

Research progress and prospects of complete ammonia oxidizing bacteria in wastewater treatment

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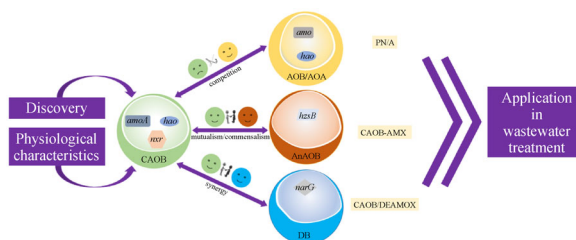
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HIGHLIGHTS

- Comammox bacteria have unique physiological characteristics.
- Comammox bacteria are widely distributed in natural and artificial systems.
- Comammox bacteria have the potential to reduce N₂O emissions.
- Coupling comammox bacteria with DEAMOX can be promoted in wastewater treatment.
- Comammox bacteria have significant potential for enhancing total nitrogen removal.

GRAPHIC ABSTRACT



ABSTRACT

Complete ammonia oxidizing bacteria, or comammox bacteria (CAOB), can oxidize ammonium to nitrate on its own. Its discovery revolutionized our understanding of biological nitrification, and its distribution in both natural and artificial systems has enabled a reevaluation of the relative contribution of microorganisms to the nitrogen cycle. Its wide distribution, adaptation to oligotrophic medium, and diverse metabolic pathways, means extensive research on CAOB and its application in water treatment can be promoted. Furthermore, the energy-saving characteristics of high oxygen affinity and low sludge production may also become frontier directions for wastewater treatment. This paper provides an overview of the discovery and environmental distribution of CAOB, as well as the physiological characteristics of the microorganisms, such as nutrient medium, environmental factors, enzymes, and metabolism, focusing on future research and the application of CAOB in wastewater treatment. Further research should be carried out on the physiological characteristics of CAOB, to analyze its ecological niche and impact factors, and explore its application potential in wastewater treatment nitrogen cycle improvement.

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1 Introduction

As a key process in the natural nitrogen cycle, nitrification is a microbial process by which ammonium (NH₄⁺-N) is sequentially oxidized to nitrite (NO₂⁻-N) and nitrate (NO₃⁻-N) by two separate organisms (Winogradsky, 1890;

Kuypers et al., 2018). The first step is ammonia oxidation (NH₄⁺-N → NO₂⁻-N, Eq. 1), mediated by ammonia oxidizing microorganisms (AOM), including ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA), which use ammonia monooxygenase (AMO) and hydroxylamine dehydrogenase (HAO) to oxidize ammonia to nitrite (Arp et al., 2002; Könneke et al., 2005). The second step is nitrite oxidation (NO₂⁻-N → NO₃⁻-N, Eq. 2), catalyzed by nitrite oxidizing bacteria (NOB), which uses nitrite oxidoreductase (NXR) to oxidize nitrite to nitrate (Teske et al., 1994). However, in 2015, the

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discovery of complete ammonia oxidizing bacteria, which can oxidize ammonium directly to nitrate in a single organism (Fig. 1), challenged our understanding of the conventional two-step nitrification process (Daims et al., 2015; Van Kessel et al., 2015). Furthermore, comammox bacteria have been detected in various environments, such as wastewater treatment facilities, drinking water systems, natural wetlands, lake sediments, and soil (Wang et al., 2017; Annavajhala et al., 2018; Xia et al., 2018). Their wide distribution has attracted great interest in their ecological role compared with other nitrifiers.

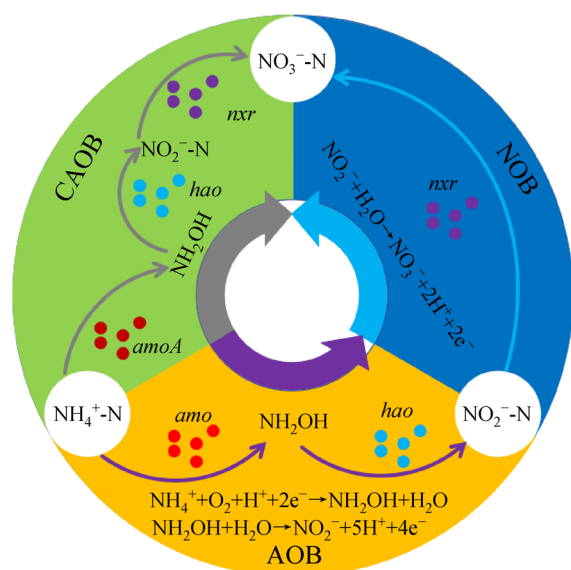


Fig. 1 Differences between traditional microbial nitrification and comammox bacteria nitrification.

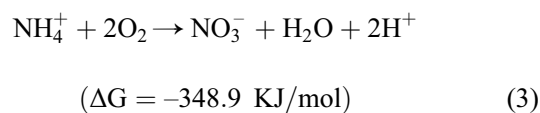
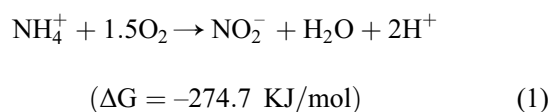
After comammox bacteria were discovered, comparisons of comammox bacteria with the *amoA* gene in the NCBI database identified a large number of highly similar sequences, in both natural (forests, plains, wetlands, etc.) and artificial systems (drinking water plants, sewage treatment plants, paddy fields, etc.) (Erwin et al., 2005; Knief et al., 2006; Jia et al., 2007; Vigliotta et al., 2007; Steenbergh et al., 2010; Daims et al., 2015; Van Kessel et al., 2015). Later research confirmed this, and found significant differences in abundance between habitats (Xia et al., 2018). The distribution difference of clades A and B had a certain relationship with the difference of the encoded genome (Palomo et al., 2018; Koch et al., 2019).

Comammox bacteria have recently been identified in water treatment systems, but their contribution to biological nitrogen removal remains unclear (Daims et al., 2015; Van Kessel et al., 2015; Pjevac et al., 2017; Wang et al., 2017; Zhao et al., 2019). They significantly contribute to nitrification in water treatment systems (Pinto et al., 2015; Xia et al., 2018), with the abundance ratio of comammox bacteria to total *amoA* genes in some wastewater treatment

plants reaching up to 100% (Zhou et al., 2021). How to utilize the physiological characteristics of comammox bacteria in aquatic ecosystems is of great significance. The integration of comammox bacteria into existing wastewater treatment processes has important research prospects and application potential for improving the effectiveness and stability of domestic wastewater treatment, reducing sewage treatment costs, and decreasing greenhouse gas emissions.

2 Discovery of comammox bacteria

Costa et al. (2006) speculated that, from a thermodynamic perspective, there was a microorganism that can completely oxidize ammonium to nitrate ($\text{NH}_4^+\text{-N} \rightarrow \text{NO}_3^-\text{-N}$, Eq. 3). This single microorganism, which can complete nitrification, is named complete ammonia oxidizing bacteria (CAOB). In 2015, two laboratories in the Netherlands and Austria found strains in the genus *Nitrospira* that can independently complete the entire nitrification process, confirming the existence of comammox bacteria (Daims et al., 2015; Van Kessel et al., 2015).



2.1 *Candidatus Nitrospira inopinata*

Daims et al. (2015) collected biofilm from the wall of a hot water pipe of a 1200 m deep oil exploration well in North Caucasus, Russia, and enriched it in an ammonium-containing inorganic medium at a constant temperature of 46 °C. The enriched culture could oxidize $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$. By detecting the specific rRNA targeting probe of nitrifying bacteria, fluorescent in situ hybridization (FISH) confirmed that the enriched culture only contained *Nitrospira*, and the rest were Betaproteobacterium without ammonia oxidation ability. A polymerase chain reaction (PCR) did not detect any known *amoA* genes or 16S rRNA genes of AOA and AOB. Metagenomic sequencing found that the relative abundance of *Nitrospira* in the enriched culture was 68%–80%. Further enrichment revealed that *Nitrospira* could oxidize $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$. Therefore, it was considered to be a microbial strain with complete ammonia oxidation ability and was named *Candidatus Nitrospira inopinata* (*Ca. N. inopinata*). In 2017, Kits et al.

(2017) obtained the pure culture strain of *Ca. N. inopinata* by enrichment culture, which was also the first pure culture strain of comammox bacteria.

2.2 *Candidatus Nitrospira nitrosa* and *Candidatus Nitrospira nitrificans*

Also in 2015, van Kessel et al. (2015) sampled the anaerobic compartment of a trickling filter connected to a recirculation aquaculture system. Under low dissolved oxygen ($\text{DO} \leq 3.1 \mu\text{mol/L O}_2$) and oligotrophic conditions, the genus *Brocadia* of anammox bacteria (AnAOB) and two different *Nitrospira* genus were enriched by culturing for 12 months. A high abundance of *Nitrospira* was rarely seen under low DO conditions. Both NOB species carried the genes (*amo* and *hao*) for ammonia oxidation as well as the *nrx* gene necessary for nitrite oxidation; therefore, these two strains were confirmed to have the metabolic potential to oxidize $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$, and are named *Candidatus Nitrospira nitrosa* (*Ca. N. nitrosa*) and *Candidatus Nitrospira nitrificans* (*Ca. N. nitrificans*), respectively. The phylogeny of 16S rRNA genes indicates that they belong to lineage II of the genus *Nitrospira*.

2.3 *Candidatus Nitrospira kreffii*

Sakoula et al. (2021) used a continuous membrane bioreactor with an effective volume of 5 L containing *Ca. N. nitrosa* and *Ca. N. nitrificans* (van Kessel et al., 2015), operated at room temperature and under suitable conditions (e.g., pH = 7.5, DO = 50% oxygen saturation) for 39 months. They obtained a novel comammox bacteria species, *Candidatus Nitrospira kreffii* (*Ca. N. kreffii*), which displayed clear differences in terms of ammonia and nitrite oxidation kinetics. For example, *Ca. N. kreffii* has a higher nitrite affinity. Furthermore, partial inhibition of ammonia oxidation at ammonium concentrations as low as 25 μM was observed in the bioreactor, indicating that differences in ammonium tolerance could potentially be a niche-determining factor for different comammox *Nitrospira*.

2.4 Summary

All identified comammox are *Nitrospira* bacteria, belonging to lineage II of the *Nitrospira* genus, and are phylogenetically divided into two branches according to the *amoA* gene: clade A and clade B. These two clades have significant differences in substrate operation and metabolism, energy transfer, and environmental adaptability (Palomo et al., 2018). In comammox bacteria enrichment cultures, genome analysis has identified the *amoA* gene, encoding AMO; *hao* encoding HAO; and *nrx* encoding NXR, which can independently perform the complete nitrification process (Daims et al., 2015; Van Kessel et al., 2015; Sakoula et al., 2021). Van Kessel et al.

(2015) found that the *amoA* gene in *Ca. N. nitrosa* and *Ca. N. nitrificans* is different from that of typical ammonia oxidizing bacteria, and belongs to two different branches phylogenetic, but is highly similar to *pmoA* of the *methane monooxygenase* of *Crenohrix polyspora*. Therefore, it is inferred that the *amoA* gene of some comammox bacteria in the previous study may be incorrectly classified as *methane monooxygenase* and could be ignored (Daims et al., 2015; Van Kessel et al., 2015).

The discovery of comammox bacteria revolutionized our understanding of the nitrification process, and the relative contribution of microorganisms to the nitrogen cycle should be reassessed.

3 Physiological characteristics of comammox bacteria

3.1 Nutrient matrix

Comammox bacteria have advantages over traditional nitrifying bacteria in oligotrophic substrates, such as low ammonia nitrogen and low DO (Kits et al., 2017). In addition to the reported AOA of marine *N. maritimus* SCM1, the ammonia half-saturation constant of AOA and AOB is much higher than that of purified and cultured *Ca. N. inopinata*, but the affinity of comammox bacteria to nitrite is much lower than that of traditional NOB (Kits et al., 2017). Zhao et al. (2021) used urea and nitrite to enrich and cultivate comammox bacteria for 390 days to increase the ratio of *amoA* gene, indicating that comammox bacteria have diverse metabolic potential.

3.2 Environmental factors

Environmental conditions, such as DO, pH, and temperature have a significant impact on the growth and activity of comammox bacteria (Kits et al., 2017; Palomo et al., 2018; Roots et al., 2019; Takahashi et al., 2020; Sun et al., 2020). The microbial oxidase of comammox bacteria is similar to cytochrome bd-type terminal oxidase, which is generally expressed under extremely low DO concentrations and has a higher affinity for DO (Borisov et al., 2011). There are currently no reports on the half-saturation constant of oxygen, but comammox bacteria are more competitive under low DO conditions. With increasing DO, AOA and AOB activities gradually increase, but comammox bacteria lose their competitiveness (Palomo et al., 2018; Roots et al., 2019). Sun et al. (2020) found that when the pH was between 6.74 and 8.65, the abundance of comammox bacteria in coastal wetlands was positively correlated with pH. In addition, comammox bacteria can show nitrifying activity at pH 3–4, indicating that comammox bacteria may be able to adapt to low pH (Takahashi et al., 2020). Shi et al. found that the abundance of comammox bacteria was relatively high in samples collected from wetland ecosys-

tems. Based on the changes in comammox bacteria species diversity in summer and winter, they speculated that temperature may be a key factor affecting the structure of comammox bacteria (Shi et al., 2020). Zhou et al. (2021) tested the abundance, activity, and metabolic characteristics of nitrifying bacteria in drinking water and wastewater treatment plants with seasonal changes and found that the network complex of comammox microorganisms in sewage treatment plants was highest in December. However, a study by Kits et al. (2017) found that the optimal growth of *Ca. N. inopinata* occurred at 37 °C, and the optimal enrichment culture temperature was 46 °C. This indicates that different comammox bacteria can adapt to growth environments of different temperatures.

3.3 Enzymes and metabolism

The comammox bacteria metabolism pathway is complex and can adapt to different nutrient substrates (Daims et al., 2015; Van Kessel et al., 2015; Shen et al., 2016; Kits et al., 2017; Han et al., 2019; Liu et al., 2020a; Wang et al., 2021; Zhao et al., 2021). Liu et al. (2020a) found that comammox bacteria contribute 34%–87% of nitrification in a typical oligotrophic environment with high pH and low temperature along the Yangtze River. Kits et al. (2017) enriched and cultivated comammox bacteria under higher temperature conditions, demonstrating that comammox microorganisms can adapt to varying and more extreme environments. Metagenomic analysis shows that the discovered comammox bacteria can use urea as a nitrogen source for energy metabolism and biosynthesis (Daims et al., 2015; Van Kessel et al., 2015; Zhao et al., 2021). Studies have found that comammox bacteria can use hydrogen sulfide, sulfide, and fermentation products for metabolism (Kits et al., 2017), while trace organic pollutants in the environment can be removed through metabolism (Han et al., 2019). Some comammox may also have the potential to oxidize formic acid and hydrogen (Palomo et al., 2018), and a variety of antibiotics have been used to effectively and selectively enrich different strains of comammox bacteria (Wang et al., 2021). In addition, comammox bacteria have a gene encoding alkaline phosphatase, which can make it more advantageous under insufficient phosphorus conditions (Shen et al., 2016).

Comammox bacteria have a complete genome of key functional enzymes in the oxidation process of $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$, and can complete the whole process of traditional two-step nitrification metabolism itself. The comammox bacteria genome encodes the nitrite reductase gene (*nir*) but lacks the nitric oxide reductase gene (*nor*). In theory, it produces NO but not N_2O during biological reactions (Kits et al., 2019). Palomo et al. (2018) found that there was no pathway related to NO_x metabolism in the known comammox bacteria genome, and the NO_x reduction reaction may not be possible. Comammox bacteria

sequester carbon through the reduced tricarboxylic acid (rTCA) cycle. The rTCA cycle is usually the mechanism of CO_2 fixation by anaerobic or aerobic microorganisms (Lücker et al., 2010); compared with the Calvin cycle corresponding to AOB, the fixed unit CO_2 of the rTCA cycle consumes less adenosine triphosphate (ATP) and requires less oxygen (Lawson and Lücker, 2018). Theoretically, the comammox bacteria oxidation unit $\text{NH}_4^+\text{-N}$ has a shorter metabolic pathway and a higher growth yield than AOB, so it is easier to obtain a competitive advantage under low DO conditions (Costa et al., 2006).

3.4 Natural habitat distribution

Similar to AOA, comammox bacteria have a high affinity for $\text{NH}_4^+\text{-N}$ and are suitable for growth under low $\text{NH}_4^+\text{-N}$ conditions. There are numerous natural systems suitable for the growth of comammox bacteria. Table 1 shows partial statistics of the distribution of comammox bacteria in natural ecosystems.

Comammox bacteria are widely distributed in nature, and the distribution further confirms that comammox bacteria like to grow under conditions of low DO and $\text{NH}_4^+\text{-N}$. AOA was first discovered in the marine environment. However, although the physiological characteristics of comammox bacteria are similar to those of AOA, Liu et al. (2020b) detected a high abundance of comammox bacteria in extremely saline sediments, showing that comammox bacteria can adapt to high-salinity environments, and a distinct differentiation of comammox *Nitrospira* groups driven by salinity has been identified (Sun et al. 2021). However, so far, comammox bacteria have not been found in the ocean (Daims et al., 2015; Kuypers, 2017; Xu et al. 2020), indicating that salinity is a key factor. Whether there are comammox bacteria in marine habitats is an important research direction.

At present, data on the nitrification kinetics of comammox bacteria are derived from pure cultured *Ca. N. inopinata*. (Kits et al., 2017). Therefore, there are some limitations for studying the nitrification kinetics of comammox. With the widespread discovery of comammox bacteria and the versatility of metabolism, research on the physiological characteristics of comammox bacteria will further understanding of the niche characteristics of comammox bacteria in the biosphere and its relative contribution to the nitrogen cycle.

4 Application in wastewater treatment

4.1 Distribution in water systems

In conventional artificial system studies, only the nitrification activity of AOM and NOB is evaluated, and the presence of comammox bacteria is rarely considered. NOB

Table 1 Comammox bacteria partial distribution in natural ecosystems

Growth environment	Country	Strains	Research method	Key finding	Reference
Atmospheric fine particles	China	<i>N. inopinata</i>	PCR ^{a)} Clone sequencing	The abundance of comammox bacteria in the atmosphere is less than AOA and greater than AOB.	Gao et al., 2016
Forest soil, lake sediments, freshwater organisms, etc.	Austria; Netherlands	/	Metagenome	A PCR primer set was developed specifically for the subunit <i>amoA</i> gene encoding the unique <i>amoA</i> of comammox bacteria.	Pjevac et al., 2017
Forest soil	China	/	qPCR	The abundance of comammox bacteria is very high. When the pH is 4.0–9.0, the abundance exceeds AOB and NOB.	Hu and He, 2017
Sediments from the Yangtze River Estuary	China	<i>N. inopinata</i> <i>N. nitrosa</i> <i>N. nitrificans</i>	Metagenome; Macrotranscriptome; qPCR	The presence of comammox bacteria was detected, and a primer set for clade A was designed to quantitatively detect the <i>amoA</i> gene of comammox bacteria.	Yu et al., 2018
Riparian soil	China	<i>N. nitrosa</i> <i>N. nitrificans</i>	qPCR; Correlation analysis	The microbial abundance of comammox in the riparian soil was 10 ⁸ copies/g, and the abundance of comammox and Nitrospira was significantly correlated under certain conditions.	Wang et al, 2019

Note: a) qPCR: quantitative polymerase chain reaction.

of the *Nitrospira* genus was mistakenly thought to exist widely in artificial systems. Metagenomic detection and analysis show that some of these have a full set of characteristic genes for ammonia oxidation, indicating that they may be comammox bacteria (Pinto et al., 2015). Table 2 shows partial statistics on the distribution of comammox bacteria in artificial systems.

Comammox bacteria are widely distributed in natural and artificial systems (Fig. 2). However, previous calculations on the contribution of ammonia oxidizing microorganisms in the nitrogen cycle ignored the role of comammox bacteria. Theoretically, the AOM/NOB value in the traditional nitrification process should be between two and three (Winkler et al., 2012), but studies have found that the abundance of *Nitrospira* in the same habitat exceeds AOM (Zeng et al., 2015), which cannot be explained by traditional nitrification theories. It has now been demonstrated that some *Nitrospira* may be comammox bacteria (Pinto et al., 2015; Tatari et al., 2017). The discovery of comammox will cause a reevaluation of the relative contribution of nitrification in the nitrogen cycle, and the structure of the flora.

4.2 Potential application in wastewater treatment

Wang et al. (2018) found that, among eight wastewater treatment plant samples investigated, activity abundance of *amoA* of the comammox bacteria in six samples was significantly higher than or comparable to that of AOB, even reaching 24 times more in some wastewater plants. The abundance of comammox bacteria to total *amoA* genes in drinking water plants and AAO wastewater treatment plants is up to 46%–100% (Zhou et al., 2021), indicating

that comammox bacteria may play an important role in wastewater treatment. Sato et al. (2021) used a segmented reactor to first ammonify organic nitrogen, and then nitrate into nitrate as a hydroponic fertilizer for plant growth. Through 16S rRNA gene sequencing and shotgun metagenomic analysis, the dominant microorganism in the nitrification process was confirmed as comammox bacteria, and qPCR analysis showed that the expression of comammox-type AMO was more than 500 times higher than that of traditional AMO. Heise et al. (2021) found that comammox bacteria participated in the removal of NH₄⁺-N at low steady-state NH₄⁺-N concentrations in an aquaponic system, indicating that comammox plays an important role in this system.

Research on the growth mechanisms and physiological characteristics of comammox bacteria can improve the treatment of urban domestic wastewater. Fig. 3 shows a common microbial denitrification process in wastewater plants. For urban wastewater partial nitrification-denitrification (PN/D) and partial nitrification-anaerobic ammonia oxidation (PN/A) denitrification systems, the growth and enrichment of comammox bacteria are one of the important reasons for the destruction of these systems (Wang et al., 2020). Controlling the growth of comammox bacteria is an important factor in realizing PN/D or PN/A of urban domestic wastewater. Purified and cultured comammox bacteria have a low affinity for nitrite (Kits et al., 2017; Xia et al., 2018). Meanwhile, comammox bacteria and anammox bacteria can form copolymers under hypoxic conditions (Van Kessel et al., 2015; Gottshall et al., 2021; Shao and Wu, 2021). In theory, the nitrite oxidation activity of comammox bacteria can be inhibited by controlling conditions to achieve stable PN/A. In the PN/

Table 2 Comammox bacteria partial distribution in artificial systems

Growth environment	Country	Species	Research method	Key finding	Reference
Wastewater treatment plants (WWTPs)	China	<i>N. inopinata</i>	Metagenome; 16S rRNA	Comammox bacteria abundance accounts for $\leq 0.1\%$. It is speculated that the contribution of comammox bacteria nitrification is small in the sewage treatment process.	Chao et al., 2016
Drinking water systems	Singapore; China; United States	<i>N. inopinata</i> <i>N. nitrosa</i> <i>N. nitrificans</i>	Metagenome	Comammox bacteria are widely distributed in drinking water systems and coexist with traditional AOM. The nitrification of drinking water systems may be mainly completed by comammox bacteria.	Wang et al., 2017
WWTPs	United States	<i>N. nitrosa</i>	Metagenome	To achieve enrichment of <i>Ca. N. nitrosa</i> , it has a higher affinity for urea.	Kits et al., 2017
WWTPs	United States United Kingdom	<i>N. inopinata</i> <i>N. nitrosa</i> <i>N. nitrificans</i>	Metagenome; qPCR	A primer set and qPCR targeting clade A were designed; comammox bacteria prefer long sludge age and attached growth, and the increase in abundance in the same habitat has no correlation with the decrease in AOB and NOB abundance.	Camejo et al., 2017
Urban lake	China	/	16S rRNA	Comammox bacteria are widely distributed in urban lakes, eutrophication may inhibit its growth.	Xu et al., 2020
Acidic soils	Japan	/	16S rRNA	When the pH is 3–4, nitrification activity of comammox bacteria is still detected.	Takahashi et al., 2020
A continuous membrane bioreactor	Netherlands	<i>N. Kneftii</i>	FISH; Metagenome;	They obtained a novel comammox bacteria species, <i>Ca. N. kneftii</i> . Moreover, they think differences in ammonium tolerance could potentially be a niche-determining factor for different comammox <i>Nitrospira</i> .	Sakoula et al., 2021
Lab-scale PN/A SBR Reactor	China	/	16S rRNA qPCR	The <i>amoA</i> gene of comammox bacteria in the PN/A system that has been running stably for more than 1,000 days accounted for $89.2 \pm 7.9\%$, achieving synergistic denitrification under hypoxic conditions.	Shao and Wu, 2021
Aquaponic system	Germany	/	16S rRNA	Comammox bacteria are found in the high-efficiency aquaponic symbiosis system, which participate in the removal of $\text{NH}_4^+\text{-N}$ at low steady-state $\text{NH}_4^+\text{-N}$ concentrations.	Heise et al., 2021

A process, $\text{NH}_4^+\text{-N}$ and DO continuously decrease as particles or biofilms develop, which may lead to the enrichment of comammox bacteria (Wang et al., 2020), competing with AOB and reducing the total nitrogen removal rate, instead of the traditional NOB proliferation effect.

In some wastewater treatment plants, comammox bacteria account for a relatively high proportion of activated sludge (Zhou et al., 2021). An important research direction in wastewater treatment is how to use the physiological characteristics of comammox bacteria to further reduce sewage treatment costs and improve treatment effects. Using comammox bacteria to treat low $\text{NH}_4^+\text{-N}$ municipal sewage under low DO conditions can reduce the aeration; therefore, the energy consumption. The generation of sludge can also be reduced, as well as the cost of post-sludge treatment and disposal. With the recent research and development of partial denitrification ($\text{NO}_3\text{-N} \rightarrow \text{NO}_2\text{-N}$) and the anaerobic ammonia oxidation coupling process (DEAMOX), simultaneous treatment of $\text{NO}_3\text{-N}$ and $\text{NH}_4^+\text{-N}$ in the same reactor has been realized.

Compared with PN/A, the DEAMOX process has many advantages, such as a more stable accumulation of $\text{NO}_2\text{-N}$, no complicated control, and theoretical 100% deep denitrification. In this process, comammox bacteria are used to couple partial denitrification-anaerobic ammonia oxidation to develop a new type of denitrification process. Hypoxic nitrification reduces aeration energy consumption, and anoxic partial denitrification-anaerobic ammonia oxidation saves carbon sources (Fig. 4). The process is stable and easy to control, with a low sludge output and a reduction in greenhouse gas emissions, and may be more suitable for saving energy, reducing consumption, and deep denitrification (Xu et al., 2021).

Traditional biological denitrification is usually achieved through nitrification and denitrification, producing N_2O in the process. N_2O is an important greenhouse gas. Comammox bacteria have no N_2O biosynthesis pathway; therefore, using comammox bacteria as the main reaction microorganism in the nitrification process may greatly reduce N_2O emissions. Comammox bacteria can oxidize low ammonia nitrogen to nitrate nitrogen under low DO

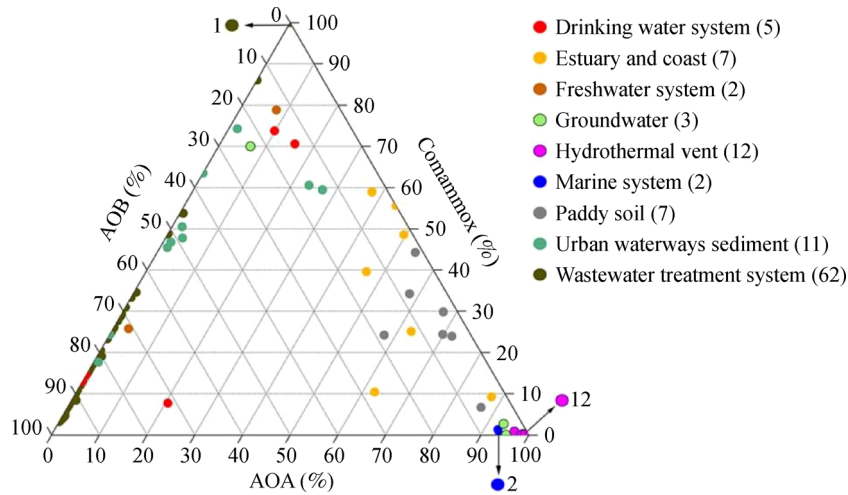


Fig. 2 Ternary plot of the proportions of comammox, AOB, and AOA in 111 metagenomic data sets of different environmental samples from the NCBI SRA database (Xia et al. 2018).

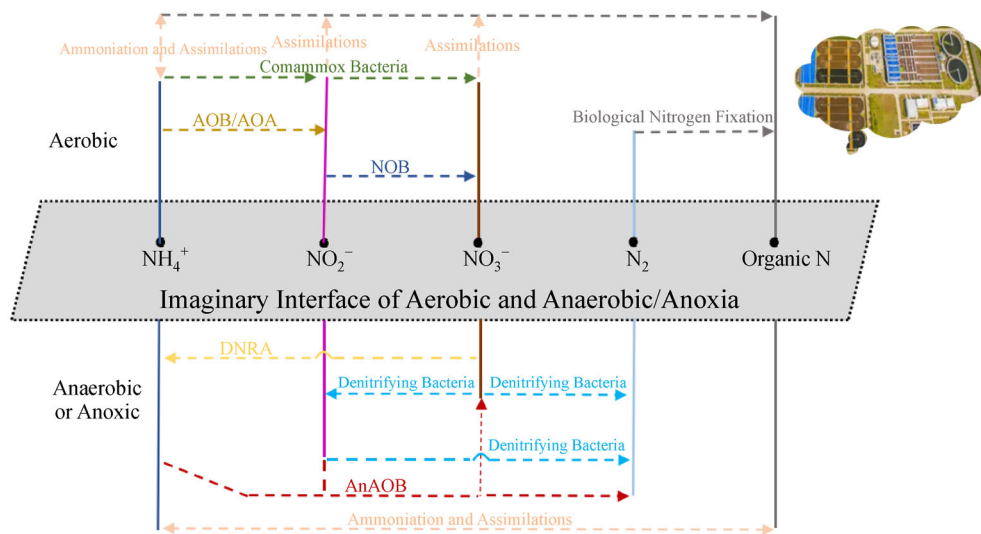


Fig. 3 Common microbial denitrification processes in wastewater treatment plants. (DNRA: dissimilatory nitrate reduction to ammonium).

conditions. Low DO meets the reaction conditions of simultaneous denitrification (Holman and Wareham, 2005). Simultaneous nitrification and denitrification can reduce internal circulation and aeration energy consumption and save space, while the nitrification process, avoiding the accumulation of nitrite, can be used to treat micro-polluted drinking water, improve treatment efficiency, and avoid the potential risk of nitrite production.

5 Conclusions and outlook

This review summarizes the discovery, physiological characteristics, and ecological distribution of comammox bacteria, and proposes potential applications in wastewater

treatment. All comammox that have been discovered are *Nitrospira* bacteria, belonging to lineage II of the *Nitrospira* genus. The affinity of comammox bacteria to ammonium is higher and is also slower growing than conventional AOB/NOB, so better adapted to oligotrophic conditions. Furthermore, comammox bacteria have a complex pathway and can live in many adverse environments, as demonstrated by their wide distribution in natural and artificial systems. Coupling with DEAMOX or enriching in oligotrophic conditions can promote total nitrogen removal, thereby reducing both costs and N_2O emissions.

The discovery of comammox bacteria has important significance and research value for calculating and evaluating the relative contribution of nitrification in the

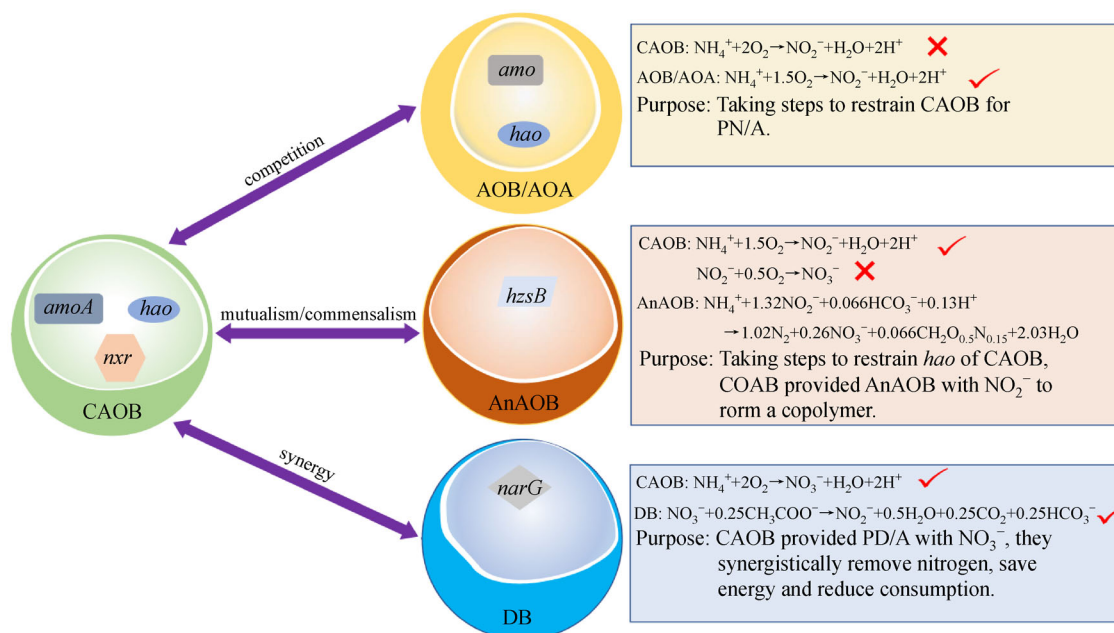


Fig. 4 Application of comammox bacteria in wastewater treatment. (DB: denitrifying bacteria).

microbial nitrogen cycle. To detect the presence and abundance of comammox bacteria using the *amoA* gene of comammox bacteria, a variety of specific primers have been developed to identify and quantify comammox bacteria of different branches and species levels. However, the accuracy and sensitivity of these need to be further improved.

Comammox bacteria are of great significance in both natural and artificial systems, but the niche differences and influencing factors between them and AOB, AOA, NOB, and other microorganisms remain unclear, and may be a research hotspot for microbial applications in the future. At present, our understanding of comammox bacteria is limited, and the in situ activity of comammox bacteria cannot be fully evaluated. There is an urgent need to develop isolation and purification methods to study the physiological characteristics of microorganisms at the single-cell level, and to explore the coupling mechanism between comammox bacteria and traditional nitrification and denitrification. For the treatment of micro-polluted water sources, it is necessary to further explore the enrichment and operating conditions of comammox bacteria. In wastewater treatment, the use of comammox bacteria can reduce aeration energy consumption, greenhouse gas N_2O emissions, and sludge production, while improving deep denitrification efficiency. It has a broad application potential and may become a new biological denitrification process in the future.

The following areas should be given priority in future studies on comammox bacteria: 1) Efficient and accurate methods that can identify the contribution of diverse microorganisms from similar functional microbes should

be developed (e.g., AOB/AOA/NOB/comammox bacteria), and the accuracy and sensitivity of specific primers need to be further improved; 2) In wastewater treatment plants, the quantity of comammox bacteria as a proportion of nitrifying bacteria varies, and the relative contribution is not clear; evaluation and calculation of this should be strengthened in the future; 3) Comammox bacteria are used to couple DEAMOX to develop a new nitrogen removal process, the potential of comammox bacteria in more sustainable nitrogen removal techniques could be assessed from the point of view of engineering applications.

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