REVIEW ARTICLE

The main anammox-based processes, the involved microbes and the novel process concept from the application perspective

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HIGHLIGHTS

- The PNA, denitratation/anammox, and DAMO/anammox process are reviewed together.
- Denitratation/anammox-based process is promising in mainstream treatment.
- DAMO and denitratation processes realize the higher nitrogen removal efficiency.
- The utilization of metabolism diversity of functional microbe is worth exploring.
- An effective waste treatment system concept is proposed.

GRAPHIC ABSTRACT
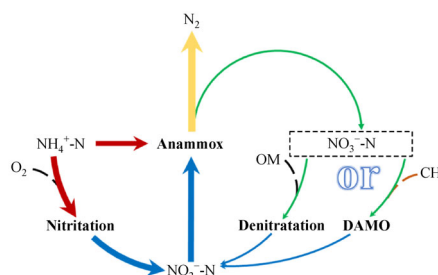
ABSTRACT

Anammox technology has been widely researched over the past 40-year from the laboratory-scale to full-scale. It is well-known that in actual applications, the solo application of anammox is not feasible. Since both ammonium and nitrite are prerequisites based on the reaction mechanism, the pre-treatment of wastewater is necessary. With the combination of anammox process and other pre-treatment processes to treat the actual wastewater, many types of anammox-based processes have been developed with distinct nitrogen removal performance. Thus, in order to heighten the awareness of researchers to the developments and accelerate the application of these processes to the treatment of actual wastewater, the main anammox-based processes are reviewed in this paper. It includes the partial nitritation/anammox process, the denitratation/anammox (PD/A) process, the denitrifying anaerobic methane oxidation/anammox (DAMO/A) process, and more complex deuterogenic processes. These processes have made the breakthroughs in the application of the anammox technology, such as the combination of nitrification and PD/A process can achieve stability and reliability of nitrogen removal in the treatment of mainstream wastewater, the PD/A process and the DAMO/A have brought about further improvements in the total nitrogen removal efficiency of wastewater. The diversity of functional microbe characteristics under the specific condition indicate the wide application potential of anammox-based processes, and further exploration is necessary. A whole waste treatment system concept is proposed through the effective allocation of above mentioned processes, with the maximum recovery of energy and resources, and minimal environmental impact.

1 Introduction

Over the past several decades, many researchers have engaged in the pursuit of the ultimate goal of the smooth and reliable application of anammox process over a broader scope (Guo et al., 2020a; Fang et al., 2021; Fu et al., 2021). The theoretical knowledge obtained from the research work on this process is extensive, from the chemical perspective, microbial perspective, engineering perspective and process application perspective (Bi et al., 2020; Ding et al., 2021; Li et al., 2021; Qiu et al., 2021; Xu et al., 2021). In practice, many installations adopting

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anammox-based processes have been constructed and operated in an attempt to achieve a more effective treatment system in the wastewater treatment sectors. However, a review of related research indicates that most of the application cases have been limited to the treatment of sidestream wastewater, like biomass digestion effluent, landfill leachate, and the advantages of this process for sidestream wastewater have been clearly shown. However, significant challenges remain in the treatment of mainstream wastewater, with performance stability and quality the main challenge in actual application of this process (Chen et al., 2019). Therefore, the main challenge in developing the anammox process is in its actual application to the treatment of mainstream wastewater, and new ways to overcome the bottlenecks are being vigorously explored. Challenges remain in the sidestream wastewater treatment field using anammox-based processes as well: the long-startup time, the limited nitrogen removal efficiency (NRE) and the fragile resistance ability of microbes to external environment fluctuation are considered insurmountable barriers in the effective implementation of this process. To some extent, these problems are caused by such extrinsic factors as the difficulty of operation and control and complex feedback conditions (Guo et al., 2020c). There are, however, other intrinsic factors which need more attention, such as the low growth rate of anammox bacteria, the low anammox specific activity at low temperature, the easily inhibition of anammox bacteria at extreme environment.

It is vital to ensure the stable and reliable acquisition of both nitrite and ammonium, both of which are reactants in the anammox reaction in the anammox-based process. However, in actual applications, the nitrogen species in wastewater differ according to the source of the wastewater (Guo et al., 2020a). In practice, in the biomass digestion effluent and the landfill leachate, the main nitrogen species is ammonium, while in the fertilizer in runoff, and in explosive production wastewater, the main nitrogen species is nitrate. Therefore, it is important that the appropriate process is adopted to deal with the actual nitrogen species in the specific wastewater, and the influent must be pre-treated to supply the nitrite and ammonium with balance for the anammox reaction. A number of different combinations of anammox process and pre-treatment processes have been developed: the partial nitration (PN)/anammox (PN/A) process (F. Qian et al., 2018), the denitratation (PD)/anammox (PD/A) process (Si et al., 2018), the denitrifying anaerobic methane oxidation (DAMO)/anammox (DAMO/A) process (Shi et al., 2013), and the dissimilatory nitrate reduction by anammox bacteria (DNRA)/anammox process (Castro-Barros et al., 2017).

As biological methods, the anammox-based processes are closely related with the function of microbes, like anammox bacteria, ammonium oxidizing bacteria (AOB), denitratation bacteria (DB), and DAMO archaea. The specific metabolism characteristics of the different microbe species need to be properly utilized in anammox-based processes because functional microbes are crucial for the successful realization of nitrogen removal. The temperature, pH, substrate concentration, salinity, and presence of hazardous substances exerts an effect on the microbes to some extent. In actual applications, unless the conditions are idea, the function of microbes will be reshaped, disturbing the whole nitrogen removal route, and compromising nitrogen removal (Guo et al., 2020c). The combination of at least two kinds of functional microbes in anammox-based processes constrains the reactor environment. Therefore, the metabolism characteristics of each functional microbe species need to be well-understood to optimize the process and successfully conduct the operation of the related installations in actual applications.

In this review, the major anammox-based processes in actual application are reviewed, and the microbe information are summarized to provide a thorough guide to the mechanisms in each process in the actual applications. Finally, a novel integrated system concept for waste treatment is proposed to maximize energy and resource recovery and minimize the detrimental effect of waste treatment on the environment.

2　The main anammox-based processes

The pre-treatment processes of actual wastewater can be divided into different categories in anammox applications. This can be done based on a number of different considerations. From the nitrite supply route perspective, the pre-treatment processes can be separated into the nitration process and PD process (Ma et al., 2020a). From the electron-donor perspective, the pre-treatment processes can be separated into the nitration process, the organic matter-based PD process (Ma et al., 2017), the thiosulfate-based PD process (J. Qian et al., 2018; Deng et al., 2019) and the methane-based DAMO process (Shi et al., 2013). With the combination of anammox and these above mentioned pre-treatment processes, many anammox-based processes have been developed toward actual application.

Based on the core mechanism and actual feasibility, three comprehensive and practical anammox-based processes have garnered most of the research attention: the PN/A process (Chen et al., 2019), the PD/A process (Ma et al., 2017; Zhou et al., 2020), and the DAMO/A process (Shi et al., 2013; T. Liu et al., 2019; Liu et al., 2020). The nitrogen removal pathway of these processes are shown in Fig. 1. Details of these processes are shown in Table 1 for comparison purposes. Among these three processes, it can be seen that the PN/A process is the most presentative and practical since, for different types of actual wastewater, the largest share of nitrogen species is the ammonium (Guo et al., 2020a). Since the performance of the PD/A process
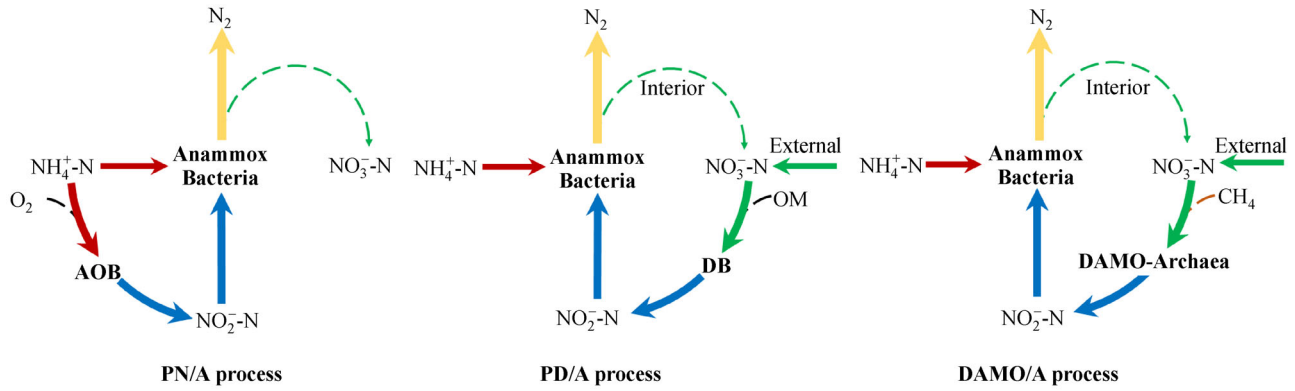
Fig. 1 The nitrogen removal pathway of three anammox-based processes.

Table 1 Characteristics of the main anammox-based processes

Process	Substrate	Reaction	Microbe	Environment	Theoretical NRE	Products
PN/A	O_2 NH_4^+-N	Nitritation Anammox	AOB Anammox bacteria	Aerobic Anaerobic	≈90%	N_2 NO_3^--N
DN/A	BOD NO_3^--N NH_4^+-N	Denitratation Anammox	DB Anammox bacteria	Anaerobic	≈100%	N_2 CO_2
DAMO/A	CH_4 NO_3^--N NH_4^+-N	DAMO Anammox	DAMO-archaea Anammox bacteria	Anaerobic	≈100%	N_2 CO_2

and the DAMO/A process is desirable in improving NRE, in recent years, these two processes are increasingly drawing more attention.

2.1 PN/A process

The PN/A process is the process most investigated at the laboratory-scale and pilot-scale, and even at the full-scale. The majority of the research is on the treatment of sidestream wastewater, like digestion effluent, landfill leachate, and specific types of industrial effluent. For a long time, this process was considered impractical for the treatment of mainstream wastewater due to the low NRE

and the instability of the performance (Cao et al., 2017; Chen et al., 2019). In recent years, more and more effort has been focused on improving the application of this process in the treatment of mainstream wastewater. As shown in Fig. 2, in this process, two kinds of bacteria are involved in the PN/A process: the AOB and anammox bacteria (Wang et al., 2021). In the case of the influent ammonium, part of which is oxidized to nitrite through the nitritation process, this nitrite and the residual ammonium are converted into nitrogen gas and a little nitrate by the anammox process.

Since there are two distinct biological reactions involved, the installations in actual applications can be
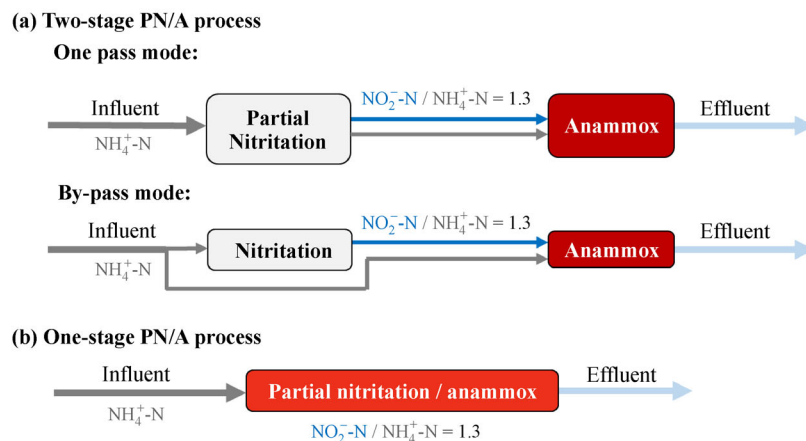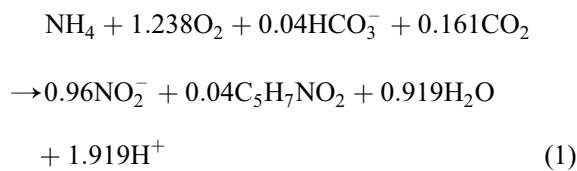

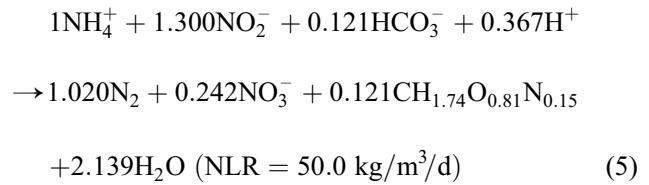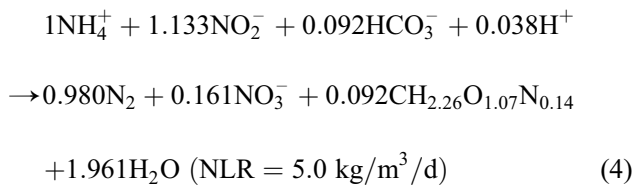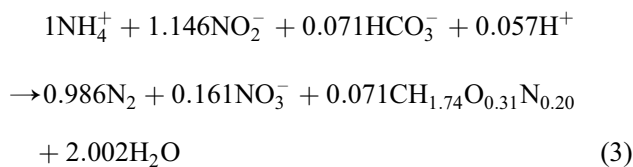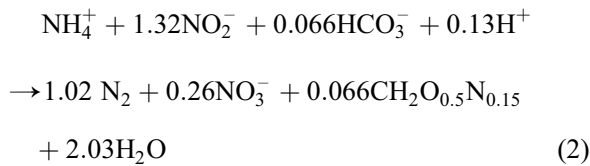**Fig. 2** The actual configuration of the PN/A process.

divided into the two-stage configuration and the one-stage configuration (Guo et al., 2020a). In the two-stage configuration, the nitritation step and anammox step occur in two sequential reactors, as shown in Fig. 2a. In the two-stage configuration, according to whether there is a bypass for the influent, the two-stage configuration can further be divided into a one-pass configuration and a by-pass configuration, as shown in Fig. 2a. In the one-stage mode, the nitritation step and anammox step occur in a single reactor, as shown in Fig. 2b.

Nitritation is conducted by autotrophic AOB with the reaction, expressed by equation (1) (Antwi et al., 2020). The anammox reaction is conducted by autotrophic bacteria, and four anammox reactions have been proposed (Guo et al., 2020a), as shown in equation (2), equation (3), equation (4) and equation (5):

Nitritation reaction:

$$NH_4 + 1.238O_2 + 0.04HCO_3^- + 0.161CO_2$$

$$\rightarrow 0.96NO_2^- + 0.04C_5H_7NO_2 + 0.919H_2O$$

$$+ 1.919H^+ \tag{1}$$

anammox reaction:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+$$

$$\rightarrow 1.02\,N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15}$$

$$+ 2.03H_2O \tag{2}$$

$$1NH_4^+ + 1.146NO_2^- + 0.071HCO_3^- + 0.057H^+$$

$$\rightarrow 0.986N_2 + 0.161NO_3^- + 0.071CH_{1.74}O_{0.31}N_{0.20}$$

$$+ 2.002H_2O \tag{3}$$

$$1NH_4^+ + 1.133NO_2^- + 0.092HCO_3^- + 0.038H^+$$

$$\rightarrow 0.980N_2 + 0.161NO_3^- + 0.092CH_{2.26}O_{1.07}N_{0.14}$$

$$+1.961H_2O \,(NLR = 5.0 \text{ kg/m}^3/\text{d}) \tag{4}$$

$$1NH_4^+ + 1.300NO_2^- + 0.121HCO_3^- + 0.367H^+$$

$$\rightarrow 1.020N_2 + 0.242NO_3^- + 0.121CH_{1.74}O_{0.81}N_{0.15}$$

$$+2.139H_2O \,(NLR = 50.0 \text{ kg/m}^3/\text{d}) \tag{5}$$

In the one-stage PN/A process, the AOB and anammox bacteria experience the same growth conditions, like temperature, pH, free ammonium (FA), and free nitrite acid (FNA). Therefore, the maximum overlap of the suitable growth environment of each bacteria is crucial for the successful and stable operation of this kind of configuration. In reverse, in the two-stage PN/A process, each kind of bacteria has its own growth environment. As such, it is feasible to create the most suitable environment for each type of bacteria, which is an effective way to maximize their ability in the nitrogen removal. However, from the achieved NRR by each process based on the previous research, as shown in Table 2, the one-stage PN/A process still have the efficiency advantage in the actual application (Guo et al., 2020a).

In actual applications, mainstream wastewater occupy the huge share of the nitrogen pollutants by the whole society. In wastewater treatment plant (WWTP), the adoption of the anammox-based process can realize the prominent reduction of the energy consumption (X. Li et al., 2017; Du et al., 2019). The granule anammox sludge can maximize the utilization of reactor volume and has the more surface area, which has the distinct advantages, like high surface area, than that of the biofilm sludge (Guo et al., 2021). However, the size of granule is too small or too big, still causing the negative effect, like the sludge washout or the decrease of the substance transfer effect (Tang et al., 2011; Tan et al., 2020). The washout was caused by the too short HRT or the gas accumulation in sludge due to the improper operation. The decrease of substance transfer effect was caused by the too big size of granule sludge. Thus, cultivating the granule sludge with small size and well settling ability is the crucial. Thus, granule sludge is the hopeful choice in the anammox application in the main-stream wastewater treatment. In mainstream wastewater treatment, the short HRT is very crucial to the nitrogen efficiency. Under the low HRT, the

Table 2 The highest NRRs reported in PN/A process research under different condition

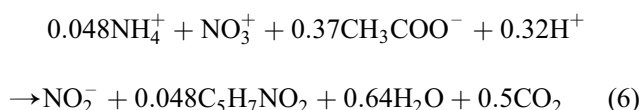| Reactor	Configuration	Scale	Temp (°C)	pH	Wastewater	NH$_4^+$-N (mg/L)	HRT (h)	NRR (kg/m^3/d)	NRE (%)	Reference
ALR	One-stage	Laboratory	25	7.6 ± 0.3	Mainstream	50	2	0.72	71.8	Chen et al., 2019
ALR	One-stage	Laboratory	25	7.8–8.2	Mainstream	63	1	1.28	78.7	Guo et al., 2021
						42	1	0.66	63.2	
ALR	One-stage	Laboratory	30	–	Sidestream	448.8	2	3.9	73	Wang et al., 2017
ALR	One-stage	Laboratory	30	7.7	Sidestream	304	1.5	3.9	80	Qian et al., 2018
ALR	Two-stage	Pilot	30	–	Sidestream	1150	48	0.6	72	Wang et al., 2017
MBR + UASB	Two-stage	Laboratory	24–32	7.5–8.5	Sidestream	900–1500	–	0.77–2.16	80	Li et al., 2017

MBR: membrane biological reactor.

substance transfer is the crucial, which can only be well realized by the granule sludge. For the application of granule sludge in mainstream wastewater treatment, there have been several breakthroughs in recent years at the laboratory-scale, as shown in Table 2. In the treatment of 50 mg/L ammonium wastewater with a micro granule-based PN/A reactor, the long-term operation results showed that NRR of 0.72 kg/m^3/d were obtained under a relatively short hydraulic retention time (HRT) of 2 h (Chen et al., 2019). In another work, by cultivating a concentrated, highly dispersive granule sludge with good settleability in a one-stage PN/A-hydroxyapatite (HAP) reactor, at an HRT of about 1.0 h and influent ammonium concentration of 63.0 mg/L, the average nitrogen removal rate (NRR) was 1.28 kg/m^3/d, which far exceeds that reported in similar studies (Guo et al., 2021). The impressive performance of these processes achieved with synthetic wastewater demands further verification in the actual treatment of mainstream wastewater.

In the application of the PN/A process to the treatment of both sidestream wastewater and mainstream wastewater, the non-negligible problem is that the maximum NRE of this process is limited by the nitrate production in the anammox reaction (Guo et al., 2020a). We know that at least 11% of influent ammonium is converted to nitrate at the ideal state. With the influent ammonium concentration of several hundred or several thousand mg/L, the effluent nitrate concentration still can reach dozens or hundreds mg/L (Guo et al., 2020a). This is not negligible. Because actual mainstream wastewater has a low ammonium concentration, the residual nitrate in effluent can still meet the discharge standard. However, in the case of the sidestream wastewater, the nitrate produced by anammox reaction cannot be ignored, and the further removal of this nitrogen species is necessary. To overcome this, the following extension process is proposed.

2.2   PD/A process

In the treatment of nitrate wastewater through the anammox process, the PD process is a cost-saving process for nitrite supply (Chen et al., 2021). The traditional denitrification process requires that the organic carbon source supply has a theoretic COD/nitrate ratio of at least 2.86. However, with the PD process, the required COD/nitrate ratio is just 1.14. It represents a COD saving of about 60%. PD is conducted by autotrophic bacteria according to the reaction shown in equation (6) (Ma et al., 2020a).

$$0.048NH_4^+ + NO_3^+ + 0.37CH_3COO^- + 0.32H^+$$

$$\rightarrow NO_2^- + 0.048C_5H_7NO_2 + 0.64H_2O + 0.5CO_2 \quad (6)$$

Developments in the PD process are suitable for three applications. One is suited for the treatment of nitrate

wastewater. In the event that both ammonium wastewater and nitrate wastewater are in one location, the joint treatment of two kinds of wastewater seems feasible. The nitrate in the wastewater can be converted into nitrite, then the nitrite wastewater and ammonium wastewater can finally converge to the anammox process, and the nitrogen species can be removed, as shown in Fig. 3a. This joint process is referred to as the PD/A process (Deng et al., 2019; Yang et al., 2020). The application of this process is limited to situations where certain conditions are met: one is the ability to secure access to both nitrate wastewater and ammonium wastewater in one location; and the other is a balance between nitrite input and ammonium input.

Another development is suited to the treatment of nitrate effluent from the PN/A process reactor in the treatment of sidestream wastewater. The PD process can convert this nitrate into nitrite, which can participate in the anammox reaction again for complete removal according to the specific process design shown in Fig. 3b. In this situation, there are three biological reactions involved in nitrogen removal during the whole process: PN, anammox, and PD. This process is referred to as the PN/A-denitratation (PN/A-PD) process. In a study of the PN/A-PD process, 80% of the nitrate in the effluent of anammox was converted to nitrite, and a maximum NRE of 94.06% was obtained with the total nitrogen (TN) in the effluent at 10.98 mg/L on average (Du et al., 2015). The discussion above leads to the conclusion that the optimal process order for the treatment of sidestream wastewater treatment to achieve the ideal NRE is PN/A-PD PN/A TND.

The third development is suited to the treatment of mainstream wastewater. The application of the PN/A process to the treatment of mainstream wastewater still faces a bottleneck due to its unstable removal performance, the difficulty of AOB inhibition, and low temperature. In recent years, a novel approach proposed is the complete ("complete" means the complete conversion of ammonium to nitrate) nitrification (CN)-PD/A (CN-PD/A) process shown in Fig. 3c (Ma et al., 2017). In this process, part of the influent is introduced into the nitrification reactor, where all the ammonium is completely oxidized to nitrate. Then, this part of flow and another part of influent are introduced into the post-set or pre-set PD/A reactor. In PD/A reactor, the nitrate is stably and reliably converted to nitrite. Meanwhile, the produced nitrite and the ammonium are converted to nitrogen gas by anammox bacteria. Then, nitrate with a low concentration is produced again. In this process, the difficulty of the PN/A process in the treatment of mainstream wastewater due to unavoidable nitrite-oxidizing bacteria (NOB) inhibition and low NRR is avoided, and the nitrogen removal performance is excellent. In a CN-PD/A process for the treatment of mainstream wastewater, an NRE of 80±4% for the influent with a low COD/NH$_4^+$-N ratio of 2.6 and a TN concentration of 60.5 mg/L was achieved (Ma et al., 2017).
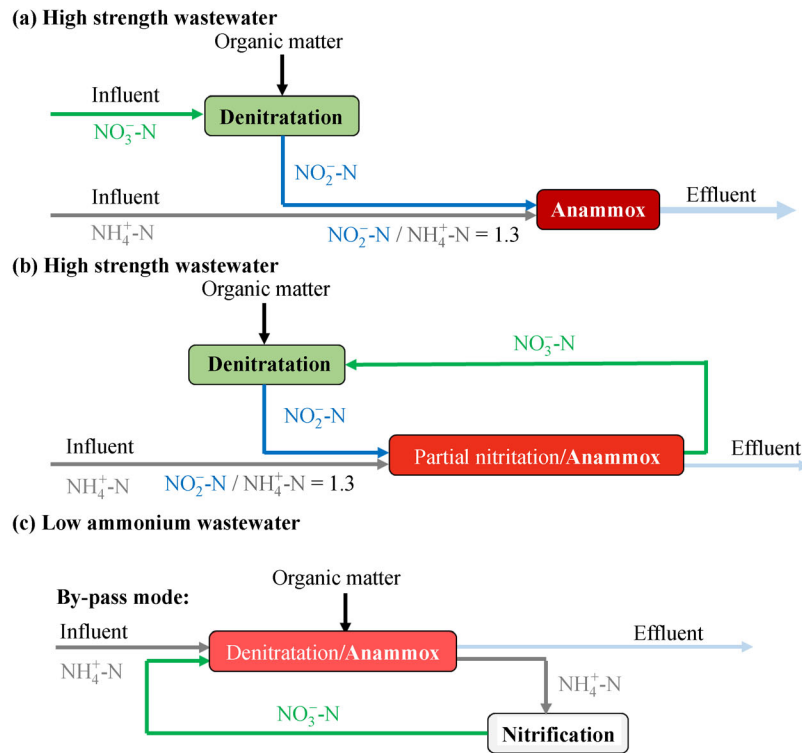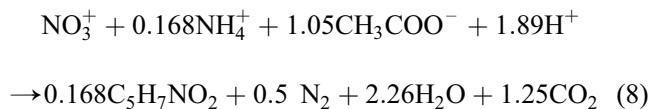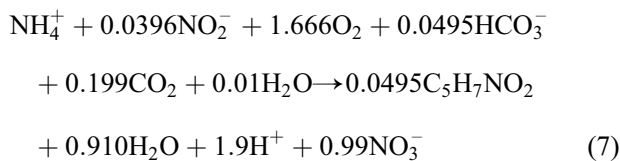
Based on the nitrification equation (7), denitrification

(a) High strength wastewater

(b) High strength wastewater

(c) Low ammonium wastewater

Fig. 3   The actual configuration of the PD/A-based process.

equation (8), the PN equation, the PD equation (Ma et al., 2020a),

$$
\begin{aligned}
&NH_4^+ + 0.0396NO_2^- + 1.666O_2 + 0.0495HCO_3^- \\
&+ 0.199CO_2 + 0.01H_2O \rightarrow 0.0495C_5H_7NO_2 \\
&+ 0.910H_2O + 1.9H^+ + 0.99NO_3^- \qquad (7)
\end{aligned}
$$

$$
NO_3^+ + 0.168NH_4^+ + 1.05CH_3COO^- + 1.89H^+
$$

$$
\rightarrow 0.168C_5H_7NO_2 + 0.5 \; N_2 + 2.26H_2O + 1.25CO_2 \quad (8)
$$

a comparison of TND process, the PN/A process and the CN-PD/A process in mainstream wastewater treatment is shown in Table 3. From table, it can be seen that although the TND process has very strong operability, the PN/A and CN-PD/A processes have obvious advantages with regard to the reduction of oxygen consumption, carbon source consumption, and the reduced sludge production. Despite the higher oxygen consumption of the CN-PD/A (57%),

the carbon source requirements (22.8%), and sludge production (39.31%) than the PN/A process, this process has distinct advantages over the TND, and the operability is strong. Since the actual application of the PN/A process in the treatment of mainstream wastewater still faces difficulty due to unstable performance, the growth of NOB, especially at low temperatures, the second best performer, the CN-PD/A process, is recommended. The nitrogen removal performance of the CN-PD/A process has been reported to be constant (Ma et al., 2020b). From the above, it can be concluded that in the treatment of mainstream wastewater, the process optimal order is CN-PD/A PN/A TND.
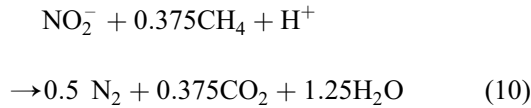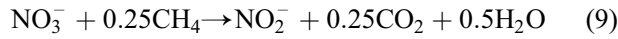
2.3 DAMO/A process

There are basically two kinds of DAMO microbes (Fu et al., 2017a): the DAMO-archaea, which convert the nitrate to the nitrite, as shown in equation (9), and the DAMO-bacteria, which concert the nitrite to nitrogen gas, as shown in equation (10). In an ideal DAMO/A-based

Table 3   The three processes for mainstream wastewater treatment

Process	Oxygen consumption	Carbon source consumption	Sludge production	Operability
TND	100%	100%	100%	Very strong
PN/A	42.3%	0	20.82%	Weak
CN-PN/A	57%	22.8%	39.31%	Strong

process, only the DAMO-archaea and anammox activity is expected for methane saving. However, in actual DAMO/A process, because there is always DAMO-bacteria in the reactor besides the DAMO-archaea and anammox bacteria, a portion of the nitrogen removed can be attributed to the DAMO-bacteria (Xie et al., 2017).

$$NO_3^- + 0.25CH_4 \rightarrow NO_2^- + 0.25CO_2 + 0.5H_2O \quad (9)$$

$$NO_2^- + 0.375CH_4 + H^+$$

$$\rightarrow 0.5 \ N_2 + 0.375CO_2 + 1.25H_2O \quad (10)$$

For the application of the DAMO/A process, methane transportation is the most important step due to the low dissolubility of methane and the unique environment requirement of DAMO microbes. The limitation of methane transfer affects the DAMO/A-based process more notably than nitrogen transfer. Two ways to supply methane to the DAMO/A-based process have been shown: one is the use of a hollow fiber membrane (Shi et al., 2013; Ding et al., 2017), which facilitates the consumption of the methane by the attached biofilm, and the second is by dissolving the methane into the influent (Fan et al., 2019). In practice, the anaerobic fermentation produces methane with the gas phase and the liquid phase. While the methane in the gas phase can be easily collected and purified, the dissolved methane is difficult to recover, especially at lower temperatures. Also, the slow release of the dissolved methane into the environment contributes to the greenhouse effect and makes it undesirable. This suggests that the DAMO/A-based process is capable of resolving both the ammonium removal and methane emission problems in the anaerobic fermentation effluent (Fu et al., 2017a).

The typical reports about nitrogen removal performance of DAMO/A process is shown in Table 4. Initially, the hollow fiber membrane biological reactor is suitable for enriching DAMO microorganisms (Ding et al., 2017). In the membrane biological fiber reactor (MBfR), it was reported that the biofilms formed on fibers surface included two layers: the inner layer was thin and dominated by DAMO-bacteria and anammox bacteria, while the outer

layer was thick made up of granules 100–200 μm in diameter and dominated by DAMO-archaea (Fu et al., 2017a). The high performance of NRR of 16.53 kg/m³/d with satisfactory effluent quality (similar to 8 mg/L) makes the practical application of DAMO/A in wastewater treatment promising (Fan et al., 2019). It has been recently shown that the granular sludge can also be successfully applied for nitrogen removal from synthetic partial nitritation effluent (Fan et al., 2019). It has been revealed that a layered structure is formed in the granular sludge with anammox bacteria in the outer layer and DAMO microorganisms in the inner layer, and a high NRR of 1.0 kg /m³/d is achieved from synthetic sidestream wastewater (Liu et al., 2021).

There are several possible applications of the DAMO/A process. Like the PD/A process, the DAMO/A process is suited for the treatment of nitrate wastewater and ammonium wastewater, as shown in Fig. 4a. It can also be applied to the two-stage PN and DAMO/A (PN-DAMO/A) process (Nie et al., 2019; Liu et al., 2021, 2020), as shown in Fig. 4b. This design is based on the DAMO microbes and the anammox bacteria, both of which require an anaerobic environment (Nie et al., 2019). As shown in Table 4, in an MBfR reactor, with biofilms consisting of a coculture of DAMO and anammox microorganisms, a NRR of 0.68 kg/m³/d was achieved, indicating that the nitrate reduction rate achieved by the DAMO process can be high enough to remove the nitrate produced by the anammox process, therefore enabling complete nitrogen removal from wastewater (Cai et al., 2015). In a two-stage PN-DAMO/A process, a high performance of NRR, at 2.5 kg/m³/d, was reported within 200 days operation, indicating the potential of the DAMO/A process in wastewater treatment applications (Nie et al., 2019). In another two-stage PN-DAMO/A process, with an HRT of 1 day, the process achieved an NRR of over 1 kg /m³/d, and nitrite and ammonium removal rates of 0.56 kg/m³/d and 0.47 kg/m³/d, respectively, with no nitrate accumulation (Xie et al., 2017). An effluent with 3 mg TN/L was achieved simultaneously by integrating anammox and DAMO reactions in mainstream wastewater treatment (Xie et al., 2018). A stable NRR of 0.13 kg/m³/d,

Table 4 The typical reports about nitrogen removal performance of DAMO/A process

Process	Configuration	Scale	Temp (°C)	pH	Wastewater	TN (mg/L)	HRT (h)	NRR (kg/m³/d)	NRE (%)	Reference
DAMO/A	MGSR	Laboratory	35±1	7.8–8.0	Sidestream	1000	1.44	16.53	99.2	Fan et al., 2019
DAMO/A	Granule	Laboratory	30±0.5	7.0±0.2	Sidestream	1050	24	1.0	94.8	Liu et al., 2021
DAMO/A	MAMBR	Laboratory	35 ± 1.0	7.0–7.5	Sidestream	5000	48	2.5	99.9	Nie et al., 2019
DAMO/A	MBfR	Laboratory	–	7.0–8.0	Sidestream	1030	24	1.0	99.9	Xie et al., 2017
DAMO/A	MBfR	Laboratory	–	7.0 ± 0.5	Mainstream	51.5	4	0.28	93.3	Xie et al., 2018
DAMO/A	MBfR	Laboratory	10	7.0 ± 0.5	Mainstream	51.5	9	0.13	90–94	Liu et al., 2020
PN-DAMO/A	MBfR	Laboratory	30 ± 1	7.0 ± 0.5	Sidestream	1030	16	1.5	98	Liu et al., 2019

MGSR: Membrane Granular Sludge Reactor; MAMBR: Membrane Aerated Membrane Bioreactor.

together with a high-level effluent quality (TN < 5.0 mg /L) was also achieved in a laboratory-scale upflow MBfR by the DAMO/A process at a temperature as low as 10°C (Liu et al., 2020). The third process, named the one-stage PN-DAMO/A, integrates PN, anammox and DAMO in a single MBfR, as shown in Fig. 4c. With sidestream wastewater feeding, the proposed one-stage PN-DAMO/A process achieved an average NRE of 98% and a NRR of 1.5 kg/m^3/d (T. Liu et al., 2019).

There are several issues in the wastewater treatment adopting the DAMO process. First, methane is characterized with the inflammability and low aqueous solubility. In laboratory-scale studies, the increased energy for sparging methane to the reactor, the additional cost in the methane transportation system and the release of methane in actual applications are the drawbacks (Shi et al., 2013). Second, anammox bacteria, DAMO-bacteria and DAMO-archaea are involved in nitrogen removal in the DAMO processes. The control of DAMO-bacteria is necessary to save methane. It has been reported that competency of DAMO-bacteria is lower in the presence of nitrite and ammonium than anammox bacteria (Stultiens et al., 2019). Therefore, operation parameter controls may need to be adopted to control DAMO-bacteria.

3   The characteristics of functional microbe species

3.1   Anammox species

To date, six anammox genera and at least 24 species have been detected, as shown in Table 5. It should be noted that few investigations have been focused on identifying most of these species, and that the environments have also been limited. For instance, species *moscowii* has only been reported once, and most species of the genus *Scalindua* have only been found in environments with high salinity. The anammox species detected in laboratory-scale reactors and WWTPs are mainly from the genus of *Anammoxoglobus*, *Brocadia*, *Jettenia*, and *Kuenenia* (Song et al., 2020). As each species has a defined but as yet unknown niche, two different anammox species can rarely be found in the same natural location (Kartal et al., 2007b). In addition, the growth and optimal temperature (Tomaszewski et al., 2017), salinity (Gonzalez-Silva et al., 2017), nitrogen loading rate (NLR) (Zhang et al., 2017), HRT (Park et al., 2015), disproportionate substrate concentration (Qiao et al., 2017), and volatile fat acids (VFAs) (Nejidat et al., 2018) are crucial parameters which
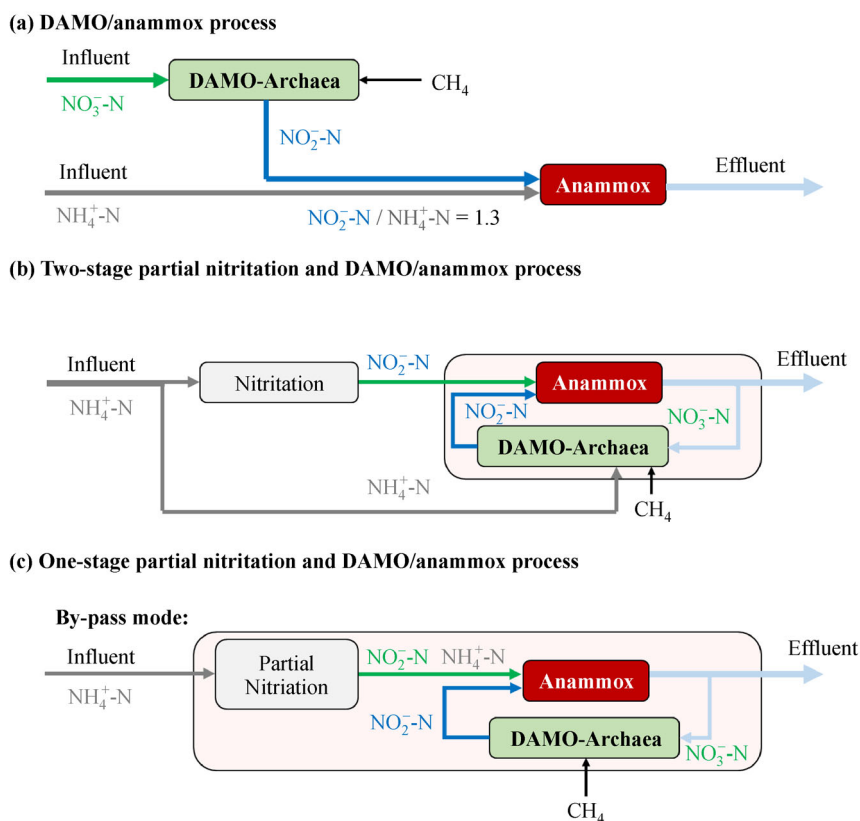

(a) DAMO/anammox process

(b) Two-stage partial nitritation and DAMO/anammox process

(c) One-stage partial nitritation and DAMO/anammox process

Fig. 4   The actual configuration of the DAMO/A-based process.

Table 5 The reported anammox species and their main features

Genus	Species	μ_{max}(h^{-1})/DB(d)	Tolerance environment Temp. (°C)/pH	Main characteristics and preferred habitat
Anammoxoglobus	propionicus	n.d	33/7−7.3	Out-compete other anammox bacteria and heterotrophic denitrifiers for the oxidation of propionate; Dominant in SNAD process.
Brocadia	anammoxidans	0.0027/n.d	20−52/6.7−8.3	The predominant species in low temperature mainstream.
	fulgida	n.d	10−52/7.2−8.3	Strong autofluorescence; Out-competed other anammox bacteria in the presence of acetate; Capable of organotrophic nitrate reduction; Become worst at salinity; Survives with IC deficit; Tolerates low temperature until 10°C.
	sinica	0.33/2.1	25−45/6.5−8.8	Dominated at high NLRs; The capacity to oxidize the VFAs; Good tolerance to salinity, low temperature, phenol and thiocyanate; Superior aggregation ability, better biomass retention as granules and consequently stable performance.
	caroliniensis	0.005/n.d	30/6.8−7.6	Enriched in nitrite shunt process.
	sapporoensis	0.0082/3.5	20−45	-
	brasiliensis	n.d	n.d	Raised significantly at low substrate concentration condition; Found in activated sludge.
Jettenia	asiatica	n.d	n.d	Found in granular sludge; Can utilize VFAs as carbon sources; Higher adaptability to propionate stress than acetate; Show tolerate to phenol; Can adapt to low temperature; An alternative lifestyle to chemolithoautotrophy.
	caeni	0.18/3.9	20−42.5/6.5−8.5	Found in membrane bioreactor; Dominated at low NLRs.
	moscovienalis	0.025/28	20−45/8.0	Extensive intracellular membrane structures.
Kuenenia	stuttgartiensis	0.004/n.d	25−52/6.5−9.0	The common species in research; Good tolerance to salinity, higher affinity for nitrite, low temperature; Has a protein surface layer as the outermost layer of the cell.
Scalindua	sorokinii	n.d	n.d	Found from Black sea; Existed in high ammonium and low nitrite conditions from Black Sea
	richardsii	n.d	n.d	Found from Black sea; Existed at high nitrite/nitrate and low ammonium from Black Sea
	brodae	n.d	n.d	Found from WWTPs; Existed in soil.
	wagneri	n.d	n.d	Found from WWTP; Comparable or even higher tolerances for high NO_2-N.
	arabica	n.d	n.d	Found from Arabian Sea.
	pacifica	n.d	n.d	*Found from Bohai Sea; Versatile life style.*
	profunda	0.002/n.d	15−45/7−8	Cyanate use for anammox reaction.
	sinooilfield	n.d	n.d	Tolerate High-Temperature in petroleum reservoirs.
	rubra	n.d	n.d	Uses compatible solutes for osmoadaptation, found in Red Sea.
	zhenghei	n.d	n.d	Found from South China Sea.
	japonica	n.d	n.d	−
Anammoximicrobium	moscowii	n.d	n.d	Enriched from river in Moscow.

determine the metabolism of each kind of anammox bacteria species. Also, some anammox bacteria species show strong adaptability to extreme environments. Therefore, it is likely that the niches of anammox bacteria are much broader than generally thought, which suggests they may be able to exert their specific roles in applications for the treatment of special wastewater. There are several categories of environment-specific species categories. They are discussed in the following section:

3.1.1 The volatile fatty acids-specific species

For some kinds of actual wastewater, such as digested effluent, VFAs are an unavoidable component (Guo et al., 2020a). In studies incorporating simultaneous PN/A and denitrification (SNAD), the dominant species in the adopted reactor was *propionicus* (Wen et al., 2017), which could out-compete other anammox bacteria, even heterotrophic denitrifiers, for the oxidation of propionate

(Kartal et al., 2007b). In the presence of acetate, The species *fulgida* also out-competed other anammox bacteria to realize organotrophic nitrate reduction (Winkler et al., 2012). Species *stuttgartiensis* cells can conduct DNRA to reduce nitrate to ammonium via nitrite (Kartal et al., 2007a). The *species fulgida* performs the DNRA/anammox reaction at a high rate in the presence of VFAs (Castro-Barros et al., 2017). Besides, it has also been reported that the species *sinica* and species *asiatica* also could oxidize the VFAs (Shu et al., 2015).

3.1.2   The temperature-specific species

The species capable of tolerating specific temperatures are detailed in Table 5. The growth temperature and optimal temperature of different species of anammox varied (Tomaszewski et al., 2017). The activation energy of the anammox reaction is higher than that of wastewater. The change in the nitrogen removal performance of the anammox-based process was attributed to reduced enzyme activity. With long-term cultivation, the adaptation of anammox bacteria at low temperature has also been observed (Lotti et al., 2015). Especially, species *sinica* was shown to have good tolerance to low temperature (J. Li et al., 2018).

3.1.3   The salinity-specific species

In a natural saline environment with a salinity of 84 g/L, the activity of anammox bacteria was still observed (Yang et al., 2012). It was found that species *sinica* and species *stuttgartiensis* had good tolerance to salinity (Gonzalez-Silva et al., 2017; J. Li et al., 2018). As expected, the genus *Scalindua* showed strong tolerance to salinity. However, the utilization of genus *Scalindua* in WWTP was poor. More exploration is required for a better understanding of this.

3.1.4   The NLR-specific species

At high NLRs, the species *sinica* was found to be dominant (Zhang et al., 2017; Cho et al., 2018; Oshiki et al., 2018). However, at low NLRs, the species *caeni* was found to be dominant (Ma et al., 2017; Zhang et al., 2017).

3.1.5   The substrate-specific species

As known to all, in the operation of anammox reactor, with the external factors, like the fluctuations of substrate and the operation parameters, being resolved, the reminding crucial aspect is inhibition effect of anammox bacteria by FA or FNA, especially the FNA. For the fresh water experiment, the genus *Brocadia* and the genus *Kuenenia* is a focus. Based on the previous theory, the genus *Brocadia*

is the R-strategy, while genus *Kuenenia* is the S-strategy. In earlier studies, there were some contradictions about this hypothesis (Tang et al., 2010). It should be noticed that it is the actual nitrite concentration in reactor that determine the selection of anammox species rather than the influent nitrite concentration. In recently years, this hypothesis were verified in many one-stage PNA reactor and anammox reactor (Chen et al., 2019; Guo et al., 2020c). Also, in our laboratory, in the long term operation of both pure anammox reactor and the one-stage PNA reactor, with the low nitrite strategy or the no nitrite accumulation strategy, the almost pure genus *Kuenenia* was selected.

In addition, large increases were reported in the species *brasiliensis* under limited substrate conditions (W. Li et al., 2017). Species *stuttgartiensis* is known to have higher affinity for nitrite (van der Star et al., 2008; Ren et al., 2016). It was reported that species *sorokinii* was found in high ammonium and low nitrite condition. In reverse, in high nitrite/nitrate and low ammonium condition, species *richardsii* was detected. The high nitrite condition is favorable for species *wagneri* (Yokota et al., 2018).

3.1.6   Hazardous substance-specific species

It has been reported that species *sinica* is well able to tolerate phenol and thiocyanate (J. Li et al., 2018a). The species *asiatica* is also found in phenol environment (Pereira et al., 2014).

3.1.7   Other environment-specific species

Species *fulgida* has the special characteristic of auto fluorescence. However, in some environments, the auto fluorescence will disappear (Böllmann et al., 2019). The shortest doubling time of anammox species was reported to be only 2.1 d by species *sinica*. Species *sinica* has a special NH$_2$OH‑dependent metabolic pathway (Oshiki et al., 2016). Under biodegradable organic matter (BOM) environment and high nitrate, species *sinica* still can grow (Shu et al., 2015; Li et al., 2016). Although some species belonging to *Scalindua* were discovered in WWTP, other species was seldom detected. The above characteristics of each species should be the basis for the exploration of proper anammox species for specific wastewater. In the quest to further the application of the annamox process, this should become a research spot.

3.2   AOB species

Five AOB genera have been detected belonging to the class *Proteobacteria*, as shown in Table 6. Four genera were classified to the *β-Proteobacteria* subclass, like *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio* and *Nitrosolobus*, while one genera, *Nitrosococcus*, belongs to the *γ-Proteobacteria* subclass. AOB characteristics and

Table 6 The reported AOB species and their main features

Class	Genus	Species	Salt requirement (%)	Max. Amm. affinity (mM)	Preferred habitat
Beta Proteobacteria	*Nitrosomonas*	*Nitrosomonas europaea*	2.3	400	Common species in WWTPs; Sewage disposal plants.
		Nitrosomonas eutropha	2.3	600	Sewage disposal plants/Eutrophic.
		Nitrosomonas halophila	5.3	400	Brackish water.
		Nitrosococcus mobilis	2.9	250	Eutrophic /Aquatic.
		Nitrosomonas communis	1.5	250	Moderate eutrophic pH neutral soils/Freshwater.
		Nitrosomonas nitrosa	1.8	100	Eutrophic freshwater/Marine environment/ WWTPs.
		Nitrosomonas oligotropha	0.8	50	Oligotrophic freshwater/Natural soils.
		Nitrosomonas ureae	1.2	200	Oligotrophic freshwater/Natural soils.
		Nitrosomonas marina	4.8	200	Marine.
		Nitrosomonas aestuarii	3.6	400	Marine.
		Nitrosomonas cryotolerans	3.2	400	Marine; Low temperature as low as 5°C).
		Nitrosomonas sp. PY1	n.d	n.d	Activated sludge.
		Nitrosomonas sp. NP1	n.d	n.d	
		Nitrosomonas sp. SN1	n.d	n.d	
		Nitrosomonas mobilis Ms1	n.d	n.d	WWTP granules.
	Nitrosopira	*Nitrosospira briensis*	1.5	200	Natural soils/Freshwater/Marine.
	Nitrosovibrio	*Nitrosovibrio tenuis*	0.6	100	Natural soils.
	Nitrosolobus	*Nitrosolobus multiformis*	1.2	50	Soils/Sewage disposal plants.
Gamma Proteobacteria	*Nitrosococcus*	*Nitrosococcus oceani*	6.4	1000	Marine.
		Nitrosococcus halophilus	10.5	500	Marine/Salt lakes.
		Nitrosococcus wardiae D1FHS	n.d	n.d	Eutrophic marine sediment.
	Nitrosoglobus	*Nitrosoglobus terrae*	n.d	n.d	Acidic soil.

predominant species vary based on the treatment configuration, substrate and experimental conditions (Aoi et al., 2000).

3.2.1 Carbon source-specific species

The common species in WWTPs *N.* is *europaea*. The AOB which has been characterized in the most detail is the bacterium *N. europaea* so far (Woebken et al., 2020). In biofilm reactor, there is an interesting phenomenon between *N. europaea* and associated heterotrophic bacteria: with a limited BOM environment, through ammonium oxidation, the *N. europaea* provide BOM to heterotrophic bacteria, which then forms a biofilm suitable for the attachment of *N. europaea* (Keshvardoust et al., 2019).

3.2.2 Salinity-specific species

In the treatment of sidestream wastewater, salinity increases from zero to 3.0%, and *N. europaea* and *Nitrosomoccus mobilis* are the salinity-tolerated AOB species. Especially, *Nitrosomoccus* has been shown to the extremely high salinity-tolerant (Guo et al., 2020c).

In the case of mainstream wastewater with low salinity, *N. marina* was dominant, while with high salinity, *N. oligotropha* was dominant (Wu et al., 2020). In a WWTP, with a high salinity level, *N. marina* were found dominant, while with low salinity level, *N. urea* became dominant (Wu et al., 2013). In treating wastewater from a thermal power plant, it was reported that *N. halophila* was responsible for ammonium oxidation.

3.2.3 Temperature-specific species

AOB benefits more from high temperatures than NOB. However, in actual application, a high-energy input is a concern for the high temperature environment. With a high temperature of 35°C–45°C and industrial wastewater treatment, *N. nitrosa* was observed (Shore et al., 2012). It was reported that *N. communis* could adapted to the high temperature in soil (Bei et al., 2021). In a sequence batch

reactor (SBR), with the temperature decreased from 25°C to 12°C and the control of FA, *N. eutropha* and *N. nitrosa* were found to be more fierce than other species (Qi et al., 2020).

3.2.4   Dissolved oxygen (DO)-specific species

The AOB has a higher affinity for oxygen than NOB. The growth yield of AOB was twice that of NOB under a limited DO environment (Guo et al., 2020c). It was found that in a high DO environment, *N. oligotropha* was dominant, while in a low DO environment, *N. europaea* were enriched (Liu et al., 2019).

3.2.5   Substrate concentration-specific species

AOB can also be divided into the k-strategists and r-strategists (Soliman and Eldyasti, 2018). Based on a study from 11 WWTPs, the dominant species were *N. europaea* and *N. eutropha* under high ammonium environment, whereas *N. ureae*, *N. oligotropha* and *N. marina* were dominant under the low ammonium environment (Otawa et al., 2006). At a low NLR, the predominant AOB species was *N. europaea*, while at a high NLR, it was *N. oligotropha* (Zhang et al., 2018). For steel wastewater treatment with a high ammonium content, *N. aestuarii* s survived for nitritation (Cho et al., 2016). In various WWTPs, *N. mobilis* is an important AOB. *N. mobilis* was shown to be able to tolerate nitrite at 300 mM (Thandar et al., 2016).

FA and FNA can inhibit AOB and NOB (Zhang et al., 2021). A proper high FA concentration favors AOB growth over NOB growth. However, it was reported that FA inhibition for NOB is reversible. It was reported that the most effective inhibition strategy for NOB was the control of FA and FNA at a temperature below 25°C.

3.3   Denitratation species

Group A and group B are the two groups of DB with BOM, (Martienssen, 1997; Phanwilai et al., 2020), as shown in Table 7. Group A can only convert nitrate to nitrite, while group B can first convert nitrate to nitrite, then after the depletion of nitrate, it further converts the accumulated nitrite to nitrogen gas. In practice, these two groups of denitratation bacteria coexist in WWTPs. BOM can also be used for denitrification, which is conducted by denitrifying glycogen-accumulating organisms (DGAOs), as shown in Table 7.

The microbe community, organic matter and pH may affect nitrate reduction by DB. It has been reported that high pH conditions were beneficial to achieve denitratation with a denitrification reactor (Si et al., 2018). The extent of the effects is also depend on the duration time (Ma et al., 2020b). Some drawbacks facing the practical application of denitratation. First, the growth rate of the anammox biomass would be significantly suppressed by DB. The higher biomass yield and growth rate of DB could prevent substrate transfer for anammox bacteria, finally reducing the NRE (Lotti et al., 2015; Zhang et al., 2017). Thus, it is crucial to control the BOM content in influent. Second, some kinds of anammox bacteria can alter their metabolism pathway with the existence of other electron donors, like propionate.

3.4   DAMO species

The microbes involved in the DAMO reaction are shown in Table 8. DAMO and anammox have some similarities: both are characterized by the slow grow rate of microbes and show potential for actual application. In the DAMO reaction, DAMO-archaea reduced nitrate to nitrite coupled to methane oxidation, while DAMO-bacteria reduce the generated nitrite to nitrogen gas (Fu et al., 2017a).

Table 7   The reported denitratation species and their main features

Carbon source type	Categories	Species	Characteristics
Exogenous carbon source	Group A	*Staphylococcus* sp.	Only capable of reducing nitrate to nitrite.
		Rhodobacter sphaeroides 2.4.1	Contains nitrate reductase but not nitrite reductase.
	Group B	*Thauera aminoaromaticaa*	Showing a progressive onset (PO) of denitrification genes, with no transcription of nirS (encoding nitrite reductase) detected until all nitrate was reduced, resulting in the accumulation of nitrite.
		Thauera phenylaceticaa	
		Thauera sp. *DNT-1*	
		Thauera terpenicaa	
Endogenous carbon sources	DGAOs	*Dechloromonas*	Very rapid in assimilation of propionate.
		Accumulibacter	Could behave as GAO under poly-P-limiting conditions.
		Comamonadaceae unclassified	Could transform carbon sources into PHAs for denitritation.
		Thermomonas	Mainly utilize nitrate under acetate conditions.
		Dechloromonas	Played a key role in carbon glycolysis and acidification of slowly biodegradable organic matter.
		Saccharibacteria	Enriched in glycerol-driven reactors.

Table 8 The reported DAMO species and their main features

Microbe	Species	Characteristics
DAMO bacteria	M. oxyfera	Rod-shaped; 0.25–0.5 × 0.8–1.1 μm; A slow doubling time of 11.5 d was estimated; Dominated in highly contaminated nitrate and oxygen environment; Harbors three methanol dehydrogenases.
	M. sinica	Enriched in the nitrite reactor; roughly coccus-shaped (0.7–1.2 μm); Grew in honeycomb-shaped microcolonies;
	M. limnetica	Dominated the planktonic microbial community in the anoxic depths of the deep stratified Lake; Constituted up to one third of all metatranscriptomic sequences in situ; The reconstructed genome encoded a complete pathway for methane oxidation, and an incomplete denitrification pathway, including two putative nitric oxide dismutase genes.
	M. lanthanidiphyla	Enrichment of 'Ca. M. oxyfera' with cerium added as trace element and without nitrate; Encode a lanthanide-dependent XoxF-type methanol dehydrogenases.
DAMO archaea	Methanoperedens nitroreducens	Comply with first order kinetic model with a rate constant of 0.019 ± 0.006 h^{-1} and a biomass-specific rate constant of 0.04–0.14 L/(h× g−VSS); Capable of independent AOM through reverse methanogenesis using nitrate as the terminal electron acceptor.

M. oxyfera is classified as a "NC10" phylum (Fu et al., 2017b). This bacterial species has a unique ultrastructure that is distinct from that of other previously described microorganisms. *M. oxyfera* reduces nitrite to nitric oxide and then achieves methane oxidation using the in situ produced oxygen from the dismutation of nitric oxide. *M. nitroreducens* reduces nitrate to nitrite with methane served as the electron supplier. In contrast to *M. oxyfera*, which employs aerobic methane oxidation, *M. nitroreducens* oxidizes methane through reverse methanogenesis. This pathway was very recently proven. *M. nitroreducens* has a slightly higher affinity constant for nitrate than that of most known denitrifying bacteria (Lu et al., 2019). It was reported that the optimized temperature for DAMO-archaea reaction is 35°C. At both pH 6.5 and 7.5, the maximum DAMO-archaea reaction rate appeared. It was reported that the DAMO-archaea is inhibited in environments with the existence of DO (Lou et al., 2020). It was also reported that in MBfR, DAMO-archaea could convert nitrate to ammonium (Nie et al., 2021).

The low activity and slow doubling times of DAMO microbes have limited its practical application. However, the strategy of adding Geobacter sulfurreducens and hydroxyapatite was shown to stimulate the DAMO process due to the acceleration of electron transfer through conductive materials (Chang et al., 2021a, 2021b). It was also reported that the DAMO activity was obviously stimulated by the nucleobase and betaine (Wang et al., 2019). Supplementation of basal medium with PQQ is also an effective strategy for the enrichment DAMO-bacteria (Hatamoto et al., 2018).

The enrichment of DAMO culture can be conducted with the SBR reactor configuration (W. Li et al., 2018). The successful cultivation of DAMO-bacteria granules was conducted with the SBR reactor (He et al., 2015a). It was reported that DAMO-archaea could be enriched by regulating the trace element iron (Lu et al., 2018), and that the cultivation of the DAMO-bacteria by the magnetically stirred gas lift reactor is also desirable.

Freshwater canal sediment, freshwater lake sediment, paddy soil, anaerobic digester sludge and return activated sludge from a sewage treatment plant have been successfully adopted as inoculum for DAMO cultivation (Shen et al., 2015). The metabolic activity and growth rate of DAMO-bacteria were affected by temperature, pH and salinity (He et al., 2015b).

It was reported that under an environment with both nitrite and ammonium, the competency of DAMO-bacteria is lower than anammox bacteria (Hu et al., 2015; Stultiens et al., 2019). However, in the one-stage PN-DAMO/A process in an MBfR, the AOB, anammox bacteria, DAMO-bacteria and DAMO-archaea coexisted in the biofilm (T. Liu et al., 2019). DAMO-bacteria has high diversity in the nitrate reactor, with one similar to *M. oxyfera*, while the DAMO-bacteria in the nitrite reactor were relatively unified and close to *M. sinica* (Fu et al., 2017b). In a high nitrate and oxygen environment, *Methylomirabilis* was the dominant population (Luo et al., 2018). It was also proposed that adopting the low-temperature operation to enhance methane dissolution, which also can reduce the cost of warming the reactor (Li et al., 2020).

With salinity as high as 84 g/L, it was also found that DAMO and anammox bacteria could co-occur (Yang et al., 2012). The optimum salinity for the metabolic activity and growth rate of DAMO-bacteria is 0 g NaCl/L. Under salinity stress of 20 g NaCl/, salinity adaption of DAMO-bacteria was also found (He et al., 2015b).

4 The existing outstanding problems

From above, it is obvious that while there has been clear progress in the anammox-based processes, there are still questions which need to be resolved from the actual application perspective and a more comprehensive perspective.

4.1 The competence relationship between PD and DAMO

It is known that in some kinds of wastewater, like digestion effluent, there is both BOM and dissolved methane in the liquid (Liu et al., 2021). For the utilization of this dissolved methane as an electron donor in the DAMO process, the DAMO process for nitrate reduction should come before the PD process since the methane easily escapes from the liquid. However, there are no reports about the effect of BOM on the DAMO process. Even in the DAMO reactor, with the synchronic existence of BOM, it remains unclear whether the DB forms the competition relationship with the DAMO-archaea for nitrate. These points should be considered in future research focused on utilizing the dissolved methane in the digestion effluent.

4.2 The release of methane from the influent

Methane is one of the least reactive organic molecules. In the treatment of digestion effluent, because the discharged effluent is exposed to the open air, it is crucial to find a way to effectively capture the methane for DAMO reaction and not allow it to be simply released to the air. With the escape of methane from liquid, not only is the DAMO activity effected, but the released methane is an environmental problem (Shi et al., 2013). This highlight the importance and complexity of operating the DAMO reactor well.

4.3 The precise control of methane and BOM supply

In actual applications, the precise control of methane and BOM in the influent from the anaerobic reactor is an important point for the healthy conversion of nitrate. The excessive supply of methane not only causes the loss of methane production in the upstream anaerobic fermenta-

tion step, but also results in greenhouse gas emissions (Guo et al., 2020a). The inadequate supply of methane and BOM in the influent results in a slowdown in the microbe metabolism. Thus, cooperation between the upstream and the downstream is necessary to balance the energy recovery and the carbon source utilization.

5 The proposed novel process system for waste treatment

Considering all the above, it is possible to propose a novel integrated system for waste treatment based on an overall perspective. This system is comprised of two process lines with the exchange of substances in order to maximize the utilization of existing substances, like nitrogen species, methane, and organic matter in wastewater. A schematic of this system is shown in Fig. 5. In the mainstream wastewater treatment process line, the organic matter in mainstream wastewater is first removed through the COD capture process, making the effluent suitable for nitrogen removal through PN/A process (Guo et al., 2020b). The settled sludge is transferred to the anaerobic fermentation reactor for methane gas production. Due to the imbalance of nutrient substances in the sludge, other biomass waste (like waste from the food industry, stockbreeding, and agriculture) can also be poured into the anaerobic reactor to enhance methane production. The discharge of the anaerobic fermentation reactor can be divided into two kinds: the solid fermentation dregs, which can be utilized as fertilizer, and the digestion liquid with a high ammonium concentration, phosphorus, and organic matter and dissolved methane, which can be transferred to the sidestream wastewater treatment line (Guo and Li, 2020). The digestion liquid is first transferred to the DAMO/
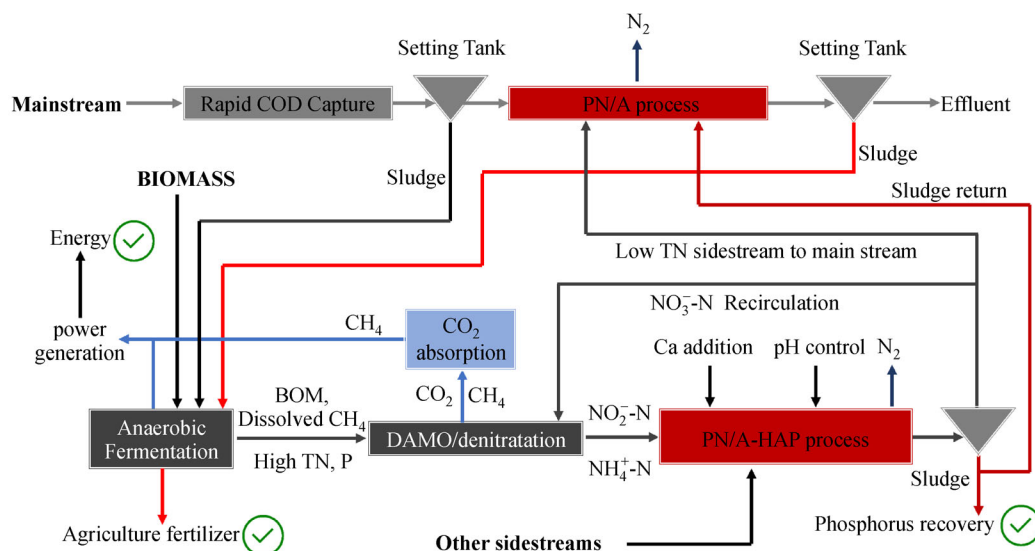

Fig. 5 The proposed future waste treatment system integrating each anammox-based processes.

denitratation reactor for methane and organic matter utilization for the removal of nitrate, which comes from the later PN/A-HAP reactor. In the DAMO/denitratation reactor, with the production of CO_2, the partial pressure of methane is further reduced according to the henry law, resulting in the escape of methane from the fermentation liquid, and the gas contain the methane and the carbon dioxide. This gas is passed through the carbon dioxide absorption equipment to recover the high quality methane gas, ensuring no detrimental greenhouse effect from the process. After removing the methane and organic matter, the digestion liquid enters the PN/A-HAP reactor for nitrogen removal and phosphorus recovery (Guo and Li, 2020). Of course, other types of sidestream wastewater (like fill land leachate, fertilizer industry, electron industry) are also well-suited to the PN/A-HAP reactor. The nitrate in the effluent produced by the anammox reaction can be partly recirculated to the DAMO/denitratation reactor for nitrite production (Du et al., 2015), which can then be sent to the PN/A-HAP reactor for removal. The formed sludge, which has a good settling property, can be transformed to the PN/A reactor for mainstream wastewater treatment (Guo et al., 2021). It is acknowledged that achieving the removal of all of the nitrogen in the PN/A-HAP reactor is not possible due to the high cost and the need for precise control. However, with the most of nitrogen species in the digestion effluent being removed, the low total nitrogen effluent can be transformed to the mainstream wastewater treatment process line for further nitrogen removal. In the PN/A reactor, both the mainstream wastewater and the effluent of PN/A-HAP reactor can be merged for further nitrogen removal. The effluent of the HAP reactor after settling can be discharged, while the settled sludge can be transformed to the fermentation reactor for methane production again. The only sludge discharge location is on the PN/A-HAP reactor. Due to the high phosphorus content in the sludge, the potential for phosphorus recovery from the sludge is promising (Guo and Li, 2020).

The future waste treatment system is not just focusing on the wastewater. The application scenario of this future waste treatment system is the mainstream, sidestream, and the organic waste, like the food waste, kitchen waste, activated sludge and so on, discharged by the whole society. It targets at the carbon, nitrogen and the phosphorus in the wastewater and the organic wastewater. Through the combination of a lot of novel processes, the most effective treatment can be achieved from many perspectives, like the energy recovery, the low-cost nitrogen removal, the phosphorus resource recovery, and the minimum environment effect.

6   Conclusions

In recent years, there have been significant developments in the anammox-based process toward its wide practical application. Many novel anammox-based processes have been developed by combining the anammox process with other processes, like denitratation and the DAMO process. Among them, the PN/A process, the DN/A process and the DAMO/A process have received considerable attention due to their practical operability in actual wastewater treatment. Novel technologies like the PN/A-HAP process and the CN-PN/A process have paved the way to mainstream wastewater treatment. While the nitrogen removal performance of the DAMO/A process has been demonstrated to be both excellent and thorough, further exploration considering reducing the cost and the complexity is required for actual application. Since there is considerable diversity in the metabolic activity of the functional microbes in the anammox-based process, further exploration to optimize the specific characteristics in actual applications is promising. Through the effective allocation of each anammox-based processes in the whole waste treatment system, it is considered feasible to maximize energy and resource recovery and minimization the detrimental effect on the environment in waste treatment.

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