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Partial-nitritation of low-strength anaerobic effluent: A moderate-high dissolved oxygen concentration facilitates ammonia-oxidizing bacteria disinhibition and nitrite-oxidizing bacteria suppression

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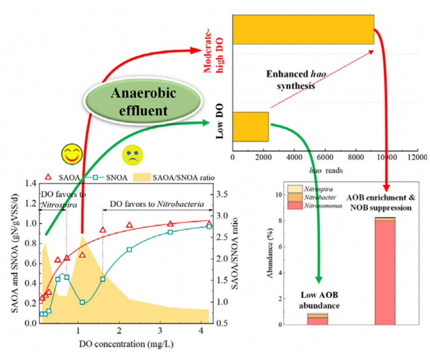
HIGHLIGHTS

- Bio-refractory organics reduce AOB activity by suppressing hydroxylamine oxidation.
- Moderate-high DO is superior to low DO for PN of mainstream anaerobic effluent.
- Moderate-high DO facilitates AOB disinhibition and NOB suppression simultaneously.

GRAPHICAL ABSTRACT



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ABSTRACT

Integrating anaerobic treatment with partial nitritation (PN)/anammox is a promising technology to achieve energy-efficient wastewater treatment, while partial nitritation of the mainstream anaerobic effluent (Aneff) was rarely reported. A PN reactor fed with low-strength Aneff was employed in this study to investigate the performance and technology bottleneck of this process. When operated at low dissolved oxygen (DO) concentration (0.30–0.43 mg/L), gene coding hydroxylamine oxidation (*hao*) was severely suppressed by bio-refractory organics, which results in a decreased ammonia-oxidizing bacteria activity and nitrite accumulation rate. The ammonium conversion and nitrite accumulation were recovered by increasing the DO concentration to a moderate-high level (1.10 ± 0.20 mg/L) and achieved long-term stable operation. At this condition, *hao* showed a dramatic increase while gene encoding nitrite oxidoreductase was appropriately suppressed; the effluent NO_2^-/NH_4^+ ratio reached 1.17, and a low NO_3^-/NO_x^- ratio of 0.38 was achieved simultaneously. The findings in this study revealed the adverse effects of Aneff on PN and supported a practical operating strategy for efficient PN of Aneff.

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1. Introduction

As a promising technology for sustainable wastewater treatment, anammox has attracted much attention in recent years (McCarty, 2018). It is characterized by three advantages compared with the conventional nitrification-denitrification process: (i) reducing the oxygen needed for ammonium oxidation by 60%, (ii) eliminating the use of

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carbon for nitrate reduction, and (iii) cutting sludge yield by 80% (Ma et al., 2020). Anammox in high-strength ammonium wastewater treatment has been widely reported in the last few decades (Kartal and Kuenen, 2010), while the application of anammox in sewage mainstream still facing many challenges (Cao et al., 2016). As the preliminary process of anammox, nitrite accumulation by partial nitritation (PN) plays a key role in the application of anammox. However, the produced nitrite can be easily oxidized to nitrate by nitrite-oxidizing bacteria (NOB) because the Gibbs free energy of nitration is much higher than that of nitritation (Eqs. (1) and (2)) (Mulder et al., 1995), which reduces the available nitrite for the subsequent anammox process and the nitrogen removal efficiency. In the treatment of high-strength ammonium wastewater, NOB can be well controlled by high free ammonia and free nitric acid under low dissolved oxygen (DO) concentration (Duan et al., 2019; Seuntjens et al., 2018), while these elements are absent when treating low-strength ammonium wastewater. Therefore, maintaining a stable PN process with efficient nitrite accumulation has been a bottleneck for the further development of mainstream anammox.

$$NO_3 + 1.5O_2 \rightarrow NO_2 + 2H_2O + H^+; \Delta G^0 = -275 \text{ kJ/mol} \quad (1)$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^-; \Delta G^0 = -74.1 \text{ kJ/mol} \quad (2)$$

Employing a combination of low DO concentration and intermittent aeration is a prevalent regime (Kornaros et al., 2010). By applying low DO concentration, NOB activity in PN system can be well controlled. In addition, the intermittent aeration is conducive to the selective enrichment of ammonia-oxidizing bacteria (AOB) because NOB needs a longer duration to respond to DO increase (Hellinga et al., 1998; Ma et al., 2015). So, regulating the PN system by employing a combination of low DO concentration and intermittent aeration has been adopted as the main strategy in many real cases.

Recently, integrating anaerobic treatment with PN-anammox for energy-efficient wastewater treatment has received extensive attention, because this process has the potential to achieve energy-neutral/positive wastewater treatment (Gao et al., 2014; Lücker et al., 2010). In this process, organics are recovered as methane in the anaerobic stage, with all of the effluent nitrogen being converted into ammonium (Chen et al., 2017), then the Aneff is further treated through PN/anammox to remove nitrogen. Although some advanced technologies (i.e., anaerobic membrane bioreactors (AnMBRs)) have been adopted in the anaerobic stage, organics will still be present in the Aneff in a concentration of 20–35 mg COD/L (Hu et al., 2018; Lei et al., 2018, 2019). However, previous studies paid much attention to the adverse effects of organics on anammox bacteria when PN/anammox technology is taken (Cheng et al., 2020), only several general results about the impacts of organics on AOB was reported (Leal et al., 2016; Zhang et al., 2019). Furthermore, current knowledge about the impacts of organics on the PN system was mainly acquired at high COD/N ratios (COD/N > 1.0 mg/L), and the organics were supplied using substances with high biodegradability (e.g., sodium acetate, alcohol, and glucose). The COD/N ratio of Aneff is generally lower than 0.75 when treating sewage, and the organics are characterized by a low biodegradability (such as humic substances and low molecular weight neutrals) (Chen et al., 2017). So, the effect of Aneff on PN may differ from general organics, and the conventional regulation strategy that relying on low DO concentration may face serious challenges.

In this study, a PN reactor fed with the Aneff was operated at room temperature for over 200 days. Nitrogen conversion, sludge properties, microbial evolution, and the functional genes involved in nitrogen conversion were monitored at different operating stages. Suppression of Aneff on ammonium oxidization was found when operated at low DO concentration, while a moderate-high DO concentration showed high-efficiency to solve this issue. The underlying mechanism based on activity tests, microbial consortia, and functional genes analysis was identified and discussed. The findings in this study revealed the adverse effects of Aneff on PN, and supported a practical operating strategy for PN of Aneff, which will expedite the development of anaerobic treatment integration with anammox for sewage treatment.

2. Materials and methods

2.1. Reactor setup and operation

A system integrating AnMBR and PN-anammox was built to achieve successive methanation and autotrophic nitrogen removal in a previous study (Lei et al., 2020). The schematic of the PN system used in this study is illustrated in Supplementary Material. Briefly, a columnar PN reactor has an inner diameter of 80 mm and a working volume of 1.0 L (total volume of 1.5 L) was employed in the current study. The reactor was batch fed using a peristaltic pump (BT100J-1A, Huiyu, China). The total cycle period was set at 7 min, it comprises an aerobic period of 3 min and an anaerobic period of 4 min. Aeration was supplied by a gas pump (VBY7506, Cheehie, China) under the control of a timer; the DO concentration was controlled by aeration rate and the on/off of the gas pump. To prevent sludge washout during decantation, a membrane module made of nylon mesh with a pore size of 40 μm was used to intercept sludge. The reactor was fed with synthetic ammonium wastewater same to a previous study during the start-up stage (NH_4^+-N = 50 mg/L, TP = 5 mg/L) (Chen et al., 2019); the influent was replaced with Aneff when the effluent NO_2^-/NH_4^+ ratio reached 1.0 (Table 1). Hydraulic retention time (HRT) of the reactor was set in a range of 0.8–1.2 h, which results in a nitrogen loading rate (NLR) of 1.0–1.5 gN/L-reactor/d. The reactor temperature was controlled at 25 ± 1 °C by a water bath, the pH and DO were monitored by pH and DO probes (HQ30d, HACH, USA), respectively.

2.2. Batch tests to determine specific activities and growth kinetics

Referring to the specific activities of AOB (SAOA) and NOB (SNOA) in the reactor, ex-situ bath tests were implemented on days 138, 150, and 195. The test was carried out in several flasks with each flash has a total volume of 250 mL; two duplicates samples were set. Before the test, 50 mL of mixed liquor taken from the reactor was used as inoculum after it was washed thrice using a phosphate-buffered solution. Then a solution containing necessary nutrients and the nitrogen source was added. The total mixed liquid volume in each bottle was set at 200 mL, with the initial NH_4^+-N concentration of 25 mg/L. Each bottle was aerated by a pump to support DO; a water bath with magnetic stirring was employed to keep the constant temperature (25 ± 1 °C) and stirring intensity (150 rpm/min). During the test, samples were collected regularly to monitor the NH_4^+-N consumption and NO_3^--N production. Finally, the SAOA and SNOA were calculated by dividing the nitrogen consumption to the sludge concentration.

The growth kinetics of AOB and NOB were tested when ammonium conversion was inhibited by Aneff. Sludge used in this study was taken from the reactor on day 215, and the Aneff was used as the nitrogen source. The detailed procedure is the same as the activity test while a

Table 1
Characteristics of the anaerobic effluent.

Parameters	Value
COD	22.8 ± 8.1 mg/L
TN (NH_4^+-N)	49.9 ± 2.4 mg/L
COD/N ratio	0.45 ± 0.17
TP	5.0 ± 0.5 mg/L
Volatile fatty acids	<5 mg/L
pH	7.5 ± 0.1
Turbidity	<1 NTU

series of DO concentrations were employed. One repeated sample was set to guarantee the reliability of the test.

2.3. DNA extraction and metagenome sequencing

On day 133, 153, 164, 195, 215, sludge sample with a volume of 2 mL was collected for microbial analysis. Total genomic DNA extraction was performed according to the manufacturer's instructions provided in the Mag-Bind Soil DNA Kit (OMEGA). The extracted DNA were assessed via agarose gel electrophoresis with ethidium bromide staining (FR-980A, Furi, China) and fluorometer nucleic acid quantification (Qubit®, Life Technologies, USA), successively. The extracted DNA was used for constructing a shotgun library and high-throughput sequencing using the Illumina HiSeq XTen platform to generate 150 bp paired-end reads (300– 600 bp mean insert size).

The quality of the sequenced raw data was evaluated and filtered to obtain relatively accurate and valid data (Bolger et al., 2014). The clean sequences were stitched and assembled into a long-sequence contig (Peng et al., 2012). According to the overlapping relationship between the reads, contigs were obtained, and the assembly results were comprehensively evaluated to select the best assembly results. Genes with a length of ≥100 bp were selected and translated into amino acid sequences. CD-HIT software (version 4.6) was used to remove redundancy to obtain non-redundant gene sets (Langmead and Salzberg, 2012). Gene abundance information in each sample was calculated based on the reads on the alignment and the gene length. The gene set was compared with the KEGG database to obtain species and genes annotation information. Functional genes and species abundance were calculated based on gene set abundance.

2.4. Sampling and analytical methods

Influent and effluent samples were collected every three days to test the NH_4^+-N, NO_2^--N, and NO_3^--N concentrations after filtering through a 0.45 μm microporous filter using Nasser's reagent colorimetry (λ = 420 nm), N-(1-naphthyl)-ethylenediamine dihydrochloride colorimetry (λ = 540 nm), and sulphamic acid spectrophotometry (λ_1 = 220 nm, λ_2 = 275 nm), respectively. The COD concentration was tested using the same samples with nitrogen analysis according to the rapid digestion-spectrophotometric method (λ = 420 nm), and was corrected by subtracting the contribution of NO_2^--N using a correction coefficient of 1.1 g COD/g NO_2^--N. A laser granularity distribution

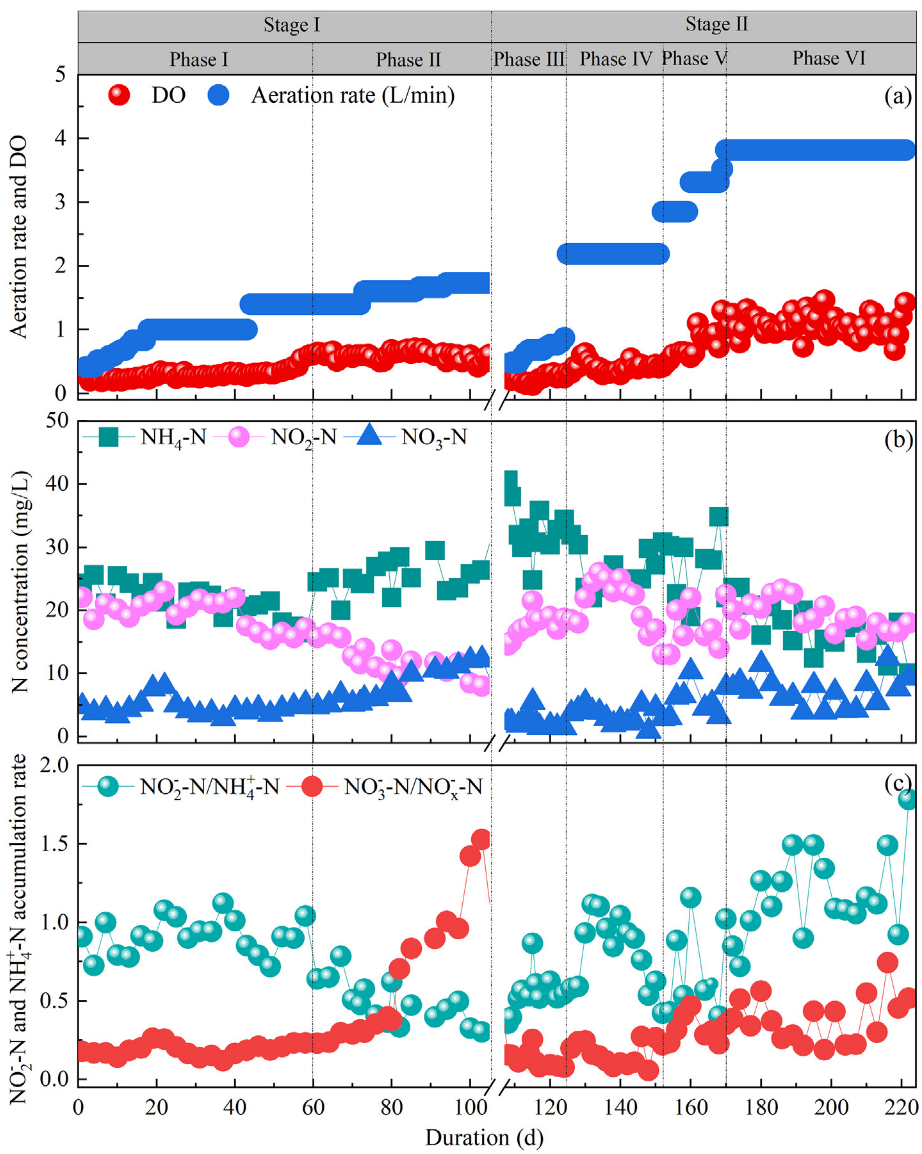

Fig. 1. System performance of the partial nitritation system. (a) Aeration rate and DO concentration, (b) nitrogen composition in the effluent, and (c) NO_2^-/NH_4^+ and NO_3^-/NO_x^- ratios in the effluent.

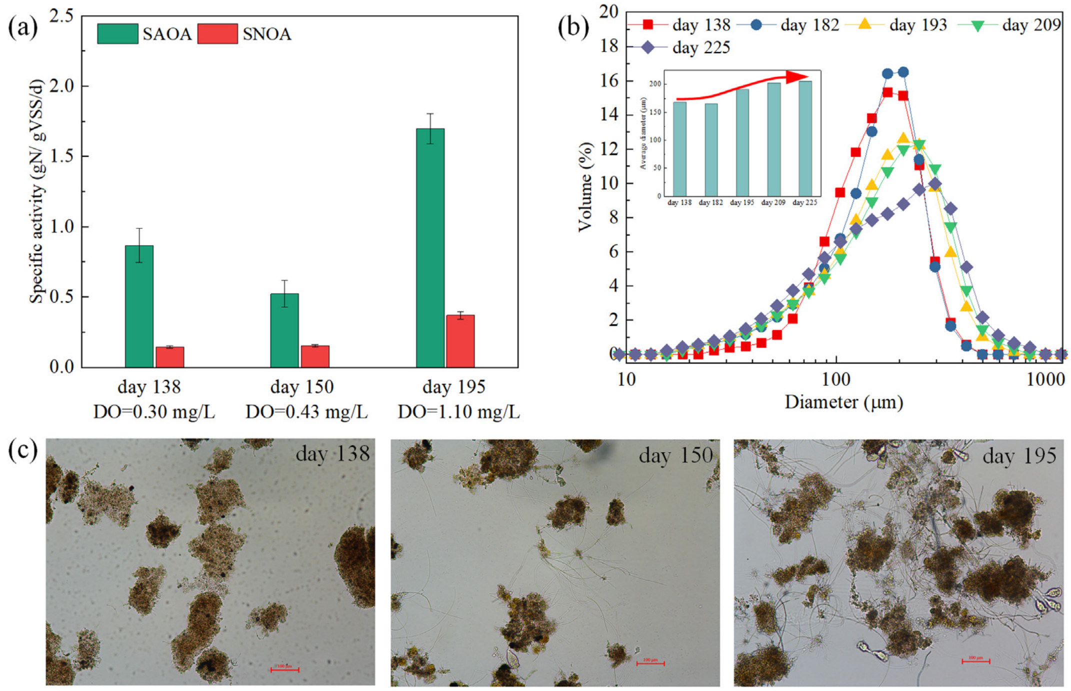

Fig. 2. Sludge property at different conditions: (a) specific activity, (b) particle size distribution, and (c) sludge morphology.

analyzer (LS 230/SVM+, Beckman Coulter, USA) was employed to monitor the sludge size variation (0–2000 μm) in the mixed liquor. The morphology of the microbes in the reactor was examined regularly using a digital microscope (Eclipse 50i, Nikon, Japan) at different operation stages.

3. Results and discussion

3.1. Suppression of anaerobic effluent on ammonium transformation and the recovery process at a moderate-high DO concentration

The PN reactor was operated at two different stages with different NLRs (or HRTs). In stage I (low NLR), the nitrite accumulation was maintained at a high level at the initial (day 0 to 40), the average effluent NO_2^--N was 19.5 ± 2.3 mg/L at a low DO concentration of 0.30 ± 0.07 mg/L. Average NO_3^--N concentration and NO_3^-/NO_x^- ratio in the effluent was 4.6 ± 1.3 mg/L and 0.23 ± 0.06, respectively, suggesting an efficient PN system was built. In the following days (day 40 to100), effluent NH_4^+-N concentration showed a progressive decrease while NO_3^--N concentration was gradually decreased, resulting in an obvious decline of NO_2^--N concentration in effluent (from 16.5 mg/L to 8.1 mg/L). The NO_3^-/NO_x^- ratio finally reached over 0.60, and the NO_2^-/NH_4^+ ratio declined to less than 0.30 (day 100), meaning that the PN system has been deteriorated. Microbial compositions showed that the deterioration of the PN system was mainly induced by the decreased AOB relative abundance (from 20.4% to 0.15%), although the enriched NOB also is one important factor in the later stage (after day 80) (Fig. S2). These results revealed that a low DO concentration cannot maintain sustainable AOB activity when treating Aneff.

The PN system was restarted and operated at a higher NLR (stage II, 1.5 gN/L-reactor/d). After one month of operation, the NO_2^--N concentration in the effluent reached 25.0 mg/L (phase III). In the following phase (phase IV), Aneff was used as the PN influent, which resulted in a dramatic decline of ammonium oxidization rate again, and the effluent NO_2^--N concentration decreased from 25.0 mg/L to 13.0 mg/L in the following 16 days. To recover ammonium oxidization efficiency, the aeration rate was increased in phase V. In this phase, the NO_2^--N concentration showed a bit of increase, while the NO_2^-/NH_4^+ ratio was still much lower than the desired value for the anammox process (1.32). So, the aeration rate was further increased, and the DO concentration reached 1.10 ± 0.20 mg/L (phase VI). NO_2^--N in the effluent reached over 20 mg/L at a short SRT (12.5 days, Table S2) in this phase, then showed a slight decrease mainly because of the presence of anammox bacteria (Fig. 3a). The average NO_2^-/NH_4^+ ratio reached 1.17 ± 0.30, while the NO_3^-/NO_2^- ratio was only 0.39 ± 0.13, suggesting that a stable PN process was achieved when operated at a moderate-high DO concentration, a similar case was also reported previously (Cui et al., 2020).

According to the system performance depicted in Fig. 1, the nitrogen conversion in the PN system was largely suppressed by Aneff. Previous studies also reported that the presence of some organics (especially bio-refractory organics, e.g. humic substance) is adverse to maintain nitrosifying consortia activity under certain conditions (Luo et al., 2019; Zhang et al., 2019). In this study, humic substances and low molecular weight neutrals accounted for over 80% of the total organic carbon in the Aneff (Table S1). They should be responsible for the suppression of Aneff on PN.

3.2. SAOA/SNOA activities and sludge morphology impacted by anaerobic effluent

The SAOA and SNOA were tested to investigate the effects of Aneff on AOB and NOB, respectively (Fig. 2a). On day 138, the SAOA reached 0.87 ± 0.12 gN/gVSS/d and the SNOA was less than 0.14 gN/gVSS/d, suggesting that the AOB activity was well developed, while NOB activity was appropriately suppressed. After the PN substrate was replaced with Aneff, SAOA dramatically declined by 40%, having a value of 0.52 ± 0.34 gN/gVSS/d (day 150), suggesting that AOB activity was suppressed because the abundance of AOB at day 138 and day 150 were comparable (Fig. 3a). The SAOA recovered to 1.70 ± 0.11 gN/gVSS/d on day 190 when the DO concentration was increased to 1.1 ± 0.2 mg/L. Under this condition, the SAOA-to-SNOA (SAOA/SNOA) ratio was around 4.7, suggesting that the NOB activity was appropriately regulated under a moderate-high DO concentration. The sludge particle size gradually increased with the average diameter was enlarged from 168 to 202 μm (Fig. 2b), this can be explained by the variation of sludge morphology.

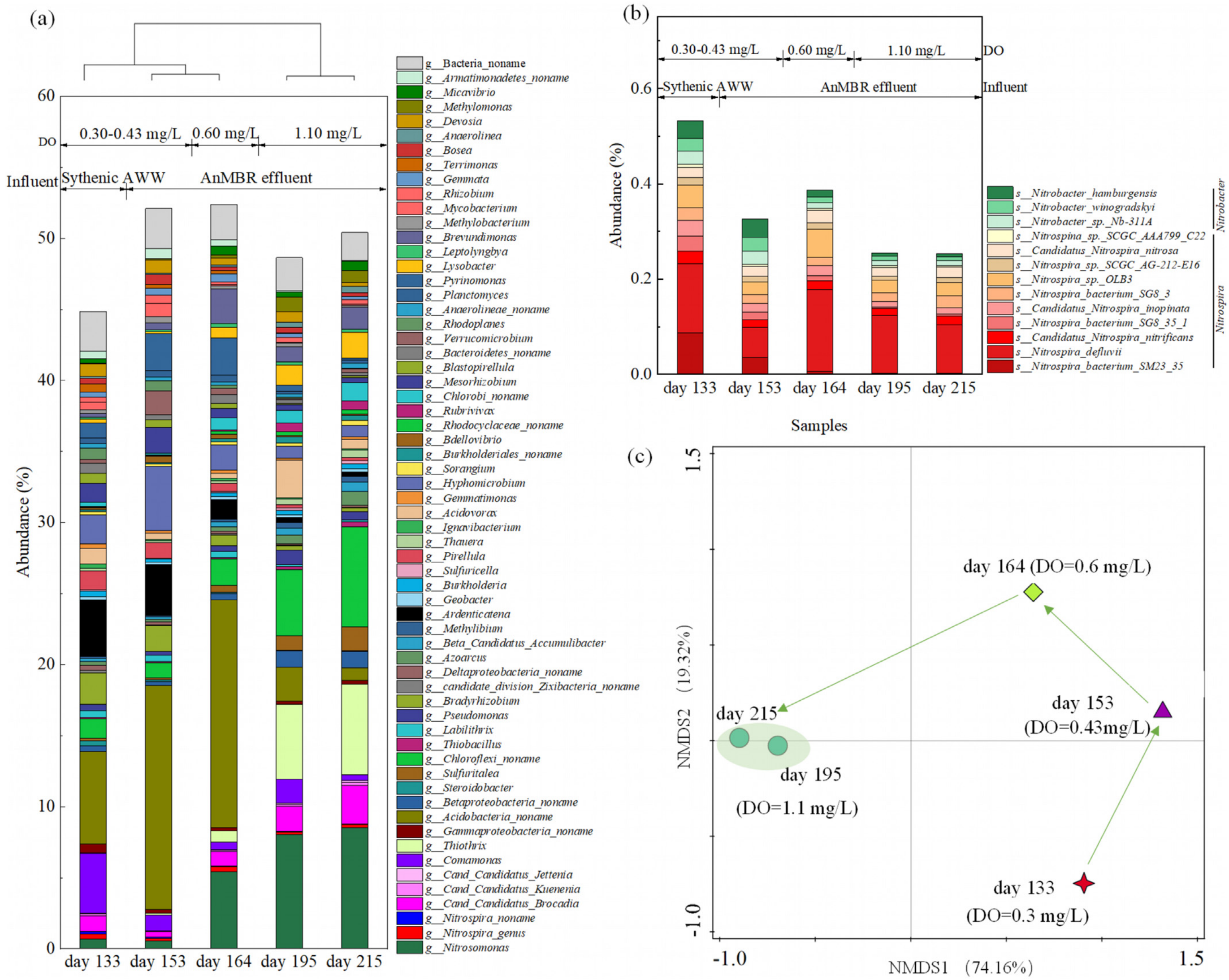

Fig. 3. (a) Relative abundance of microbes at the genus level, (b) NOB community at species level, (c) microbial consortia evolution based on NMDS at the genus level. Presented as >0.5% at the genus level of the sequence reads in at least one sample in (a) and (c). AWW: ammonium wastewater.

Sludge flocs showed a smooth margin after 30 days of operation in phase III, when the synthetic ammonium wastewater was fed. Filamentous microbes occurred only after 9 days of operation when the Aneff was fed, and was largely enriched after day 195, suggesting that the Aneff will induce the overgrowth of filamentous microbes. In a previous study (Wu et al., 2019), the filamentous microbes were found to hinder oxygen transfer and compete for DO with AOB, which can explain why a moderate-high DO concentration is needed to maintain the activity of AOB.

3.3. Microbial consortia evolution at different operating conditions

Clustering based on *Pearson* correlation indicates the microbial consortia were greatly impacted by different substrates (synthetic ammonium wastewater and anaerobic effluent) and DO concentrations (Fig. 3a). *Nitrosomonas*, the main AOB identified in the reactor, showed a slight decrease from day 133 to 153 (abundance decreased from 0.69% to 0.55%), suggesting that the AOB multiplication was inhibited when the Aneff was fed (Fig. 3a). In contrast, abundance of *Nitrosomonas* dramatically increased to 5.41% on day 164 and reached to around 8.0% on day 195. *Acidobacteria_noname*, which accounted for 6.49% of the total microbes on day 133, rose to almost 16.0% (day 153–164), then declined to less than 1.0% on day 215. This may be because *Acidobacteria_noname* was outcompeted by anammox bacteria for available iron ions. Some other prevalent genera, including *Hyphomicrobium*, *Mesorhizobium*, *Pyrinomonas*, and *Chloroflexales_noname*, showed a contrary trend with *Nitrosomonas*. These microorganisms are all heterotrophic, their proliferation was promoted mainly because organics was supplied when using Aneff. The majority of them are involved in denitrification and prefer to anoxic conditions (Isaka et al., 2012; Lv et al., 2020; Tang et al., 2018); so, their abundance declined to a low level (<1.0%) when a moderate-high DO concentration was maintained (from day183). In comparison, abundance of genus *Thiothrix* dramatically increased from less than 0.1% (day 153) to over 6.4% on day 215. Bacteria classified to this genus are typical filamentous microorganisms in the nitrification unit during wastewater treatment and reported to interfere with oxygen transfer (Wu et al., 2019). The NOB abundance was controlled to less than 0.6% in stage II, suggesting that NOB was effectively suppressed under a moderate-high DO concentration (Fig. 3a). Genus *Nitrospira* accounted for over 80% of the total NOB (Fig. 3b), species of this genus are K-strategy microbes with high activity and low population (Lücker et al., 2010), which explains why the NO_3^--N concentration in the PN effluent was still needed to be considered under a low NOB abundance (Fig. 1). Interestingly, an obvious NOB decline was observed from day

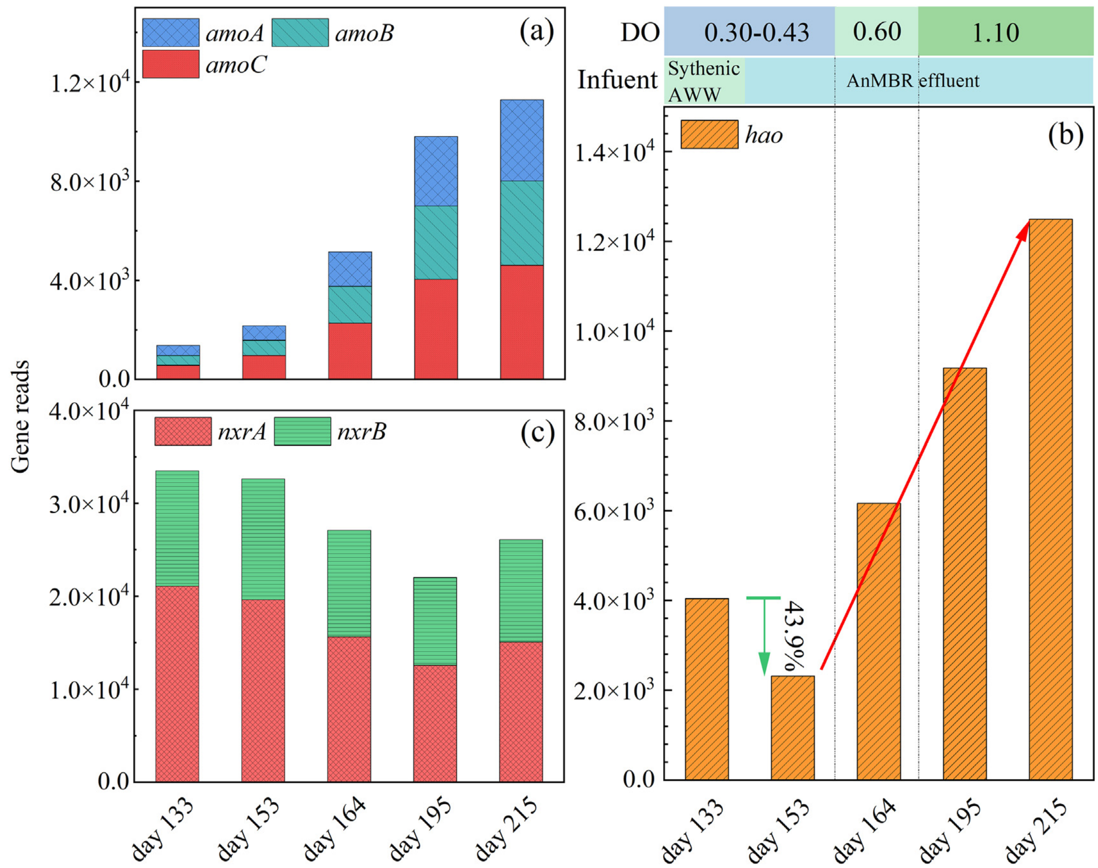

Fig. 4. Gene reads of (a) *amo*, (b) *hao*, and (c) *nxr* at different DO concentrations. AWW: ammonium wastewater; *amo*A: ammonia monooxygenase; *hao*: hydroxylamine oxidation; *nxr*: nitrite oxidoreductase.

164 to 195, this may be because of the largely shortened SRT (30 → 15 days), which promoted the NOB washout due to their low growth rate.

The cluster analysis based on non-metric multidimensional scaling (NMDS) shows that the microbial consortia was significantly affected by different substrates and DO concentrations (Fig. 3c). Samples of day 133, day 153, and day 164 were mutually dispersed, and are far from samples from day 195, indicating the difference of microbial consortia among these samples. In contrast, samples of day 195 and day 215 were agminated, meaning that the microbial consortia in these samples were highly similar, which is consistent with the cluster in Fig. 3a.

3.4. Functional genes involved in nitrogen metabolism at different operating conditions

The number of genes encoding the ammonia monooxygenase (*amo*) was only 1.37×10^3 on day 133 and increased slightly with time, reaching 2.16×10^3 on day 153 (Fig. 4a). Using Aneff showed no obvious effect on *amo* synthesis; *amo* sequence increased to 9.80×10^3 on day 195 and then remained stable, indicating a moderate-high DO concentration has the potential to promote *amo* synthesis (Fig. 4a). Taxonomic annotations suggest that *amo* originated from *Nitrosomonas* only accounted for a small proportion of total *amo* on day 133 (Fig. 5), and the sequence number dramatically increased from day 133 (1.4×10^2) to 195 (8.2×10^3), along with the increase of DO concentration, meaning that a moderate-high DO concentration promotes this pathway. In comparison, the number of gene encoding NH_2OH oxidation (*hao*) declined from 4.04×10^3 to 2.31×10^3, then increased to 1.25×10^4 on day 215; among which, *hao* derived from *Nitrosospira* decreased from 5.0×10^2 to 3.8×10^2, then reached a high level of 5.01×10^3 on day 195. As a typical AOB, the variation of *hao* derived from *Nitrosospira* might be correlated to the specific activity of NH_4^+ oxidation, suggesting a moderate-high DO concentration could promote NH_4^+ conversion. As the two procedures of nitritation shown in Eqs. (3)–(4), half of the electrons generated by *hao* are transferred back to *amo* as the catalytic energy source, and the remaining half is consumed as growth energy through generating nicotinamide adenine dinucleotide phosphate and adenosine triphosphate (Nishigaya et al., 2016). So, suppression of NH_2OH oxidation will also turn back to inhibit the NH_4^+-N oxidization. On day 195, *hao* synthesized by *Nitrosospira* accounted for over 60% of the total *hao*, indicating that this genus played a key role in NO_2^--N production, although its abundance was only 0.16%. Based on these results, *Nitrosomonas* mainly participated in NH_4^+-N oxidation while *Nitrosospira* played a key role in NH_2OH oxidation, so the nitritation in the PN reactor was achieved by the synergy of *Nitrosomonas* and *Nitrosospira*.

$$NH_3 + 2H^+ + O_2 + 2e^- \overset{amo}{\rightarrow} NH_2OH + H_2O \quad (3)$$

$$NH_2OH + H_2O \overset{hao}{\rightarrow} NO_2{}^- + 5H^+ + 4e^- \quad (4)$$

Differing from genes involved in nitritation, gene involved in nitrite oxidoreductase (*nxr*) showed a stepwise decrease from day 133 to day 164 and then remained stable. Genes encoding nitrate reductase (*nar*) and nitrous-oxide reductase (*nos*) showed a similar variation trend with gene *nxr* (Fig. S3); *nar* and *nos* mainly derived from *Acidovorax*, and reached a high content on day 153, suggesting that Aneff was beneficial to the occurrence of denitrification, although the *Acidovorax* abundance was very low (0.52%). At a moderate-high DO concentration, *nxr* hosted by the dominant NOB (*Nitrospira*) only accounted for less

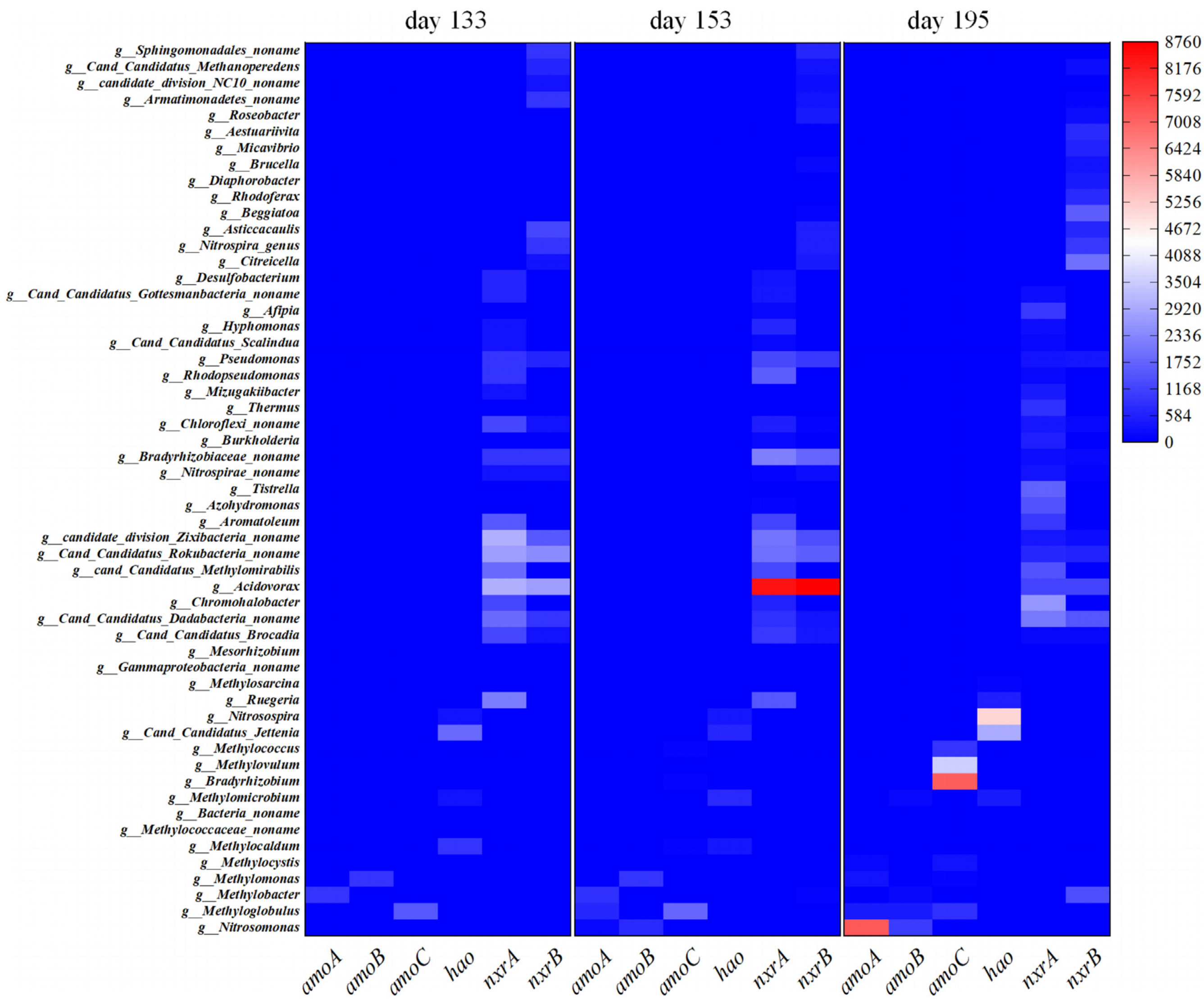

Fig. 5. Relationship between the genes involved in nitrogen conversion and genera at different DO concentrations. *amo*A: ammonia monooxygenase; *hao*: hydroxylamine oxidation; *nxr*: nitrite oxidoreductase.

than 1% of the total *nxr* number (Fig. 5) due to the low RA of *Nitrospira*, indicating that nitration (NO_2^--N → NO_3^--N) dominated by *Nitrospira* was not promoted by the increased DO concentration.

3.5. PN deterioration induced by organics in anaerobic effluent

Organics have been widely reported to suppress AOB activity when presented at a high concentration or high COD/N ratio (Racz et al., 2010; Remmas et al., 2016; Zhang et al., 2019). However, the findings of this study suggest the suppression of organics on AOB may be highly correlated to the organic properties and operating conditions. Although the concentration of the organic in the Aneff was only 22.8 ± 8.1 mg/L, it was from an AnMBR with efficient COD removal, all the organics with high biodegradability has been removed, nonbiodegradable carbons accounted for over 80% of the organics in the Aneff. When the PN reactor was operated at a low DO concentration (<0.3 mg/L), AOB activity was largely suppressed because AOB is more sensitive to these bio-refractory substances than common organics (Zhao et al., 2015). Besides, nonbiodegradable organics in Aneff can be absorbed by sludge flocs, which results in higher organics concentration in mixed liquor and hence promotes the multiplication of heterotrophic bacteria, so the available DO and space for AOB will be compressed, which further reduces AOB activity (Nogueira et al., 2002). When a moderate-high DO concentration is supplied, AOB growth will be promoted according to the growth kinetics (Fig. 6). Also, the accumulation of organics in the reactor will be mitigated, which restrained the multiplication of heterotrophic bacteria and support more favorable conditions for AOB growth.

3.6. Mechanism of moderate-high DO concentration facilitate the successful operation of PN treating anaerobic effluent

Operating at a moderate-high DO concentration (1.10 mg/L) has been proved effective in facilitating AOB enrichment and NOB washout in this study (Figs. 1, 2b, 3a, and 4), and it may be also suitable for other PN systems fed influent with low-strength nitrogen. Microbial consortia evolution revealed that *Nitrosomonas* and *Nitrospira* are the dominant AOB and NOB, respectively, in this study. *Nitrosomonas* featured a high half-saturation coefficient of 1.10 mgO_2/L, it is much higher than that of *Nitrospira* (0.16 mgO_2/L), according to growth curves in Fig. 6, and this agrees with the findings in previous studies (Law et al., 2019; Regmi et al., 2014). The maximum growth rate of *Nitrosomonas* was much higher than that of *Nitrospira* (0.126 h^{-1} vs 0.09 h^{-1}), meaning that *Nitrosomonas* has the potential to outcompete *Nitrospira* under moderate-high DO concentration. It is worth to be noticed that SRT regulation also played a crucial role in reducing NO_3^--N production in this

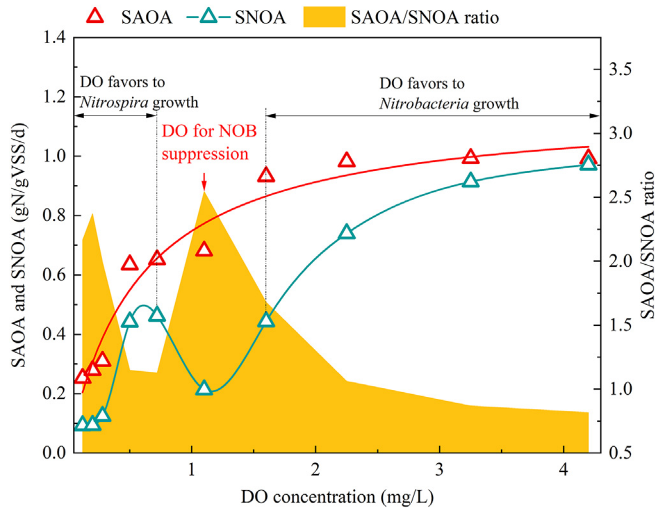

Fig. 6. Mechanism of the out-selection of NOB over AOB at high DO. Data were collected from batch test results using sludge taken from the PN reactor on day 215. The red curve represents the dissolved oxygen Monod curve based on the SAOA data. The half-saturation coefficient of AOB was determined as 1.13 ± 0.12 mgO_2/L.

process because the NOB needs to be weeded out in this way. An SRT of 12.5 d was maintained in phase VI, although this SRT is still longer than the suggested SRT in some papers (SRT 5–8 days), we consider it aggressive enough because a reasonable sludge concentration was maintained under this condition. Besides, bacteria classified to the genus *Nitrospira* were reported to lack primary protection mechanisms to oxidative stress (Lücker et al., 2010), which further promoted the selective enrichment of AOB at a moderate-high DO concentration. However, the aforementioned discussion is not meaning that DO concentration should be maintained at a very high level, a DO concentration over 2.25 mg/L is not recommended in real applications (Fig. 6). Some researchers reported that a high DO of over 3.0 mg/L can be used for a short period to suppress NOB (Law et al., 2019; Wang et al., 2020), while this strategy may be not suitable for AD effluent treatment at long-term operation. On one side, the AOB activity needs to be maintained when the reactor is fed with Aneff due to the adverse impact from the organics in the effluent. Especially during the startup stage, maintaining moderate-high DO concentration benefits the rapid enrichment of AOB. On another side, a DO concentration of over 2.25 mg/L will result in a rapid increase of NOB activity (Fig. 6). Although *Nitrobacteria* only accounted for a low proportion of total NOB in sewage mainstream (20% in this study), they still have the potential to result in a high SNOA because the maximum SNOA of *Nitrobacteria* is almost nine times that of *Nitrospira* (Kim and Kim, 2006). So, operating at a moderate-high DO concentration may be a practical option for long-term stable operation of the PN system treating Aneff. Notably, the strategy proposed in this study is based on the knowledge that *Nitrosomonas* displays higher maximum growth rates than *Nitrospira* at mesophilic temperatures (>20 °C) (Laureni et al., 2019). For real applications, the temperature will be another important factor that impacts the feasibility of this strategy.

4. Conclusions

Stable and efficient ammonium oxidization is the bottleneck during partial nitritation of the anaerobic effluent. When operating at low DO concentration, bio-refractory organics in Aneff suppressed nitrite generation by restraining NH_2OH oxidation and stimulated proliferation of filamentous microbes. Stable partial nitritation of Aneff can be achieved by simultaneous ammonia-oxidizing bacteria disinhibition and nitrite-oxidizing bacteria suppression at moderate-high DO concentration. It facilitates to mitigate the adverse effects of Aneff by promoting NH_2OH oxidation and suppressing heterotrophic multiplication in long-term operation. This study broadens our knowledge on the PN process regulation in Aneff treatment and supports a practical strategy for the PN process regulation.

CRediT authorship contribution statement

Zhen Lei: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft. **Lianxu Wang:** Methodology, Investigation, Data curation. **Jun Wang:** Methodology, Investigation. **Shuming Yang:** Investigation, Data curation. **Zhaoyang Hou:** Investigation, Data curation. **Xiaochang C. Wang:** Conceptualization, Supervision. **Rong Chen:** Project administration, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.145337.

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