



Review

A review of anammox-based nitrogen removal technology: From microbial diversity to engineering applications

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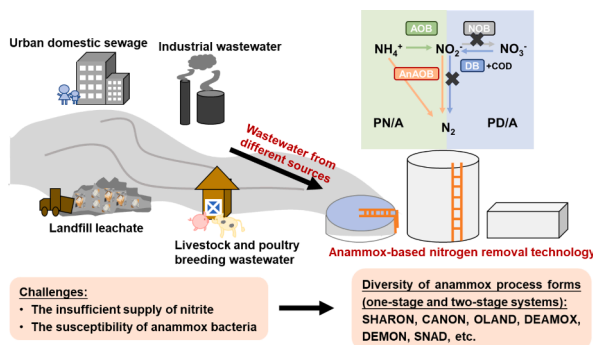
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HIGHLIGHTS

- The habitats and characteristics of different anammox bacteria were compared.
- The diversity of anammox process forms was compared and analyzed.
- The application of nitrogen treatment from different sources was reviewed.
- The obstacles and regulation strategies of anammox technology were prospected.

GRAPHICAL ABSTRACT



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ABSTRACT

The anaerobic ammonium oxidation (anammox) process has the advantages of high efficiency and low energy consumption, so it has broad application prospects in biological denitrification of wastewater. However, the application of anammox technology to existing wastewater treatment is still challenging. The main problems are the insufficient supply of nitrite and the susceptibility of anammox bacteria to environmental factors. In this paper, from the perspective of the diversity of anammox bacteria, the habitats and characteristics of anammox bacteria of different genera were compared. At the same time, laboratory research and engineering applications of anammox technology in treating wastewater from different sources were reviewed, and the progress of and obstacles to the practical application of anammox technology were clarified. Finally, a focus for future research was proposed to intensively study the water quality barrier factors of anammox and its regulation strategies. Meanwhile, a combined process was developed and optimized on this basis.

1. Introduction

In recent years, increasingly strict nitrogen discharge standards have promoted the development of nitrogen pollutant removal technology.

Traditional nitrification and denitrification biological nitrogen removal techniques have been widely used as effective methods for treating nitrogen pollutants in domestic sewage and industrial wastewater. A large amount of energy consumption promotes the continuous development

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of traditional nitrogen removal technology. Anaerobic ammonium oxidation (anammox) has been considered one of the most effective nitrogen removal processes since it was proposed in the Netherlands in 1990 (Lackner et al., 2014). Compared with traditional nitrification and denitrification processes, this process can directly produce nitrogen under anoxic conditions using ammonium as an electron donor and nitrite as an electron acceptor (Strous et al., 1998). Therefore, anammox has the advantages of having no additional organic carbon source, a high nitrogen removal load, a low operating cost and a small footprint (Lawson et al., 2017).

In recent years, substantial laboratory results have been obtained in research on anammox processes. However, the application of the anammox process to actual wastewater treatment remains a large challenge. On the one hand, anammox bacteria grow slowly (doubling time 10–12 days) and have a long start-up cycle and low cell yield (Wu et al., 2022). On the other hand, due to the complex composition of actual wastewater, there are often insufficient electron acceptors. At the same time, anammox bacteria are sensitive to environmental conditions, and actual wastewater often contains anammox inhibiting substances, such as antibiotics (Zhang et al., 2021c), heavy metals (Ma et al., 2021), and salinity (Zhang et al., 2021b). These problems limit the large-scale application of anammox processes in practical engineering.

Due to the complex composition of actual wastewater, different types of wastewater present specific challenges for sensitive anammox bacteria. This limitation emerges as a big challenge for application of anammox technology in the field of wastewater treatment. Currently, most anammox processes need to be combined with other processes to provide substrates for anammox bacteria while simultaneously addressing more complex wastewater. Partial nitrification (PN) can utilize ammonia oxidizing bacteria (AOB) to provide nitrite as a key substrate for anammox bacteria. Since both AOB and anammox bacteria are autotrophic, partial nitrification/anammox (PN/A) does not require the addition of additional organic carbon, it can save approximately 60 % of the aeration cost (Wang et al., 2022). However, low influent ammonia concentrations and temperature conditions are a challenge to maintaining the activity and growth rate of AOB and anammox (Wang et al., 2022). At the same time, if the C/N ratio in urban sewage is too high, then anammox bacteria are susceptible to the adverse effects of organic carbon. In recent years, researchers have found that partial denitrification (PD) can provide a more stable nitrite for the anammox process (Du et al., 2016). In conclusion, there are many problems to be clarified about the applicability of anammox coupling process to various types of wastewater.

At present, the actual field application of the anammox process is still limited by the actual sewage temperature, ammonia nitrogen concentration, organic concentration and other factors. Its coupling with PN, PD and other processes is also in the experimental stage. Based on the above problems, it is necessary to systematically summarize the application examples and experiences implementing anammox processes in recent years to promote the further industrial application of anammox processes to ensure this process plays a more active role in the field of wastewater denitrification treatment. This review introduces the diversity of anammox bacteria and anammox processes. According to different types of sewage water quality conditions, the selection of integrated or split process types and the theoretical key factors for the stable operation of the process are discussed. The application of anammox technology in the treatment of various kinds of wastewater are reviewed. Also, the advantages and limitations of anammox denitrification technology were fully evaluated based on the laboratory and engineering application.

2. Biodiversity of anammox bacteria

Anammox bacteria that mediate the anammox process were first found in wastewater, and since then, anammox bacteria have been found to be widely distributed in anoxic areas in natural ecosystems.

These areas include marine sediments (Rios-Del Toro et al., 2017), mangrove areas (Dai et al., 2021), oxygen minimum zones (Karthäuser et al., 2021), etc.

As major players in the global nitrogen cycle, anammox bacteria belong to the phylum Planctomycetes (Ahmad et al., 2020). Up to now, six genera of anammox bacteria have been discovered, currently including *Candidatus* Jettenia, *Candidatus* Anammoximicrobium, *Candidatus* Anammoxoglobus, *Candidatus* Scalindua, *Candidatus* Kueningenia and *Candidatus* Brocadia (Lodha et al., 2021). Anammox bacteria are a special group of bacteria with the characteristic anammoxosome structure (Moss Frank et al., 2018). Sinninghe Damsté et al. (2002) analyzed the membrane structure of anammoxosomes and found that there was a special component, ladderane lipids, on the membrane. Ladderane lipids have a dense structure and poor permeability, which can prevent the leakage of toxic substances produced in the process of anammox and prevent toxic effects from occurring on the anammox bacteria themselves (Moss Frank et al., 2018).

Anammox bacteria of different genera have different physiological and ecological characteristics. It was found that *Candidatus* Brocadia is more suitable for living environments with high $\text{NH}_4^+\text{-N}$ and $\text{NO}_2\text{-N}$ concentrations due to its low affinity for substrates. *Candidatus* Kueningenia is on the opposite situation. *Candidatus* Jettenia shows high nitrite tolerance compared with that of others (Oshiki et al., 2016). *Candidatus* Anammoxoglobus can oxidize formic acid and propionic acid and is suitable for survival in living environments containing organic matter such as sludge digests, landfill leachates and livestock and poultry breeding effluents. The niche of *Candidatus* Scalindua is characterized by a high salinity level and low temperature (Dale et al., 2009). The suitable temperature range of *Candidatus* Scalindua is also lower than that of the other genera (Kouba et al., 2022). Therefore, the application of nitrogen removal in industrial wastewater with high salinity has certain advantages. Table 1 lists the habitat conditions of anammox bacteria. The ecological diversity of anammox bacteria is determined by their metabolic diversity. Because of this, anammox is very important in the global nitrogen cycle. It also has inherent advantages and inestimable potential to be applied to the treatment of nitrogen-containing wastewater of different water qualities.

3. Diversity of anammox process forms

There are a variety of processes based on the principle of anammox, including two-stage systems and one-stage systems. The two-stage system mainly includes the single reactor for high-activity ammonia removal over nitrite (SHARON)-anammox process. The one-stage system includes completely autotrophic nitrogen removal over nitrite (CANON), oxygen limited autotrophic nitrification and denitrification (OLAND), denitrifying ammonium oxidation (DEAMOX), aerobic deammonification (DEMON), single-stage nitrogen removal using anammox and partial nitritation (SNAD) and other processes.

Fig. 1 shows the microbial principle diagram of the new biological nitrogen removal process. The SHARON process can provide $\text{NH}_4^+\text{-N}/\text{NO}_2\text{-N}$ with a ratio close to 1 for the anammox process through PN. Compared to those of traditional processes, the advantages of the SHARON-anammox combined process are a 60 % reduction in aeration, no additional requirement of organic carbon and low residual biomass yield. This process has a better treatment performance on high ammonia nitrogen and low nitrite wastewater (van Dongen et al., 2001a). The CANON process refers to the process that combines PN and anammox processes in a single aeration reactor and is the anammox process with the most research and application in the world (Wang et al., 2019). Compared to SHARON-anammox, the CANON process with a single reactor allows a savings of 63 % oxygen and 100 % organic carbon consumption, with a lower footprint and investment costs (Pérez et al., 2014). Compared with traditional nitrification and denitrification processes, SHARON-anammox and CANON have the characteristics of low sludge yield and no need to control pH and chemical oxygen demand

Table 1
Biodiversity of anammox bacteria.

Genera	Species	The morphology and size of cells	Optimal temperature (°C)	Optimal pH	The volume of anammoxosome in cells	Sources	References
Brocadia	<i>Candidatus Brocadia anammoxidans</i>	coccoid (0.7–1.0 μm)	20–43	6.7–8.3	34 %	wastewater	(Gottshall et al., 2021; Oshiki et al., 2016)
	<i>Candidatus Brocadia fulgida</i>		30–33	7.2–8.3	63 %	synthetic wastewater	(Qiao et al., 2017; Waki et al., 2021)
	<i>Candidatus Brocadia sinica</i>		37	7.6–7.8		synthetic wastewater	(Okabe et al., 2021)
	<i>Candidatus Brocadia brasiliensis</i>		34–35	7.5		synthetic wastewater	(Araujo et al., 2011)
	<i>Candidatus Brocadia caroliniensis</i>		30	6.8–7.6		wastewater	(Meng et al., 2022; Qiao et al., 2017)
Kuenenia	<i>Candidatus Kuenenia stuttgartiensis</i>	coccoid (1.0 μm)	25–37	6.5–9.0	45 %	wastewater	(Russ et al., 2014; Soler-Jofra et al., 2020)
Scalindua	<i>Candidatus Scalindua sinooilfield</i>	coccoid (1.0 μm)	20–33	6.0–8.5	56 %	oil field	(Li et al., 2010)
	<i>Candidatus Scalindua zhenghei</i>			2.0–4.0		marine water	(Li et al., 2010)
	<i>Candidatus Scalindua richardsii</i>					marine water	(Fuchsman et al., 2012)
	<i>Candidatus Scalindua brodae</i>			7.0–8.0		wastewater	(Speth Daan et al., 2015)
	<i>Candidatus Scalindua wagneri</i>		22–25	7.0–8.0		wastewater	(Osaka et al., 2012; Vipindas et al., 2020)
	<i>Candidatus Scalindua sorokinii</i>					marine water	(Wang & Gu, 2013)
	<i>Candidatus Scalindua arabica</i>		22–25	7.0–8.0		marine water	(Han et al., 2013)
	<i>Candidatus Scalindua profunda</i>		15–45	7.0–8.0		marine water	(Oshiki et al., 2016; Schwartz-Narbonne et al., 2020)
	<i>Candidatus Anammoxoglobus propionicus</i>		33	7–7.3	24 %	synthetic wastewater	(Oshiki et al., 2016)
Jettenia	<i>Candidatus Jettenia asiatica</i>	coccoid or irregular shape (0.7–1.0 μm)	30–35	8.0–8.2		synthetic wastewater	(Shu Chuan et al., 2014)
	<i>Candidatus Jettenia asiatica</i>		30–35	8.0–8.5		wastewater	(Ishimoto et al., 2021; Lilley et al., 2017)
Anammoximicrobium	<i>Candidatus Anammoximicrobium moscowii</i>		19–22	7.8–8.3		wastewater	(Wang et al., 2021)

(COD) (Manipura et al., 2005). Different from CANON, OLAND realizes NH_4^+ -N removal by AOB, through the combination of PN and anammox at low C/N ratio wastewater. The process is carried out in a rotating biological disk system under the condition of strict oxygen restriction (Courten et al., 2014). The DEAMOX process was proposed in 2004 and uses sulfide as an electron donor to reduce nitrate to nitrogen gas (Kalyuzhnyi & Gladchenko, 2009). The process is suitable for sulfuration of wastewater containing nitrogen pollutants. At the same time, SNAD processes rely on the synergistic effect of three functional bacteria, AOB, anammox bacteria and heterotrophic denitrification bacteria, to achieve effective removal of nitrogen and COD (Liu et al., 2022).

Fig. 2 shows the competition and cooperation of anammox bacteria, nitrifying bacteria and denitrifying bacteria. In the PN stage, AOB partially oxidizes ammonia nitrogen to nitrite, which provides the necessary growth substrate for anammox bacteria. At the same time, nitrate denitrification can also provide another way for anammox bacteria to obtain nitrite. Therefore, when the wastewater contains both ammonia and nitrate, the combined process of PD/A has certain application prospects (Guo et al., 2021). PD is achieved by denitrifying bacteria, and from the perspective of gene expression, it is due to the transcription of Nir genes caused by denitrifying bacteria without the regulation of C/N ratio (Zhang et al., 2020). Low concentration of COD can stimulate the growth of heterotrophic microorganisms and improve the nitrogen removal rate through denitrification. But at the same time,

high COD will inhibit the activity of anammox bacteria and increase the competition of nitrite between anammox bacteria and denitrifying bacteria. Therefore, it is very important to control the concentration of organic matter and dissolved oxygen in the influent to exert the competition and cooperation between AOB, anammox bacteria and denitrifying bacteria. According to the characteristics of each functional microorganism, the corresponding regulatory strategies were selected to achieve stable and efficient nitrogen removal.

The one-stage systems are gaining popularity as engineering experience increases. In addition, a one-stage system has lower infrastructure costs and a smaller footprint and is easier to operate, and it can avoid nitrite inhibition (Vlaeminck et al., 2012). Whereas, the start-up time of the one-stage process is long. The ecological relationship between the microorganisms in the reactor is complex, and it easily becomes unstable when subjected to load shocks. One-stage systems tend to have higher environmental sensitivity, such as pH and temperature, making NOB control more difficult (Li et al., 2018). The advantages of two-stage processes mainly lie in low environmental sensitivity and stronger thallus amplification ability. However, the operation condition setting and operation of the process are often more complicated. In other words, these two types of processes have their own characteristics and applicability. The application should be based on specific conditions, such as water quality, site, and management level.

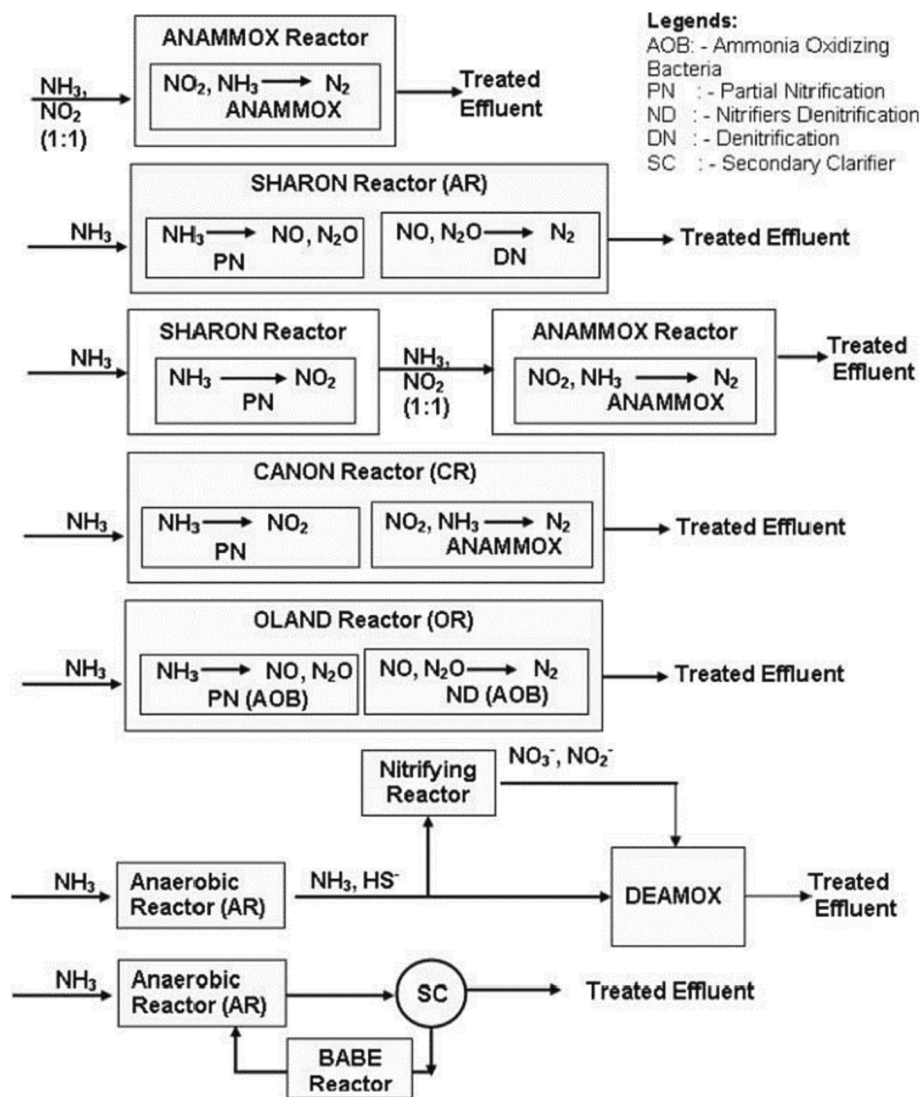
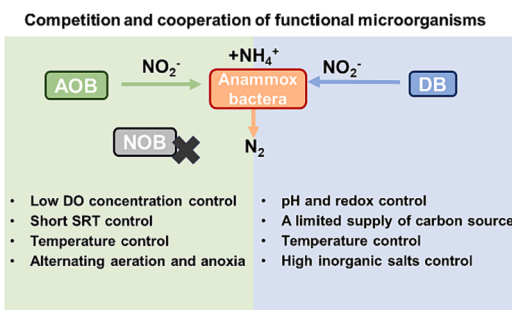
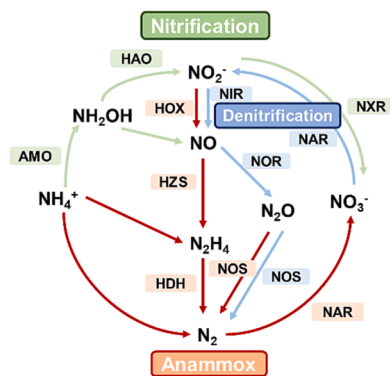


Fig. 1. Schematic figure of microbial principles in some of the new biological nitrogen removal processes (Bagchi et al., 2012).



- Low DO concentration control
- Short SRT control
- Temperature control
- Alternating aeration and anoxia
- pH and redox control
- A limited supply of carbon source
- Temperature control
- High inorganic salts control

Fig. 2. The relationship between cooperation and competition of functional microorganisms during combined application of anammox-based nitrogen removal technology. (AOB: ammonia oxidizing bacteria; NOB: nitrite oxidizing bacteria; AMO: ammonia oxidase; HAO: hydroxylamine oxidoreductase; NOR: NO oxidase; NAR: nitrite reductase; NIR: nitrite reductase; NOS: N₂O reductase; HZS: hydrazine synthase; HDH: hydrazine dehydrogenase; NOR: NO oxidase; HOX: hydroxylamine oxidase; NXR: nitrite oxidoreductases).

4. Diversity of applications

Table 2 lists some representative engineering examples. With the popularity of anammox process, this novel nitrogen removal technology has been successfully applied to treat a variety of wastewaters, including sludge liquid with a high NH₄⁺-N concentration and low C/N ratio, black water, landfill leachate, etc. Among the technologies, the most widely

used are sludge digesters and sludge pressure filtration liquid. The promotion of this technology in the treatment of industrial wastewater and landfill leachate, such as those from tanning, semiconductors, and food processing, has also been gradually carried out. However, there are still relatively few applications in treating high NH₄⁺-N concentration industrial wastewater.

Table 2
Examples of engineering application of anammox.

Process form	Treatment process type	Process address	Reactor type	Treatment object	Seed sludge type	Reactor effective volume (m ³)	Start-up time (d)	Ammonia nitrogen load (kg NH ₄ ⁺ -N•m ⁻³ •d ⁻¹)	Total nitrogen load (kg N•m ⁻³ •d ⁻¹)	NRR (kg N•m ⁻³ •d ⁻¹)	NRE (%)	References	
one-stage process	MBBR	WWTP Sjölanda, Sweden	MBBR	sludge water	anammox sludge + partial nitrification sludge	200	120		1.20	1.20	90	(Christensson et al., 2013)	
		China	IFAS	sludge dewatering liquor	anammox sludge + partial nitrification sludge	15,000	100			0.21	85 %	(Han et al., 2020)	
	ANITA™Mox	Finland	MBR	residential and industrial wastewater	anammox sludge + partial nitrification sludge	487	160			0.62	80–85	(González-Martínez et al., 2021)	
		China	IFAS	sludge digester filtrate	anammox sludge + partial nitrification sludge	15,000				0.15	86	(Yang et al., 2021)	
				SBR	hydrolysis and acidification effluent	anammox sludge + partial nitrification sludge	116.6	77		0.33–0.57	0.17–0.70		(Zuo et al., 2020)
		SNAD	Japan		swine wastewater	anammox sludge				0.13	0.09	61–78	(Ishimoto et al., 2020)
two-stage process		WWTP Klagshamn, Sweden	MBBR	anaerobic digester	anammox sludge + partial nitrification sludge	256	111			1.80	80	(Dimitrova et al., 2020)	
	SHARON-ANAMMOX®	Rotterdam, Netherlands		municipal sludge	anammox sludge + nitrification sludge	1500 + 70	1260		9.50	6.00–10.00		(van der Star et al., 2007)	
	SHARON-ANAMMOX®	Netherlands	UASB	semiconductor production wastewater	anammox sludge + anaerobic granular sludge	300 + 58	86		1.04–3.29	1.04–3.29		(Tokutomi et al., 2011)	
	CIRCOX-ANAMMOX®	Lichtenvoorde, Netherlands	ABR	leather industry waste		150 + 75			1.70–1.90			(Lackner et al., 2014)	
		Dokhaven WWTP, Netherlands		Municipal wastewater		4		0.15	0.10–0.22	0.10–0.22		(Hoekstra et al., 2019)	

Moving-bed biofilm reactor (MBBR); membrane bioreactor (MBR); Up-flow anaerobic sludge blanket (UASB); anaerobic baffled reactor (ABR).

4.1. Treatment of sludge liquid

Sludge digestion liquid and sludge pressure filtration liquid are typical wastewater types with low C/N ratios. Among them, sludge digestion liquid contains 500–2000 mg·L⁻¹ NH₄⁺-N and approximately 2000–3000 mg·L⁻¹ organic matter with poor biodegradability (Wang et al., 2017). Because the pH value of sludge liquid is generally 7.0–8.5, the temperature is generally 30–37 °C. Therefore, the high basicity and temperature of sludge digestion liquid are generally the optimal growth conditions for the growth of anammox bacteria and AOB (Wang et al., 2017).

van Dongen et al. (2001b) first explored the feasibility of PN and anammox processes for the treatment of digested sludge supernatant from the Dokhaven sewage treatment plant in the Netherlands. They achieved a remarkable denitrification effect, with more than 80 % of the NH₄⁺-N converted to N₂. Given this information, in 2002, researchers directly scaled up the reactor to build the world's first productive PN/A combined reactor. The process was already in operation at the Dokhaven sewage treatment plant. The anammox reactor had a volume of 70 m³ and a processing capacity of 750 kg N·d⁻¹ (van der Star et al., 2007). Since that time, the use of the anammox process for sludge treatment has become popular in Europe. Wang et al. (2017) achieved a stable PN process by controlling the concentrations of free ammonia and free nitrous acid. An NRR of 1.23 kg N·m⁻³·d⁻¹ was achieved in the pilot-scale PN/A reactor. For wastewater with NH₄⁺-N concentrations of 2100–2200 mg·L⁻¹, the treatment efficiency reached 98.82 %.

Sludge liquid has become the initial treatment object of anammox because of its high water temperature, low water volume, high NH₄⁺-N concentration and low C/N ratio. Approximately 75 % of the world's anammox engineering units are used to treat sludge liquid, and the anammox process has matured and been used in this field. However, there are still some urgent technical problems that need to be addressed, such as the effect of anaerobic digestion of sulfide in water on anammox reaction systems, nitrogen oxide generation links and emission reduction measures.

4.2. Treatment of landfill leachate

Landfill leachate is a kind of wastewater with complex components that is characterized by a high concentration of organic matter, a high content of toxic substances such as heavy metals, great variation in water quality, a high content of NH₄⁺-N, a high salt content and poor biodegradability (Iskander et al., 2018). Compared with traditional physical and chemical methods, biological treatment technology can achieve nitrogen and organic matter removal in sewage more effectively and is often more economical and environmentally friendly (Ren et al., 2022).

For a one-stage treatment process, Jiang et al. (2021) established a PN/A and PD/A combined process by using a sequencing batch biofilm reactor (SBBR). The main contribution of PN/A to nitrogen removal was 76.04 %, and the NRR and NRE were 2.83 ± 0.06 kg N·m⁻³·d⁻¹ and 98.6 ± 0.2 %, respectively. Ren et al. (2022), based on the integrated fixed-film activated sludge (IFAS) PN/A process, established a step feed continuous plug-in flow system to treat mature landfill leachate. At 301–405 days of operation, 98.1 % of the total inorganic NRE and 52.9 % of the COD removal rate were achieved. For mature landfill leachate and waste activated sludge, Zhang et al. (2022a) used three independent SBR reactors were used to combine PN and integrate fermentation–denitrification and PN/A. They developed a new PN, in situ fermentation, and anammox (PNFA) system. Finally, an NRE of 99.2 ± 0.1 % was achieved.

At present, there are generally many studies on the anammox process to treat landfill leachate, and the PN/A process is commonly used. At the same time, researchers continue to try various combination technologies. This is mainly because this leachate contains more toxic substances, such as heavy metals, which inhibit anammox activity to a certain

extent. Therefore, the inhibition of these inhibitory substances in landfill leachates of different ages on microorganisms, the effect of the bacterial community and the regulatory countermeasures need to be further studied.

4.3. Treatment of livestock and poultry breeding wastewater

Livestock and poultry breeding wastewater has a complex composition, large fluctuations in water quality and quantity, high COD concentrations and partial organic nitrogen (Xian et al., 2010). At the same time, antibiotic pollution in livestock and poultry wastewater has been widely studied. Due to the simultaneous threat of antibiotic-resistant bacteria and antibiotic resistance genes, the operation and performance of wastewater treatment processes can be affected (Ma et al., 2022). The traditional nitrification and denitrification treatment of this kind of high NH₄⁺-N aquaculture wastewater has many disadvantages, such as high energy consumption, poor nitrogen removal effect, and the need to supplement carbon source or add an alkali. Therefore, anammox is expected to be an alternative process for denitrification of aquaculture wastewater.

Recently, researchers have developed different PN/A processes to treat livestock and poultry wastewater. For swine wastewater, a nitrogen loading rate of 3.27 ± 0.13 g N·L⁻¹·d⁻¹ was achieved, but the denitrification efficiency was not stable (Chini et al., 2020). Yamamoto et al. (2008) found that an NRR of 0.22 kg N·m⁻³·d⁻¹ was reached after 70 days of operation of the reactor, and the color of anammox sludge changed to grayish black. Chen et al. (2021) successfully applied an anammox-based combined biological nitrogen removal process (CBNR) to a full-scale anaerobic-anoxic-oxic (A²/O) system. A running strategy of intermittent aeration and nitrate-based carbon dosage was employed. The removal efficiency of total nitrogen in the first and second stages was 65.5 ± 6.0 % and 83.5 ± 6.7 %, respectively.

At present, there are many problems, such as low NRR and unstable operation, in the treatment of anaerobic digestion liquid from pig farm wastewater by anammox. In addition, organic compounds, heavy metals, antibiotics and other components in wastewater may inhibit anammox bacteria. Therefore, we should focus on researching process optimization and seek countermeasures to eliminate the inhibition obstacles.

4.4. Treatment of monosodium glutamate wastewater

Monosodium glutamate wastewater is characterized by a high concentration of suspended solids, high COD, high biochemical oxygen demand (BOD), high NH₄⁺-N concentration, high SO₄²⁻ concentration and low pH value (approximately 2). Treating this type of wastewater is expensive, and it is a difficult industrial wastewater to treat (Shen et al., 2012). Physical and chemical methods and traditional biological treatment measures were used for this kind of wastewater treatment. Because monosodium glutamate wastewater has complex components and certain biological toxicity, the removal effect of nitrogen pollutants by biological denitrification systems is poor.

Shen et al. (2012) studied the impact of anammox bacterial enrichment in landfill leachate treatment plants, municipal sewage treatment plants and monosodium glutamate wastewater treatment plants on start-up of a monosodium glutamate wastewater treatment. After 360 days of operation, the maximum specific anammox activities were 0.11 kg·kg VSS⁻¹·d⁻¹, 0.09 kg·kg VSS⁻¹·d⁻¹ and 0.16 kg·kg VSS⁻¹·d⁻¹, respectively. At present, the volume of the anammox reactor of the TongLiao Meihua monosodium glutamate wastewater Phase I project is up to 6600 m³, and it is the largest anammox project in the world (Driessen & Reitsma, 2011). However, the high concentration of sulfate in monosodium glutamate wastewater (5000–5500 mg·L⁻¹) produces strong osmotic pressure, which greatly reduces the activity of microorganisms. At the same time, sulfate can also be converted to hydrogen sulfide by sulfate-reducing bacteria, significantly inhibiting anammox bacteria. Therefore,

anammox is generally not used for direct treatment but only for subsequent treatment. Thus, the changes in these pollutants in the combined process and their influence on the anammox process remain to be studied.

4.5. Treatment of urban domestic sewage

With the development of urbanization, the regeneration of urban sewage and energy recovery have increasingly become the focus of research. The application of the autotrophic anammox process is expected to enable municipal wastewater plants to achieve energy self-sufficiency (van Loosdrecht & Brdjanovic, 2014). Mainstream urban sewage has the problems of low $\text{NH}_4^+\text{-N}$ concentrations, unstable nitrogen loads, and temperature changes with the season.

Hu et al. (2013) adopted the process of integrated PN/A. The SBR (5 L) originally operated at 25 °C adapted to the low-temperature environment of 12 °C in only 10 days and ran stably under this temperature condition. For more than 300 days, there was no accumulation of nitrite, and the NRE exceeded 90 %. The study of Jin et al. (2013) showed that the NRR of a laboratory-scale anammox reactor operating at 35 °C could be as high as 6.61 $\text{kg N}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ at 9.1 °C by means of gradual cooling and acclimation or adding a low-temperature protective agent (glycine betaine). In Gao et al. (2022), a step-feed anoxic–oxic process combined with a PD/A process was adopted to maintain stable treatment efficiency in municipal wastewater treatment at a medium temperature (26.8 °C to 13.1 °C).

At present, the anammox process at room temperature and a low temperature has a certain research basis, and the pilot study has achieved phased success, which is expected to make the wastewater treatment plant realize energy self-support. However, to maintain the competitive advantage of AOB and NOB at a low temperature and low substrate concentration through actual engineering, the researchers still need to do further research. For example, thallus activity improved at a low temperature, thallus amplification was achieved at low substance concentrations, and thallus retention was achieved at high flow rates.

4.6. Treatment of saline wastewater

Some industrial effluents, such as those from seafood processing, textile printing and dyeing, pharmaceutical and petrochemical industries, tanning, farming and landfill leachate, contain large amounts of $\text{NH}_4^+\text{-N}$ and salts (Lin et al., 2021). Studies have shown that increasing the K^+ concentration in saline wastewater can improve the treatment performance of the reactor (Jin et al., 2007).

In terms of practical applications, a combined process coupling nitrification with an anammox system was used to evaluate the nitrogen removal performance of freshwater nitrifying anammox sludge under high salinity conditions, and a nitrification-anammox process gradually adapted to 3 % saline wastewater by enhancing aeration (Ge et al., 2019). At a salinity of 5 %, anammox bacteria are inhibited, causing the system to collapse. Xu et al. (2022) developed a salt-tolerant PD/A process. It was found that both partial denitrification sludge and anammox sludge maintained high activity at a salinity of 10 $\text{g}\cdot\text{L}^{-1}$. A denitrification efficiency of 90 % was achieved by this process, which provided a new research scheme for the treatment of industrial wastewater containing salt. However, the study of Ma et al. (2012) showed that an impact load of 30 $\text{NaCl}\cdot\text{L}^{-1}$ was the threshold that the anammox reactor could tolerate while stably operating. Although the salinity load threshold that the anammox system can tolerate varies at present (30–75 $\text{NaCl}\cdot\text{L}^{-1}$), it has great potential to utilize the anammox process to treat industrial wastewater with high $\text{NH}_4^+\text{-N}$ concentration and salinity through long-term acclimation and the addition of compatible solutes (Liu et al., 2014).

4.7. Treatment of other types of wastewater

Zhang et al. (2022b) indicated for the first time that a PD/A SBBR reactor can effectively treat pharmaceutical wastewater containing bismuth glutamate and bismuth potassium citrate. The results showed that the total NRR could reach 80.8 %, and the contribution rate of anammox accounted for 83.6 %. Li et al. (2019) coupled anammox and sulfur autotrophic reactors to process semiconductor wastewater, and the NRR of the anammox process reached 3.1 $\text{kg N}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. Tokutomi et al. (2011) transformed the original nitrification–denitrification process into a PN/A-denitrification process. PN was achieved by selective inhibition of nitrite oxidation with a high concentration of bicarbonate. The NRR of the anammox reactor was 1.04–3.29 $\text{kg N}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$, and the effluent was treated by denitrification. Daverey et al. (2013) used the CANON process to treat photoelectric industrial wastewater with a high $\text{NH}_4^+\text{-N}$ concentration. An 18 L SBR was used to control the basicity of 850 $\text{mg CaCO}_3\cdot\text{L}^{-1}$ by adding NaHCO_3 . When the influent $\text{NH}_4^+\text{-N}$ concentration reached 3636 $\text{mg}\cdot\text{L}^{-1}$, the nitrogen loading rate was 0.909 $\text{kg N}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$, and the $\text{NH}_4^+\text{-N}$ removal rate reached 98 %. Anammox processes have been applied to various wastewaters at the laboratory and pilot scales, and they have also shown wide applicability for use in other wastewater treatments (Table 3).

5. Future perspectives

Wastewater composition is complex. Livestock breeding wastewater contains heavy metal ions and antibiotics. Landfill leachate contains relatively high content of heavy metals. Coking and petrochemical wastewater contains cyanide, tar, phenols, etc. Tannery wastewater contains many organic nitrogen and heavy metal ions. Pharmaceutical wastewater contains biological inhibitors. Sulfide is also contained in wastewater from seafood processing, tanning, papermaking, and fermenting alcohol. Aquaculture wastewater, fecal sewage, municipal wastewater, chemical fertilizer, and pharmaceutical wastewater contain phosphates at varying concentrations. The abovementioned obstacles are the key factors restricting the application of anammox technology in the field of high-concentration ammonia nitrogen industrial wastewater treatment. Although the current study involved multiple factors inhibiting anammox bacteria. However, due to different strains or experimental conditions, the inhibitory thresholds of inhibitors differ, and whether they are reversible is also controversial. The combined effect has not yet been proven, and research on related regulatory strategies is very minimal. Moreover, for wastewater of different water qualities, the theoretical discussion on the choice of integrated and split process types has not been finalized, and practical proof still lacks operational data support.

Nowadays, the sidestream anammox process has been widely established at full-scale. However, the problems of long start-up period and low nitrogen removal efficiency in sidestream wastewater treatment have not been solved well. The mainstream process is faced with the problems of low stability, high C/N ratio, poor retention of anammox bacteria and difficulty in inhibiting NOB activity, etc. At present, the mainstream applications of this technology were mainly at laboratory scale (Trinh et al., 2021). In addition, low influent ammonia concentration and low temperature were unfavorable to the retention of bacterial biomass in anammox. Therefore, the problems of low nitrogen removal efficiency and low effluent quality still need to be improved (Poot et al., 2016). Researchers need to adopt effective strategies to remove high organic matter concentrations, maintain AOB growth activity and control nitrite and free ammonium concentrations to promote the development of mainstream anammox technology (Miao et al., 2020).

In addition, anammox as a new biological denitrification process does not indicate the end of the traditional process, but it should be used as a supplement to the existing process and a bridge to the development of new processes. Due to the strict reaction conditions of anammox, the

Table 3
Anammox process applied to various wastewater in laboratory and pilot scale.

Treatment object	Main water quality barrier factors	Reactor type	Reactor effective volume (L)	Seed sludge type	Hydraulic retention time (d)	Temperature (°C)	pH	Total nitrogen load (kg N•m ⁻³ •d ⁻¹)	NRE (%)	References
sludge digestion	Low C/N ratio, insufficient organic matter and alkalinity	MBBR	832.0	NOB + anammox sludge	2.0	27–30	6.8–7.3	0.41	70.0	(Xu et al., 2018)
animal manure landfill leachate	high COD and high suspended solids, high metal content, poor biodegradability	IFAS	16.5	anammox sludge + activated sludge	0.5/2.0	17–30	6.5–8.8	0.06–0.57	94.3–95.0	(Ren et al., 2022)
swine wastewater	high concentration of organic matter, containing heavy metal ions	SBR	4.0	activated sludge	0.3/6.0	21–24	7.4–7.8	1.68–1.91	90.0	(Huang et al., 2020)
aquaculture wastewater	high COD	SBR	10.0	anammox sludge + denitrification sludge	0.8	environment temperature	7.0–7.5		80.8	(Zhang et al., 2022b)
coking wastewater	contains large amounts of organic matter, phenols, cyanide, thiocyanide, tar and polycyclic aromatic hydrocarbons	EGSB	18.0	EGSB sludge for treating coking wastewater treatment of synthetic wastewater sludge + landfill leachate treatment plant sludge	0.5 + 0.5				73.3	(Dong et al., 2020)
monosodium glutamate wastewater	high suspended solids, high COD, high BOD, high NH ₄ ⁺ -N and SO ₄ ²⁻ concentration, low pH	SBR	5.0	anammox sludge	4.0	17–37	7.8–8.0	0.91	89.0	(Daveray et al., 2013)
photoelectric wastewater	high alkaline, high NH ₄ ⁺ -N concentration	UASB + SBR	5.0 + 4.0	anammox sludge	0.5	35/25	7.6–8.0	0.50	85.0–89.0	(de Graaff et al., 2011)
black wastewater	Organic load, high nitrogen and phosphorus content, containing complex types of micro pollutants	SBR	1.8	anammox sludge	0.5	12	7.3		greater than 90.0	(Li et al., 2005)
municipal waste	low temperature, low NH ₄ ⁺ -N concentration, low C/N ratio	SBR	1.8	anammox sludge	1.2–3.6	10–20	7.0–7.5	0.34–2.40	48.0–75.0	(Lotti et al., 2014)
		SBBR	10.0	anammox sludge + activated sludge	0.2	25			87.9	(Zhang et al., 2021a)

Continuously stirred tank reactor (CSTR).

influence of and regulation strategies for different water quality obstacles should be deeply studied to improve the engineering value of anammox. It is recommended that in-depth research on the following aspects be carried out in the future:

- (1) Development of a multiflora coupling process based on the anammox process.
- (2) Development of a combined process with anammox as an intermediate biological unit.
- (3) Long-term, short-term and compound effects of wastewater quality barrier factors from different sources on each part of the combined anammox processes.

6. Conclusion

In this paper, according to the habitat characteristics of anammox bacteria, the quality characteristics of wastewater from different sources and the main inhibitory factors, one-stage and two-stage processes were compared to illustrate the applicability of different processes. At the same time, combined with laboratory research and engineering application of anammox technology in the treatment of wastewater from different sources, the progress and obstacles of anammox technology in practical application were clarified. Finally, some suggestions were

given for the application of anammox technology in actual wastewater treatment.

CRediT authorship contribution statement

Zhi-Qi Ren: Conceptualization, Visualization, Writing – original draft. **Hao Wang:** Data curation, Formal analysis. **Li-Ge Zhang:** Visualization. **Xue-Ning Du:** Visualization. **Bao-Cheng Huang:** Supervision, Conceptualization, Writing – review & editing. **Ren-Cun Jin:** Supervision, Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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