New perspectives on microbial communities and biological nitrogen removal processes in wastewater treatment systems

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Abstract

Biological nitrogen removal (BNR) is a critical process in wastewater treatment. Recently, there have new microbial communities been discovered to be capable of performing BNR with novel metabolic pathways. This review presents the up-to-date status on these microorganisms, including ammonia oxidizing archaea (AOA), complete ammonia oxidation (COMAMMOX) bacteria, anaerobic ammonium oxidation coupled to iron reduction (FEAMMOX) bacteria, anaerobic ammonium oxidation (ANAMMOX) bacteria and denitrifying anaerobic methane oxidation (DAMO) microorganism. Their metabolic pathways and enzymatic reactions in nitrogen cycle are demonstrated. Generally, these novel microbial communities have advantages over canonical nitrifiers or denitrifiers, such as higher substrate affinities, better physicochemical tolerances and/or less greenhouse gas emission. Also, their recent development and/or implementation in BNR is discussed and outlook. Finally, the key implications of coupling these microbial communities for BNR are identified. Overall, this review illustrates novel microbial communities that could provide new possibilities for high-performance and energy-saving nitrogen removal from wastewater.

Keywords: Biological nitrogen removal (BNR); ammonia oxidizing archaea (AOA); complete ammonia oxidation (COMAMMOX); anaerobic ammonium oxidation (ANAMMOX); denitrifying anaerobic methane oxidation (DAMO)

1. Introduction

Biological nitrogen removal (BNR) is one of the crucial processes in wastewater treatment plants (WWTP) (Cui et al., 2019). Conventional BNR process consists of two steps, nitrification and denitrification (Eskicioglu et al., 2018, Thakur and Medhi, 2019). Nitrification consists of two sub-reactions. These two reactions are catalysed by physiologically distinct clades of microorganisms. Initially, ammonia is oxidized into nitrite by ammonia-oxidizing bacteria (AOB), and the biochemical process is illuminated by research progress on the genera *Nitrosomonas* and *Nitrosospira.* Then nitrite is oxidized into nitrate by nitrite-oxidizing bacteria (NOB) and the biochemical process is enlightened by the investigation on the genera *Nitrobacter* and *Nitrosospira*. The denitrification process is mainly undertaken by heterotrophic bacteria, such as *Paracoccus denitrificans* and *Pseudomonas stutzeri*, which require organic carbon resources as electron donor to reduce nitrogen oxides to nitrogen gas.

Due to the oxygen supply to nitrification process and the organic matter requirement for denitrification process, the conventional BNR process has become an energy-intensive process (Mannina et al., 2019). Although the nitritation and denitritation process has been developed to reduce oxygen consumption and organic carbon by 25% and 40%, respectively (Eskicioglu et al., 2018, Li et al., 2018c), the reduced oxygen concentration does not guarantee the selective inhibition of NOB. The genera *Nitrospira* has unexpected lower half-saturation constant (Km) values for dissolved oxygen (DO) as compared to *Nitrosomonas europaea*, and therefore it can thrive at low DO concentration. If DO is further reduced for washing out NOB, the ammonia oxidation rate by AOB would be impaired by limiting the electron acceptor and the electron flow to the ammonia monooxygenase (AMO) (Li et al., 2018a).

Such dilemma of BNR could be eliminated with the advances in the exploration of microbial communities involved in nitrogen cycle. Hitherto, the spectrum of microbial communities has been expanded and new versatilities in electron donors or acceptors in nitrogen removal have been discovered (Fu et al., 2017, Hu et al., 2019a, Wang et al., 2018). The prokaryotes microorganisms, ammonia oxidizing archaea (AOA), have also been discovered to harbour archaeal ammonia monooxygenase genes to encode corresponding enzyme for catalysing ammonia oxidization (Tian et al., 2018, Treusch et al., 2005, Könneke et al., 2005, Park et al., 2006, Islam et al., 2019). Complete ammonia oxidation bacteria (COMAMMOX) can catalyse both ammonia and nitrite oxidation with ammonia and nitrite exploited as electron donor (Daims et al., 2015, Daims et al., 2016, van Kessel et al., 2015, Cotto et al., 2020). It has also been identified that ferric iron could be exploited as electron acceptor for ammonium oxidation by anaerobic ammonium oxidation coupled to iron reduction (FEAMMOX) bacteria (Shin et al., 2015, Yin et al., 2019). Anaerobic ammonium oxidation (ANAMMOX) process can use ammonium as the electron donor and reduce nitrite into N_2 autotrophically (Van de Graaf et at., 1995, Mulder et al., 1995, Li et al., 2018a, Miao et al., 2020). Additionally, in denitrifying anaerobic methane oxidation (DAMO) process, methane could be utilized as the electron donor and coupled to denitrification (Luesken et al., 2011, Ettwig et al., 2010, Jiang et al., 2018a, Wang et al., 2017, Versantvoort et al., 2018).

The above-mentioned microbial communities with the ability of utilizing alternative electron acceptors or donors, could potentially provide substantial advantages over canonical nitrogen removal process, such as less N_2O production, energy saving in aeration and organic carbon saving in anoxic denitrification. Also, they can be better to confront the adverse environment such as low-DO or ammonia-deficiency conditions. Therefore, in the present study, advances in perspective microbial communities are reviewed by focusing on the advantages of each

microbial communities over canonical nitrifiers or denitrifiers. The corresponding application perspectives are further discussed and outlooked.

2. Advance in ammonia oxidizing archaea (AOA)

2.1 AOA

AOA are prokaryotes that contain archaeal AMO genes for synthesising AMO and performing ammonia oxidization which has been widely identified in activated sludge systems. One of the dominant species of AOA in the WWTPs is *Nitrosopumilus maritimus* (Limpiyakorn et al., 2011, Ren et al., 2019).

As shown in Figure 1, it has been suggested that *N. maritimus* harbors archaeal AMO genes to transcript the presumptive archaeal AMO and perform ammonia oxidation (Tian et al., 2018, Treusch et al., 2005). While it lacks the genes coding for the HAO enzymes to catalyse the conventional hydroxylamine oxidation processes, it has been proposed that nitroxyl could be an alternative archaeal pathway for ammonia oxidation (Walker et al., 2010). In this proposed pathway, nitroxyl could be generated through the spontaneous decay of HNOHOH, which is produced from insertion of two oxygen atoms into ammonia by archaeal AMO. Then nitroxyl is oxidized into nitrite by nitroxyl oxidoreductase (NXOR). When the electron extracted by NXOR transfers into the quinone pool, the proton motive force would be produced for ATP generation. Furthermore, NO is also proposed as the intermediate product in the archaeal pathway of ammonia oxidation. In this process, NO is served as the redox shuttle. By adding ammonium, a NO transient accumulation in *N. maritimus* culture was observed by Martens-Habbena et al. (2009b).

2.2 Advantages of AOA over canonical AOB on ammonia removal

It has been suggested that AOA possess advantages over canonical AOB from various aspects (Roy et al., 2017). Such advantages could be accredited to the uniqueness gene composition of AOA, which confers AOA the ability of adapting to extremely low electron

donor/acceptor condition and making it equipped with an energy-efficient carbon fixation pathway to conserve more energy and obtain higher growth yield.

The affinity for electron donor of *N. maritimus* (i.e., AOA) is higher than that of *N. europaea* (i.e., canonical AOB). A higher abundance of *N. maritimus* has been found in the deep marine environment, where the ammonia concentration is deficient. This supports that under low ammonia concentration, the growth of AOB could be outcompeted by AOA (Martens-Habbena et al., 2009a).

As shown in Table 1, due to the half-saturation constant (Km) of *N. maritimus* for ammonia is as low as 0.002 mg NH_3-N/L (Martens-Habbena et al., 2009b), *N. maritimus* can adapt to extremely substrate deprived environment. Horak et al. (2013) also found the Km of *N. maritimus* for ammonia as low as 0.0016 mg NH_3-N/L. In comparison, the Km values of *N. europaea* for electron donor (0.077-0.55 mg-NH_3-N/L) are much higher than that of *N. maritimus* (Table 1) (Hunik et al., 1992, Wahman et al., 2005, Yu et al., 2020). The reason that AOA could adapt to low ammonia environment is the ammonia permease genes for encoding the high-affinity ammonia transport ter Amt2 and the S-layer proteins. Under ammonia limited condition, to maintain assimilation cell synthesis and growth, ammonia is preferentially channelled for anabolic and catabolic pathways by the high-affinity ammonia transport ter Amt2 and the S-layer proteins (Nakagawa and Stahl, 2013).

The oxygen affinity of *N. maritimus* is also higher than that of *N. europaea*. As shown in Table 1, the Km values of *N. maritimus* for DO are in the range of 0.064 – 0.128 mg/L O_2 (Li et al., 2016b, Martens-Habbena et al., 2009b, Park et al., 2010, Qin et al., 2017), while the Km values of *N. europaea* for oxygen are generally around one order of magnitude higher than that of *N. maritimus* (Martens-Habbena et al., 2009b, Park et al., 2010, Law et al., 2019, Yu et al., 2020). The oxygen oxidase contained by *N. maritimus* is COX1-type terminal oxidase, the typical Km for oxygen of that oxidase is 0.06 mg/L O_2.

Autotrophic CO_2 fixation is an essential anabolic process of autotrophic bacteria for biomass synthesis. The CO_2 fixation cycle utilized by *N. maritimus* is hydroxypropionate/hydroxybutyrate (HP/HB) cycle, which enables AOA to thrive and outcompete AOB under extremely low electron donor/acceptor concentrations. From the genomic analysis, Könneke et al. (2014) identified genes encoding the key enzymes in HP/HB cycle is 3-hydroxypropionyl-CoA dehydratase, which enable the CO_2 fixation to become more energy-efficient and reduce the cost of protein biosynthesis due to its versatility in catalysing multiple reactions of HP/HB cycle. The HP/HB cycle requires only one-third energy in comparison to Calvin-Benson cycle exploited by AOB. Therefore, more energy can be exploited for biomass formation in *N. maritimus*. Such advantage confers that AOA has higher growth yield than AOB (Zhang et al., 2010).

2.3 Application perspectives of AOA

As the high substrate affinity of AOA determines the niche partitioning from AOB, it is perspective to exploit the disparities and apply for BNR from wastewater (Eskicioglu et al., 2018). The high affinity of *N. maritimus* to ammonia, as indicated by the low Km value, makes it a competitive candidate for thriving in the wastewater with low ammonia concentration. Roy et al. (2017) found AOB was outnumbered by AOA in a moving bed bioreactor (MBBR) where ammonia concentration is low. This result implies that AOA contribute more nitrification under ammonia limited condition than high ammonia condition. Due to the slow growth rate of AOA, biomass retention is crucial for high rate performance. Biofilms can retain microorganisms with very slow growth kinetics and therefore the applications of AOA are mainly in biofilm processes (Roy et al., 2017). Liu et al. (2016) evaluated the performance of AOA in a single stage membrane aerated biofilm reactor (MABR) and found that AOA were more stable than AOB when controlling oxygen supply for selective inhibition the activity of NOB. It is also suggested to operate MABR in oxygen

limited condition for achieving better performance, which could potentially decrease oxygen supply by 5% and result in a substantial annual operation saving by 2-3% when it applies to the entire WWTP. In addition, AOA system has substantially wider operating window for high-level total nitrogen removal in terms of the variations of oxygen and ammonia loadings.

2.4 Outlook of AOA

It has been reported from previous studies that micropollutants could be biodegraded in nitrification process (Ma et al., 2018). Helbling et al. (2012) demonstrated that the activity of AMO could be induced by micropollutants by positing into the hydrophobic pocket of AMO for reacting with oxygen-activating site, and therefore micropollutants could be oxidized in the presence of ammonia. AOA contain archaeal AMO genes for encoding archaeal AMO, suggested the possibility that micropollutant degradation by AOA. Men et al. (2016) found *Nitrososphaera gargensis* (AOA) could biotransform several micropollutants, such as tertiary amines, mainserin and ranitidine during ammonia oxidation, in which the biotransformation rates are higher than that of AOB under the same condition. Yet, *Nitrososphaera gargensis* is not the dominating AOA in wastewater. Therefore, future research interest could shift to micropollutant biodegradation kinetics of *N. maritimus*.

Furthermore, as indicated by a metagenomic study of AOA, the presence of *ureC* gene provide AOA alternative pathway to obtain energy besides the ammonia oxidation (Wang et al., 2019). The presence of *nirK* gene show the ability of AOA to catalyse nitrite reduction which could suggest possible N_2O production pathway by AOA. Moreover, the genes for transport phosphate and heavy metals, such as manganese, zinc and iron were also found in AOA genomes which suggested its versatility in utilizing inorganic phosphate and adaptation of heavy metal pollution (Wang et al., 2019). Future research could study the treatment of wastewater containing heavy metal by AOA.

3. Advance in complete ammonia oxidation bacteria (COMAMMOX)

3.1 COMAMMOX bacteria

Previously, ammonia oxidation to nitrate was widely believed to be catalysed by two physiologically distinct clades of microorganisms (i.e., ammonia oxidation to nitrite by AOB and nitrite oxidation to nitrate by NOB). While, based on the kinetic theory, the existence of a complete ammonia oxidizer with a lower maximal growth rate but a higher growth yield was postulated (Costa et al., 2006). Until recently the novel *Nitrospira* species were identified to be capable of performing complete oxidation of ammonia to nitrate (Daims et al., 2015, van Kessel et al., 2015). The species were provisionally named as *Candidatus Nitrospira inopinata* (Daims et al., 2015), *Ca. N. nitrosa* and *Ca. N. nitrificans* (van Kessel et al., 2015). Different from other species of genus *Nitrospira*, a full set of AMO and HAO genes has been detected in the genomes of COMAMMOX bacteria (van Kessel et al., 2015).

3.2 Advantages of COMAMMOX bacteria over canonical AOB

The ecological niche-partition between the COMAMMOX *Nitrospira* and canonical AOB results from the differences of functional potential in substrates utilization. COMAMMOX *Nitrospira* is proposed to be capable of outcompeting over canonical AOB under substrate deficient condition (Wang et al., 2018). For example, Kits et al. (2017) reported that the half-saturation constant of COMAMMOX *Nitrospira* on ammonia is 0.026 μg-NH_3-N/L, three orders of magnitude lower than that of AOB *N. europaea* (i.e., 0.077 mg-NH_3-N/L, Table 1) (Hunik et al., 1992), indicating the ammonia affinity of COMAMMOX *Nitrospira* is significantly higher than that of canonical AOB. Although, the study on the oxygen affinity of COMAMMOX *Nitrospira* is limited, there are evidences indicating COMAMMOX *Nitrospira* could adapt low DO conditions. Camejo et al. (2017) found COMAMMOX *Nitrospira* were enriched under oxygen limited condition (0.2 μg/L O_2), a value lower than the oxygen half saturation constant for canonical AOB (Table 1).

By comparing with *N. europaea*, COMAMMOX *Nitrospira* is more versatile in substrate utilization (Liu et al., 2019a). Under substrate limited condition, COMAMMOX *Nitrospira* can catalyse urea degradation for ammonium production. As shown in Figure 2, COMAMMOX *Nitrospira* contains the high-affinity urea transporters, the outer membrane porin (fmdC), and an urea carboxylase-related transporter (uctT), which contribute to harvesting the urea at low concentrations. Agmatinase gene was also detected in COMAMMOX *Nitrospira* which can encode agmatinase and hydrolyzes agmatine and produce urea (Palomo et al., 2018). Such a unique metabolic versatility in harvesting substrates confers the advantage of COMAMMOX *Nitrospira*.

COMAMMOX *Nitrospira* is better to withstand the copper deficiency than *N. europaea*. The AMO of *N. europaea* is encoded by *amoCAB* operon and belongs to the family of copper membrane monooxygenase (CuMMO) enzyme. The copper deficiency will result in a decrease in the AMO activity of *N.europaea* (Bennett et al., 2016, Wagner et al., 2016). However, as shown in Figure 2, the Cu^{2+} homeostasis genes (*copCD* and *copAB*), were detected in COMAMMOX Nitrospira (Palomo et al., 2018). These genes can encode proteins with higher Cu^{2+} uptake efficiency and copper tolerance. As compared to the low-affinity aa3-type heme-copper oxidase of AOB, the high-affinity cytochrome bd-like oxidases harboured by COMAMMOX *Nitrospira* can outcompete AOB under copper limited condition (Figure 2). Additionally, under oxidative stress or oxygen-limited conditions, the 2/2 hemoglobin type II (TrHb2) gene detected in the COMAMMOX genomes could confer the advantages of COMAMMOX *Nitrospira* for competing electron acceptor.

Another advantage that possessed by COMAMMOX bacteria is less N_2O production than canonical AOB. Although, similar to canonical AOB, *Ca. N. inopinata* harbours a complete set of genes for encoding AMO and β-subunit of HAO, it lacks canonical genes for encoding nitrite reductase, which suggests that the N_2O production by *Ca. N. inopinata* could come

from abiotic NH_2OH decay, rather than the nitrifier denitrification pathway. Therefore, the N_2O production from COMAMMOX bacteria could be significantly lower that from canonical AOB, which is mostly from the nitrifier denitrification pathway (Ren et al., 2019).

3.3 Application perspectives of COMAMMOX

COMAMMOX could both catalyse ammonia oxidation and nitrite oxidation. The main advantages of COMAMMOX bacteria over AOB are the ability to degrade urea for ammonium production, the better adaptability under copper-limited condition and high substrate affinities (Hu and He, 2017, Palomo et al., 2018).

The COMAMMOX application offers the essential merits in terms of energy-saving and nitrogen polishing due to its high affinity for oxygen and ammonium (Liu et al., 2019a). There have been various studies that confirmed the COMAMMOX bacteria could be the dominant nitrifying community in the bioreactor (Camejo et al., 2017, Daims et al., 2015, Roots et al., 2019). From the experiments of Roots et al. (2019), under micro-aerobic, low ammonium environment and prolonged sludge retention time (SRT), COMAMMOX *Nitrospira* became the 94% of total ammonia-oxidizing microbial communities in the reactor, and achieved average ammonium removal rate of 58.6 mg N/L/d. Such factors are identified as the pivotal conditions for the proliferation of COMAMMOX *Nitrospira*.

Also, different from synthetic wastewater, real wastewater is usually lack of certain microelements, i.e., copper. Such copper deficiency can neutralize the function of AMO for AOB and hamper the conventional nitrification process during wastewater treatment (Thakur and Medhi, 2019). In contrast, COMAMMOX *Nitrospira* could maintain its effectiveness under copper-limited environment (Fowler et al., 2018). In addition, the COMAMMOX *Nitrospira* has the metabolic capacity to degrade urea for ammonium production, which can be utilized for certain industrial urea removal process (Hu and He, 2017, Koch et al., 2019, Poghosyan et al., 2019).

3.4 Outlook of COMAMMOX

Although it has been suggested that COMAMMOX *Nitrospira* has higher DO affinity than canonical AOB and could thrive under low DO concentration, the studies on kinetics of COMAMMOX *Nitrospira* are limited at this stage (Wang et al., 2018). In order to achieve effective energy saving while removing nitrogen in wastewater treatment, it is necessary to investigate the half saturation constant of COMAMMOX *Nitrospira* for oxygen, and therefore, the aeration intensity could be better controlled with the key parameters available. The energy demand of COMAMMOX *Nitrospira* for nitrification process could be further reduced by selective inhibiting the activities of enzymes that catalyse nitrite oxidation, then nitrite would be accumulated as partial nitrification process. Transient nitrite accumulation has been observed from previous experiments (Daims et al., 2015, Kits et al., 2017), which suggested the possibility to realize nitrite accumulation of COMAMMOX *Nitrospira* by controlling environmental factors. In the future, more efforts could concentrate on the manipulation of enzyme activities of COMAMMOX *Nitrospira*. Then, with nitrite accumulation, the application versatility of COMAMMOX *Nitrospira* would be expanded. The coexistence of COMAMMOX *Nitrospira* in partial nitrification may affect the nitrogen removal efficiency due to its competition with AOB on ammonia. COMAMMOX *Nitrospira* has higher substrate affinity than AOB, thereby leading to nitrate rather than nitrite accumulation. On the other hand, such competition may benefit to N_2O mitigation from partial nitrification, as N_2O production by AOB is deprived by COMAMMOX *Nitrospira*. Therefore, it is necessary to perform further research on this aspect.

Recently, the metagenomic study of two novel COMAMMOX *Nitrospira* retrieved from terrestrial subsurface detected the full gene set for ammonia oxidation, including genes *amoCAB* and AMO subunit *amoEDD2*, and all subunits of the nitrite oxidoreductase for nitrite oxidation (Poghosyan et al., 2019). Besides, the novel COMAMMOX *Nitrospira* also

contains genes for encoding 3b bidirectional [NiFe] hydrogenase, and formate uptake and oxidation, which can oxidize hydrogen or formate as alternative energy sources. Moreover, high-affinity SulP/SLC26-type transporters could transport a broad range of substrates, including sulfate, bicarbonate and chloride. These new findings indicate the enhanced genomic and metabolic versatility of COMAMMOX *Nitrospira* (Poghosyan et al., 2019), which could be the new future research direction.

4. Advance in iron-dependent anaerobic ammonium oxidation to nitrate (FEAMMOX)

4.1 FEAMMOX bacteria

Besides the conventional electron acceptor, it has been suggested that ferric iron could serve as electron acceptor, and the electron is transferred extracellularly while obtaining energy from ammonium oxidation (Huang and Jaffe, 2018, Yang et al., 2018, Yin et al., 2019). By using ferrihydrite or goethite as the electron acceptor, Huang & Jaffe (2015) found a previously unreported species *Acidimicrobiaceae bacterium* A6 that can anaerobically oxidize ammonium. This study confirmed that *Acidimicrobiaceae bacterium* A6 plays a significant role in FEAMMOX process, as the ammonium oxidation and iron reduction only occurred when *Acidimicrobiaceae bacterium* A6 is presented.

4.2 Advantages of FEAMMOX bacteria over canonical AOB

With the unique metabolic characteristics, FEAMMOX bacteria have advantages over canonical AOB (Huang and Jaffe, 2015). One is the elimination of aeration demand, the other one is the reduced N_2O production. Since the reaction is catalysed under anaerobic condition and iron is used as the electron acceptor instead of oxygen, therefore, the oxygen demand is obviated. One molecule of oxygen is required to oxidize per molecule of ammonia for aerobic ammonia oxidation process catalysed by *N. europaea* (Liu et al., 2019a), in contrast, three molecules of ferric oxide are required to oxidize per molecule ammonium for

FEAMMOX reaction (Huang and Jaffe, 2018). Therefore, less energy is required for FEAMMOX to oxidize ammonium.

Although, to date, the N_2O production analysis on FEAMMOX bacteria *Acidimicrobiaceae bacterium* A6 has not been thoroughly investigated, it has been suggested that N_2O could be produced abiotically through NH_2OH decomposition to N_2O by coupling ferric iron reduction, with the major product being nitrite (Huang and Jaffe, 2015). This is different from *N. europaea*, which harbours genes *nirS* and *nirK* for encoding nitrite reductase, with N_2O being the major product of the nitrifier denitrification pathway (Ren et al., 2019).

4.3 Application perspectives of FEAMMOX

FEAMMOX bacteria play a pivotal role in natural iron and nitrogen cycle (Yin et al., 2019). The application of *Acidimicrobiaceae bacterium* A6 in the constructed wetland with high iron stratum led to a higher ammonium removal (i.e., 25.0 ± 7.3%) as compared to the stratum with lower iron concentration (i.e., 11.0 ± 9.7%) (Shuai and Jaffé, 2019).

Also, Yang et al. (2018) investigated the FEAMMOX process for wastewater treatment. By adding ferrihydrite into an anaerobic digestor, anaerobic ammonium oxidation was induced and the ammonium removal efficiency increased by 33%. The abundance of FEAMMOX bacteria was also increased, which suggested the possibility of applying FEAMMOX in BNR from wastewater. In order to improve the efficiency of FEAMMOX, Ruiz-Urigüen et al. (2018) designed a novel method of substituting anodes with ferric iron as electron acceptor as a mean to promote ammonium removal efficiency of FEAMMOX bacteria. The results identified *Acidimicrobiaceae bacterium* A6 can transfer electrons onto electrodes and ammonium removal could be enhanced.

4.4 Outlook of FEAMMOX

Through a metagenomics analysis on the genus *Acidimicrobiaceae*, *Acidimicrobiaceae bacterium* A6 is the only species that can use ammonium as electron donor for ferric iron

reduction. Besides, there is also a group of oxygenase related genes found in the enrichment culture. Such the monooxygenase related genes may be induced by the oxidation of ammonium under iron reducing conditions and participate co-metabolic degradation of chlorinated ethane such as trichloroethylene and tetrachloroethylene (Ge et al., 2019). These new findings conferred the new versatility of FEAMMOX bacteria to degrade organic contaminants. Furthermore, *Acidimicrobiaceae bacterium* A6 has been suggested to harbour genes that can utilize uranium U (VI) in addition to ferric iron as electron acceptors for ammonium oxidation (Gilson et al., 2015). The ability of coupling U (VI) reduction with ammonium oxidation provides new lights on remedying metal contaminated area with limited carbon sources.

Also, the enrichment of FEAMMOX bacteria deserves more efforts. There have been previous studies testing the abundance of FEAMMOX bacteria (Huang and Jaffe, 2018), which suggested that increasing iron concentrations or providing more powerful electron acceptors can increase the abundance of FEAMMOX bacteria. Yet, the studies were undertaken in the constructed wetland rather than WWTP. In the future, more efforts could focus on the enrichment of FEAMMOX bacteria in wastewater environment.

For biological nitrate reduction, ferrous iron could be used as electron donor to reduce nitrate by autotrophic bacteria, which has been observed in wastewater treatment process with nitrate removal efficiency at 46.6% (Zhang et al., 2016). The FEAMMOX bacteria can oxidize ammonium by reducing ferric iron into ferrous iron, which could provide electron donor for the nitrate-dependent ferrous iron oxidizing bacteria to reduce nitrate. Then, the oxidized ferric iron could be used by FEAMMOX bacteria again. Such coupling could offer new lights on application perspective.

5. Advance in ANAMMOX

5.1 ANAMMOX bacteria

With the unique ecophysiology properties, ANAMMOX bacteria has been widely investigated by various researchers (Li et al., 2018b, Wang et al., 2019a). Five genera of ANAMMOX bacteria have been identified as: *Candidatus Brocadia*, *Candidatus Kuenenia*, *Candidatus Jettenia*, *Candidatus Scalindua* and *Candidatus Anammoxoglobus* (Ma et al., 2016, Peeters and van Niftrik, 2019), which play a critical role in the global nitrogen cycle. The cell of ANAMMOX bacteria is divided into three compartments, which are the anammoxosome, cytoplasm and periplasm. It also contains a unique membrane lipids named as ladderanes, which enable the membrane of ANAMMOX bacteria to be less permeable and more rigid. It has been hypothesized that the anammoxosome compartment is where the energy metabolism located, in which the proton motive force, used for ATP synthesis, arisen from coupling anammox reaction to an electron transport chain in the anammoxosome membrane. ANAMMOX bacteria acquire energy from reducing nitrite anaerobically and using ammonium as electron donor, in which nitrite is firstly reduced to nitric oxide, then nitric oxide is combined with ammonium to form hydrazine by hydrazine synthase, and finally, hydrazine is oxidized to dinitrogen gas by dehydrogenase (Peeters and van Niftrik, 2019).

For different ANAMMOX species, metagenome studies suggested that there might be various ANAMMOX metabolic pathways (Wang et al., 2019b). For example, it has been reported that ANAMMOX bacteria *Ca. Brocadia* lacks the genes for encoding canonical nitrite reductase (NirS or NirK), which can reduce nitrite into nitric oxides. Instead, *Ca. Brocadia* could contain genes for encoding unidentified nitrite reductase that reduce nitrite into hydroxylamine instead of nitric oxide, and then hydrazine is synthesized from hydroxylamine and ammonium and further oxidized into dinitrogen gas. Moreover, Hu et al. (2019b) found that ANAMMOX bacteria *Ca. K. stuttgartiensis* could utilize nitric oxide as an alternative electron acceptor in the absence of nitrite and perform anaerobic ammonium

oxidation by coupling nitric oxide reduction. *Ca. K. stuttgartiensis* contains gene kuste3160 that can encode nitric oxide reductase, flavoprotein norVW. Therefore, with the versatility of ANAMMOX bacteria, it could withstand the adverse environment and perform more stable BNR than the heterotrophic denitrifiers.

5.2 Advantages of ANAMMOX bacteria over heterotrophic denitrifiers

Given the unique metabolism of ANAMMOX bacteria, there could be significant advantages of ANAMMOX bacteria over heterotrophic denitrifiers, especially energy saving (Lei et al., 2018). In terms of carbon requirement, ANAMMOX bacteria are chemoautotrophic bacteria that oxidize ammonium by using nitrite as electron acceptor and CO_2 as the main carbon source. In this process, nitrate is formed by oxidizing nitrite to nitrate while reducing carbon dioxide to organic carbon during synthesis (McCarty, 2018), thus, the requirement of organic substrate for denitrification is obviated. In contrast, organic carbon is required by heterotrophic denitrifiers, their electron transport system requires organic carbon as electron acceptor to accommodate reducing nitrogen oxides.

ANAMMOX bacteria could out-compete traditional heterotrophic denitrification bacteria due to the high nitrite affinity and strong tolerance to nitrite inhibition (Ma et al., 2016). As shown in Table 2, previous studies have suggested that the typical half-saturation constant for nitrite of ANAMMOX bacteria is from 0.035 – 0.2mg-N/L, which is 1 – 2 orders of magnitude lower than that of heterotrophic bacteria. Although an extremely higher half-saturation constant for nitrite of ANAMMOX bacteria (i.e., 21 mg-N/L) was reported by Tang et al. (2013), it could be explained by the limited substrate diffusion in granular sludge. Such result is still lower than that of heterotrophic denitrification bacteria (i.e., 37 mg-N/L) (Tang et al., 2013). Therefore, ANAMMOX bacteria have a higher affinity for nitrite and could play a dominant role in nitrite reduction. Such a high affinity could be accredited to the reaction intermediate. For the reaction of ANAMMOX process, hydrazine is the intermediate

product. Hydrazine is an energy-rich compound that can provide electrons for ferredoxin reduction. The proton motive force could be generated by this process, which could result in the highest reaction rate at lower substrate concentration (Peeters and van Niftrik, 2019).

It has been suggested that ANAMMOX bacteria could withstand higher nitrite toxicity as comparing to heterotrophic denitrifying bacteria (Ma et al., 2016), as the half nitrite inhibition constant of ANAMMOX bacteria (i.e., 179 mg N/L) is higher than that of heterotrophic denitrifiers (i.e., 0.047 mg N/L) (Tang et al., 2013). Due to the ladderane anammoxosome membrane of ANAMMOX bacteria is equipped with strained linearly concatenated cyclobutane moieties that can prevent the permeations of FNA (Carvajal-Arroyo et al., 2014, Lotti et al., 2012). Therefore, ANAMMOX bacteria could have higher tolerance on FNA than heterotrophic denitrifiers.

5.3 Application perspectives of ANAMMOX

ANAMMOX system has been successfully implemented in full-scale BNR application. Over 100 full-scale sidestream deammonification plants handling high-ammonia used water have been in successful operation worldwide since ANAMMOX bacteria were first discovered in the 1990s (Lackner et al., 2014, Li et al., 2018b). However, its application for mainstream BNR is still limited. Considering the significant advantages of ANAMMOX bacteria over conventional heterotrophic denitrifying bacteria, its mainstream application could convert the wastewater treatment process to an energy-neutral or -producing process with the combination of anaerobic treatment for carbon source (Li et al., 2018a). Since ANAMMOX process does not require organics for denitrification, organics are better converted into methane for energy production, and methane production could be increased by 47% (McCarty, 2018). Also, the aeration demand could be substantially curtailed by better controlling of aeration systems. This can lead to 50% reduction of energy to supply air by comparing with conventional BNR process (McCarty, 2018).

One of the key barriers for applying ANAMMOX process in mainstream is how to selectively remove NOB but keep AOB (Ma et al., 2016). There are three genera of NOB commonly found in WWTPs which are genus *Nitrobacter*, genus *Nitrospira* and a novel NOB genus *Candidatus Nitrotoga*. The selective inhibition of NOB through manipulating environmental factors has been studied based on different NOB genus (Liu et al., 2019b, Ma et al., 2016, Wei et al., 2018). Genus *Nitrobacter* and genus *Nitrospira* have different substrate affinities. Genus Nitrobacter (*Nitrobacter hamburgensis* and *Nitrobacter winogradskyi*) are r-strategists that can adapt to with high nitrite and high DO condition, while genus *Nitrospira* (*Nitrospira sp*. and *Candidatus Nitrospira defluvii*) are K-strategists with higher substrate affinity, which can thrive in low nitrite and low DO condition (Liu et al., 2015). Both the activities of *Nitrobacter* and *Nitrobacter* could be inhibited by increasing free ammonia (FA) concentration, while the tolerance of *Nitrobacter* to FA is higher than that of *Nitrobacter* (Liang et al., 2015a). Both the genus *Nitrobacter* and genus *Nitrospira* could be suppressed by free nitrous acid (FNA), while genus *Nitrobacter* is more tolerant to FNA as comparing with genus *Nitrospira* (Wang et al., 2017). Yet, the novel NOB genus *Ca. Nitrotoga* was reported to have a substrate affinity coefficient higher than that of *Nitrospira* and within the range reported for *Nitrobacter* (Figdore et al., 2018). It has been suggested that the growth of *Ca. Nitrotoga* could be benefited under high nitrite/FNA concentrations (Ma et al., 2015). An important feature of *Ca. Nitrotoga* is that it can grow at low temperatures between 4 °C and 17 °C (Liang et al., 2015b). Therefore, FA and FNA are vital in ANAMMOX process (Liu et al., 2019b). Wei et al. (2018) achieved an ideal influent for ANAMMOX bacteria with a nitrite accumulation rate at 87.8% by controlling both FA and FNA concentrations at 38 mg-NH_3/L and 0.623mg/L, respectively. These results suggest that FA and FNA could play major inhibitory roles on the activity of NOB, and provide suitable inlet for ANAMMOX process in BNR from mainstream wastewater.

5.4 Outlook of ANAMMOX

For the mainstream application of ANAMMOX bacteria, the changes of temperature could significantly affect the activity of ANAMMOX bacteria and BNR performance. It has been suggested that low temperature could reduce the growth rate of ANAMMOX bacteria, and may impair the BNR performance (He et al., 2018). De Cocker et al. (2018) investigated the activity of ANAMMOX bacteria under low temperature (<15 °C), and found that the adaptation of ANAMMOX bacteria could be achieved by minimizing competition and maximizing growth space for ANAMMOX bacteria. Also, a shift of ANAMMOX community was observed, in which the dominant ANAMMOX genus shifted from *Ca. Brocadia* to *Ca. Kuenenia*. These results showed the potential of application of ANAMMOX bacteria for BNR from mainstream wastewater. Future research could focus on the low temperature adaptation strategies under mainstream wastewater condition, and identify the specific ANAMMOX community that prefers lower temperature.

It is recently identified that nitric oxide could be served as an alternative electron acceptor than nitrite for ANAMMOX bacteria (Hu et al., 2019b). The addition of NO could be reduced as N_2H_4, the proton motive force to drive the reaction and alleviate the inhibition of nitrite in sensitive regions. More studies about ameliorating the inhibition effect on ANAMMOX bacteria could be performed. Moreover, using nitric oxide as electron acceptor for ANAMMOX bacteria could obviate the nitrite accumulation, which is one of essential triggering factor for N_2O production from BNR process. Therefore, future research could focus on research of stable supply of nitric oxide for ANAMMOX bacteria.

6. Advances in denitrifying anaerobic methane oxidation (DAMO)

6.1 DAMO microorganism

It has been suggested that anaerobic methane oxidating microorganisms could use nitrogenous compound, such as nitrite or nitrate, as electron acceptor (Hu et al., 2019a). From

the experiments of He et al. (2013), in which methane and nitrate were consumed simultaneously under anoxic condition without inputting other carbon sources.

There are two types of microorganisms that can catalyse DAMO process (Gupta and Goel, 2019). For DAMO bacteria, two bacterial species have been identified to be capable of coupling methane oxidation to the reduction of nitrite to N_2, one is *Candidatus Methylomirabilis oxyfera*, and the other one is *Candidatus Methylomirabilis Lanthanidiphila*. Based on the 16S rRNA gene sequences, both the *Ca. M. oxyfera* and *Ca. M. lanthanidiphila* are categorized in clade A of NC10 phylum. From the metagenomic study, the genes for encoding membrane/periplasmic bounded nitrate reductase, nitrite reductase, nitric oxide dismutase and nitric oxide reduction are found in both *Ca. M. oxyfera* and *Ca. M. lanthanidiphila*. For methane oxidation, *Ca. M. oxyfera* harbors genes for encoding three methanol dehydrogenases, while *Ca. M. lanthanidiphila* only harbours genes endoce a lanthanide-dependent XoxF-type methanol dehydrogenase (Versantvoort et al., 2018), which renders *Ca. M. lanthanidiphila* could hardly contribute for BNR process since lanthanide is a rare earth element.

It has been suggested that the mechanism of the DAMO process by *Ca. M. oxyfera* is inter-aerobic pathway and *Ca. M. oxyfera* contain complete genes for encoding essential enzymes of aerobic methane oxidation. In this pathway, nitrite is firstly reduced into nitric oxide by nitrite reductase, then, a proposed nitric oxide dismutase that can split two nitric oxide molecules into oxygen and dinitrogen. Then, oxygen would become available to oxidize methane aerobically by particulate methane monooxygenase (Ettwig et al., 2010, Shen et al., 2015).

Besides the bacterium *Ca. M. oxyfera*, an archaeon *Candidatus Methanoperedens nitroreducens*, was also capable of oxidizing methane while performing denitrification (Hu et al., 2011). By using the terminal electron acceptor (nitrate or nitrite) as the selective factor,

DAMO archaea was only found in the culture feed with nitrate (Hu et al., 2011). The metagenomic study of DAMO archaea revealed that *Ca. M. nitroreducens* harbours genes encoding membrane/periplasmic bounded nitrate reductase including NarGHJ and NapH (Arshad et al., 2015), which can encode nitrate reductase narHG and utilizing nitrate as terminal electron acceptor (Haroon et al., 2013).

6.2 Advantages of DAMO microorganisms over canonical heterotrophic bacteria

As comparing to the canonical heterotrophic bacteria, DAMO microorganism possesses two main advantages. One is the higher affinity to the electron acceptors, i.e. nitrite for DAMO bacteria and nitrite for DAMO archaea. The other one is the utilization of greenhouse gas methane by DAMO microorganism and bypassing N_2O production in denitrification pathways of DAMO bacteria.

To date, the experimental results of kinetics parameters of DAMO microorganism are limited. As shown in Table 3, He et al. (2013) obtained half saturation constant of DAMO bacteria being 6.37 mg $N\text{-}NO_2^-$/L from a series of batch activity testes. However, the activity of DAMO *Ca. M. oxyfera*-like bacteria has been confirmed in natural environment where nitrite concentration could be less than 70 μg $N\text{-}NO_2^-$/L (Hu et al., 2014). Therefore, *Ca. M. oxyfera* could have higher nitrite affinity than the reported values in Table 3. The first experimental study on the kinetics of nitrate reduction by DAMO archaea *Ca. M. nitroreducens* was conducted by Lu et al. (2019). The result indicates that the nitrate reduction kinetics of *Ca. M. nitroreducens* is 2.1±0.4 mg $N\text{-}NO_3^-$/L. By comparing the affinity of electron acceptor with heterotrophic bacteria, both DAMO bacteria and archaea could outcompete canonical heterotrophic bacteria in BNR process.

Methane is a potent greenhouse gas and 20-fold stronger than CO_2 in heat-trapping (Liu et al., 2015, Ma et al., 2017b). For both DAMO microorganism, methane is oxidized into CO_2 either by inter-aerobic pathway of *Ca. M. oxyfera*, or by reversed methanogenesis pathway of

Ca. M. nitroreducens (Wang et al., 2017). Thus, DAMO microorganism could help to reduce methane production (Ma et al., 2017b). Moreover, the production of N_2O is also obviated in the methane oxidation pathway of *Ca. M. oxyfera* (Ettwig et al., 2010). From the genome analysis of *Ca. M. oxyfera* conducted by Ettwig et al. (2010), except the gene *nosZDFY* for encoding enzymes for N_2O reduction, all genes for encoding enzymes catalyse conventional denitrification were detected in *Ca. M. oxyfera*. It seems logical to consider *Ca. M. oxyfera* as N_2O resource due to the lack of ability for conventional N_2O reduction. However, a putative enzyme NO dismutase was proposed to convert two molecules of NO into O_2 and N_2, therefore, the reaction of quinol-dependent NO reductase could be bypassed and N_2O production is eliminated.

6.3 Application perspectives of DAMO

It has been suggested that the DAMO bacteria could be feasibly enriched in bioreactors and perform BNR by feeding with effluent from anaerobic digestion (Fu et al., 2017, Luesken et al., 2012, Shi et al., 2013b). The main obstacle for applying DAMO bacteria is the long doubling time (25 days) (Ettwig et al., 2010, He et al., 2013, Wang et al., 2017), and therefore, an effective way of retaining sufficient biomass is required for reactors. Biofilm reactors can retain microorganisms with slow growth kinetics and biomass could be naturally accumulated on the biofilm, which could be a suitable bioreactor configuration to enrich DAMO microorganisms. There have been different attempts of using reactors configured with membranes to cultivate DAMO microorganisms. Hu et al. (2019a) utilized immobilized biologically activated carbon, which allows DAMO bacteria to grow on the surface and form biofilms. This achieved two magnitude higher abundance of DAMO bacteria abundance than the controlled sludge-based reactor. By using the biofilm reactor and feeding with synthetic wastewater, Shi et al. (2013a) found DAMO microorganisms become the dominant microbial

community (20% - 30%) in the reactor. This result indicated that DAMO microorganisms in the reactor played a significant role in reducing nitrite into N_2.

6.4 Outlook of DAMO

The competition between DAMO archaea and DAMO bacteria for methane could impair the reduction of nitrate and nitrite, as both the processes require methane as electron donor. Although the methane affinity of DAMO bacteria (i.e., 1.472 mg-CH_4/L) has been tested by He et al. (2013), the comparison of methane affinity between DAMO bacteria and archaea were rarely studied. Therefore, further efforts could concentrate on the methane affinity of DAMO archaea and the effects of competition for methane on the BNR process.

A stable and sufficient supply of methane for DAMO process is critical for nitrite/nitrate reduction (He et al., 2013, Hu et al., 2019a). In WWTPs, a possible source of methane could be anaerobic digester. However, the composition of biogas from anaerobic digester also contains H_2S, therefore further research should focus on the impact of H_2S on DAMO process.

7. Novel BNR processes through the coupling of different microbial communities

7.1 Coupling DAMO with ANAMMOX

DAMO and ANAMMOX microorganisms could co-exist and remove nitrogen collaboratively (Fu et al., 2017). As shown in Figure 3, in the ANAMMOX process, nitrate could be produced from ammonia oxidation. DAMO archaea could utilize nitrate as the electron acceptor and reducing nitrate into nitrite. Therefore, nitrite produced from DAMO archaea could be either captured by ANAMMOX bacteria for accepting electron (Li et al., 2018a), or reduced by DAMO bacteria with coupling methane oxidation (Fu et al., 2017). There have been experiments on coupling these two microbial communities for BNR process (Fu et al., 2017, Hu et al., 2015, Shi et al., 2013b, Xie et al., 2018). It has been shown that nitrogen removal performance by coupling DAMO and ANAMMOX processes in membrane

biofilm reactor achieved more than 90% of total nitrogen removal, and as high as 190 mg NO_3-N/L/d (Shi et al., 2013b, Xie et al., 2018).

However, the main barrier that impede the application of this process is the control of oxygen concentration in the reactor. In order to maintain a stable nitrite supply for both ANAMMOX and DAMO bacteria, ammonium oxidation needs to be catalysed by AOB with the presence of oxygen. However, oxygen inhibition on ANAMMOX activity was observed at DO of 0.08 – 1.44 mg/L (Strous et al., 1997). The oxygen inhibition on DAMO bacteria was also observed, and the expression of genes participated the methane oxidation were down-regulated under 2 – 8 % exposure of oxygen (Luesken et al., 2012). Therefore, it is necessary to control the oxygen supply during ammonium oxidation.

Also, the mechanism of aerobic granular sludge could shed a light on the proposed combination (Fu et al., 2017, Li et al., 2019a). The aerobic microorganisms that require oxygen as electron acceptor concentrate on the outer surface, and anaerobic methane/ammonium oxidizers are more towards the inner layer of the granule. Zhu et al. (2011) demonstrated the feasibility of cultivating the co-culture of DAMO bacteria and ANAMMOX bacteria by using granular sludge. The abundance of DAMO cells increased from $(5.19 \pm 0.23) \times 10^7$ cells to $(1.22 \pm 0.18) \times 10^{10}$ cells after one-year operation. Thus, it is perspective to apply granular sludge to the combination of DAMO bacteria and ANAMMOX bacteria.

7.2 Coupling AOA with ANAMMOX

AOA often thrive at DO levels of 0.1 mg/L and can achieve higher ammonia oxidation under oxygen-limited conditions (Liu et al., 2016, Qin et al., 2017, Roy et al., 2017). Therefore, AOA are likely being a better partner with ANAMMOX than canonical AOB in autotrophic nitrogen removal process. The critical aspect of AOA/ANAMMOX cooperation is careful control of substrates. Yan et al. (2012) provided the first direct evidence of cooperation

between AOA and ANAMMOX bacteria, in which AOA and ANAMMOX bacteria were cultivated under oxygen limited condition. Due to the high oxygen affinity for AOA, the limited oxygen was consumed by AOA which prevented the ammonia oxidation activity of ANAMMOX bacteria (22 μg NH_4^+/g protein/min) from being impaired by exposure to oxygen. Pan et al. (2016) further proved the feasibility of coupling AOA and ANAMMOX bacteria for treating low strength ammonium wastewater. The results indicate that AOA play a significant role when ammonium concentration lower than 1.3 mg-N/L. It has been shown that the oxygen concentration could be controlled so that nitrite produced by AOA could be consumed by ANAMMOX at the same rate (Straka et al., 2019). It is necessary to maintain an oxygen balance to ensure both two microbial communities could strive. To date, there are studies indicating that the intermittent aeration mode could promote the proliferation of AOA, while maintain the activity of ANAMMOX bacteria. Both Qiu et al. (2019) and Li et al. (2019b) detected the high contributions of AOA and ANAMMOX bacteria of BNR under such condition.

7.3 Coupling COMAMMOX with DAMO/ANAMMOX

With high substrate affinities for ammonia and oxygen, COMAMMOX *Nitrospira* could become the dominating ammonia oxidizer under low oxygen condition, thereby reducing energy demand for aeration (Roots et al., 2019).

It is perspective to incorporate COMAMMOX *Nitrospira* with DAMO/ANAMMOX process for a sustainable BNR scheme. The final product of ammonia oxidation by COMAMMOX *Nitrospira* is nitrate, which is undesired for the nitrite dependent DAMO or ANAMMOX processes. However, DAMO archaea could utilize nitrate as the electron acceptor and reduce it into nitrite for the metabolic reactions of DAMO and ANAMMOX bacteria. But due to the high affinity of COMAMMOX *Nitrospira* to nitrite, nitrite reduced from DAMO archaea could be outcompeted by COMAMMOX *Nitrospira* over DAMO and ANAMMOX bacteria.

A ping-pong interaction between COMAMMOX *Nitrospira* and DAMO archaea could be formed (Daims et al., 2016). Therefore, it is necessary to separate COMAMMOX *Nitrospira* from DAMO and ANAMMOX bacteria into two reactors, so that the competition for nitrite could be minimized. Also, a transient accumulation of nitrite by COMAMMOX *Nitrospira* was observed at certain conditions (Daims et al., 2015, Kits et al., 2017). This suggests that the metabolic activity of COMAMMOX *Nitrospira* could be manipulated, so that nitrite oxidation could be selectively inhibited and then a stable supply of nitrite to DAMO and ANAMMOX bacteria could be assured.

In addition, nitrate has been reported to be capable of ameliorating the inhibition effect from nitrite on ANAMMOX bacteria (Li et al., 2016a). It was found that addition of exogenous nitrate attenuated the nitrite toxicity with 80% of ANAMMOX activity recovered. Therefore, the elevation of external nitrate concentration by COMAMMOX *Nitrospira* would trigger the nitrate/nitrite antiporter to pump out nitrite from sensitive regions of cells and therefore alleviate the toxic effect on Anammox in this novel coupling (Li et al., 2016a).

8. Conclusion

This paper systematically reviewed recent advances in some newly identified microbial communities related to novel BNR processes, including AOA, COMAMMOX, FEAMMOX, ANAMMOX and DAMO. Their particular metabolic characteristics in nitrogen cycle and potential BNR applications were reviewed and discussed. Overall, these microbial communities have unique advantages over canonical nitrifiers or denitrifiers in BNR, such as higher substrate affinities, better environmental tolerances and/or less greenhouse gas emission. These facilitate to better development and implementation of novel and sustainable BNR processes to achieve higher nitrogen removal with much lower energy and cost requirements, especially through the coupling of some collaborative microbial communities.

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Table Captions

Table 1. The comparison of electron affinity between AOB and AOA

Table 2. The comparison of nitrite affinity between ANAMMOX bacteria and heterotrophic denitrifying bacteria

Table 3. The comparison of electron acceptor affinity between DAMO microorganism and heterotrophic denitrifying bacteria

Figure Captions

Figure 1. Presumptive archaea ammonia oxidation pathway.

Figure 2. Illustration of metabolic versatility of COMAMMOX Nitrospira in harvesting substrate.

Figure 3. The conceptual combination of ANAMMOX and DAMO Processes.

Table 1. The comparison of electron affinity between AOB and AOA

	AOB		AOA	
	Nitrosomonas europaea	Reference	*Nitrosopumilus maritimus*	Reference
Km for Electron donor (ammonia)	0.156 mg-NH_3-N/L	(Wahman et al., 2005)	0.002 mg-NH_3-N/L	(Martens-Habbena et al., 2009b)
	0.077 mg-NH_3-N/L	(Hunik et al., 1992)	0.0016 mg-NH_3-N/L	(Horak et al., 2013)
	0.55 mg-NH_3-N/L	(Yu et al., 2020)		
Km for Electron acceptor (DO)	0.3 mg/L O_2	(Law et al., 2019)	0.11 mg/L O_2	(Qin et al., 2017)
	0.66 mg/L O_2	(Martens-Habbena et al., 2009b)	0.12 mg/L O_2	(Martens-Habbena et al., 2009b)
	0.42 mg/L O_2	(Yu et al., 2020)	0.084 mg/L O_2	(Li et al., 2016b)
	0.8 mg/L O_2	(Park et al., 2010)	0.064 mg/L O_2	(Park et al., 2010)

Table 2. The comparison of nitrite affinity between ANAMMOX bacteria and heterotrophic denitrifying bacteria

Microbial community		Km for Electron acceptor (nitrite)	Culture	References
ANAMMOX bacteria	*Candidatus Brocadia*	0.035mg N/L	Suspended cell	(Lotti et al., 2014)
		<0.1mg N/L	Aggregates	(Strous et al., 1999)
		<0.052mg N/L	Sludge	(Dalsgaard and Thamdrup, 2002)
		0.15 mg N/L	Modelling	(Straka et al., 2019)
		0.2 mg N/L	Suspended cell	(Wisniewski et al., 2019)
	Candidatus Kuenenia stuttgartiensis	0.657 mg N/L	Granular sludge	(Chen et al., 2011)
Heterotrophic denitrifying bacteria	*Paracoccus denitrificans*	60 mg N/L	Sludge	(Medhi et al., 2017)
	-	37mg N/L	Granular sludge	(Tang et al., 2013)

Table 3. The comparison of electron acceptor affinity between DAMO microorganism and heterotrophic denitrifying bacteria

Microbial community		Affinity of Electron acceptor	Culture	References
DAMO bacteria	*Candidatus 'Methylomirabilis oxyfera'*	6.37 mg-N/L	Pure culture	(He et al., 2013)
DAMO Archaea	*Candidatus Methanoperedens nitroreducens*	2.1 mg-N/L	Pure culture	
Heterotrophic denitrifying bacteria	*Paracoccus denitrificans*	60 mg-N/L	Sludge	(Medhi et al., 2017)
	--	37mg-N/L	Granular sludge	(Tang et al., 2013)

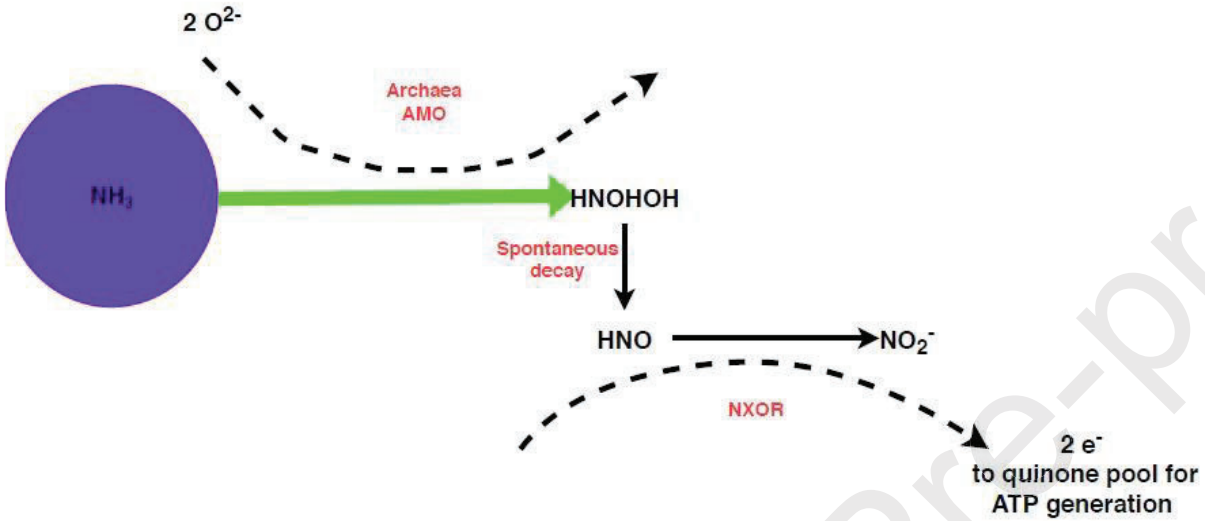


Figure 1. Presumptive archaea ammonia oxidation pathway.

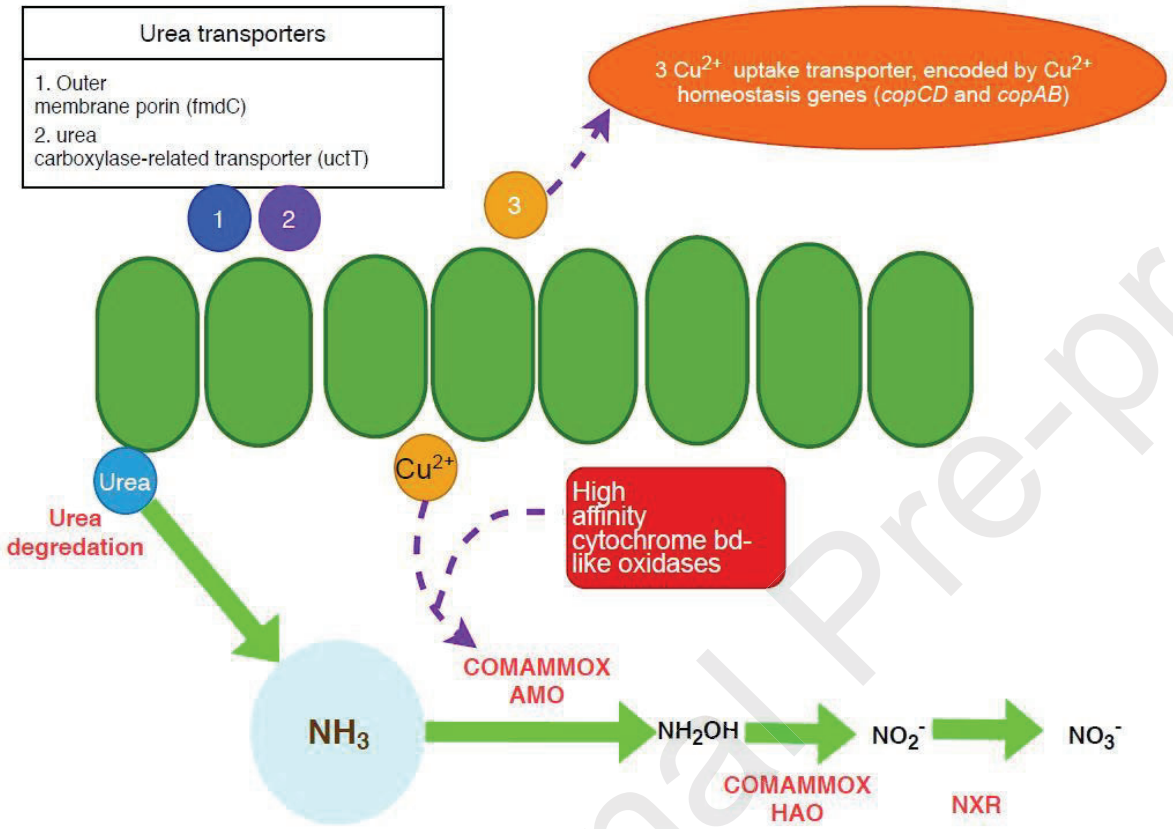
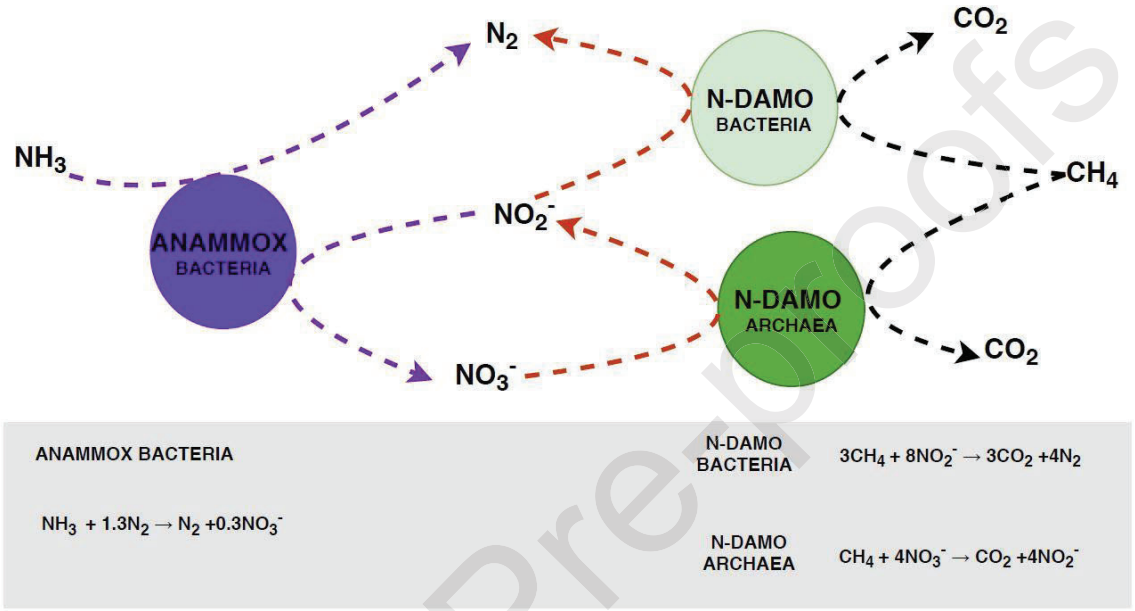


Figure 2. Illustration of metabolic versatility of COMAMMOX Nitrospira in harvesting substrate.



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