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A strategy for starting and controlling nitritation-denitrification in an SBR with DO and ORP online monitoring signals

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ABSTRACT

Nitritation-denitrification is a high efficiency and energy saving nitrogen removal approach in wastewater treatment, but it faces the risk of nitrite oxidizing bacteria (NOB) proliferation. This study adopts a control strategy for ammonium oxidizing bacteria (AOB) improvement and NOB repression by adopting the first derivatives of DO and ORP via the nitritation-denitrification process in an SBR. The control strategies optimized the length of the aerobic and anoxic phases, which may improve nitrogen removal and inhibit NOB growth. Under the controlled operation conditions (temperature: 29°C–30°C, pH: 8–9, SRT: 14 d), a control system for the lab-scale nitritation-denitrification SBR treatment of ammonium-rich synthetic wastewater (approximately 500–750 mgN-NH_4^+/L) is presented. According to the experimental results, the SBR demonstrated better performance in nitrogen removal and NOB inhibition during the whole operation period; specifically, N-NH_4^+ was less than 1 mg N/L in the effluent, and the nitrite accumulation rate (NAR) was more than 98%. Therefore, this lab-scale study demonstrates the possibility of the application of a real-time control strategy for out-competing NOB in SBR conditions and providing relatively high nitrogen removal rates.

Keywords: Nitritation-denitrification; SBR; NOB inhibition; DO; ORP

1. Introduction

Biological nitritation-denitrification is an effective method for nitrogen treatment, and this process consists of two successive stages: aerobic autotrophic nitrification and anoxic heterotrophic denitrification [1,2]. In contrast to the traditional nitrification-denitrification process, this process can reduce oxygen consumption in the nitrification stage by 25% and reduce the additional organic carbon source requirement in the denitrification stage by 40% [3]. Meanwhile, this process has a smaller sludge production, and denitrification rates with nitrite are usually 1.5–2 times faster than with nitrate [4,5].

This two-step reaction is carried out by two kind of autotrophic bacteria, namely, ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), which belong to the *Nitrosomonas* and *Nitrobacter* species, respectively [6]. Because both AOB and NOB are autotrophic aerobic bacteria,

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oxygen and inorganic carbon are necessary for their growth [7]. To obtain a stable nitritation-denitrification process, the out-competition of NOB in the SBR during the aerobic phase is key, as NOB is responsible for converting nitrite to nitrate [8,9]. The establishment of the nitritation pathway is realized by a gradual reduction in the amount of nitrite that is available to provide energy for the growth of NOB, finally leading to the elimination of NOB from the system [10].

There are several factors that have been identified as promoting the nitrite pathway by selectively inhibiting or limiting the growth of NOB over AOB. To be specific, this can be achieved by an operating system with a high pH, high free ammonia (FA) and free nitrous acid (FNA), low dissolved oxygen (DO), high temperature and a low solids retention time (SRT) [4,11–13].

Therefore, the objective of this work is to study the control strategies for the nitritation-denitrification process in an SBR. A lab-scale SBR was studied for the treatment of synthetic wastewater with the control system. Compared with the traditional control system based on a fixed time, in this study, the control system adopts real-time control strategies based on DO and ORP online monitoring signals.

2. Materials and methods

2.1. Experimental setup

Experiments were performed in a lab-scale aerobic/anoxic SBR, with a total volume of 12 L, that was made from plexiglass and equipped with a feeding and effluent pump, an aeration system (fine bubble injection), a mechanical stirrer (Rushton type with constant stirring rate of 465 rpm) and a heat tape for temperature control (maintained at 30°C ± 0.5°C). The air flow rate was imposed with a rotameter. The pH (EasySense pH 31, METTLER TOLEDO, Switzerland), dissolved oxygen (DO) (EasySense O_2 21, METTLER TOLEDO, Switzerland) and oxidation-reduction potential (ORP) (EasySense ORP 41, METTLER TOLEDO, Switzerland) were monitored during the entire study period. A control cabinet connected to a computer allowed the management of the stirring, aeration and all inputs and outputs of the reactor. The configuration of SBR used in this study is shown in Fig. 1.

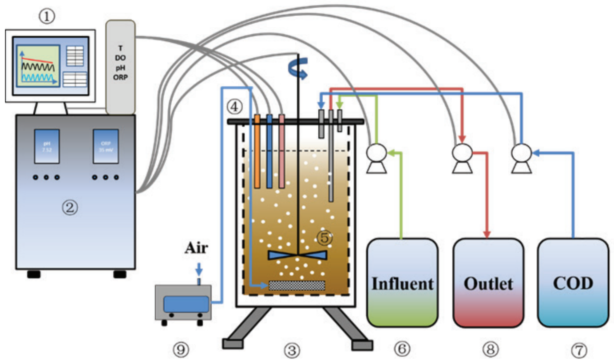


Fig. 1. Configuration of the SBR reaction system: (1) Computer; (2) PLC Control Cabinet; (3) SBR Reactor; (4) Online Probe; (5) Agitator; (6) Feeding Tank; (7) CH_3COONa Tank; (8) Outlet Tank; and (9) Air Pump.

2.2. SBR operation

The SBR was inoculated with floccular sludge that was domesticated from the anaerobic digester of the WWTP of the Henan Lian Hua Gourmet Powder CO. LTD (Zhoukou, China). Initially, 3 L of the seed sludge and 7 L synthetic wastewater were added into the SBR. The initial concentration was approximately 2.3 gVSS/L.

The SBR was operated for 60 d in a sequencing batch mode with a cycle composed of: aerobic feeding with ammonium-rich wastewater (100 mL/min, 10 min, for a volumetric exchange ratio of 10%), a successive aerobic reaction phase and anoxic reaction phase (with secondary carbon feeding), a settling phase (20 min) and withdrawal (10 min). A solution of sodium acetate (76.88 NaAC g/L) was used as a secondary carbon source during the anoxic phase, with the amount being adjusted to the nitrite load to be denitrified (feeding during 1–2 min, a minimal COD: N ratio of 3.5 was needed for nitrite denitrification).

The experimental period was divided into three phases according to the operation conditions and results. In Phase I (day 1–20) and Phase II (day 21–35), the reactor was fed with medium containing ammonium (500 mg $N\text{-}NH_4^+/L$) using a time control mode to establish a stable system for combined nitritation and denitrification, which was accompanied by controlling DO at 0.2–0.3 mg O_2/L and 0.4–0.5 mg O_2/L in Phase I and Phase II, respectively. The aerobic and anoxic phase lengths were fixed to 100 min and 180 min, respectively. Then, an automatic control of aerobic and anoxic durations was implemented based on DO and ORP signals with a real-time control strategy in phase III (day 36–65); the medium containing ammonium in the feeding phase was increased to 750 mg $N\text{-}NH_4^+/L$, and the DO was kept between 0.7 and 0.8 mg O_2/L. Sludge was manually wasted for maintaining a sludge retention time (SRT) of 14 d during the experiment.

The synthetic N-rich wastewater was stored in a 25-L tank at ambient temperature. This solution was composed of (for 1 L with a total N concentration of 250–1,000 mg $N\text{-}NH_4^+/L$): 1.17–4.71 g of $(NH_4)_2SO_4$, 3–12 g of $NaHCO_3$, 0.194 g of $CaCl_2$, 0.46 g of $MgSO_4 \bullet 7H_2O$, 0.042 g of KH_2PO_4 and 2 ml of a trace elements solution. $(NH_4)_2SO_4$ was used as the ammonium source during the final month of the study period. The molar ratio of inorganic carbon to ammonium was maintained at 2:1. The trace elements solution [14] was prepared as followed: 1.25 g/L EDTA, 0.55 g/L $ZnSO_4 \bullet 7H_2O$, 0.4 g/L $CoCl_2 \bullet 6H_2O$, 1.27 g/L $MnCl_2 \bullet 4H_2O$, 0.4 g/L $CuSO_4 \bullet 5H_2O$, 0.05 g/L $Na_2MoO_4 \bullet 2H_2O$, 1.25 g/L $FeCl_3 \bullet 6H_2O$, 1.37 g/L $CaCl_2 \bullet 2H_2O$ and 44.4 g/L $MgSO_4 \bullet 7H_2O$. The complementary solution (sodium acetate) that was used as a source of organic carbon for denitrification had a COD concentration of 60 g COD/L.

2.3. Analytical methods

During the study, samples were collected from the SBR influent and the treated effluent; moreover, some samples were collected in the reactor during specific kinetic tests. All samples were filtered through Millipore filter units (0.45 µm pore size). The ammonium concentration was measured by means of water quality determination of ammonia nitrogen using Nessler's reagent in spectrophotometry

(National standard of PRC). Nitrite and nitrate ($N\text{-}NO_2^-$ and $N\text{-}NO_3^-$) were quantified by ionic chromatography (ICS-900, 2003, DIONEX). COD, total suspended solids (SS) and volatile suspended solids (VSS) were measured according to standard methods.

2.4 Calculations

The concentrations of free ammonia (FA) and free nitrous acid (HNO_2, noted FNA) were calculated using the following equation, with nitrite concentration (mgN-NO_2^-/L), temperature (T in Kelvin) and pH [15]:

$$\mathrm{FA} = \frac{\left[\mathrm{N-NH_4^+}\right] \times 10^{\mathrm{pH}}}{\left[e^{6344/(273+T)} + 10^{\mathrm{pH}}\right]} \tag{1}$$

$$\mathrm{FNA} = \frac{\left[\mathrm{N-NO_2^-}\right]}{\left[1 + e^{-2300/(273+T)} \times 10^{\mathrm{pH}}\right]} \tag{2}$$

With FA (mg N-NH_3/L): free ammonia; FNA (mg N-HNO_2/L): free nitrous acid; [N-NH_4^+]: ammonium concentration (mg N-NH_4^+/L); [N-NO_2^-]: nitrite concentration (mg N-NO_2^-/L).

The nitrite accumulation ratio was estimated regularly by measuring the nitrite, nitrate and ammonium in the reactor at the end of the aerobic phase. The nitrite accumulation ratio (NAR, %) was calculated by dividing the produced nitrite by the sum of nitrite and nitrate.

$$\mathrm{NAR} = \frac{\left[\mathrm{N-NO_2^-}\right]}{\left[\mathrm{N-NO_2^-}\right] + \left[\mathrm{N-NO_3^-}\right]} \tag{3}$$

where [N-NO_2^-] is the nitrite concentration (mg N-NO_2^-/L); and [N-NO_3^-] is the nitrate concentration (mg N-NO_3^-/L).

3. Results and discussion

3.1. Reactor performance and NOB repression

The SBR was launched using the sludge of the nitrification-denitrification system and worked 3–6 cycles every day, and one cycle was selected for the batch experiment. Fig. 2 shows the reactor performance over time over the course of the experiments (64 d).Regarding the effluent quality, the ammonium concentration rapidly decreased to less than 1 mg N-NH_4^+/L at the beginning of experiment. Even though the initial ammonium concentration increased to approximately 60 mg N-NH_4^+/L after day 36, the ammonium concentration was still less than 2 mg N-NH_4^+/L. Very strong performance was achieved throughout the entire study, as the ammonium removal rate was higher than 97%.

As described previously, a more efficient ammonia nitrogen removal rate was obtained in this study, which benefited from the inoculation of the well-nitrified sludge. Before inoculation, the original sludge was cultured in the other SBR with synthetic wastewater under aerobic conditions for two weeks, which prompted the development of AOB and NOB in the sludge. Moreover, because the sludge was domesticated from an anaerobic digester, it contained many denitrifying bacteria. Consequently, this process was beneficial to the quick start-up of the nitrification-denitrification process in the SBR.

In this study, the nitritation process was established quickly after SBR start-up. Fig. 3 shows the level of nitrite pathways achieved in the SBR; at the beginning of experiment, most of the ammonium was converted to nitrate by NOB during the aerobic phase. The nitrite accumulation ratio (NAR) increased from 0% to 90% in 20 d and maintained at more than 98% until the end of the study. According to the results, nearly all the NOB was inhibited after 20 d of operation.

To start the nitritation process, NOB needs to be suppressed and washed out from the system during the aerobic phase, as NOB is responsible for the second step of nitrification (convert nitrite to nitrate). The establishment of the nitritation pathway is realized by a gradual reduction in the amount of nitrite that is available to provide energy for

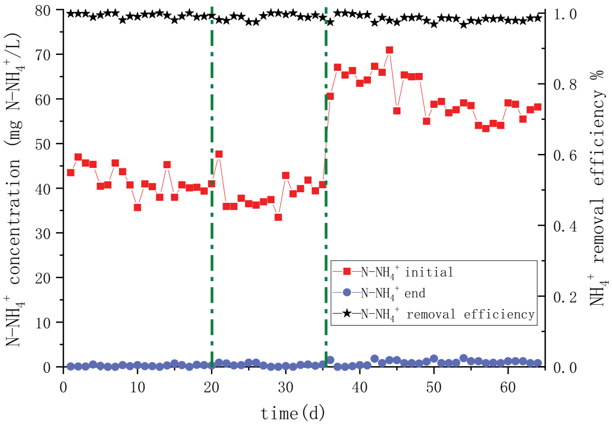


Fig. 2. Long-term performance of the SBR process.

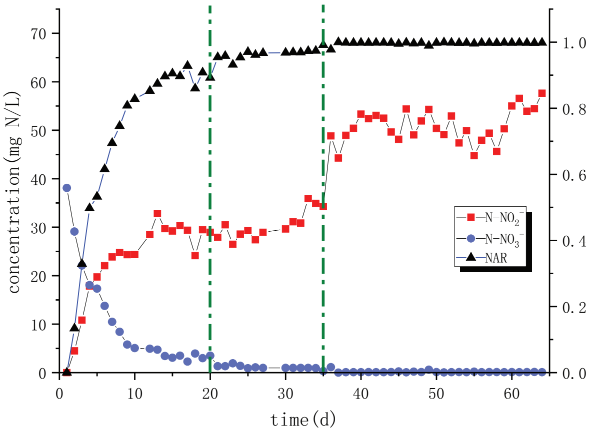


Fig. 3. Degree of nitrite accumulation in the aeration stages.

the growth of NOB, which finally leads to the elimination of NOB from the system [16]. There are several factors that have been identified to promote the nitrite pathway by selectively inhibiting or limiting the growth of NOB over AOB. To be specific, this can be achieved by an operating system with a high pH, high free ammonia (FA), free nitrous acid (FNA), low dissolved oxygen (DO), high temperature and low solids retention time (SRT) [4,11,12,17].

To repress NOB activity during the aerobic phase, the SBR was operated in controlled conditions. In the reactor, the initial pH was controlled to nearly 8, DO was maintained at approximately 0.3 mg/L, and then the concentration of DO was gradually increased to 0.5–0.8 mg DO/L after the setup of nitritation. Moreover, thanks to the combined effect of the aerobic phase control and cycle time control, the nitrite pathway was well maintained during the study. The control system was implemented for controlling the SBR operation, which also allowed for control of the aeration duration. Avoiding excessive aeration is an effective strategy for inhibiting NOB. Lastly, the SBR was run under a controlled pH (higher than 7.8), temperature (29°C–30°C) and ammonium concentration (40–50 mg N-NH_4^+/L); high free ammonia (FA) was achieved in the SBR, which was also one key factor in inhibiting NOB [18,19]. During the aeration period, FA was kept higher than 1 mg NH_3/L in the process, and the maximum value was 9.3 mg NH_3/L. This range of FA inhibited NOB but did not affect AOB. Due to these conditions, the nitritation-denitrification in the SBR started quickly.

3.2. Trends in the variation of DO and ORP during the reaction

Based on the analysis of online signals (DO and ORP) during the process of nitritation-denitrification, there was obvious variation in the characteristics of DO and ORP in the online curves. The two parameters also showed periodic variation in the cycle of the reaction. Fig. 4 presents the DO and ORP online curves during three successive SBR cycles, and the two signals showed the same trend. In the pre-aeration period (period 1, 4, 7), DO temporarily maintained a lower level of 0.1–0.2 mg/L, and then it suddenly rose and reached approximately 5.5 mg/L. This peak indicated that the remaining COD from the last cycle was almost consumed; at the same time, ORP increased from –550 to 50 mV. During the aerobic period (period 2, 5, 8), due to the consumption of DO by AOB, the DO decreased rapidly and maintained at approximately 0.5–0.6 mg/L. At the end of the aerobic phase, DO rose slowly from 0.5 mg/L to 0.8 mg/L. When the nitritation reaction was nearly finished, DO have a characteristic peak and rose rapidly to approximately 6.2 mg/L. Meanwhile, ORP showed the same variation trend but had smaller amplitude. Then, the SBR operation switched to the anoxic denitrification phase, and DO decreased rapidly and was maintained at less than 0.01 mg/L. Simultaneously, ORP quickly declined to –550 mV, but two bending points were detected that represented the termination of denitrification.

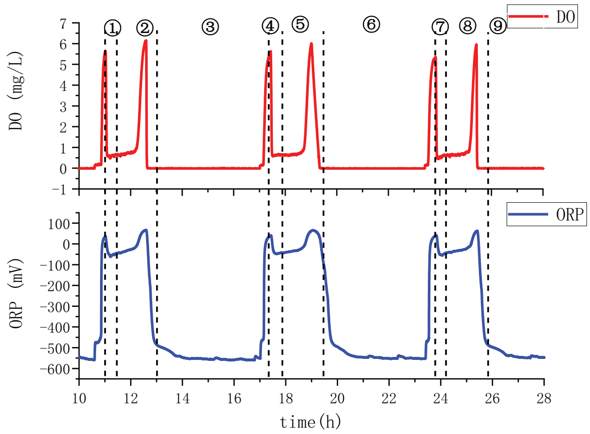


Fig. 4. Trends of variation of DO and ORP.

In one SBR cycle, DO rise along with ORP, and vice versa. This is because the variation in ORP relates to the biochemical reaction, such as oxidation and reduction reactions [20,21]. A high positive value indicates an environment that favours an oxidation reaction, such as that produced by free oxygen, while a low negative value indicates a strong reducing environment, such as that produced by free metals [22,23]. Therefore, an ORP increase was accompanied by a DO rise during the aerobic period, and ORP decreased during the anoxic period because of a cease in aeration. Therefore, the monitoring and regulation of online parameters and N-containing pollutants can be helpful in operating SBR.

3.3. First derivatives of DO and ORP

The regulation of measured online parameters (DO and ORP) and N-containing ions (N-NH_4^+, N-NO_2^- and N-NO_3^-) during a typical SBR cycle is shown in Fig. 5. This batch experiment shown in Fig. 5 was carried out in Phase III. The DO signal increased rapidly as soon as the cycle began, and then stabilized during nitritation period and increased again when ammonia was depleted. The point α was determined by a threshold limit on the DO first derivative (Fig. 5(c)), which corresponded to the decrease in the nitritation rate due to ammonia limitation (or depletion). Meanwhile, ORP decreased during the anoxic period, and this decreasing rate accelerated as soon as the nitrite was depleted. The point β was detected with a threshold limit on the first derivative of ORP (Fig. 5(d)). Threshold values used for the control of the cycle presented on Fig. 5(d) were 0.018 mgO_2/ (L.S) and –2.1 mV/S for α and β, respectively.

According to the experimental results, DO and ORP had regular variation trends with ammonium removal, which can be adopted to automatically control SBR operations. In the aerobic period, the nitritation reaction was carried out, and the oxidation of N-NH_4^+ by AOB needed oxygen consumption. In the condition of a dynamic equilibrium between aeration supply and oxygen consumption by AOB, DO remained nearly constant and was kept at a low level. With a decrease in concentration of N-NH_4^+, the nitritation rate and oxygen consumption were reduced gradually; therefore, the DO increased slowly [24]. At the end of aerobic period, N-NH_4^+ was oxidized almost completely, and there was a very weak aerobic reaction in the reactor. While maintaining constant aeration, DO would suddenly increase greatly, this caused a large increase in DO and its first derivative. During the anoxic phase, ORP declined rapidly after aeration stopped, which resulted from the transformation of N-NO_2^- and N-NO_3^- to N_2 after adding COD. The main reason for the decrease of ORP was the rapid reduction of the oxidized substrate [25]. As denitrification continued, the oxidizing substances gradually decreased, and the reducing of ORP slowed down until a small platform was presented, which meant the denitrification was complete. Meanwhile, the first derivative of ORP reached a minimum value. Therefore, the minimum value of the first derivative of ORP can be used as a threshold indicating the forthcoming termination of denitrification.

Based on this experiment, the threshold values of the first derivatives of DO and ORP used for the control of the cycle were determined. To study the stability of the two threshold values, four batch experiments described in Table 1 were carried out. This group of batch tests were designed for investigating the influence of different feeding concentrations (250–1,000 mg N-NH_4^+/L) to the first derivatives of DO and ORP.

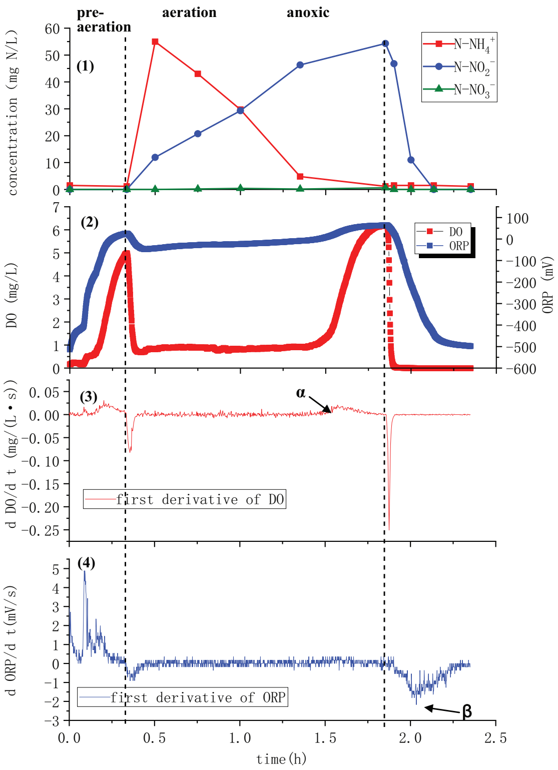


Fig. 5. Results of one typical batch experiment: (a) The variation in N-NH_4^+, N-NO_2^- and N-NO_3^-; (b) the variation in DO and ORP; (c) the variation in the first derivative of DO; and (d) the variation in the first derivative of ORP.

The controlling aeration rate was 6 L/min, and the influence of four feeding concentrations on the first derivative curve was measured on day 50, 51, 52, and 53. The specific experiment results are shown in Table 2.

According to the results shown in Table 2, a variation in the feeding concentrations would not affect the variation rule of the first derivatives of DO and ORP. Under the four different feeding concentrations, N-NH_4^+ was nearly degraded completely, the NAR was over 98%, and no accumulation of N-NO_3^- was found. This indicated that the reactor was able to resist the impact load of N-NH_4^+. At the end of nitritation and denitrification, the first derivative of DO and ORP both had extreme values, which indicated the possibility and stability of using the first derivative extreme values for SBR real-time control. Moreover, we observed that under the different feeding concentrations, the extremums were stable and did not fluctuate considerably with the variation in the feeding concentration. Therefore, using the extreme values of the first derivatives of DO and ORP as the control threshold is feasible.

3.4. Comparative analysis of bacterial community compositions

According to the SBR operation results, the average SBR ammonium removal efficiency was higher than 97%, which was related to the compositions of the dominant bacterial community. To comprehensively analyse the microbial communities during the SBR operation, three activated sludge samples were collected from seed sludge, the nitrification-denitrification stage and the nitritation-denitrification stage for Illumina MiSeq sequencing and were marked as 1#, 2# and 3#, respectively. Through an analysis of 16S rDNA test for samples, the similarities and differences of the three bacterial community compositions were described by the community heat map (Fig. 6).

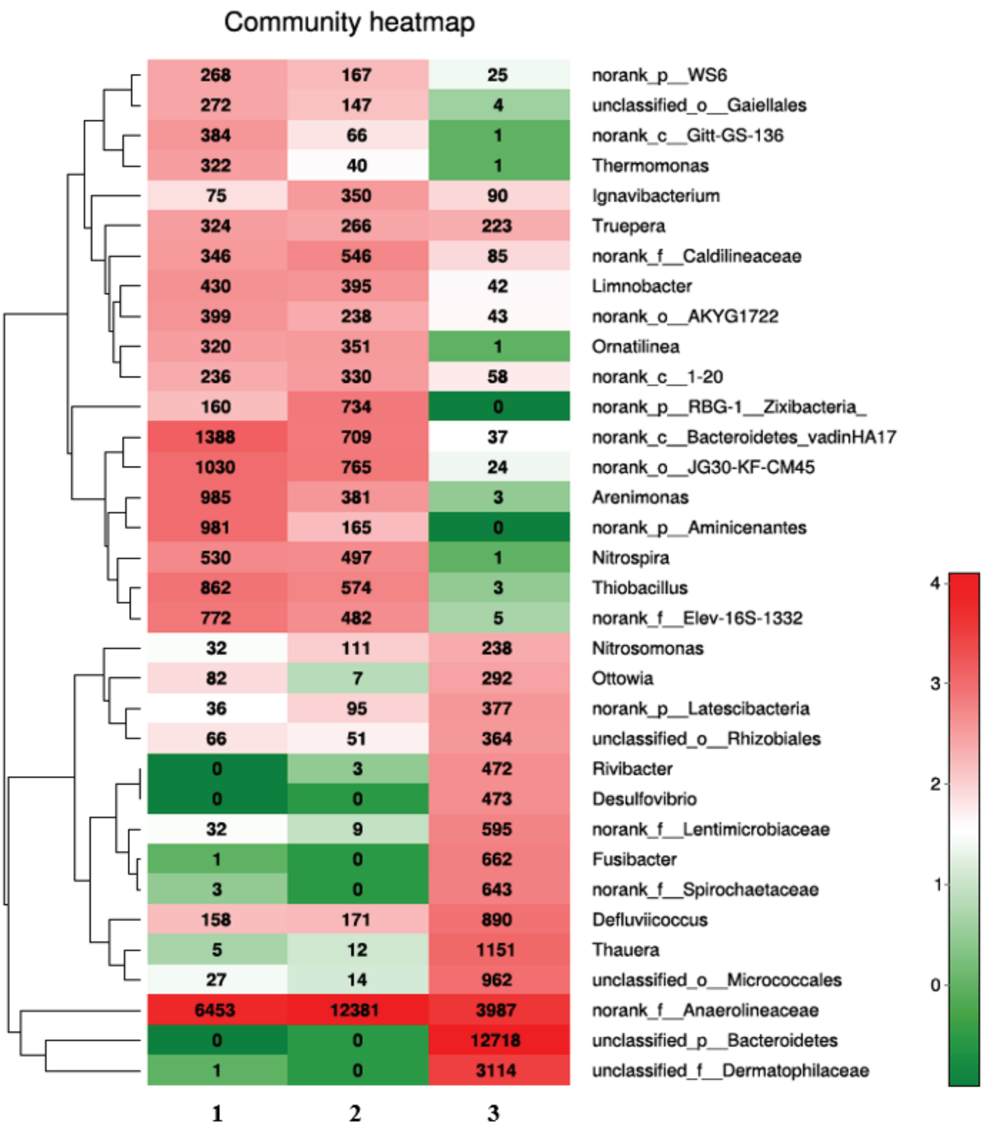
The bacterial community structures were identified by the relative abundance of the bacterial community of sludge

Table 1
Batch experiments demonstrating the influence of different feeding concentrations on the first derivatives of DO and ORP

Feeding concentration (mg/L)	Test time (day)	Aeration rate (L/min)
250	50	6
500	51	6
750	52	6
1000	53	6

Table 2
Results of batch experiments on the influence of different ammonium concentrations in feeding on the values of the first derivatives of DO and ORP

Feeding concentration (mg/L)	N-NH_4^+ removal efficiency (%)	NAR (%)	First derivative of DO (mg/(L·s))	First derivative of ORP (mV/s)
250	98	99	0.019	–2.2
500	99	99	0.018	–2.1
750	99	98	0.018	–2.1
1,000	99	99	0.02	–2



	1	2	3
norank_p__WS6	268	167	25
unclassified_o__Gaiellales	272	147	4
norank_c__Gitt-GS-136	384	66	1
Thermomonas	322	40	1
Ignavibacterium	75	350	90
Truepera	324	266	223
norank_f__Caldilineaceae	346	546	85
Limnobacter	430	395	42
norank_o__AKYG1722	399	238	43
Ornatilinea	320	351	1
norank_c__1-20	236	330	58
norank_p__RBG-1__Zixibacteria_	160	734	0
norank_c__Bacteroidetes_vadinHA17	1388	709	37
norank_o__JG30-KF-CM45	1030	765	24
Arenimonas	985	381	3
norank_p__Aminicenantes	981	165	0
Nitrospira	530	497	1
Thiobacillus	862	574	3
norank_f__Elev-16S-1332	772	482	5
Nitrosomonas	32	111	238
Ottowia	82	7	292
norank_p__Latescibacteria	36	95	377
unclassified_o__Rhizobiales	66	51	364
Rivibacter	0	3	472
Desulfovibrio	0	0	473
norank_f__Lentimicrobiaceae	32	9	595
Fusibacter	1	0	662
norank_f__Spirochaetaceae	3	0	643
Defluviicoccus	158	171	890
Thauera	5	12	1151
unclassified_o__Micrococcales	27	14	962
norank_f__Anaerolineaceae	6453	12381	3987
unclassified_p__Bacteroidetes	0	0	12718
unclassified_f__Dermatophilaceae	1	0	3114

Fig. 6. Relative abundances of the bacterial community of sludge samples at genus level (1) seed sludge; (2) nitrification-denitrification sludge; and (3) nitritation-denitrification sludge.

samples at the genus level (Fig. 6), and the analysis results clearly showed that the samples derived from the seed sludge (1#) and nitrification-denitrification stages (2#) have a similar composition. After the establishment of the nitritation process, the character of the nitritation-denitrification sludge composition (3#) was different from that of the other two samples. In this study, *Nitrosomonas* were regarded as the dominant AOB and *Nitrospira* were the NOB in the SBR [26]; other AOB and NOB genera were extremely rare in this reactor.

Based on the genus level distributions of the three samples illustrated in Fig. 6, *Nitrosomonas* were found in all the three samples. Because of the existence of *Nitrosomonas*, high ammonium removal efficiency was achieved at the SBR start-up stage. Meanwhile, there was a high relative abundance of *Nitrospira*; in the reactor, the nitrite produced by AOB was converted to nitrate by NOB. Therefore, nitrate was detected at the end of the aerobic phase in the first stage of SBR operation. The last sample (3#) was taken on day 60 when the stable nitritation performance was achieved in stage 3, and no *Nitrospira* were detected in this sample. Based on the analysis results, the NOB was successfully inhibited and washed out from the SBR by applying the control strategies mentioned in this study. *Nitrosomonas* played a significant role in ammonium removal, for which a stable nitritation process and high NAR were maintained at the end of the aerobic phase.

4. Conclusion

An SBR was run under controlled DO (less than 0.8 mg/L), pH (higher than 7.8), temperature (29°C–30°C) and ammonium concentration (40–50 mg $N\text{-}NH_4^+$/L) conditions. NOB was inhibited, and the nitritation-denitrification in the SBR was successfully started in 20 d. During the operation period, ammonium removal was higher than 97%, and NAR was maintained at approximately 98%. In addition, an increase in N-NH_4^+ concentration during feeding enhanced the inhibition effect on NOB without affecting the NAR.

In the nitritation-denitrification process, DO and ORP signals appeared as the periodic trends of variation. Moreover, there were obvious feature points at the end of aerobic and anoxic periods, respectively. These may be adopted to indicate the end of the reaction. By calculating the first derivatives of DO and ORP, the maximum value of the first derivative of DO can be adopted as a threshold indicating the end of nitritation. In the denitrification stage, the minimum value of the first derivative of ORP can be used as a threshold indicating the end of denitrification.

In the stable nitrification-denitrification SBR system, the variation of feeding concentrations did not affect the trends of the first derivatives of DO and ORP, and the variation of feeding concentrations did not affect the extreme values of the first derivatives of DO and ORP. Furthermore, the two first derivatives may be used for the controlling parameters of an automatic operation.

According to the results of Illumina MiSeq sequencing and q-PCR for the biomass samples, *Nitrosomonas* were regarded as the AOB and *Nitrospira* were the NOB in this study. When nitritation was established in SBR, *Nitrospira* were hardly detected, which verified the control strategies applied in this study are effective in inhibiting NOB.

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