Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2023.30147

315 (2023) 355–363
December

Inhibition strategies of nitrite oxidation bacteria in biological nitrogen removal system: a mini review

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Received 24 July 2023; Accepted 20 November 2023

ABSTRACT

In the partial nitrification and anaerobic ammonia oxidation (Anammox), it was necessary to control ammonia oxidation, aiming to inhibit the activity of nitrite-oxidizing bacteria (NOB) to achieve the accumulation of NO_2^-–N. This review summarized methods of NOB inhibition which had been reported in the current literature. These methods included changing the dissolved oxygen concentration and sludge retention time, adjusting the free nitrite acid and free ammonia levels, adding chemical inhibitors such as hydroxylamine (NH_2OH) and hydrazine (N_2H_4), and using other inhibition methods such as electromagnetic radiation and ultrasound. Most study revealed that NOB could be effectively inhibited by combining different inhibition methods and real-time control strategies. Concurrently, the utilization of biofilm and granular sludge and multi-bacterial cooperation processes associated with anammox and denitrification could reduce or eliminate the effect of NOB on the performance of the biological nitrogen removal system. This review aimed to provide valuable information for optimizing the biological nitrogen removal process to ensure highly efficient and stable operation.

Keywords: Biological nitrogen removal; Anammox; Nitrite-oxidizing bacteria inhibition; Multi-bacteria collaboration

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1. Introduction

The volume of domestic wastewater and the difficulty of its treatment were constantly increasing with the growing economy. The total nitrogen content of the receiving waters increased as a result. This not only resulted in nutrient enrichment of the water body and pollution of the ecological environment but was also harmful to human health [1]. Presently, mainstream wastewater nitrogen removal techniques included traditional methods such as nitrification–denitrification and derived processes rooted in anammox, such as partial nitritation-anammox.

For the traditional nitrification–denitrification process reported by the study of Ran et al. [2], shown in Fig. 1, NH_4^+–N was oxidized to NO_2^-–N by ammonia-oxidizing bacteria (AOB) and then to NO_3^-–N by nitrite-oxidizing bacteria (NOB) [2]. Subsequently, NO_3^-–N was reduced to NO_2^-–N by denitrifying bacteria. The partial nitritation-anammox (PN/A) process involved the conversion of half of the NH_4^+–N to NO_2^-–N by AOB, followed by the utilization of the generated NO_2^-–N and the remained NH_4^+–N by anaerobic ammonium oxidizing bacteria (AnAOB) in a series of metabolic pathways for nitrogen removal [3]. Compared with the nitrification–denitrification process, the PN/A process

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could reduce about 60% of aeration, 80% of sludge production, and 83% of N_2O emission [4]. In recent years, the PN/A process was successfully applied in the treatment of sludge digestate, food industry, breeding, and animal husbandry wastewater, and presented excellent nitrogen removal performance [5].

To reduce wastewater treatment costs, the stability and adequality production of NO_2^-–N was the main obstacle in the short-cut nitrification and denitrification process or the partial nitritation (PN) process. In the activated sludge system, NOB would compete with AOB for dissolved oxygen (DO) and with AnAOB for NO_2^-–N. It was noted that the value of Ks-NH_4^+ in AOB with the existence of NOB was three times higher than that without NOB [6]. This indicated a synergistic interaction between AOB and NOB, facilitating the oxidation of NH_4^+–N [7]. Thus, inhibiting the activity of NOB rather than eliminating NOB would be necessary.

Recently, some effective methods were explored to inhibit the activity of NOB. The aims of this review were to summarize (1) methods of inhibiting the activity of nitrite-oxidizing bacteria and (2) mechanisms underlying these inhibition strategies briefly.

2. Methods and mechanisms of NOB inhibition

In order to inhibit the activity of NOB, researchers had explored various methods. This review divided these methods into three aspects, that is, change operation parameters of reactor, addition chemical inhibitor and other strategies.

2.1. Change operation parameters of reactor

2.1.1. Dissolved oxygen

The oxygen saturation constant of AOB was 0.25–0.50 mg/L, while that of NOB was 0.72–1.84 mg/L [8]. This suggested that AOB could more efficiently acquire oxygen because of its lower oxygen saturation constant when compared to NOB [9]. Therefore, limitation the concentration of DO had been commonly used to inhibit NOB activity. The specific operating parameters of the reactor that inhibit NOB activity by controlling dissolved oxygen were summarized in Table 1.

Common methods of controlling DO concentrations were reduced aeration and intermittent aeration [10]. Zhu et al. [11] found that the production rates of NO_3^-–N decreased from 33.80 to 5.80 mg·N/(L·d) with dissolved oxygen reduction from 0.80 to 0.60 mg/L in a PN/A rotating biological contactor. Yao et al. [12] found that maintaining an aeration rate of 3.3 L/(min·L) resulted in the nitrite accumulation ratio (NAR) reaching more than 99% during PN process operation. In addition, Chen et al. [13] found that NOB activity could be suppressed using intermittent aeration at 8 L/h. Because NOB was inhibited in the anoxic stage, it was difficult to recover rapidly in the aerobic stage. In addition, the study of Wang et al. found that the relative abundance of NOB was lower under intermittent aeration conditions compared to continuous aeration [14]. It was noted that intermittent aeration was not applicable to all reactors. For example, intermittent aeration was unable to alter the dissolved oxygen distribution within the biofilm, resulting in the inability to suppress NOB activity [15].

Moreover, the method for suppressing NOB activity by controlling DO require further refinement and optimization. The previous study found that under prolonged low dissolved oxygen conditions, the dominant NOB population shifted from *Nitrobacter* to *Nitrospira*. *Nitrospira* demonstrated a greater ability to adapt to low dissolved oxygen conditions, which contributed to this shift [16]. This change would increase the activity of NOB and further enhance the abundance of NOB, which ultimately impeded the stable operation of the PN/A process. In addition. some studies had shown that the PN process failed to initiate successfully under low dissolved oxygen conditions [17–20]. This might be due to the presence of substances such as refractory organic matter in the influent water or competition for DO by heterotrophic bacteria leading to inhibition of AOB. Therefore, the dissolved oxygen concentration needed to be rationally selected according to the actual situation.

2.1.2. Sludge retention time

For the biological nitrogen removal process, sludge retention time (SRT) was an essential control factor. Controlling sludge retention time had been commonly used to inhibit NOB metabolism due to the low cost. NOB washout could be achieved by selecting an SRT shorter than the NOB generation time but longer than the AOB generation time. For example, in Changi wastewater treatment plant, inhibition of NOB activity was achieved by shortening the sludge retention time [21].

However, a shorter SRT could reduce the biomass in the system and even cause it to crash [22]. In addition, nitrogen loading rate (NLR) and temperature both affected the effectiveness of this strategy. At the lower NLR, SRT shortening might not inhibit NOB activity [23]. At temperatures between 20°C–35°C, the growth rates of NOB were much lower than that of AOB, while the opposite was observed at temperatures below 20°C [24]. Therefore, the adjustment of SRT could be employed in conjunction with other strategies to yield improved results.

2.1.3. Free ammonia and free nitrite acid

Previous studies showed that free ammonia (FA) and free nitrite acid (FNA) affect nitrification. The PN process could be achieved by selecting the appropriate concentration range since free ammonia and free nitrite acid exhibit different inhibitory concentrations for AOB and NOB. The FA and FNA concentrations varied with pH. In the PN system, the optimal accumulation of free ammonia and free nitrite acid occurred at pH 8.30 and 6.30, respectively, resulting in a PN efficiency of 84% and the dominance of AOB in the sludge [25]. Generally, the FA inhibitory concentrations of common NOB such as *Nitrobacter* and *Nitrospira* were 6.00–9.00 and 0.04–0.08 mg·NH_4^+–N/L, respectively [25]. The dominant NOB population shifted from *Nitrospira* to *Nitrobacter* after the long-term application of the FA strategy, rendering the FA strategy ineffective due to the change in the NOB population. In response, increasing the concentration of free nitrite acid was considered to inhibit NOB activity,

Table 1
Reactor operating parameters for nitrite-oxidizing bacteria suppression by dissolved oxygen control

Reactor	Water source type	Temperature (°C)	Influent NH_4^+–N (mg/L)	Dissolved oxygen	Treatment method	Nitrite accumulation	References
Automatic recycling reactor	Synthesis wastewater	25	50.00 ± 5.00	<0.26 mg/L	Continuous aeration	Yes	[21]
Sequencing batch reactor	Domestic wastewater	25	50.20–80.40	1.80 ± 0.32 mg/L	Intermittent aeration: aeration 8 min, anoxia 21 min	Yes	[22]
Sequencing batch reactor	Domestic wastewater	32 ± 1	36.50–79.51	0.40–0.60 mg/L	Intermittent aeration: aeration 10 min, anoxia 11 min	Yes	[23]
Sequencing batch reactor	Domestic wastewater	25 ± 3	62.87	8.00 L/h	Intermittent aeration: aeration 30 min, anoxia 30 min	Yes	[13]
					Intermittent aeration: aeration 30 min, anoxia 20 min	Yes	
					Intermittent aeration: aeration 30 min, anoxia 15 min	Yes	
					Intermittent aeration: aeration 30 min, anoxia 10 min	Yes	
Up-flow anaerobic sludge bed	Synthesis wastewater	30 ± 2	70.00–85.00	0.35 ± 0.15 mg/L	Continuous aeration	No	[15]
				0.20–0.10 mg/L	Intermittent aeration: aeration 1 min, anoxia 5 min	Yes	
Sequencing batch reactor	Domestic wastewater	30 ± 1	33.00–76.90	0.80–1.20 mg/L	Intermittent aeration: aeration 21 min, anoxia 8 min	Yes	[24]

primarily because free nitrite acid had a greater inhibitory effect on *Nitrobacter* [26]. However, increasing the levels of both FA and FNA in the matrix resulted in changes in the dominant NOB bacteria and their resistance to treatment [27]. As an alternative approach, initiating the PN process could be achieved by alternately increasing the amounts of free nitrite acid and free ammonia in the substrate [26].

Indeed, the method of inhibiting NOB activity using FA and FNA has not been systematically studied to date. Further research should focus on investigating the mechanisms through which FA and FNA inhibit NOB activity continuously, and provide scientifically accurate method to achieve NOB inhibition.

2.2. Chemical inhibitor addition

2.2.1. Hydroxylamine (NH_2OH)

Hydroxylamine (NH_2OH) was an intermediate product of the nitrification process performed by AOB. Previous studies found that the addition of NH_2OH to the reactor promoted the accumulation of NO_2^-–N [28–30]. The NAR achieved above 95% after addition of NH_2OH, resulting in the rapid initiation of PN within 5 d [31]. Furthermore, when the value of mole ratio of NO_3^-–N production to NH_4^+–N removal was more than 25%, exogenous 2 mg/L NH_2OH could effectively restore the performance of nitrogen removal. Furthermore, it was observed that the inhibitory effect on NOB persisted even after the long-term addition of NH_2OH was discontinued [32]. However, the activities of AOB and NOB could be inhibited by prolonged high NH_2OH addition (10–15 mg/L) [33], and the relevant data were shown in Table 2.

Some studies suggested that the addition of NH_2OH might only temporarily inhibit NOB activity [34,35]. Zhao et al. [36] observed that the relative abundance of *Nitrospira* reduced with the increasing of NH_2OH concentration in the PN system. Then, the NOB activity returned to its initial level after NH_2OH addition was stopped. This was mainly because *Nitrospira* inhibition by NH_2OH was reversible.

Regarding the inhibitory mechanism of NH_2OH on NOB, Feng et al. [29] found that NH_2OH might inhibit the expression of the *nxrB* gene, the gene encoding nitrite oxidase, and thereby inhibit the activity of NOB [29], as shown in Fig. 2. Finally, the addition of NH_2OH increased NO generation in the activated sludge system [32]. As shown in Fig. 2, NOB (e.g., *Nitrospira*) was particularly susceptible to inhibition by NO due to its high affinity for NO_2^-–N [37].

2.2.2. Hydrazine (N_2H_4)

Hydrazine (N_2H_4), an intermediate product of anammox, to which NOB was less adapted than AOB [38,39]. The addition of N_2H_4 became an effective measure to inhibit NOB activity. It was found that sludge morphology affected the inhibition of NOB by N_2H_4, for example, flocculated sludge was superior to granular sludge system [40]. With respect to the addition model, the study of Xiang and Gao [41] found that the intermittent administration mode had a more stable inhibitory effect on NOB activity.

For the mechanism of NOB activity inhibition by N_2H_4, it had been suggested that the applied N_2H_4 might react

Table 2
Partial reactors operation of hydroxylamine concentration control

Reactor	Volume	HRT	Temperature	Treatment method	Influent (mg/L)		Water source type	Result	References
	(L)	(H)	(°C)		NH_4^+–N	COD			
A^3-IFAS	42.00	12	21.00	Adjust NH_2OH content to 5.00 mg·N/L	39.80 ± 6.60	234.20 ± 57.90	Domestic wastewater	TNRE: 82.50%	[41]
Sequencing batch reactor	10.00	12	25.00	Adjust NH_2OH content to 4.50 mg·N/L	65.90	–	Domestic wastewater	TNRE: 98.63% NAR: 93.30% ± 1.15%	[34]
Sequencing batch reactor	2.00	24	30.00	Adjust NH_2OH content to 5.00 mg·N/L	120.00	–	Synthesis wastewater	NAR: 95.08%	[39]
Sequencing batch reactor	80.00	30	34.00	Adjust NH_2OH content to 20.00 mg·N/L	796.83 ± 41.02	130.20 ± 41.00	Domestic wastewater	TNRE: 83.02% ± 5.10%	[36]
Sequencing batch reactor	5.00	24	33.00–35.00	Adjust NH_2OH content to 2.00 mg·N/L	400.00	–	Synthesis wastewater	TNRE: 45.58% MRNN: 20.92%	[35]
Sequencing batch reactor	10.00	6	19.50–28.20	Adjust NH_2OH content to 5.00 mg·N/L	61.00–82.00	–	Domestic wastewater	NAR: 97.20% ± 1.70%	[38]

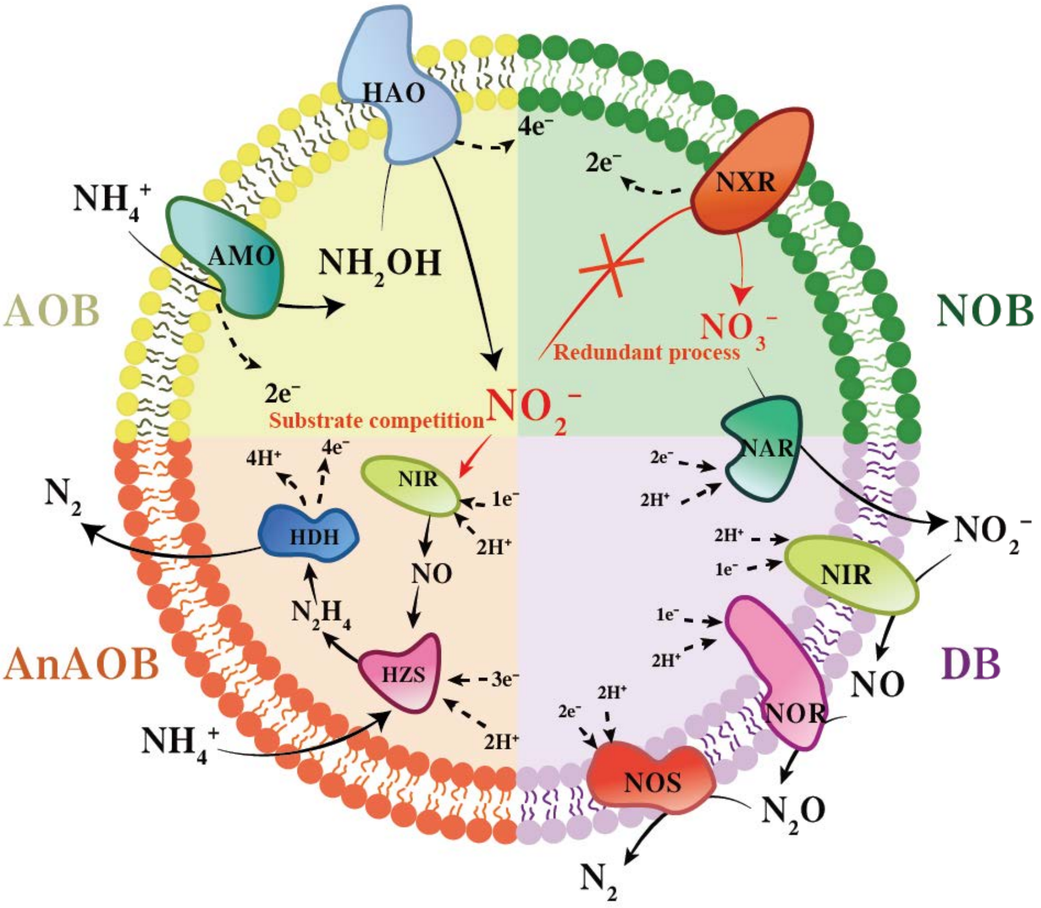


Fig. 1. Nitrogen transformation pathways in the biological wastewater treatment system.

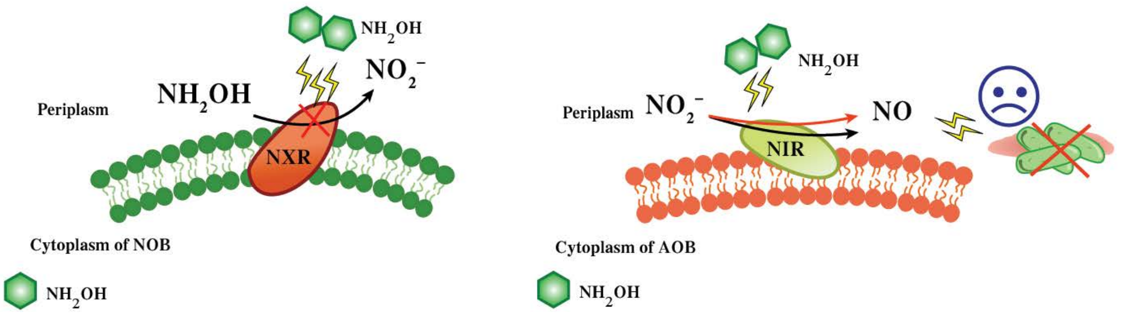


Fig. 2. NH_2OH inhibiting NOB activity.

with NO_2^-–N to produce azide compounds that could inhibit *nxrB* gene expression to reduce NO_3^-–N production [42], as shown in Fig. 3a. In addition, the redox potential decreased rapidly after adding N_2H_4, with a longer oxidation time for nitrite than ammonia, weakening the activity of NOB at this time, as shown in Fig. 3b [32,43].

2.2.3. Other inhibitors

In addition to the aforementioned intermediates involved in the nitrogen removal process, the inclusion of other substances such as sulfide and formic acid could also inhibit the activity of NOB.

Sulfide improved the nitrogen removal performance while inhibiting NOB. Semi-inhibitory concentrations of sulfide for AOB and NOB were 20.60 and 15.80 mg·S/g·VSS, respectively. Seuntjens et al. [44] suggested that sulfide selectively inhibited NOB activity while enriching AOB. However, the study of Kouba et al. [45] reported that the addition of sulfide prolonged the oxidation time of NH_4^+–N. Therefore, the strategy of adding sulfide was only used to initiate or restart short-course nitrification processes.

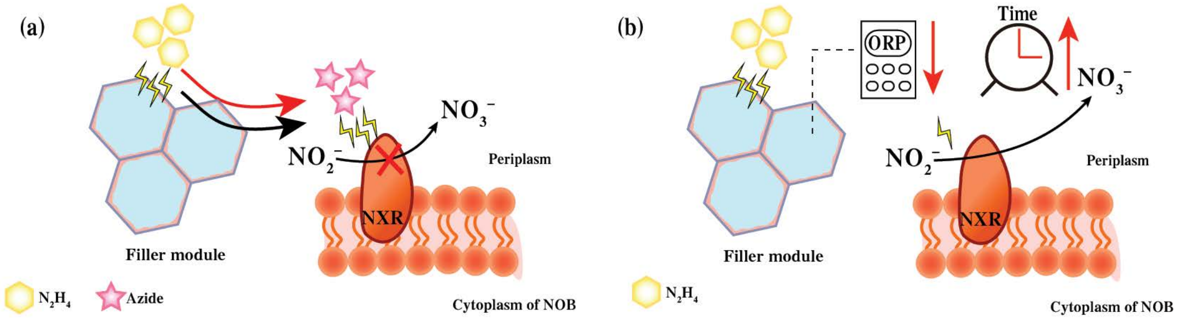


Fig. 3. Mechanism of NOB activity inhibition by N_2H_4, (a) inhibition the *nxrB* gene expression and (b) reduction the redox potential.

Formic acid was also observed of inhibiting NOB activity. The study of Wang et al. [46] found that formic acid could inhibit the expression of the *nxrB* gene. In addition, a stable PN process was achieved in the reactor treated with formic acid [46]. However, it was necessary to domesticate the sludge before choosing this inhibition method, as formic acid was an organic substance that could only be used by some bacteria, such as methylotrophic bacteria and aerobic denitrification bacteria.

2.3. Other methods

2.3.1. Electromagnetic radiation

In the 1980s, Moore [47] suggested that the presence of a magnetic field both inside and outside an organism could affect its metabolism, referred to as a biological effect of magnetism. Recent studies confirmed that introducing an electromagnetic field inhibited NOB activity [48,49]. Wang et al. [50] introduced a 0.06 μT magnetic field into the sequencing batch reactor (SBR) system, resulted in an overall 71.43% decrease in SOUR-NO_3^-–N and a 32.05% increase in SOUR-NO_3^-–N. The result showed that NOB activity was inhibited while AOB activity was promoted with the introducing of magnetic field [50]. In addition, it was shown that under weak magnetic field conditions, the relative abundance of functional bacteria, except for Betaproteobacteria to which AOB belongs, appeared to decrease [49]. Jia et al. [51] showed that a 15 mT electromagnetic field reduced the abundance of the *nxrB* gene, which affected the metabolic activity of NOB. In general, electromagnetic radiation could inhibit NOB activity, but the appropriate range of radiation intensity need to be further investigated.

2.3.2. Ultrasound

Studies showed that low energy (0.15 W/mL) interval ultrasonic exposure for 10 min on PN sludge could effectively improve AOB activity while suppressing NOB metabolism, further increasing NAR to 85% [52]. Zheng et al. [53] discovered utilization of low energy density ultrasound (0.066 kJ/mg·VSS) effectively reduced the relative abundance of NOB in the SBR reaction. In addition, the structure of the activated sludge was loosened after the ultrasonic treatment, making it easier to transfer the substrate within the activated sludge.

However, long-term ultrasonic treatment of activated sludge was observed to inhibit the *Nitrososphaera* genus, which belonged to AOB. Interestingly, *Nitrospira* (a genus of NOB) would adapt to ultrasonic conditions, potentially diminishing the ultrasonic inhibitory [54]. Consequently, ultrasonication could be considered as one of the options when used in combination with other NOB inhibition methods.

2.3.3. Change in sludge morphology

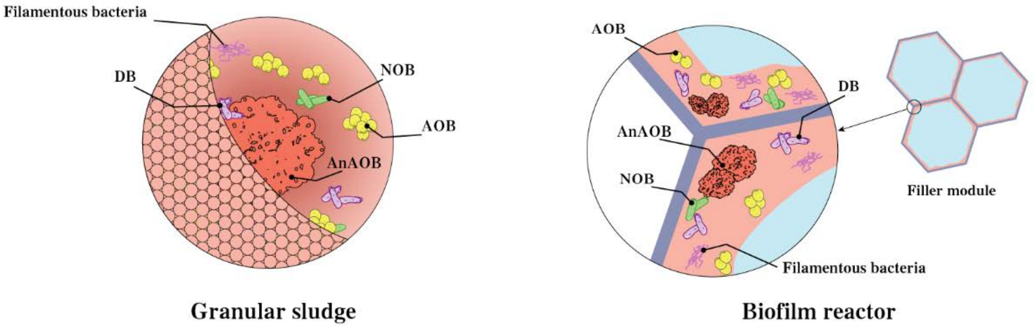
Common forms of sludge used in wastewater treatment include flocculent sludge, biofilm and granular sludge. Li et al. [22] found that in the PN/A granular sludge system, NOB was mainly present in the floc sludge, and the abundance of NOB could be effectively reduced by eluting the floc sludge. This phenomenon might be attributed to the lower Ks-O_2 (oxygen half-saturation constant) of NOB compared to AOB. NOB in flocculated sludge displayed a greater affinity for oxygen than AOB, resulting in their enrichment within the floc sludge [55].

Additionally, biofilm and granular sludge had specific microbial distributions as shown in Fig. 4. NOB located outside the biofilm or granular sludge faced intense competition for oxygen from other bacteria. Conversely, those NOB situated within the biofilm or granules had restricted access to oxygen, which ultimately inhibited their activity [56,57]. To sum up, sludge forms like granular sludge or biofilm were more effective in suppressing NOB, thus aiding in the successful implementation of the PN process.

2.3.4. Improved multi-bacteria cooperation

Common forms of activated sludge included flocs, granular sludge, and biofilms. Zhang et al. [58] conducted covariance network analysis and found that NOB was associated with other microorganisms, suggesting that NOB might be involved in a complex symbiotic relationship in activated sludge.

Quorum sensing was a communication mechanism between microorganisms, whereby microorganisms interacted by secreting and sensing signaling molecules [59]. Acyl-homoserine lactone (AHLs) were common signaling molecules in microbial quorum sensing. It was found that AOB and NOB might secrete signaling molecules involved in quorum sensing [60]. Different signaling molecules could



Granular sludge

Biofilm reactor

Fig. 4. Distribution of bacteria in anaerobic granular sludge and biofilm reactor.

produce different effects, for example, C4-HSL could promote NO$_2^-$–N accumulation, whereas C8-HSL could inhibit AOB activity [61]. In addition, Ma et al. [62] observed that while AHLs did not affect the bacterial abundance of NOB, it did induce changes in nitrite oxidation kinetics. In summary, quorum sensing played a pivotal role in regulating the balance between AOB and NOB populations. However, further research should be conducted on population sensing or signaling molecules to effectively inhibit NOB activity.

3. Conclusion and prospects

3.1. Conclusion

(1) Suppression of NOB activity could be achieved by controlling the operating conditions of the reactor by adjusting the DO concentration, SRT, FA, and FNA.
(2) NOB suppression could be achieved by administering nitrogen removal process intermediates such as hydrazine and hydroxylamine.
(3) NOB suppression could also be achieved by exogenously applied electromagnetic radiation and ultrasound.

3.2. Prospects

Various combinations of suppression methods had been devised. While some of these methods could effectively inhibit the activity of NOB, the durability of this inhibition was closely linked to reactor operational parameters, the composition of the wastewater, the structure of activated sludge, and the composition of functional microbes. Consequently, there is a demand for the development of a combined suppression strategy to tackle the problem of NOB resilience in the future.

Besides regulating operating parameters and imposing external measures to inhibit NOB activity, microbial quorum sensing was the direction of extensive research attention. This meant that inhibition of NOB could be achieved by regulating the signaling between microorganisms. Additionally, apart from the inhibition of NOB activity, researchers were currently investigating alternative processes such as simultaneous partial nitrification-anammox-denitrification to achieve nitrogen removal with low energy consumption and high efficiency by modulating microbial colony collaboration. Therefore, in future research, attempts could be made to study the quorum sensing mechanism of microorganisms. The collaborative regulation between NOB and other bacteria could be utilized to achieve the continuous development of nitrogen removal technology.

Credit authorship contribution statement

Shaoqing Mo: writing the paper. Lingjie Liu: Writing – review & editing. Shaopo Wang: Writing – review & editing, Supervision. Chenchen Wang, Nannan Liu, Dong Wang and Chunsheng Qiu: Resources, 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.

Acknowledgments

This work was supported by the Natural Science Foundation of Tianjin, China (No. 22JCQNJC00020); the Tianjin Research Innovation Project for Postgraduate Students (No. 2022SKYZ172) and the Key Project of Tianjin Natural Science Foundation, China (No. 18JCZDJC10080).

Abbreviations

A^3-IFAS — Alternating anoxic/aerobic with integrated fixed-film activated sludge
AHL — Acyl-homoserine lactone
Anammox — Anaerobic ammonia oxidation
AnAOB — Anaerobic ammonium oxidizing bacteria
AOB — Ammonia-oxidizing bacteria
DB — Denitrifying bacteria
DO — Dissolved oxygen
EPS — Extracellular polymeric substances
FA — Free ammonia
FNA — Free nitrite acid
Ks — Half saturation constant
MRNN — Mole ratio of NO$_3^-$–N production to NH$_4^+$–N removal
NAR — Nitrite accumulation ratio
NLR — Nitrogen loading rate
NOB — Nitrite-oxidizing bacteria
NXR — Nitrite oxidase
PD/A — Partial denitrification-anammox
PN — Partial nitritation

PN/A — Partial nitritation-anammox
SAA — Specific anammox activity
SBR — Sequencing batch reactor
SNAD — Simultaneous partial nitrification-anammox-denitrification
SRT — Sludge retention time
TNRE — Total nitrogen removal efficiency
UASB — Up-flow anaerobic sludge bed

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