 4 h	22 d (66 cycles)
III	40	0.04–1.10	0.59	Preset 5 h	20 d (60 cycles)
IV	40	0.04–1.71	0.62	Real-time control	88 d (182 cycles)

Table 2
Composition of the domestic wastewater

Contents	COD ($mg \cdot L^{-1}$)	NH_4^+-N ($mg \cdot L^{-1}$)	NO_3^--N ($mg \cdot L^{-1}$)	NO_2^--N ($mg \cdot L^{-1}$)	pH	C/N	Alkalinity ($CaCO_3$) ($mg \cdot L^{-1}$)
Average	188	65.6	0.15	0.08	7.61	2.05	521.9
Number of samples	120	120	120	120	120	120	120
Standard deviation	37	11	0.05	0.02	0.3	0.5	45

monitored online using DO/pH meters (WTW Multi 340i, Germany). The TN removal efficiency (E_{SND}) during 1–84 days was calculated as the following equation [27]:

$$E_{SND} = \left(1 - \frac{\rho(NO_x^- - N)}{\rho(NH_4^+ - N)} \times 100\%\right), \quad (1)$$

where $\rho(NO_x^-$-N) is the total concentration of NO_3^--N and NO_2^--N in the effluent; $\rho(NH_4^+$-N) is the concentration of oxidized NH_4^+-N in nitrification. Since day 85, NO_3^--N concentration both during nitrification and in the effluent was always below 0.5 mg/L and was considered negligible. Therefore, E_{SND} was calculated as the following equation:

$$E_{SND} = \left(1 - \frac{\rho(NO_2^- - N)}{\rho(NH_4^+ - N)} \times 100\%\right), \quad (2)$$

where $\rho(NO_2^-$-N) is the NO_2^--N concentration in the effluent.

Nitrifying bacteria in sludge samples were semi-quantified by FISH according to Amann [28]. Table 3 lists the 16S rRNA-targeted oligonucleotide probes used in this study [29–31]. The FISH images from random-selected 50 fields of each sludge sample were quantified using the software of Image-Pro Plus 6.0.

3. Results and discussion

3.1. Achievement of nitritation through low DO and real-time control

Fig. 2 shows the variations of NH_4^+-N removal and nitrite accumulation rates throughout the experimental period. In the start-up period of nitritation, due to the relatively lower wastewater temperature of 19 ± 1°C and seed sludge with complete nitrification-denitrification, the preset short-cycle control of aeration time was applied in phases I–III to encourage AOB growth, i.e., aeration was preset and aeration was stopped before the pH ''ammonia valley'' detected. NH_4^+-N was still left in the effluent of phases I–III, but the concentration gradually declined and NH_4^+-N removal was continuously improved when aeration time was prolonged from 3 h to 4 h and 5 h. Due to the seed sludge with complete nitrification-denitrification, no nitrite was detected and nitrite accumulation rate was almost 0 at the beginning of start-up period. However, only on the 3rd day of phase I nitrite could be detected and nitrite accumulation rate reached 18.1%. Reasons for nitrite accumulation in the initial start-up period were the selective inhibition of low DO concentration to NOB, as well as NOB having a lag time in nitritation. In the subsequent 30 days, the nitrite accumulation rates fluctuated and showed a non-stability state, which suggested that NOB activities were partially suppressed, whereas AOB competitive dominance

Table 3
16S rRNA-targeted oligonucleotide probes used in this study

Probe	% Formamide	Sequence	Fluorochrome-labeled	Specificity
EUB_{mix}[1]	35(with NSO1225)	EUB338:GCTGCCTCCCGTAGGAGT	FITC	Eubacteria
	40 (with NIT3)	EUB338II: GCAGCCACCCGTAGGTGT		
	35(with Ntspa662)	EUB338III: GCTGCCACCCGTAGGTGT		
NSO1225	35	CGCCATTGTATTACGTGTGA	Cy^3	Ammonia-oxidizing β-Proteobacteria
NIT3	40	CCTGTGCTCCATGCTCCG	Cy^3	Nitrobacteria
Ntspa662	35	GGAATTCCGCGCTCCTCT	Cy^3	Nitrospira

[1]EUBmix is an equimolar mixture of probes EUB338, EUB338-II and EUB338-III.

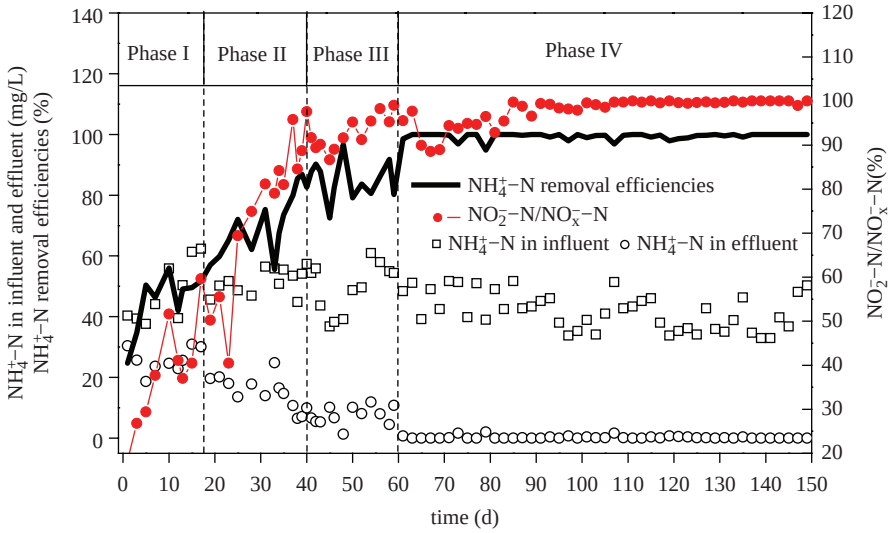


Fig. 2. Variations of NH_4^+-N removal and nitrite accumulation rates.

was not still established. In the middle-end of phase II, the nitrite accumulation rates rose stably indicating the AOB dominance further enhanced. In the phase III, the nitrite accumulation rates reached 95% and maintained at above 95% till the end of experimental period.

In phase IV, aeration time was real-time controlled based on the monitoring of pH ''ammonia valley''. Under this control strategy, nitritation tended to be stable with nitrite accumulation rate above 95%. Therefore, the operation with low DO concentrations under limiting aeration was feasible to achieve nitritation.

3.2. Simultaneous nitritation-denitritation (SND via nitrite) under limiting aeration

Obvious TN loss occurred in the aerobic period under limiting aeration. Typical cyclic studies on the 85th and 139th day were performed to gain a better insight into SND via nitrite (Fig. 3). Fig. 4 shows the variations of TN removal throughout the experimental period.

As shown in Fig. 3, TN concentration gradually declined with the decrease of NH_4^+-N concentration in both cyclic studies. However, as a result of incomplete SND, the declining rate of NH_4^+-N was more rapidly than that of TN. The NH_4^+-N was almost consumed, whereas TN was still left.

As presented in Fig.4, in the initial period under limited aeration, a lower TN removal (0.74–6.93%) was found resulting from microbial assimilation. In this study, TN removal efficiency below 5% caused by assimilation was excluded from SND efficiency. As a result of longer SRT (50–66 d), both biomass yield and assimilating nitrogen removal declined, whereas it should be noted that TN removal tended to be rising. And thus SND occurred in the reactor. In the effluent of phase I, both nitrite and nitrate could be detected.

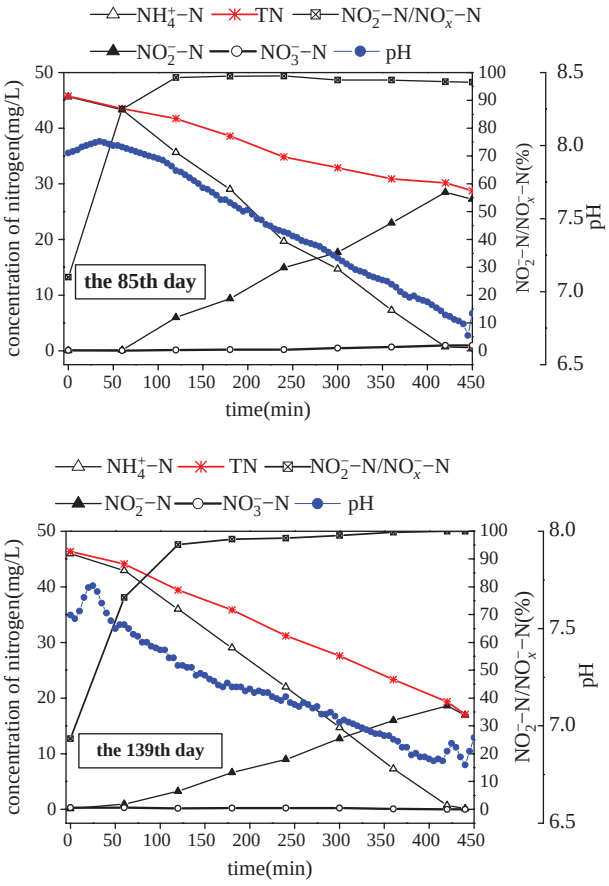


Fig. 3. Cyclic studies on the 85th and 139th day.

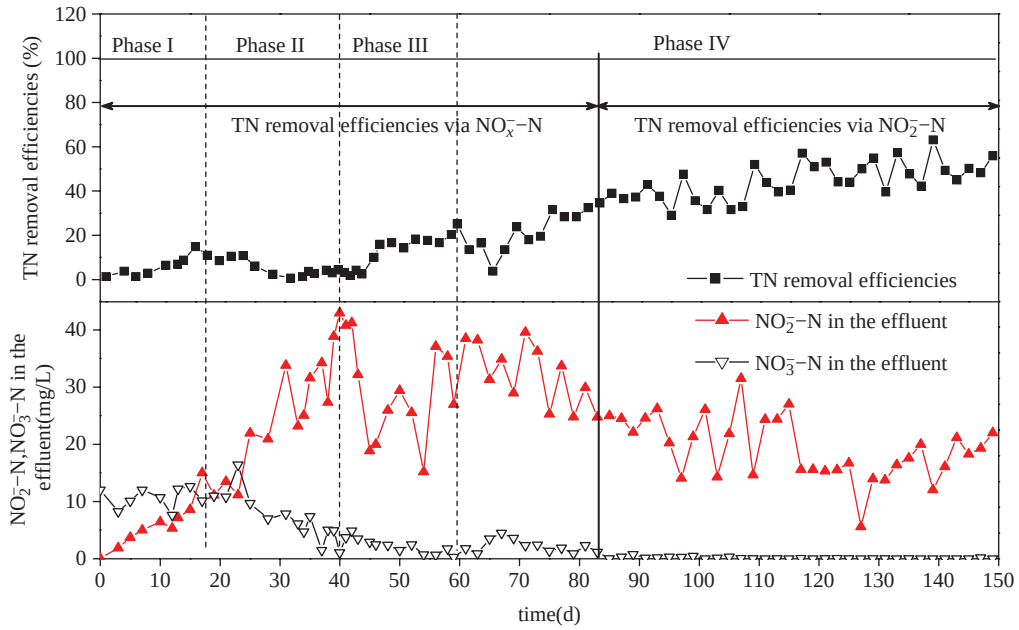


Fig. 4. Variations of TN removal throughout the experimental period.

During phases II and III, nitrate concentration significantly decreased corresponding to the increasing of nitrite and improving of TN removal, which implied SND not only by nitrate, but also by nitrite pathway. Since the 85th day in phase IV, very few nitrate (<0.5 mg/L) could be detected in the effluent. Cyclic studies on day 85 and day 139 (Fig. 3) also show that the nitrate concentrations throughout the aerobic period closed to 0 mg/L. As shown in Fig. 2, in phase III the nitrite accumulation rates reached 95% and maintained at above 95% till the end of experimental period. Therefore, it could be concluded that from day 85, SND was achieved mainly through nitrite pathway. The SND efficiency via nitrite was 52% on average, and the maximum was up to 63%.

Fig. 5 shows COD removal during experimental period under limited aeration. COD removal efficiencies were maintained at 72–97% and 86% on average

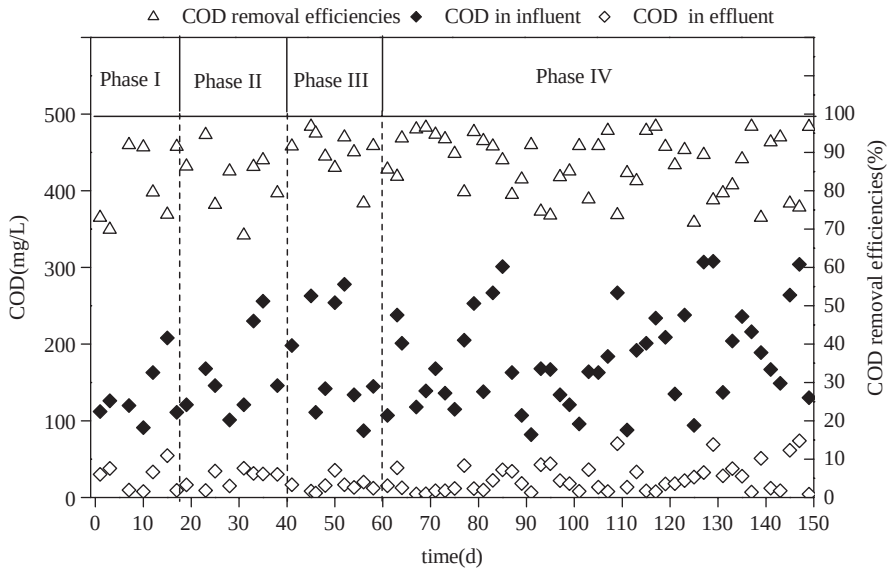


Fig. 5. COD removal during experimental period under limited aeration.

with an average influent COD concentration of 180 mg/L and effluent COD concentration of 25 mg/L. The performance of COD removal was not impacted under limited aeration.

3.3. Mechanisms of SND via nitrite

SND has been explained by two major theories: one is the establishment of anoxic micro-environment, i.e., the restricted diffusion of oxygen into the sludge floc to form anoxic niches and promote SND; the other is distinct metabolic pathway of the microbial populations involved, i.e., certain microorganisms have a denitrification capability under aerobic conditions [7]. In this study, SND via nitrite resulted from the combination of anoxic micro-environment and distinct metabolic pathway, and DO was the major factor. The possible mechanisms for SND via nitrite in this study are discussed as follows.

3.3.1. Mechanism 1: Competition between AOB and NOB under low DO conditions

Partial nitrification to nitrite is the preliminary step for SND via nitrite, which can be achieved through appropriate regulation of temperature, free ammonia (FA) concentration, DO concentration, pH and SRT, as well as aeration time control [11–16]. In this study, due to real domestic wastewater with low C/N ratios treated at normal temperature ($19 \pm 1^{\circ}C$), DO concentration was the major regulating factor, which kept under 1 mg/L throughout the experimental period. Table 4 presents the FISH semi-quantification results of AOB and NOB in seed sludge and four operational phases. Under limiting aeration, AOB percentage increased from initial 1.98% to above 10% and became the dominant nitrifying bacteria in the middle-end of experimental period, whereas NOB percentage decreased from initial 8% to below 0.2%. Previous studies have reported that oxygen affinity constant of AOB and NOB was 0.25–0.5 mg/L and 0.72–1.84 mg/L, respectively [32]. Thus, AOB have a stronger DO affinity than NOB. Especially at low DO concentrations, AOB outcompete NOB. In this study, the average DO concentration was only 0.46–0.62 mg/L with aeration rate of 40 L/h. This low DO concentration promoted AOB growth and prevented NOB activity.

3.3.2. Mechanism 2: Establishment of anoxic micro-environment under limiting aeration

SND via nitrite implies that any nitrite produced by nitrification should be reduced to N_2 rather than oxidized to nitrate. Nitritation is performed under aerobic conditions, whereas denitritation is usually an anoxic process. These two processes are conflictive in term of oxygen demand and hard to occur concurrently in one reactor, which is the main reason for non-stability of TN removal under SND conditions.

In this study, firstly, the existence of anoxic micro-environment under limiting aeration was the major factor causing SND via nitrite. Experimental measurement showed that when MLSS concentration was above 3,200 mg/L with DO controlled at 0.3–0.54 mg/L, anoxic micro-environments appeared in the flocs, where nitrite was preferably reduced. Although the oxygen in the bulk liquid kept stable by DO controller, in the flocs the presence of ammonia caused the additional oxygen consumption for oxidation and DO level to a lower, and thus resulted in localized oxygen depleted micro-environments inside the flocs. Furthermore, under low DO conditions, the competition for oxygen between AOB and NOB suggests that less oxygen is available for nitrite oxidation.

Secondly, the large and compact activated sludge flocs were formed when the reactor was long-term operated under a longer SRT (50–66 d), which favored the establishment of anoxic micro-environment. Previous studies showed that in SBR operated under longer SRT, the biomass yield declined and much more remaining inorganic substances, non-biodegradable constituents, intracellular stored poly-hydroxybutyrate (PHB) and polyphosphate promoted

Table 4
FISH semi-quantification results of nitrifying bacteria

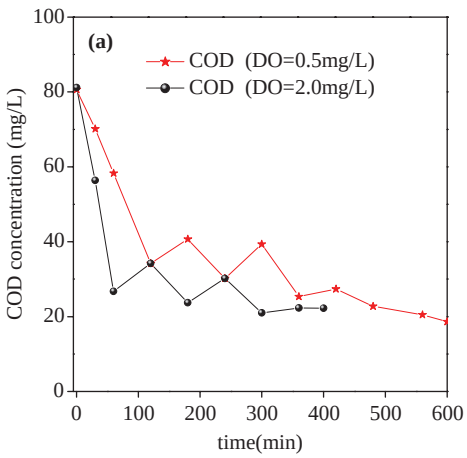
Samples	AOB (%) (probe: NSO1225)	NOB (%)	
		Nitrospira (probe:Ntspa662)	*Nitrobacteria* (probe:NIT3)
Seed sludge	1.98 ± 0.21	8 ± 0.63	0.625 ± 0.28
End of phase I	4.36 ± 0.74	5.35 ± 0.52	No signal
End of phase II	9.97 ± 1.01	0.49 ± 0.17	No signal
End of phase III	10.13 ± 0.93	<0.2%	No signal
End of phase IV	10.9 ± 0.54	<0.2%	No signal

the flocculation of activated sludge flocs [33]. Moreover, it should be noted that during the initial operational period, a slight sludge bulking occurred due to limited aeration. A small amount of filamentous organisms could work as the backbone of microstructure to form a strong and compact macrostructure. Previous research reported that when the average diameter of sludge flocs increased from 40 to 80 μm, TN removal was improved from 21% to 52% [33]. The large and compact flocs restricted diffusion of oxygen and provided an oxygen shield to the inside of the flocs. It was more important that an oxygen consumption from the bacteria found on the surface, such as nitrifying bacteria and aerobic heterotrophs. These aerobic bacteria prevented deeper zones of the flocs to receive oxygen. The larger the flocs were, the less available was the oxygen in the center of the flocs, which favored TN removal.

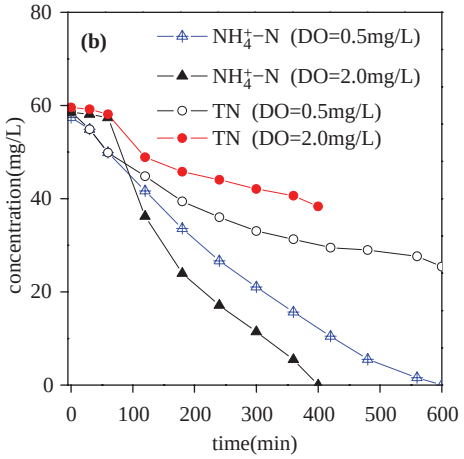
3.3.3. Mechanism 3: Characteristics of COD and NH_4^+-N biodegradation under low DO conditions promoting denitrification

Most denitrifying bacteria are heterotrophs and obtain energy from the oxidation of organic substrate using NO_x^--N as electron acceptor. Therefore, the amounts of organic substrate or C/N ratio are very important for SND and N-removal. With regard to the domestic wastewater used in this study, the soluble COD is typically limiting with an average C/N ratio only of 2:1. Typically cyclic studies on the 125th day in phase IV with a stable TN removal via nitrite were carried out to investigate the characteristics of COD, NH_4^+-N and TN biodegradation, which were compared with the performance under normal DO level of 2 mg/L (Fig. 6).

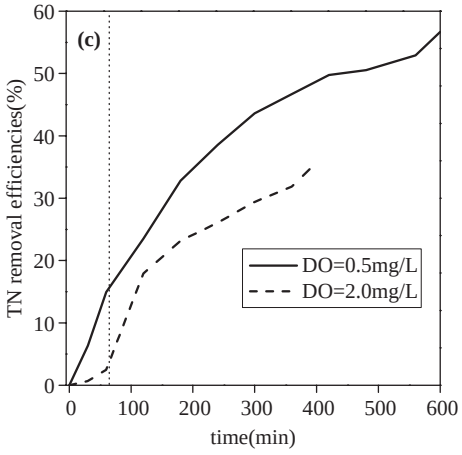
As shown in Fig. 6(a), the biodegradation rate of organic substrate at DO = 0.5 mg/L was much lower



(a) COD variations in one cycle



(b) The variations of NH_4^+-N and TN in one cycle



(c) TN removal efficiencies in one cycle

Fig. 6. Characteristics of COD, NH_4^+-N and TN biodegradation at DO = 2 and 0.5 mg/L. (a) COD variations in one cycle. (b) The variations of NH_4^+-N and TN in one cycle. (c) TN removal efficiencies in one cycle

than that at DO = 2 mg/L. In Fig. 6(b), TN removal at DO = 0.5 mg/L was obviously better than that at DO = 2 mg/L. Fig. 6(c) presents the variations of TN removal efficiencies in one cycle at different DO levels. Under DO level of 0.5 mg/L, TN removal was 14.9% after one hour and finally reached 57%; in contrast, under DO level of 2 mg/L, TN removal was only 2.5% after one hour and finally closed to 36% (Fig. 6c). Furthermore, with DO =2 mg/L, organic substrate degradation was prior to NH_4^+-N oxidation and most COD was depleted during the first hour, while NH_4^+-N oxidation just began. At DO = 0.5 mg/L, both of them concurrently occurred resulting in organic substrate and NH_4^+-N simultaneously declining.

The reasons for COD and NH_4^+-N degradation under low DO conditions promoting denitrification are the followings. Firstly, under limited and fixed aeration, DO level in the initial period is the lowest, which favors the establishment of anoxic micro-environment and promotes denitrification occurring at the beginning of aerobic period. Secondly, due to denitrification beginning in the initial aerobic period, a large amount of organic substrates from influent provide the carbon sources for denitrification. Thirdly, under low DO conditions, due to the rate of organic substrate degradation slowing down, the remaining COD can be used for subsequent denitrification. However, under high DO conditions, due to organic substrate degradation prior to NH_4^+-N oxidation, denitrification cannot occur in the initial period without NO_2^--N as electron acceptor despite of the existence of organic carbon sources. Subsequently, organic substrates are rapidly depleted resulting in carbon sources deficient for denitrification.

3.3.4. Mechanism 4: AOB contributing to denitrification

There are some reports on AOB, such as *Nitrosomonas europaea* [34] and *Nitrosomonas eutropha* [35], contributing to denitrification through oxidizing ammonia to produce N_2O, NO or N_2 under low DO conditions. Although the denitrification rate of AOB is relatively lower in comparison to heterotrophic denitrification rate, this possibility of AOB contributing to denifitrication could not be excluded in this study because the occurrence of simultaneous nitritation-denitritation was accompanied by AOB competitive dominance. This possibility needs further experimental confirmation before any firm conclusions can be made.

4. Conclusions

A lab-scale SBR was operated to treat domestic wastewater with low C/N ratios at normal temperature of 19 ± 1°C. Under limited aeration with an average DO concentration below 1 mg/L, simultaneous nitritation-denitritation was achieved. The average TN removal by SND via nitrite was maintained at 52% with a maximum of 63.1%.

At normal temperature of 19 ± 1°C, AOB competitive dominance was enhanced by the combination of low DO operation (<0.8 mg/L) and the preset short-cycle control of aeration time. After 60 d operation, nitritation was started up with nitrite accumulation rate above 95%. Significantly, the real-time control strategy of aerobic durations based on on-line monitoring of pH ''ammonia valley'' maintained the stable performance of nitritation with nitrite accumulation rate above 95% and NH_4^+-N removal over 97%.

Without the addition of external carbon sources, low DO concentration was the major factor for achievement of SND via nitrite, which enhanced AOB dominance, favored the establishment of anoxic micro-environment as well as altered the characteristics of COD and NH_4^+-N biodegradation to promote the occurrence of SND via nitrite.

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