REVIEW

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Anammox and partial denitrification coupling: a review

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As a new wastewater biological nitrogen removal process, anammox and partial denitrification coupling not only plays a significant role in the nitrogen cycle, but also holds high engineering application value. Because anammox and some denitrifying bacteria are coupled under harsh living conditions, certain operating conditions and mechanisms of the coupling process are not clear; thus, it is more difficult to control the process, which is why the process has not been widely applied. This paper analyzes the research focusing on the coupling process in recent years, including anammox and partial denitrification coupling process inhibitors such as nitrogen (NH_4^+, NO_2^-), organics (toxic and non-toxic organics), and salts. The mechanism of substrate removal in anammox and partial denitrification coupling nitrogen removal is described in detail. Due to the differences in process methods, experimental conditions, and sludge choices between the rapid start-up and stable operation stages of the reactor, there are significant differences in substrate inhibition. Multiple process parameters (such as pH, temperature, dissolved oxygen, redox potential, carbon-to-nitrogen ratio, and sludge) can be adjusted to improve the coupling of anammox and partial denitrification to modify nitrogen removal performance.

1 Introduction

With the development of the economy and public awareness of environmental protection, the pollution of water environments has become the central topic of academic discussion. The deterioration of water quality caused by the accumulation of inorganic nitrogen is a typical and serious pollution source, and it can influence human and animal health directly or indirectly.[1,2] In recent years, high nitrogen removal has been a focus point in water treatment. NO_3^-, NO_2^- and NH_4^+ are the main living style of the cycle network of inorganic nitrogen in the biosphere (Fig. 1) in nature. Nitrogen removal in sewage treatment means that different forms of nitrogen are converted into nitrogen and discharged into air. Familiar nitrogen removal processes include nitrification and denitrification, partial nitrification and denitrification, the anammox process, and the anammox and partial denitrification coupling process. In these processes, anammox and partial denitrification coupling is a clean and environment-friendly approach, which can remove ammonia nitrogen, nitrate nitrogen and nitrous nitrogen at the

same time. In one case, a novel partial denitrification anammox coupling process achieved a high total nitrogen (TN) removal rate of 97.8%.[3] The development of the anammox and partial denitrification coupling technology shows great significance for
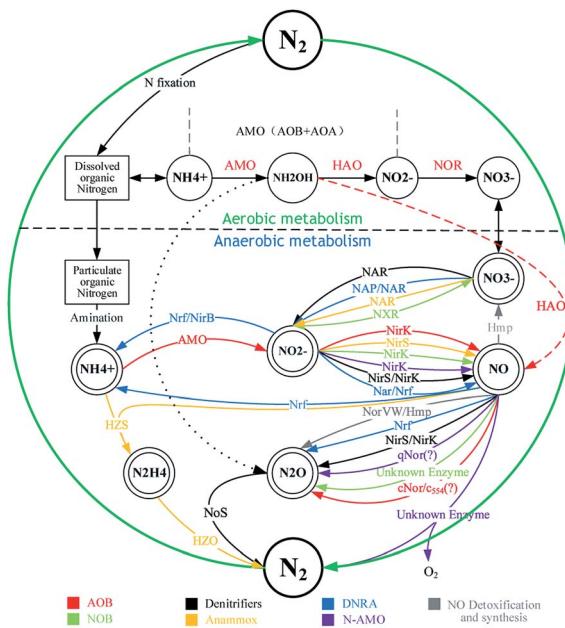

Fig. 1 Nitrogen biosphere cycle network.

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the establishment of a relatively complete denitrification mechanism and a new nitrogen cycle system.[4] The coupling mechanism is shown in Fig. 2: (1) Denitrification provides carbon sources and substrates for anammox. During the denitrification process, NO_3^-, as an electron acceptor, generates NO_2^- under enzymatic action and inhibits NO_2^- reductase to reduce N_2O production, where organic matter is consumed and CO_2 is generated.[2,5] (2) Partial denitrification consumes dissolved oxygen (DO) during the consumption of organic matter to provide a low-oxygen environment for anammox;[6] CO_2 from denitrification is used as a carbon source for anammox. (3) Anammox consumes the substrate NO_2^-, reducing the effects of NO_2^- accumulation on denitrification and avoiding the further reduction of NO_2^- to N_2O. Anammox bacteria use NO_2^- and NH_4^+ as substrates that are reduced and oxidized to N_2, and the released energy is used for CO_2 synthesis of organic matter and life activities.[7,8]

In 1998, Strous *et al.*[9] cultivated anammox bacteria in a sequencing batch reactor and proposed a stoichiometric formula for the anammox metabolism (eqn (1)). Although many quantitative methods of anammox metabolism have been proposed in recent years, such as that by Lotti *et al.*,[10] the growth mechanism of suspension of anammox bacteria and the study of the kinetics of eqn (2) and (1) have a wide range of applications and are readily accepted by scholars.

$$NH_4^+ + 1.32NO_2^- + 0.66HCO_3^- + 0.13H^+ \rightarrow$$
$$0.066CH_2O_{0.5}N_{0.15} + 1.02N_2 + 0.26NO_3^- + 2.03H_2O \quad (1)$$

$$NH_4^+ + 1.146NO_2^- + 0.071HCO_3^- + 0.057H^+ \rightarrow$$
$$0.071CH_{1.74}O_{0.31}N_{0.20} + 0.986N_2 + 0.161NO_3^- + 2.0002H_2O \quad (2)$$

$$NO_3^- + 0.083C_6H_{12}O_6 \rightarrow NO_2^- + 0.5CO_2 + 0.5H_2O \quad (3)$$

Fig. 2 Anammox and partial denitrification coupling nitrogen removal matrix flow direction.
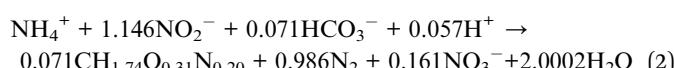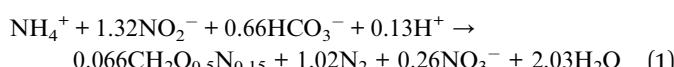
As shown by eqn (1) and (2), NO_3^- produces about 11.2% of the total nitrogen after consuming 1 mol of NH_4^+. Although nitrogen in sewage cannot be eliminated, it supplements the consumption of NO_3^- in the denitrification process.[6] Therefore, the operating costs of wastewater treatment plants (WWTPs) are reduced.[11–14] Through the anammox and partial denitrification coupling process, the NO_3^-–N removal efficiency can reach 95.8%, while the NH_4^+–N removal rate reaches 92.8% and the anammox contribution to TN removal is 78.9%.[13] The process meets the requirements of smaller space, simple control and environmental friendliness; furthermore, it creates no secondary pollution.[7]

2 Anammox and partial denitrification coupling process and application

Anammox and partial denitrification coupling nitrogen removal is divided into separated and combined processes (Table 1).

2.1 Separated process

The separated process of anammox and partial denitrification coupling is inoculated with denitrifying bacteria and anammox with absolute advantages in two mutually independent reactors and simultaneously generates NO_2^-–N and nitrogen removal processes, which can effectively avoid space competition. Organic matter is fully utilized by denitrifying bacteria in the denitrification reactor, reducing the concentration of organic matter flowing to the anammox reactor. Wastewater containing nitrate (such as municipal wastewater, industrial wastewater, and aquaculture wastewater) first flowed into the partial denitrification reactor to reduce nitrate to NO_2^-–N while consuming organic matter as the electron donor. Then, the wastewater NO_2^- was adjusted before flowing into the anammox reactor (NO_2^-–N/NH_4^+–N = 1.32) and was used to remove nitrogen, and part of the effluent returned to the partial denitrification reactor for deep nitrogen removal.[13]

Cao *et al.*[15] used a separated reactor to treat high-concentration nitrogen-containing wastewater (NO_3^-–N = 820 mg L^{-1}) in which the TN in the effluent was less than 20 mg L^{-1}, and the TN removal rate was as high as 97.8%. It is demonstrated that the removal rate of NH_4^+–N peaks at 96.7% when treating domestic sewage, and this process can effectively solve the problem of nitrogen residues in sewage. Du *et al.*[13] optimized the mixed volume ratio of influent NO_3^-–N and sewage and the amount of organic matter to treat high-nitrogen wastewater (NO_3^-–N = 1000 mg L^{-1}); the TN in the effluent was less than 11 mg L^{-1}, and the removal rate of TN was as high as 95.8%. Meanwhile, the contribution rate of anammox to TN nitrogen removal was 78.9%. In addition, Wang *et al.*[16] proposed a new process, EPDPR (endogenous partial denitrification and denitrifying phosphorus removal), in which the removal rate of phosphorus is as high as 92.3% and the output of 79.2% of NO_2^-–N is the best for the post-anammox process. The suitable substrate ratio (NO_2^-–N/NH_4^+–N = 1.32) and high purification capacity (TN < 6 mg L^{-1}, TP < 0.4 mg L^{-1}) provide new ideas for removing nitrogen, phosphorus and other
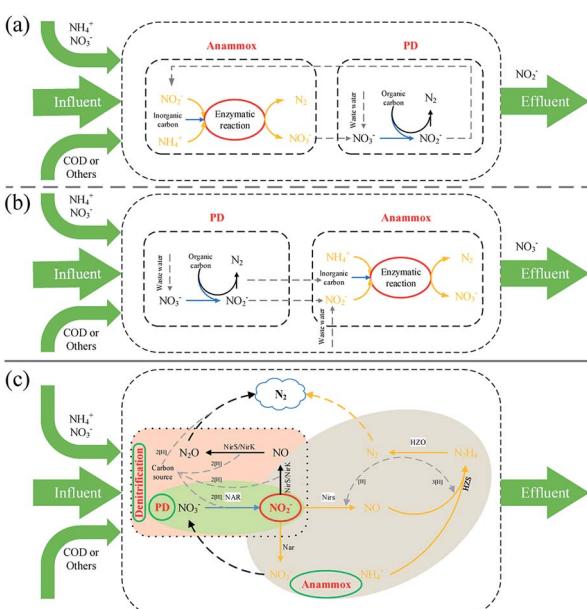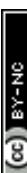
Table 1 Experimental studies on separated and combined processes

| Process | Operating parameter | | | | | Nitrogen removal effect | | | | | Reference |
	Reactor	C/N	Carbon source	Influent	$T/^\circ C$	Effluent TN/ mg L^{-1}	NO$_3^-$–N removal rate/%	NTR	Anammox denitrification contribution rate/%	TN removal rate/%	
Separated process	PD (SBR) Anammox (UASB)	3.0/2.8/2.6	Sewage	NO$_3^-$–N = 40 NH$_4^+$–N = 59.3	18.7–27.8	4	97.9	85.6	78.2	—	7
	PD (SBR) Anammox (UASB)	Low	Sewage	NH$_4^+$–N = 166 NO$_3^-$–N = 50	12.9–15.1	8	89.5	55.1	70	—	12
	PD (SBR) Anammox (UASB)	0.5–1.7	Septic tank	NH$_4^+$–N = 40 NO$_3^-$–N = 125	14.8–28.2	11	95.8	80	78.9	—	13
	PD (SBR) Anammox (USB)	3.0	Sodium acetate	NO$_3^-$–N = 30–400	23.6–28.8	—	92.5	83.3	—	—	30
	PD (SBR) Anammox (UASB)	2.9	Sodium acetate	NH$_4^+$–N = 15 NO$_3^-$–N = 20	25 ± 3	5.8	—	80	49.8	90	17
	PD (SBR) Anammox (UASB)	3.0	Septic tank	NH$_4^+$–N = 70.1 NO$_3^-$–N = 20	28	≤20	—	80	—	97.8	15
Combined process	UASB	3.0/3.4	Sodium acetate	NH$_4^+$–N = 30 NO$_3^-$–N = 30	17.3–28.8	10	89.5	97.1	9.23	93.2	25
	SBR	3.0	Landfill leachate	NH$_4^+$–N = 47.5 NO$_3^-$–N = 93.7	30 ± 1	≤20	—	75.6	—	84.8	21
	UASB.	1.6	Septic tank	NH$_4^+$–N = 67.7 NO$_3^-$–N = 70–180	26 ± 2	<10	83.3	—	95.6	93.1	27
	SBR	3.0	Acetate solution	NH$_4^+$–N = 50 NO$_3^-$–N = 50	12.7–29.2	15	91.5	87	95	93.6	18
	SBBR	2.0–2.5	Sodium acetate	NH$_4^+$–N = 151 NO$_3^-$–N = 165	25–35	12.5	>90	—	—	93	19
	UASB	1.1	Sodium acetate	NH$_4^+$–N = 280 NO$_3^-$–N = 280	34–36	9.3	—	97.4	85.8	96.2	20

nutrients from sewage. A mainstream literature search showed that the reason why the separated process is stable and the TN removal rate is high is that anammox makes a greater contribution to the TN removal rate.[7,12,13,17]

2.2 Combined process

The combined process of anammox and partial denitrification coupling is started by inoculating the optimal partial denitrification floc sludge and anammox granular sludge,[18–20] and it has also been reported that the inoculated partial denitrification sludge is acclimated from PN sludge.[21]

Zhang et al.[20] reported a new process called completely anaerobic ammonium removal over nitrite (CAARON); they compared the simultaneous addition of acetate at low C/N with that of asynchronous addition and confirmed that the asynchronous addition of acetate optimized the reactor. The distribution of the bacterial nitrate-to-nitrite transformation ratio (NTR) was 97.4%, and the anammox nitrogen removal contribution rate was 85.8%. Also, Du et al.[22] carried out the DEAMOX reaction in an SBR reactor, and supplying acetate to the reactor at different periods also achieved a nitrogen removal rate of up to 95.8%. Zhang et al.[23] studied the engineering feasibility, economic feasibility, and potential challenges of the PN/AMX and PDN/AMX processes. The researchers noted that bacteria showed good capture of soluble COD; this is another way to control the effects of organic matter on anammox bacteria. Qin et al.[24] focused on the effects of glucose on DEAMXO nitrogen removal performance in an upstream continuous consideration reactor and confirmed that dissolved COD had a significant effect on the biological communities. When the concentration of glucose was 56.4 mg L^{-1}, the system TN removal rate was 98.6%.

2.3 Reactor management

The method of managing the reactor in the separated and combined processes is as follows:

(1) Selection of reactor type and seed sludge. Given that the processing has low carbon demand and minimal sludge production, researchers usually use SBR or UASB as the anammox and partial denitrification coupling starting reactor.[18,20,21,25] Anammox granular sludge and partial denitrification floc sludge are inoculated as seed sludge, or anammox sludge is domesticated to obtain the target strain.

(2) Optimization of hydraulic retention time (HRT) and proper ventilation. Properly increasing wastewater HRT in the reactor can afford higher TN and COD removal rates, and even difficult-to-degrade COD will be consumed in a higher HRT. This method effectively enriches a sufficient amount of partial denitrification and anammox functional microorganisms.[13,19,25] Optimizing aeration can avoid excessive production of extracellular polymers (EPS), which is beneficial to the stability of the microbial community and nitrogen removal.[25]

(3) Selection of organic matter feeding methods and addition of microbial carriers. Organic matter feeding methods are distributed continuously and intermittently. The richness and metabolism of continuous feeding of organic matter anammox

bacteria will be limited, reducing the nitrogen removal ability of the reactor. Intermittent feeding is widely used in the combined process and performs well, with a high TN load nitrogen removal effect.[20,22] Partial denitrification and anammox bacteria can be effectively retained on the microbial carrier, expand the specific surface area of the microorganism, increase the mass transfer rate, and enhance the nitrogen removal effect.[26,27]

2.4 Engineering application

Research of the anammox and partial denitrification coupling process is still in its infancy. The process has not been popularized or applied to nitrogen removal for municipal wastewater treatment; therefore, it has been rarely reported to date. Although the engineering application of anammox has been increasing gradually in the past 10 years, the development of anammox and some denitrification coupling processes have lagged, and laboratory-scale research[7,13,25,28] has a positive effect on engineering applications.

Wang et al.[29] reported simultaneous partial nitrification, anaerobic ammonium oxidation and denitrification (SNAD) treatment of landfill leachate in a full-scale (192 m³) sewage treatment plant. The TN removal was around 83.9%, which confirms that mass balance of the system is the best approach for the evaluation of anammox and nitration processes and that molecular tools and tracer studies with labeled nitrogen are useful approaches for the evaluation of anammox. Based on an AAO process, Li et al.[26] added carriers to the reactor to enrich anammox bacteria in the anoxic zone, forming the An-AAO process. Anammox species enrichment in situ increases the nitrogen removal rate, and it has been proven that anammox has a high nitrogen removal contribution rate and is the key to the stability and engineering application of the denitrification coupling process, which was successfully used in urban sewage treatment plants.

3 Inhibition factors of the coupling process

3.1 Nitrogen inhibition

NH_4^+, NO_2^- and NO_3^- are important monitoring indicators of wastewater (including domestic sewage, landfill leachate, mustard wastewater, aquaculture wastewater and printing and dyeing wastewater). NO_2^- and NO_3^- act as key raw materials and inhibitors in the anammox and partial denitrification coupling process. Their inhibition mechanism and concentration will affect the coupling process, operating conditions and operating strategies; therefore, it is imperative to understand the nitrogen inhibitory concentration and mechanism.

3.1.1 Free ammonia inhibition. One study found that a high concentration of NH_4^+ has no influence on the anammox process; Strous et al.[31] noted that the anammox process showed no inhibition when treating high-concentration ammonia nitrogen wastewater at 1000 mg L^{-1} NH_4^+. Isaka et al.[32] also mentioned that a high concentration of ammonia (NH_4^+, 690 mg L^{-1}) shows no significant inhibition of the anammox process during the process of ammonia plant wastewater

treatment. However, with deep research into anammox and partial denitrification coupling,[33–35] it was found that the above situation is likely caused by the fact that the content of FA (free ammonia, NH_3) in the sewage is less than the requirement of the inhibition value; therefore, the inhibitory effect on the anammox process is FA instead of NH_4^+.

Compared with NH_4^+, FA can diffuse more easily into biological cells through the lipid membrane. In the mechanism of FA inhibition, the FA changes the pH (as eqn (4)) of the intracellular environment after it enters the cells[6,35] (intracellular pH < extracellular pH). High or low pH destroys the transmembrane potential and changes the substance exchange selectivity between the cells and the external environment; this will interfere with the synthesis of enzymes, leading to cell inactivation or even death.

$$C_{FA} = \frac{17}{4} \frac{C_{t,NH_3} \times 10^{pH}}{[\exp(6334/(273+T)) + 10^{pH}]} \quad (4)$$

Fernández et al.[36] analyzed the short-term and long-term effects of FA on the anammox process. As a result, with short-term exposure, as the concentration of FA increased from 38 to 100 mg L^{-1}, the metabolic activity was inhibited by 50% to 80%; these inhibition concentrations (ICs) were recorded as the IC_{50} and IC_{80}, respectively. However, in their study of long-term effects, anammox showed decreased nitrogen removal performance when the FA concentration was only 35 to 40 mg L^{-1}. Summarized from many related research studies (Table 2), the activity threshold (initiation inhibition concentration) of FA on anammox is 20 to 25 mg L^{-1}, and FA < 5 mg L^{-1} can afford higher nitrogen removal efficiency.

The inhibition concentration of FA for denitrifying bacteria was only 7.5 mg L^{-1} as reported by Vadivelu et al.[37] However, Nair et al.[151] and Waki et al.[38] both noted that a certain concentration of FA can enhance the activity of microorganisms, especially in the denitrification process. Higher concentrations of FA (exceeding the activity threshold) will inhibit biological activity, and long-term loading of denitrifying bacteria and anammox strains will cause large-scale deaths. It can be concluded that FA mainly affects biological activity (inhibition of reversibility) by affecting the biome structure.[39,40] FA inhibition can be reduced by changing sewage operating conditions, such as pH, HRT, sludge type and sludge status (particles, biofilm or flocculation). He et al.[41] stated that the safe

concentration of FA in the reactor increased to 15 mg L^{-1} (IC_{10}) after pH elevation. Compared with a granular sludge reactor, Yang et al.[42] added a composite support to the anammox reactor to increase reactor stability and FA tolerance and proved that nitrogen removal performance could be properly restored by reducing the NH_4^+ concentration and increasing the hydraulic retention time (HRT). In addition, it is possible to domesticate bacteria under high concentrations of FA by applying restrictive conditions to the bacteria and obtain highly active anammox bacteria.

3.1.2 Free nitrous acid inhibition. In the coupling process of anammox and partial denitrification, both anammox and denitrifying bacteria were inhibited under a certain concentration of NO_2^-, and the inhibition of anammox was more obvious.[11] Numerous studies[49–52] have shown that NO_2^- inhibition is actually derived from the toxic effects of free nitrous acid (FNA), and the FNA concentration is affected by temperature, pH and NO_2^- concentration (eqn (5)).[53] The FNA inhibition rate is about 3 times that of FA; Zhang et al.[54] proved that FNA is the largest inhibitor of the coupling process, and the long-term stable operating concentration of the reactor should be controlled to less than 1.5 μg L^{-1}.

$$C_{FNA} = \frac{47}{14} \frac{C_{t,NO_2}}{[\exp(-2300/(273+T)) \times 10^{pH}] + 1} \quad (5)$$

In the mechanism of FNA inhibition, NO_2^- acts as a decoupling agent to diffuse into the membrane of prokaryotic cells; the permeability expansion of the cell membrane affects the enzymes involved in electron transport and proton transfer, thereby affecting the inhibition of the synthesis of adenosine triphosphate (ATP).[55,56] Finally, it will affect the ATPase-catalyzed reaction and reduce biological activity. Studies have shown that in order to ensure safe and stable operation of the reactor, the IC_{10} value can usually be used as a safe value,[54,57,58] and the FNA concentration should be controlled between 1.5 and 5 μg L^{-1}. In this research, the coupling nitrogen removal efficiency could be improved with proper heating (>25 °C) and low pH (<5.6), which could reduce the effects of the FNA concentration to ensure high activity of the strains.[59]

FNA inhibits the reduction of N_2O to cause partial denitrification and accumulate NO_2^-. According to the research by Alinsafi et al.,[60] the amount of N_2O released during wastewater treatment is positively correlated with the concentration of

Table 2 Research on the inhibitory threshold concentration of FA for anammox and denitrifying bacteria

Reactor	$T/°C$	pH	HRT/d	Sludge source	$NH_4^+/(mg L^{-1})$	Time/d	Threshold	Reference
UASB	33–35	—	0.375–0.5	Nitrification/denitrification 1 : 1	50–400	187	29.65 mg L^{-1}	42
SBR	29	—	1.35–2.57	ELAN granular sludge	500–1000	175	23.55 mg L^{-1}	43
MBR	25 ± 1	8.0	0.5	Anammox reactor	100	800	16–23 mg L^{-1}	44
MBBR	34 ± 0.8	—	2	Laboratory MBBR	700 ± 100	275	25.5 mg L^{-1}	45
SBR	32–35	7.9	0.5	EGSB reactor	429–513	220	40 mg L^{-1}	46
SBR	33/30	7.8	—	SBR reactor	70–2800	200	35–40 mg L^{-1}	36
SBR	30–35	7.8	—	UASB reactor	1615 ± 68.5	131	77 mg L^{-1}	47
EGSB	32	7.7	—	—	84–420	70	>50 mg L^{-1}	48

NO_2^-, which indirectly proves that an increase in FNA concentration can promote N_2O reduction. Among the studies of FNA inhibition on denitrifying poly-phosphate accumulating organisms, Zhou et al.[61] considered FNA as the main reason for the effects on N_2O produced by the denitrification process, and they found that its inhibitory concentration was 0.7 to 1.0 µg L^{-1}. A low concentration of FNA provides mild coupling conditions for denitrification and anammox.

Niu et al.[62] proposed that the IC_{50} of FNA for anammox inhibition was in the range of 1.08–2.81 µg L^{-1}, and it showed a higher nitrogen removal rate under FNA <2 µg L^{-1}. The inhibition of FNA can be reduced by changing the spatial structure (filler or carrier) of the biome, sludge state and operating conditions. Yang et al.[42] used shale ceramic particles and suspended spheres as carrier-loaded functional bacteria; their research showed that the initial value of FNA inhibition was as low as 77.02 µg L^{-1} and the anti-toxic performance was much lower than the anammox granular sludge IC_{50} (117 µg L^{-1}), which matched the research results of Puyol et al.[52] He et al.[55] observed that the inhibition concentrations of anammox and some denitrifying bacteria communities ranged from 1.5 to 213 µg L^{-1} (Table 3).

3.2 Organic inhibition

Organic matter is of great significance to anaerobic and denitrifying bacteria. It acts as a carbon source for the denitrification process and as an inhibitor of the anammox process. There are numerous organic substances in sewage; these can be roughly classified into concentration inhibition type and toxicity inhibition type according to their inhibition mechanisms. Studies on the concentration and inhibition mechanisms of organics on the coupling process will help researchers to adjust the operating conditions of denitrification processes and improve their efficiency.

3.2.1 Concentration inhibition. There are two separate explanations for the mechanism of concentration-inhibiting organic inhibition. The first one is the inhibition of space competition. Within the inhibition of space competition, the organic matter is used by denitrifying bacteria as the carbon source, causing the proliferation rate of denitrifying bacteria to become much higher than that of anammox bacteria. Because the anammox bacteria have reduced living space, the number of bacteria and the nitrogen removal efficiency of anammox

experience great reductions.[67–69] The second explanation is the inhibition of metabolic pathway diversity. Scholars consider anammox to be the dominant strain in the system under a high concentration of organic matter; however, the metabolic mode can change. In the process, anammox bacteria uses organic matter as a substrate instead of NO_2^- and NH_4^+, which reflects the diversity of anammox metabolism.[6,67,70] The two processes of anammox and organic oxidation are independent, and the anammox process can be re-expressed under inorganic-dominated conditions.[67]

As shown in many studies, high concentrations of organic matter can inhibit the coupling nitrogen removal process, while lower concentrations can promote the growth and coupling of biomes.[67,70,71] However, because the lack of a carbon source will inhibit the growth of denitrifying bacteria, the proper concentration of organic matter is vital to the anammox and coupling denitrification process; the effects of concentration on the anammox and denitrifying bacteria are shown in Table 4. Du et al.[13] calculated the contribution rates of several nitrogen removal processes. It was found that the anammox nitrogen removal contribution rate was 78.9%, and the domination of the anammox process was the real reason for the coupling and stable operation. When treating high-concentration organic wastewater (landfill leachate, mustard wastewater, etc.), it is possible to conclude that the appropriate addition of non-toxic organic substances (such as acetate, glucose and skim milk powder) can improve the activity of anammox and denitrifying bacteria and improve their coupling effects.[16,72,73] The largest contribution rate of anammox during treatment of landfill leachate was only 23.4%, recorded by Wei et al.[28] The main reason is that functional COD in sewage is beneficial to the growth of denitrifying bacteria and inhibits the development of the anammox community. Continuing to inoculate sludge and optimize the influent parameters can promote the growth and reproduction of anammox bacteria and shorten the reactor startup time. Feng et al.[74] studied the metabolism of anammox bacteria and found that under the condition of higher organic concentration, more energy consumption was required to produce EPS and amino acids accumulated in the cells, which reduced the contribution rate of anammox to nitrogen removal.

3.2.2 Toxicity inhibition. Inhibition of toxic organics occurs due to irreversible poisoning of microorganisms or inactivation of key enzymes. Several common toxic organics are alcohols, aldehydes, phenols and antibiotics.

Table 3 Research on the effects of FNA on anammox and denitrifying bacteria

Reactor	T/°C	pH	HRT/h	NO_2^-–N mg L^{-1}	FNA/µg L^{-1}	Sludge	Effect	Time/d	Reference
UASB	35 ± 1	7.5–7.8	12–1.5	130–260	0.67/2.81	Granule sludge	IC_{10}/IC_{50}	900	62
EGSB	35 ± 1	7.1–7.3	1.5–3	117.8–710	15	Digested sludge	IC_{10}	400	41
UASB	35 ± 1	7.5–7.9	1.5–3	229.7	17	Granule sludge	IC_{10}	700	63
AAFEB	35	7.5–8.5	3	533.8	12–19	Biofilm/granule	IC_{10}	300	64
CSTAn	25 ± 3	7.0–7.3	12–24	300–400	70 ± 10	Digested sludge	Restrain	180	65
EGSB	30 ± 2	6.5–7.3	—	160	19	Granule sludge	IC_{10}	—	52
UASB	35 ± 1	7.5–8.2	4	210–420	6	Anammox	No	600	66
SBR1/2	30/33	7.8	24	70–420	11	Biofilm	IC_{50}	200	36

Table 4 Research on the inhibitory effects of the organic matter concentration on anammox and denitrifying bacteria

Reactor	$T/°C$	pH	HRT/d	Sludge	Carbon source	c (mg L^{-1})	Effect	Time/d	Reference
MBfR	22 ± 2	7.0–8.0	1.1	Biofilm	Methane	0.24–1.21 atm	Increased activity	70	75
UASB	35	7.8–8	1.5	Granule sludge	Skim milk powder	0–400	C/N threshold value 3.1	30	76
—	20	7.4	2.6	Activated sludge	Pig wastewater	2200/6.4	No	25	38
USB	28–35	7–8	—	Mixed sludge	Landfill leachate	1400–2000	Restraint	90	14
UASB	25–32	7.9	0.67	Activated sludge	Livestock wastewater	254 ± 115	No	190	68
EGSB	35 ± 1	—	—	Anammox sludge	Glucose	C/N: 1.6	Increased activity	91	77
—	30 ± 1	7.2	1–2	Granule sludge	Sodium acetate	0–40	Increased activity	400	73
SBR	35	7.5	—	Biofilm	Methane	Enough	Restraint	200	78

Table 5 Inhibition of anammox and denitrification by antibiotics

Type	Antibiotic	$T/°C$	pH	c (mg L^{-1})	Effect	Reference
β-Lactam	Penicillin G-Na	20 ± 1	7.0 ± 0.05	4946.4–5282.4	−50%	82
G-Antibiotic	Polymyxin B sulfate			1.8–18.6	−50%	
Amide alcohol	Chloramphenicol			333.7–486.1	−50%	
Aminoglycosides	Kanamycin sulfate			3934.4–6573.8	−50%	
Macrolides	Erythromycin	25 ± 1	7.8 ± 0.1	20	−50.6%	83
Quinolones	Norfloxacin	24 ± 1.9	8.2 ± 0.07	0.001	−39.6%	84
Macrolides	Erythromycin	24 ± 1.3	8.1 ± 0.04	0.001	A few	
Tetracycline	Oxytetracycline	35 ± 1	8.4 ± 0.11	2	−81.3%	85

(1) Alcohols, aldehydes and phenols. The main toxic alcohols are methanol and ethanol. The inhibition mechanisms are that methanol will be converted to aldehydes by methanol dehydrogenase, and ethanol will be converted to acetyl coenzyme before being oxidized to acetaldehyde.[79] As noted in previous research, there was no accumulation of NO_2^- in a reactor with added methanol; the contribution rate of anammox denitrification continuously decreased, and the coupling system was seriously inhibited. Peng *et al.*[80] analyzed the methanol in the reactor experimentally; they found that anammox had no activity at 3 to 4 mmol methanol, and the inhibition was irreversible.

Phenols originate from manufacturing wastewater (such as paper, textile and pharmaceutical); also, there are small amounts of phenols in urban sewage. Lyu *et al.*[81] stated that when the phenol concentration was set at 400 mg L^{-1} and 1000 mg L^{-1} in intermittent experiments, the anammox removal rate decreased by 56% and 76%, respectively. Through inhibiting the activity of anammox strains and changing the microbial community structure, the experiment reduced the nitrogen removal rate. Studies by Peng *et al.*[80] pointed out that the inhibitory strength of benzenes is related to their substituent groups, and the relationships between them are as follows: −COOH < −SO₃−H < −H < −OH < −CH₃ < −CHO < −OCH₃ < −Cl. When benzene, toluene, phenol and benzoic acid are taken as carbon sources, their IC$_{50}$s are about 9.21 (7.80 to 10.62), 35.28 (30.16 to 40.40), 124.55 (113.51 to 135.59) and 3833.12 (3481.57 to 4184.67) mg L^{-1}, respectively.

(2) Antibiotics. Antibiotics are biopharmaceuticals and are found in wastewater, such as aquaculture wastewater, livestock

Table 6 Research on the effects of salinity on anammox and denitrifying bacteria

Reactor	HRT/d	Salt type	c (g L^{-1})	Effect	Time/d	References
SBR	1.4–2.6	NaCl	1.75–18.33	Anammox activity −94%, nitrogen removal rate −70%	215	43
SBR	0.9–1.3	NaCl	3.4–12.2	Nitrogen removal rate −60%	450	89
SBR	—	Seawater	3.5–35	Short-term 30 g L^{-1} activity −30%, long-term 35 g L^{-1} activity −42%	3 year	90
SBBGRs	2	NaCl	0–5%	Activity −40%	241	91
PN-An	0.1	NaCl/KCl/Na₂SO₄ = 1 : 1 : 1	0.11–2.4%	25 g L^{-1} activity −60%, >24.1 g L^{-1} nitrification rate −50%	150	92
Batch	—	NaCl	25–125	100 g L^{-1} AOB, no effect, 125 g L^{-1} start suppression	—	93
EGSB	—	NaCl	5–12	Increased heat resistance	2 year	94
—	0.25–0.5	NaCl	0/3/25/45	Inhibition 0/23.43/48.89/67.94	120	95

Table 7 Inhibitory concentrations of metal ions on anammox bacteria

Experimental conditions						IC_{50} (mg L^{-1})							Reference
Sludge	pH	$T/°C$	Domestication time (h)	Main strain	Experiment type	Cu^{2+}	Cr^{2+}	Pb^{2+}	Zn^{2+}	Ni^{2+}	Cd^{2+}	Mn^{2+}	
PD/An granule 3.2 mm	7.8	30	12	*Brocadia Fulgida*	Gas	19.3	26.9	45.6	59.1	69.2	174.6	175.8	87
Mixed sludge	7.9 ± 0.1	35 ± 0.5	24	*Candidatus Brocadia*	Liquid	—	—	—	15.1	6.0	—	—	96
An granule 2.6 ± 0.6 mm	7.8 ± 0.12	35 ± 1	0	—	Liquid	—	—	—	—	—	—	7.33	100
Anammox granule	7.5 ± 1	30	2	*Brocadia*	Liquid	4.2	—	—	7.6	48.6	11.2	—	101
Anammox sludge	7.0 ± 0.2	37 ± 1	—	Planctomyces	Liquid	—	9.84	2.3	As^{3+}: 10.4	7.0	—	—	102
Anammox sludge	7.5 ± 0.1	35 ± 0.5	1.8	*Candidatus Kaenenia*	Liquid	—	—	—	—	14.6	—	—	103
Anammox granule	8.1 ± 0.1	35 ± 1	0.8	—	Liquid	12.9	—	—	—	—	—	—	104
KSU-1 bacteria	7.0 ± 0.2	35 ± 1	24	KSU-1	Liquid	Hg^{2+}: 60.4 ± 2.5	Ag^{+}: 11.5 ± 0.5	—	11.7 ± 0.4	—	—	—	105

wastewater and surface water. Because of their antibiotic actions, microorganisms are inhibited or killed, and antibiotics have obvious inhibitory effects on anammox and denitrifying bacteria in sewage treatment (Table 5). Ding et al.[82] studied the effects of different antibiotics on anammox and found that the order of toxicity of various antibiotics was as follows: polymyxin B > chloramphenicol > penicillin G-Na > kanamycin sulfate. The IC_{50} inhibition concentrations of these antibiotics were 5114.4 (4946.4 to 5282.4), 10.2 (1.8 to 18.6), 409.9 (333.7 to 486.1) and 5254.1 (3934.4 to 6573.8) mg L^{-1}, respectively; moreover, the simultaneous toxicity of multi-component antibiotics shows synergism. According to the research by Zhang et al.[83] on the effects of erythromycin on the coupling process, transient inhibition occurred at low concentration (expressed as low-dose addition inhibition), but the activity could be restored in a short time; however, the inhibitory effect was irreversible when a higher concentration was added.

3.3 Salt inhibition

Salt will affect the infiltration of the inside and outside environments of microbial cells. This process creates an osmotic pressure difference that will cause cell death or cleft of microorganisms. This phenomenon exists in some wastewater, such as seafood processing wastewater, mustard production wastewater, dyeing wastewater, and petroleum production wastewater.[86] Salt-tolerant microorganisms can be obtained by high salinity acclimation or directly from the ocean.

For example, Giustinianovich et al.[43] found that the activity of anammox was inversely proportional to the salt concentration in the treatment of canned fish wastewater. When the concentration of NaCl increased from 1.75 to 18 g L^{-1}, the activity of anammox decreased by 94%, while the coupling nitrogen removal rate of anaerobic ammonia nitrogen was reduced to 30%. However, direct exposure of anammox to 18 g L^{-1} NaCl for acclimation resulted in higher activity of anammox bacteria and a higher denitrification rate. In another study, the microbial community was subjected to short-term high load salinity (3.4 to 12.2 g L^{-1}),[87] and it was found that anammox and denitrifying bacteria were inhibited when the salt was added; however, they could restore their activity within a short time and maintained stable operation in wastewater. Additionally, the influences of the concentration values of different salts are dissimilar; Dapena et al.[88] stated that the IC_{50}s of Na$_2$SO$_4$, NaCl, and KCl were 11.36, 13.46, and 14.9 g L^{-1}, respectively (Table 6).

3.4 Other inhibition

3.4.1 Metal inhibition. Metals can inhibit microbial metabolism in wastewater treatment processes, especially heavy metals. They affect the microbial activity, cell structure, biological species and population structure by changing cell membrane permeability, synthesizing specific expressed proteins and small organic acids, and forming poorly soluble inorganic substances. Research on heavy metals has achieved abundant outcomes (Table 7); most metals have inhibitory effects on microorganisms. Val del Río et al.[87] studied the IC_{50} values of Cu^{2+}, Cr^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+} and Mn^{2+} on

Table 8 Recent application research of anammox and partial denitrification coupling

Reactor	Operating parameters	Experimental results	Conclusion and evaluation	Time	Reference
UASB (2L)	1. Influent NH_4^+-N and NO_3^--N about 30 mg L^{-1} 2. Carbon source: acetic acid, C/N: 2.0–3.4 3. Sludge source: DEAMOX-SBR reactor 4. Temperature: not controlled, seasonally changing	1. Effluent TN: 6.56 mg L^{-1}, nitrogen removal rate 89.1%, NO_3-N to NO_2^--N conversion rate 90%	1. Adjust liquid circulation and increase nitrogen removal performance 2. The protein structure and composition of EPS play important roles in sludge stability 3. The mass transfer limitations affect anammox and PD activity	225 d	131
UASB (8L)	1. C/N: 0.2–0.6, NO_2^-/NH_4^+ ratio about 1.32 2. DO: 0.2–0.4 mg L^{-1}, temperature: 16–30 °C 3. TN: 51.3 ± 8.4 mgN L^{-1}, COD: 25.5 ± 6.9 mg COD·L^{-1}	1. TN <5 mg N L^{-1} 2. TN removal rate 90% 3. Nitrogen removal rate 2.28 kg N (m³ d)$^{-1}$	1. In the model establishment and experimental comparison, the uniform particle size model cannot reflect the real situation, and it is necessary to establish a model to accurately describe the granular anammox system	200 d	127
SBR (5L), UASB (3.2L)	1. NO_3^- : 1000 mgN L^{-1} : NH_4^+: 58.3 mg L^{-1} 2. Carbon source: acetic acid, COD: 82.5 mg L^{-1} 3. Sludge source: PD reactor (NTR about 80%) 4. Temperature: seasonal change (14.8–28.2 °C)	1. NO_3-N removal rate 95.8% 2. NH_4^+-N removal rate 92.8% 3. High COD (76.5–98.6 mg L^{-1}) anammox contributed 78.9% to TN removal	1. The strain produces a high concentration of SMP in the absence of substrate, inhibiting the growth of the strain 2. Anaerobic amoxidase activity is sensitive to high concentrations of organic matter	215 d, 500 d	13
SPNAD (10L)	1. Influent NH_4^+-N, COD and TN: 1000 ± 250 mg L^{-1}, 1100 ± 200 mg L^{-1} and 1300 ± 75 mg L^{-1} 2. Temperature: 28 ± 3 °C, DO < 0.5 mg L^{-1}, pH: 8 ± 0.2 3. Sludge source: pilot SBR and laboratory anammox	1. NRE: 98.7%, NRR: 0.23 kg (m³ d) 2. Anammox contributes 69.3% to nitrogen removal, denitrification contribution rate 15.7%	1. Different kinetics have different effects on the anammox community 2. Microorganisms with different functionalities (richness): *Nitrosomonas* (3.0%), *Cadillata* (2.13%) and *Spindle ella bacteria* (25.3%) coexist	200 d	108
UASB (1.96L)	1. Sludge source: anammox sludge according to UASB reactor (120 d), conventional sludge from WWTPs 2. Temperature: 33 °C, influent pH: 7.5–8.0	1. TN removal rate 92.47% 2. The dominant species are *Thauera* 25.30% and *Candidatus Brocadia* 6.85%	1. Anammox and PD gene expression differs with time 2. NarG can produce the nitrite required by anammox. NirS is a key gene for the	180 d	150

Table 8 (*Contd.*)

Reactor	Operating parameters	Experimental results	Conclusion and evaluation	Time	Reference
			reduction of nitrite to produce nitric oxide. Its activity plays an important role in denitrification and anammox reaction		
SBR (10L), UASB (3.2L)	3. NO_3^-/NH_4^+: 1.6, C/N: 2.0, carbon source: acetate 1. Influent: NH_4^+: 57.8 mg N L^{-1}, COD: 175.8 mg L^{-1} 2. Sludge source: NTR about 80% 3. Influent: NO_3^- (20–40 mg N L^{-1}). The three stages of C/N are 3.0, 2.8 and 2.6. Temperature: 18.7 °C~27.8 °C	3. Anammox and PD gene richness 1. NO_3–N removal rate 97.9% 2. NH_4^+ and COD removal rate 95.2% and 81.6% 3. Effluent TN, COD: 4.0 mg N L^{-1}, 30.1 mg L^{-1}	1. *Candidatus Brocadia* is a dominant species in the reactor sludge biome 2. The PD-Anammox process has the advantages of low N_2O and low CO_2 and is environment-friendly	224 d	7

anammox bacteria, and the respective results were as follows: 19.3, 26.9, 45.6, 59.1, 69.2, 174.6, and 175.8 mg L^{-1}. Kalkan *et al.*[96] also focused on the short-term impact of anammox IC$_{50}$. However, Chen *et al.*[97] added some metal ions to the reactor, namely Fe^{3+} (6.61 mg L^{-1}), Cu^{2+} (1.18 mg L^{-1}), and Ni^{2+} (1.11 mg L^{-1}); it was found that the anammox nitrogen removal rate increased by 50%, and this phenomenon showed that metal ions may have positive influences on anammox bacteria.[98] Studies have found that low concentrations of Cu^{2+} (0.05 to 0.56 mg L^{-1}) will inhibit the activity of denitrifying bacteria, while Zn^{2+} will promote the growth and reproduction of denitrifying bacteria at lower concentrations.[66] Moreover, higher concentrations (>30 mg L^{-1}) will produce inhibition,[99] and when two kinds of metal ions impact denitrifying bacteria, the superimposed effect on denitrifying bacteria is slightly weaker.

3.4.2 Sulfide and phosphate inhibition. Sulfides and phosphides are common stepless inhibitors of the anammox and denitrification process. In the coupling denitrification process, SO_4^{2-} and NO_2^- are simultaneously reduced by anammox bacteria, which function as electron acceptors to produce H_2S and N_2. The produced sulfide (H_2S) will inhibit the anammox process and may be the dominant species (sludge inoculation). There are differences between the operating conditions and the inhibition effects in each study.[88,106,107] Zhang *et al.*[108] studied the effects of phosphate on different morphologies of sludge (anammox particles and flocs) through batch experiments and found that phosphate can reduce the abundance of bacteria in the sludge but has less effect on the dominant strain (*Candidatus Kuenenia stuttgartiensis*). Moreover, according to the study, the IC$_{50}$ values of the phosphate medium for anammox particles and flocs were 1664.7 ± 114.7 and 1205.9 ± 7.5 mg L^{-1}, respectively. In studies of the effects of sulfides and phosphates on anammox and denitrification, the bacterial strains used are relatively few, and other bacteria should be studied in the future.

4 Operational regulation of the coupling process

In order to operate the coupling process efficiently and economically, the coupling parameters should be adjusted to increase the denitrification rate and reduce the inhibition effect. The adjusting methods include: (1) Mechanical treatment (sedimentation or filtration). (2) Operating parameter control (pH, DO or T). (3) Sludge control (sludge selection, domestication or residence time). (4) Modification of the reactor operation strategy.

4.1 Parameter control

4.1.1 pH and T. pH is the main factor affecting the concentration of FA and FNA; it has independent effects on FA and FNA inhibition. Low pH will increase FNA concentration (eqn (5)), and high pH will increase FA concentration (eqn (4)). By adjusting to the appropriate pH, FA and FNA inhibition can be reduced. According to eqn (1) and (3), the anammox and partial denitrification coupling nitrogen removal process will

consume H$^+$ (alkali production process) and the pH will increase.[109] In order to ensure better metabolic and propagation conditions and a higher nitrogen removal rate, the pH of the inlet water should be adjusted to neutral or weakly acidic. If the amount of alkali production is uncertain, slightly alkaline inlet water is more suitable.[110,111] It is suitable for slightly alkaline inlet water.

According to the study by Strous et al.,[9] the pH should be controlled within the range of 7.0 to 8.0; the optimal physiological pH range of anammox in a single system is 6.7 to 8.3, while the optimal growth pH of denitrifying bacteria is 6 to 9 and the optimal coupling pH is 8.0. Similar studies[111] have found that a suitable range for anammox bacteria growth is pH 7.0 to 8.5; denitrifying bacteria have little effect at pH 7.0 to 8.0, while the optimal pH load for anammox and partial denitrification coupling is 7.0 to 8.0. If the pH is lower (<5.06) or higher (>9.00), it will affect the concentration of substrates and inhibitors in the water environment, influencing the growth and reproduction of anammox and denitrifying bacteria. Thereby, the electrolyte balance in the cell is destroyed to achieve the purpose of inhibiting the bacteria.[112] Puyol et al.[52] confirmed that high pH is more serious than FA inhibition, and maintaining an appropriate pH value is crucial for the stability of the bacterial community structure. Strous et al.[9] pointed out that adding HCO$_3^-$ to water is an effective method to regulate pH, and proper pH conditions will enrich the bacterial community and lead to a higher nitrogen removal rate.

The survival temperature of anammox bacteria is between 20 °C and 40 °C, and the optimal temperature range for nitrogen removal is 30 °C to 40 °C.[111,113] Higher temperature (>45 °C) will irreversibly inhibit the activity of anammox bacteria, and the life activity of bacteria at lower temperature (<10 °C) is completely stopped.[111] The optimal growth temperature range of denitrifying bacteria is 20 °C to 35 °C; excessively high or low temperature will cause changes in the physical structures of cell membranes (lipids and proteins).[113] Much research has shown that maintaining the temperature at 30 °C to 35 °C is optimum for anammox and partial denitrification coupling.[45,55,114]

Although the bacteria can be inactivated at high temperature, the demand and application of high temperature denitrification cannot be hindered. Zhang et al.[94] increased the salinity appropriately to improve the heat resistance of anaerobic microorganisms (anammox). The optimum temperature is extended to 40 °C to 50 °C. In recent years, low and medium temperature coupling technology for denitrification has become a research focus point. Cheng et al.[115] studied urban river sludge and found that the anammox rate is higher in winter than in summer, while the denitrification rate is higher in summer than in winter; this phenomenon provides an opportunity for the application of coupled nitrogen removal technology. The studies by Gilbert et al.[116] showed that the temperature effect is closely related to the shape of the biomass. It was also found that the activities of the granular and carrier biofilm species (anammox) can be maintained at a temperature below 13 °C; however, the activity of suspended species begins to be suppressed at temperatures below 15 °C. The comparison

shows that the thicker the biofilm in the low temperature environment, the higher the environmental adaptability and the stronger the resistance to temperature impact. According to the studies by Lotti et al.,[117] the anammox and partial denitrification coupling process can work well at low and medium temperatures (15 °C to 25 °C). Anammox bacteria that are biologically active in high or low temperature environments (85 °C and −2 °C) are also found in nature,[118,119] which provides the possibility of denitrification in wastewater at different temperatures. However, the anammox and denitrifying bacteria that survive at higher or lower temperatures cannot meet the optimal temperature of enzyme activity. Further exploration of the optimal temperature requirement of enzyme activity coupling with anammox and denitrification is needed. In addition, the temperature affects the FA and FNA concentrations. As shown in eqn (4) and (5), temperature is an important parameter for stable operation control of the reactor.

4.1.2 DO and ORP. Dissolved oxygen (DO) is one of the key parameters for coupling control of anammox and partial denitrification. The living environment of DO for anammox bacteria is usually controlled at 0.6 to 0.8 mg L^{-1}. Generally, lower DO (<2% air saturation) will inhibit the life activity of anammox bacteria (reversible), while higher DO (>18 air saturation) will irreversibly inhibit the bacteria.[120] Anammox bacteria will die when exposed to an excessive DO environment, and the living environment of DO for denitrifying bacteria is close to zero; therefore, the coupling of anammox and some denitrification bacteria needs to occur in a low oxygen environment.[58] Due to the mutual difference between oxidase and nitrite reductase, higher DO will inhibit nitrate reduction.[106] When anammox and partial denitrification are coupled, the DO should be controlled below 0.7 mg L^{-1} to facilitate the coupling.

Oxidation–reduction potential (ORP) is used to characterize the macroscopic redox properties exhibited by substances in aqueous solution, and the ORP values are related to DO, pH, and organic matter concentration.[121,122] It has been noted that when anammox is coupled with partial denitrification, an ORP between −50 and +50 mV is beneficial for denitrification.[106] ORP is widely used for online monitoring and parameter control of anammox, nitrification, and denitrification processes. A crowd of studies have shown that compared with other parameters (DO or pH), ORP is a more consistent process control parameter.[123] The ORP provides reliable control operating parameters for the stable operation of the anammox and partial denitrification coupling process.[124]

4.1.3 C/N and NO$_2^-$/NH$_4^+$. C/N is the key to start an anammox and partial denitrification coupling reactor, and it is also an important control parameter to maintain stable reactor operation and efficient nitrogen removal.[19,30,125] The presence of organic matter is generally believed to have adverse effects on the anammox process because the heterotrophic denitrifying bacteria grow at a much higher rate and with a higher cellular synthesis growth yield compared to autotrophic anammox bacteria.[106]

Anammox bacteria are autotrophic bacteria, and the participation of organic carbon will inhibit bacterial growth. Sheng et al.[77] studied the effects of different organics and C/N on the

SAD process; although the biological community believed that in a low COD environment, the anammox bacteria were dominant (C/N = 0.8), they found that the denitrifying bacteria grew faster under a high carbon–nitrogen ratio (C/N = 1.2). Wang et al.[129] performed batch tests and indicated that anammox collaborates well with denitrification at low C/N (C/N = 1.0). Miao et al.[126] found that when the C/N of the reactor inlet water was adjusted from 1.1 to 2.5, the increase of C/N promoted the growth of denitrifying bacteria and inhibited the growth rate of anammox bacteria (the abundance and activity of anammox bacteria remained stable). Influent C/N directly affects the competition between autotrophic and heterotrophic microorganisms, and most denitrifying bacteria are heterotrophic microorganisms; therefore, a higher influent C/N is beneficial to the growth of denitrifying bacteria. Miao et al.[126] reported that C/N low-load wastewater was adjusted as the C/N increased from 1.0 to 2.5; the contribution of denitrification to TN removal gradually increased, and denitrifying bacteria also accumulated. Cao et al.[12] reported that the contribution of denitrification to TN removal is directly proportional to C/N, confirming the effects of C/N on denitrifying bacteria.

Studies have shown[126] that the C/N the reactor can accept is related to the thickness of the biofilm. Thicker biofilms can maintain a good nitrogen removal rate even with high C/N (C/N = 10). When the biofilm is thin and sensitive, low C/N (C/N = 2) can also inactivate microorganisms. C/N has a significant effect on the accumulation of $NO_2^- -N$. Therefore, in the treatment of sewage with less organic matter, an additional carbon source must be used to determine the $NO_3^- -N$ content in the sewage. In the study of separated and combined partial denitrification and anammox coupling processes, a large number of literature reports have noted that the optimal range of C/N adjustment for the startup and operation of the reactor is 2.0 to 3.0.[7,12,13,21,25]

NO_2^-/NH_4^+ is a direct parameter to measure the optimal removal rate of TN. Studies have shown that the optimal stoichiometric ratio (1.32 : 1, often used as the nitrogen source addition ratio to start the coupling reactor) will reduce the TN removal rate.[9,127] When NO_2^-/NH_4^+ is more than 1.3 during the nitrogen removal process, the NO_2^- in the reactor will gradually accumulate and affect the bacterial activity; when NO_2^-/NH_4^+ is less than 1.3, despite the high denitrification rate, the anammox process is inhibited and the TN removal rate is reduced by 20% to 60%.[128] Wang et al.[129] stated that when NO_2^-/NH_4^+ is between 0.1 and 0.5, anammox and denitrifying bacteria can coexist; however, the denitrification contribution rate is significantly higher than that of anammox. The calculation of the nitrogen path shows that during the coupling process,[13] anammox is the main denitrification method (accounting for 78.9% of the total denitrification amount), which is the real reason for the stable operation of the coupling process. Maintaining a suitable NO_2^-/NH_4^+ value with influent water (stoichiometry ratio 1.32 : 1) is an important parameter for the rapid start-up and stable operation of the coupling reactor.

4.1.4 Other parameters. The light effect is the inhibition by light of the activity of photosensitive microorganisms.[9] Because anammox bacteria are light-sensitive microorganisms, light energy can inhibit the activity of anammox bacteria, reducing the nitrogen removal rate by 30% to 50%. Therefore, protective measures should be taken in the laboratory environment to increase the nitrogen removal rate.[26]

Hydraulic residence time (HRT) is the average residence time of sewage to be treated in a reactor. In the process of anaerobic ammonia oxidation and partial denitrification coupling, proper increase of HRT can improve the TN removal rate.[13] When the nitrogen removal process (or bacteria) is inhibited, adjusting to the appropriate HRT can effectively restore nitrogen removal performance (or bacterial activity).[12,130,131]

4.2 Sludge

4.2.1 Sludge domestication. Anammox and partial denitrification coupling methods can be divided into split and integrated processes. In the split process, the sludge of absolute dominant bacteria (anammox and denitrification bacteria) is inoculated into two different reactors to remove nitrogen, which effectively avoids competition between the substrates and living space of microorganisms.[132] The coupling process mainly starts by inoculating part of the denitrifying floc sludge and anammox granular sludge.[22,132,133] It has been reported that denitrifying sludge is obtained by acclimation of nitrifying sludge.[122] The advantage of this process is that the anammox activity and denitrification activity are positively related, and the synergistic metabolism can greatly improve the TN removal rate.[134–136] Because the screening of the type of sludge is largely determined by the coupling process, the screening of functional anammox and denitrifying bacteria is often performed by adding screening factors (such as pH, T and salt) to the sewage during process operation and commissioning.[31] Because of the large difference in demand for sludge-dominant bacteria for sewage treatment performance goals, sludge must be domesticated and cultivated.

The most common sludge forms are biofilm, floc sludge (suspended state), and granular sludge. Raudkivi et al.[137] stated that the tolerance performance of the three states during domestication is as follows: granular sludge > biofilm > flocculent sludge. Granular sludge and biofilm have high mass transfer resistance, which alleviates the inhibitory effects caused by transient impact and provides sufficient adaptation time for microorganisms. Therefore, in order to shorten the domestication time and achieve more bacteria load, it is important to choose appropriate sludge domestication.

4.2.2 Sludge retention. In order to maintain a stable anammox and partial denitrification coupling denitrification process, the sewage treatment system needs a sufficient sludge residence time (SRT). Jenni et al.[138] pointed out that when a sufficient SRT is ensured, anammox and denitrifying bacteria can coexist even at high C/N. Anammox and denitrifying bacteria can only be guaranteed when the SRT is longer than the doubling time of sludge. In order to obtain better denitrification performance, the typical doubling time of anammox is 15 to 30 days.[139] It is necessary to ensure an appropriate SRT in the reactor, which is vital to achieve a high nitrogen removal rate in the coupling process.[13,139]

4.3 Reactor quick start strategy

The optimized reactor operating parameters will shorten the reactor startup time and also increase the speed of the actual industrial application process of anammox and partial denitrification coupling. Research on anammox and partial denitrification coupling technology is listed in Table 8. Methods of quick start strategy parameter control include sludge selection, adjustment of initial conditions and reactor commissioning.

4.3.1 Sludge selection. According to Zhou et al.,[140] adding quality sludge can shorten both the time of anammox and denitrification and the success time of the reactor. Moreover, when less active matrix anammox was added, the anammox and partial denitrification coupling reactor was successfully started and the start-up time was reduced by 5 days. Wang et al.[141] inoculated anaerobic granular sludge (AGS) and activated sludge (AS) in a laboratory-scale reactor, and the reactor to which AS was added was successfully started; the reactor start-up time shortened from 21 days to 5 days (TN removal rate was 70%), while the reactor inoculated with AGS failed to start. Zhang et al.[142] added 5 different sludge EPS in the interval test of anammox activity; they noted that denitrifying sludge EPS (DS-EPS) can increase the activity of anammox bacteria and shorten the startup time of the reactor.

4.3.2 Initial condition adjustment. When the reactor is started, the characteristics of the operating environment of the anammox and denitrifying bacteria (such as T, pH or NO_2^-/NH_4^+) are used as direct control variables for fast startup. Zhou et al.[61] inoculated non-deoxygenated anammox and denitrifying sludge at room temperature from 24 °C to 30 °C and returned the washed-out sludge to the reactor to increase the residence time; this played an important role in the reactor startup and performance recovery. Yin et al.[143] found that adding a small amount of reduced graphene oxide (RGO) to a started anammox reactor will increase the anammox bacteria activity, thereby shortening the reactor startup time and improving the nitrogen removal rate. Jara-Muñoz et al.[144] also quickly started the coupling reactor under two conditions of hypoxia and higher temperatures (30 °C to 40 °C), with shortened the microbial doubling time. A number of studies have shown that during the coupling reactor start-up process, controlling the NO_2^-/NH_4^+ ratio at 1.32 : 1 is conducive to promoting the rapid growth of bacteria and shortening the reactor startup time.[9,106,145]

4.3.3 Reactor operation. During the operation of the reactor, it is necessary to regularly monitor the operating parameters (DO, NO_2^-, NO_3^-, etc.) to ensure better treatment performance (TN, COD removal rate, sludge concentration, etc.) and to debug the reactor operation parameters. These are conducive to the rapid startup and stable operation of the coupling reactor.

4.3.4 Improved reactor performance. Charles Glass et al.[146] observed that denitrification was inhibited at around pH 6.5, and the NAR increased significantly when the pH was adjusted from 7.5 to 9.0. It is worth noting that in the long-term and short-term effects of pH on denitrification, a high pH value is beneficial to achieve partial denitrification of nitrite accumulation.[147–149] The SPDAF (synergizes the partial denitrification,

anammox and *in situ* fermentation) collaborative process established by Ji et al.[30] has the disadvantages of slow degradation of organic matter and low utilization rate. The sludge in the fermentation tank was inoculated into the UASB reactor to promote denitrification. The bacteria consumed a large amount of organic matter to produce more nitrite and increased the rate of nitrogen removal by anammox. Zhang et al.[19] added sponge bio-carriers to facilitate the enrichment of microorganisms and the formation of EPS. The richness of the biofilms increased by 6 times and the TN removal rate was maintained at 93%, which solved the problems of immature biofilms and insufficient anammox bacteria. Niu et al.[27] investigated the stability of FA and FNA to the process and found that both FA and FNA affect the TN removal rate; therefore, the operating conditions were optimized to establish a clear parameter control range (pH 7.5 to 8.5, FA below 10 mg/100 mg NH_4^+–N and an FNA below 0.005 mg/100 mg NO_2^-–N), ensuring high nitrogen removal rate and effluent quality.

5 Conclusion

This paper discussed the coupling principles and inhibition mechanisms of anammox and summarized the coupling parameter control and optimization (or rapid) startup strategy. This paper focused on reviewing many practical issues that arise during the startup and nitrogen removal of anammox and some denitrification coupling reactors, summarizing the inhibition mechanisms and concentration control ranges of inhibition parameters (organics, salts, etc.), the influence and adjustment ranges of basic parameters (pH, T, DO, etc.), sludge differences (dominant bacteria), and reactor startup control strategies. Because anammox and partial denitrification coupling is affected by multiple parameters, it is utilized less in common sewage-treatment processes. In order to improve the denitrification performance and develop the coupling denitrification process, it is recommended that further studies on the coupling denitrification process focus on the following aspects:

(1) Research on the inhibition mechanisms. Studies on the inhibition performance and mechanisms of inhibitors, such as substrates, organic matter and salt.

(2) Optimization of operating parameters. Based on the anammox and partial denitrification coupling reactor, combined with the actual bio-dominant species (or characteristic strains), the optimal operating parameters should be adjusted to guide the rapid startup and stable nitrogen removal of the reactor.

(3) Research on the mass transfer mechanism. Based on the state of the sludge (particles, flocculent, etc.) and the distribution of strains in the sludge, studies can be conducted about the mass transfer mechanism under different conditions, the mass transfer relationship between the state of the sludge and the distribution of the species community.

(4) Recovery kinetics after inhibition. When the coupling is inhibited, some additives are often added to restore the activity of the partially coupling strains; therefore, the work process of the recovery process needs to be further explored.

(5) Mathematical model for the coupling process. A unified mathematical model should be established for the coupling process of anammox and denitrification, which is helpful for grasping the mechanism of sewage treatment, predicting the state of sewage treatment, and optimizing the parameter of sewage treatment.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

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