

## Review Article

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# Anammox Process for Wastewater Nutrient Removal: Recent Trends and Future Prospects

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Anammox (an abbreviation for anaerobic ammonium oxidation) is a reaction where specialized microorganisms with anamoxosomes carry out coupled oxidation reduction process where ammonium is oxidized, and nitrite is reduced to form dinitrogen. Here, nitrite is used as the electron acceptor under anoxic conditions. Anamoxosomes (phylum Planctomycetes) are strict anaerobes hence oxygen is not required for this process for treating nitrogen-rich wastewater. The Anammox process was discovered by Mulder in 1995 to avoid the need to add additional COD (chemical oxygen demand) to the system, it functions as an effective and affordable biological nutrient removal procedure in wastewater treatment. It is mainly done to protect the quality of the water body that it is discharged into. Algal bloom which is caused by fixed nitrogen such as ammonium and nitrate is avoided. Anammox is used as a better alternative approach for the removal of nitrogen from wastewater treatment as the bacteria requires less energy, reduces CO<sub>2</sub> emissions, produces less excess sludge. It is also known as a low-energy consuming and ecofriendly technology. We can also observe increasing importance of anammox process. Therefore, this study reviews and discusses the current developments in anammox combined processes and its impact on wastewater treatment techniques.

## Introduction

Anaerobic ammonia oxidation (anammox) is known to be one of the most ingenious discoveries fabricated for the treatment of wastewater having high ammonia nitrogen concentrations. Due to the rapid development and urbanization, nitrogen pollution has become a greater cause of concern in

recent years (Wen *et al.*, 2020). The consumption of nitrogen fertilizers and discharge standards of sewage are the mainstream causes of factors affecting terrestrial and aquatic environments further leading to a possibility to acidification at a global scale. (Gruber and Galloway, 2008). The fundamental nitrogen removal process is the traditionally used nitrification and denitrification

process. Nitrification, a two-step process, is generally carried out by aerobic, autotrophic bacteria that oxidize ammonium to nitrite ion and nitrite ion to nitrate ion with molecular oxygen as electron acceptor. It is catalysed by chemolithoautotrophic microorganisms oxidizing either ammonia or nitrite (Helder and De Vries, 1983). Nitrite and nitrate are then reduced to dinitrogen by denitrifying bacteria, which brings the complete removal of nitrate to harmless nitrogen gas as the end product. The denitrifying bacteria contributing to nitrate removal which are functioning in nitrite accumulation and/or complete denitrification. The different requirements of nitrifiers and denitrifiers have led to a number of reactor combinations for the removal of nitrogen from wastewater (Hu *et al.*, 2011). There is a huge risk, and these traditional methods can therefore not be used due to several issues impending large-scale application of bio-denitrification (Wang and Wang, 2013).

Moreover, there are several limitations of the traditional nitrification and denitrification process that have gradually perceived and emerged, these include, extremely high energy consumption, high operational expenses and merely any removal of ammonia nitrogen (Jetten *et al.*, 2001). Hence, the Anammox process has significant advantages for energy saving and sludge reduction, also having a significant effect on the capital costs and greenhouse gasses emissions being reduced (Blackburne *et al.*, 2008).

This study reviews the research and evolution of anammox process, along with the microbial and technological aspects of the treatment. According to the current situation of wastewater treatment, the future development of anammox is also proposed and the potential application of anammox is prospected.

### **Stoichiometric Equations of Anammox Reaction**

The anammox method is primarily focused on removal of ammonium from wastewater. It has two distinct steps when applied in the wastewater

treatment. The first phase is ammonia oxidising bacteria partial nitrification (nitritation) converting half of the ammonium to nitrite:



The resultant ammonium and nitrite are transformed by anammox bacteria into dinitrogen gas and water through a proportionating reaction:

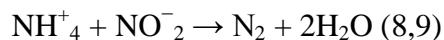


Figure 1 shows the above reactions that take place inside an anammox bacterial cell, along with the help of various enzymes.

### **Microbial Wastewater Treatment**

Anammox microorganisms are autotrophs, anaerobes (as oxygen is not required for this metabolic process) and belong to phylum-*Planctomycetes* and order-*Planctomycetales*.

Six potential genera, namely *Candidatus brocadia*, *Candidatus kuenenia*, *Candidatus jettenia*, *Candidatus anammoxoglobus*, *Candidatus anammoximicrobium* and *Candidatus scalindua*, are included in the category of anammox bacteria (Zhang *et al.*, 2017). Table 1 includes a few examples of such researched anammox bacterial species in wastewater treatment.

These bacteria are specialized microorganisms having a membrane-bound compartment inside their cells called anammoxosome where the anammox reaction takes place (van Niftrik *et al.*, 2004). The anammox reaction proceeds with the help of enzymes in the membrane catalyzing the oxidation and reduction process in which ammonium is oxidized and nitrite is reduced to form dinitrogen. It is known that the anaerobic oxidation of ammonia proceeds via hydrazine ( $\text{N}_2\text{H}_4$ ) and hydroxylamine, a volatile and toxic intermediate. Hydrazine is confined within anammoxosome membrane, thus avoiding the toxic effect it can cause on the anammox microorganism if it lasts for a longer period of time. Further, it is surrounded by a

ladderane lipid membrane which is highly compact (5.10F: Anammox [Internet]). In primary wastewater treatment, the major forms of nitrogen present in effluent are organic nitrogen and ammonium. However, in the secondary treatment these two forms are converted to nitrate by nitrifying bacteria, such as ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB).

The anammox bacteria consists of a well-structured and systematic procedure that removes high concentrations of ammonium compounds from industrial and urban wastewater and convert them to dinitrogen (Zhang *et al.*, 2015). The process seems to be the most promising for energy-neutral or energy-generating wastewater plant treatment (WWPTs). (Gruber and Galloway, 2008; Reimann *et al.*, 2015) Microbial treatment plays an essential role in both conventional autotrophic nitrogen removal and advanced nitrogen removal approaches which are discussed further.

### **Technologies Involved in Nutrients Removal**

Nitrogen and phosphorus are nutrients that are a natural part of the aquatic ecosystem. It supports the growth of algae and aquatic plants, which provides food and habitat for marine organisms. The removal of nitrogen and phosphorus in wastewater treatment is a huge concern and should be controlled due to the damage caused by it. It plays an important role in protecting the quality of the water body that it is discharged into. Fixed nitrogen (such as ammonium and nitrate) produces algal bloom which is also eliminated completely. Presence of untreated or partially treated effluent containing nitrogen and phosphorus often results in depletion of dissolved oxygen in water bodies (Gonzalez-Martinez *et al.*, 2018; Hasan *et al.*, 2021).

For many years, nitrogen/phosphorus removal is done by using conventional methods but they are not exactly effective as it does not help in reducing the concentration according to permissible limits set by Central Pollution Control Board of India (Delgadillo-Mirquez *et al.*, 2016). These treated

effluents using conventional methods do not meet the standard unit, thus becoming a cause of pollution (Gonzalez-Martinez *et al.*, 2018; Hasan *et al.*, 2021). Wastewater treatment done by activated sludge is being widely used and largely recognized which comprises nitrification and denitrification as biogeochemical steps. Nitrification refers to the aerobic conversion process of ammonia or any reduced nitrogen compound to easily absorbable form of nitrogen such as, ammonium to nitrite (in oxic condition) whereas denitrification is the process of releasing nitrogen compound back to atmosphere using reduction, under anoxic condition (Orhon *et al.*, 2009). Both these processes require a catalyst also called nitrifiers- ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), ammonia oxidizing archaea (AOA) (Gonzalez-Martinez *et al.*, 2015; Gonzalez-Martinez *et al.*, 2016). This has accelerated the need for nutrient removal technology, which is cost effective, eco-friendly, and sustainable. It can include biological processes, in variation or combination referred to as autotrophic nitrogen removal technology such as ANAMMOX-anaerobic ammonium oxidation, along with its combinations like DEMON- deammonification, CANON- completely autotrophic nitrogen removal over nitrate, ANITA Mox, PN-ANAMMOX - partial nitritation with anammox. These technologies have high potential efficiency, negligible sludge biomass production, low energy and oxygen required, and no external additional carbon is used therefore these solutions are regarded as the most important technology involved in removal of nutrients.

### **Nitrogen Removal Technologies**

Several types of promising alternatives have recently emerged for removal of nitrogen from wastewater treatment. These technologies are based on the principle of metabolism of anammox bacteria and are incredibly useful with great advantages like low sludge generation, cost effectiveness and many more. The application of these technologies are implemented on a large scale including DEMON, CANON, and ANITA MOX systems consisting of

one bioreactor. On the other hand, PN-ANAMMOX has two bioreactors used for the removal of nitrogen in wastewater treatment WWT (Rahimi *et al.*, 2020).

Partial nitritation anammox PN/A also commonly known as Partial Single reactor system for High activity Ammonium Removal Over Nitrite (pSHARON) refers to the formation of nitrite from the ammonium present in wastewater, through oxidation. The oxidation of nitrite to nitrate must be prevented to only achieve partial nitrification. This is achieved by metabolism of autotrophic ammonium-oxidizing microorganisms (AOB)- *Nitrosomonas europaea*, *N. eutropha* and *Nitrosospira*. (Rahimi *et al.*, 2020) PN/A process is an efficient and economical method for nitrogen removal in wastewater treatment as ammonium and nitrite can be further converted to dinitrogen gas by anammox bacteria through autotrophic nitrogen removal process.

DEMON technology (the Deammonification system) aids in reducing high strength ammonia levels by utilizing granular anaerobic ammonium oxidizing bacteria biomass in a single bioreactor. It is operated by alternating cycles of aeration as well as no aeration that are controlled by pH maintaining a balance between aerobic and anaerobic conditions and biomass selection and retention causing stability. This technology uses less aeration and attains 25% of cost and energy saving in relation to conventional nitrogen removal systems, making it remarkably energy friendly (Demon Deammonification [Internet], 2023).

The CANON system that stands for Completely Autotrophic Nitrogen Removal Over Nitrite, potentially removes ammonium from wastewater in a single, oxygen-limited treatment step. Ideal environmental conditions required for the anammox reaction involving the limitation of oxygen that leads the oxidation of ammonium to nitrite, is created. However, it avoids the oxidation of nitrite to nitrate (Third *et al.*, 2001). ANITA Mox technology is a single-stage nitrogen removal process that includes the presence of carriers. This is

performed in two steps; aerobic nitration (through AOBs) and anoxic ammonia oxidation performed by anammox bacteria (Christensson *et al.*, 2013).

To summarize, there are several technologies based on Anammox processes used for the removal of nitrogen from wastewater providing maximum efficiency and fulfilling the environmental and economic factors. There is a huge scope to experiment and research on such technologies.

## Phosphorus Removal Technologies

The possible pathway for removal of phosphorus is usually the same as that of nitrogen. But it can also be achieved through chemical removal which involves addition of calcium, iron or aluminium salt to obtain a precipitate, advanced biological treatment that consists of uptake of phosphorus in excess of normal bacterial metabolic requirements or often a combination of both is used (Yeoman *et al.*, 1988).

## Operational Studies on Anammox Processes

Research on Anammox is majorly focused on making the removal of nutrients from wastewater more efficient. Examples of such progresses include:

### Controlling denitrification for achieving denitrification

Denitrifying biocommunities often comprise microorganisms with highly varied nitrate and nitrite reductase activity. Denitrifiers are classified into three types based on their capacity to decrease nitrate or nitrite - groups A, B and C (Martenssen, 1997). In general, all three categories of denitrifiers (A, B, and C) coexist in activated sludge or biofilms (Ma *et al.*, 2020).

Increasing the quantity of denitrifiers from groups A and C in the reactor might result in nitrite build up during denitrification. High pH conditions aided in reaching this aim (Qian *et al.*, 2019; Shi *et al.*, 2019; Si *et al.*, 2018). Denitrification is a self-alkalization

process, therefore high pH conditions in an anoxic denitrification zone are readily maintained, which is helpful to nitrite buildup in denitrification. Until now, nitrite buildup has primarily been accomplished in a single SBR, USB, or biofilm reactor supplied with nitrate, ammonia, and organic matter (Cao *et al.*, 2016; Cui *et al.*, 2017; Du *et al.*, 2016; Ma *et al.*, 2017).

Controlling denitrification in a plug-flow wastewater treatment system, such as the commonly used anoxic/oxic (A/O) process, may be accomplished by creating a denitrifying biofilm and restricting it to the anoxic zone with the greatest pH throughout the

operation (Ma *et al.*, 2020; Li *et al.*, 2019).

Nitrite accumulation can be reduced by denitrifiers using an external or endogenous organic carbon source. To control denitrification, denitrification should be finished as soon as denitrification is complete. This could be achieved via two methods:

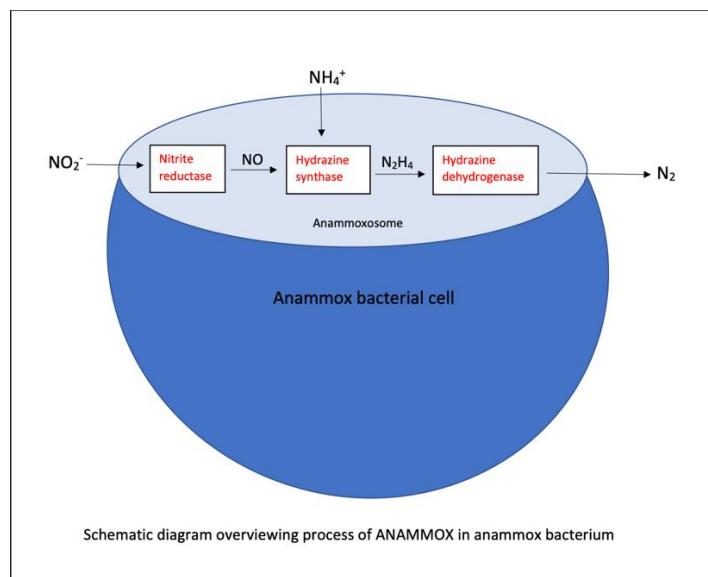
Controlling influent Carbon / Nitrogen ratio to a low level

Controlling hydraulic retention time (HRT) (Ma *et al.*, 2020)

**Table.1** Examples of Anammox Bacterial Species

S. No.	Name	Remarks	Reference
1.	<i>Candidatus Brocadia anammoxidans</i>	was the first organism to be identified that could oxidise ammonium in an anaerobic environment.	(12)
2.	<i>Candidatus Brocadia fulgida</i>	Is not only competent anammox bacterium but also its biofilm aggregates exhibit strong autoinflurescence.	(13)
3.	<i>Candidatus Kuenenia stuttgartiensis</i>	thrives not only in aerobic zones but also in summer and winter circumstances, which helps wastewater treatment by improving nitrogen removal.	(14)
4.	<i>Candidatus Anammoximicrobium moscowii</i>	was able to maintain activity while surviving for a long time without nutrients. For instance, after 90 days of no substrate being fed into the laboratory flow reactor, the rate of ammonium oxidation with nitrite was recovered after two days.	(15)

**Fig.1** Schematic diagram overviewing process of Anammox in Anammox Bacterium(10)



### Retention of Anammox Biomass

Given that the bacterial population's growth rate is slow, maintaining anammox biomass is essential for the one-stage anammox process to operate consistently (Kartal *et al.*, 1979). Typically, the number of anammox bacteria directly affects the output of an anammox bioreactor (Strous *et al.*, 1999). According to recent research, growing granular sludge and using carrier materials to produce biofilms can be effective ways to maintain anammox biomass (Jia *et al.*, 2017; Lv *et al.*, 2016; Li *et al.*, 2018).

### Anammox Biofilms

Both biofilm and granular sludge are highly aggregated. However, connected carriers are required for biofilm formation. Different carriers have been extensively researched in order to improve the wall growth characteristics of anammox bacteria (Li *et al.*, 2018). For example, zeolite particles were used as a carrier material to construct the biofilm, increasing anammox biomass enrichment and lowering biomass wash-out in the effluent to as low as 3 mgVSS/L (Fernández *et al.*, 2008). A polyethylene sponge carrier was used to remediate the leachate in the laboratory-scale

anammox-SBR (Miao *et al.*, 2016) and pilot-scale IFAS (Zhang *et al.*, 2015; Zheng *et al.*, 2016). The nitrogen removal efficiency of this carrier was high, and the biofilm protection boosted the proportion of anammox genes from 1.3% to 13.3% (Li *et al.*, 2018; Miao *et al.*, 2016).

### Anammox Granular Sludge

Granular sludge, which is made up of compact particles, has a faster settling velocity and better biomass retention, reducing the quantity of biomass developing in the suspension. Attaining a high nitrogen removal rate (NRR) (Fernández *et al.*, 2008) and lowering infrastructure costs are the primary benefits of forming anammox granular sludge. So far, a laboratory-scale anammox granular sludge system (Li *et al.*, 2018) has obtained the maximum NRR of 74.3 - 76.7 kgN/(m<sup>3</sup>d) (Tang *et al.*, 2011).

### Combined ANAMMOX process for Nitrogen Removal

Organic nitrogen, NH<sub>4</sub><sup>+</sup>-N, and a tiny quantity of NO<sub>2</sub><sup>-</sup>-N and NO<sub>3</sub><sup>-</sup>-N are the primary types of nitrogen in urban home sewage. However, the concentration of NO<sub>2</sub><sup>-</sup>-N cannot fulfil the need of

anammox. Furthermore, because AnAOB creates nitrate throughout the anabolic phase, the anammox process's potential denitrification efficiency is only 88%.

The limits of substrate varieties and the buildup of nitrate nitrogen limit the anammox process's extensive utilisation. In light of the aforementioned issues, a combination approach centred on anammox arose as the times demanded, effectively resolving these two issues (Jiaxiu *et al.*, 2022).

The common anammox combined processes include:

### **Partial Nitrification Anammox (PNA)**

Major functional organisms of this process include: *Nitrosomonas*, *Candidatus Brocadia*, *Candidatus Kuenenia*, and *Nitrospira*. Advantages of this process include: low operating cost, stable performance and decrease NO<sub>2</sub> emission.

However, it is not suitable for environments with low temperature. Its nitrogen removal rate lies between 46% to 95% (Chen *et al.*, 2021; Akaboci *et al.*, 2018; Lotti *et al.*, 2014; Sumino *et al.*, 2006).

### **Short Range Denitrification Anammox (Partial Denitrification Anammox [PDA])**

The PDA process, along with the short-range denitrification process for limiting the denitrification to the stage of NO<sub>3</sub><sup>-</sup>-N conversion to NO<sub>2</sub><sup>-</sup>-N and combining the anammox process was proposed in 2006. This lead to high total nitrogen removal efficiency (84.8% - 97.8%), strong impact load resistance as well as cost effective (Jiaxiu *et al.*, 2022; Sumino *et al.*, 2006).

### **Denitrification methane oxidation – Anammox process (DAMO)**

The denitrifying anaerobic methane oxidation (DAMO) process, which relies on NO<sub>3</sub><sup>-</sup>-N/NO<sub>2</sub><sup>-</sup>-N, can remove NO<sub>3</sub><sup>-</sup>-N/NO<sub>2</sub><sup>-</sup>-N and CH<sub>4</sub> at

the same time, and the final products are just N<sub>2</sub> and CO<sub>2</sub>, making it a potentially green biological denitrification method (Raghoebarsing *et al.*, 2006). This process has very high total nitrogen removal efficiency as well (90% - 99%). The poor growth rate of DAMO functional bacteria, on the other hand, is a significant limiting factor in this coupled process (Cai *et al.*, 2015).

## **Future Prospects**

The anammox process has a great potential in nutrients removal form wastewater and can be used in full-scale Wastewater treatment plants. In fact, various such plants have already been set up and are under investigations (Muñoz-Palazon *et al.*, 2018).

However, there are certain problems in the implementation of this technology that have the scope to be improved through further research. Long start-up time of this technology, because of the high multiplication time of annamox bacteria and little yield of biomass is one such issue (Lotti *et al.*, 2014). This area hence requires further research and development of new, efficient technologies.

Further, it is essential to address some important issues so as to make Anammox systems more accessible. Application anammox techniques at lower temperatures (10°C - 18°C) as well as lower nitrogen concentrations is the next step towards this goal (Gonzalez-Martinez *et al.*, 2018; Muñoz-Palazon *et al.*, 2018).

It is also essential to understand the exact responses of anammox communities to its environment, including different organic components and pollutants (such as drugs and antibiotics), as these are compounds are known to be resistant during the biodegradation process (Gonzalez-Martinez *et al.*, 2018; Muñoz-Palazon *et al.*, 2018). Through further research and studies, the aforementioned issues can be resolved and the anammox process can be made an integrate part of wastewater treatment.

The Anammox process is an competent and cost –

effective mode of removing high concentrations of nutrients such as nitrogen and phosphorus from waste water obtained from both urban as well as industrial areas. It is a biological process which utilizes specialized microorganisms in nutrient removal. Recent studies have suggested various methods that can further aid increasing the productivity, adaptability, and feasibility of the anammox systems. These methods include controlled denitrification, anammox biomass retention and various combined processes along with the standard anammox process. However, there still remains scope of further investigations to improve the Anammox system and make it ideal in wastewater treatment plants.

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### Conflict of Interests

The authors declare no conflict of interests.

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