Anammox Process: the Principle, the Technological Development and Recent Industrial Applications

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Abstract
The ANAerobic AMMonium OXidation or Anammox process, nowadays becomes an important technology for industrial wastewater treatment plants, especially for an effective denitrification process. Anammox process has been widely known for its high efficiency in nitrogen removal in various kinds of wastewater. This review described the details of Anammox process, including biochemical characteristics, technical development, and its applications based on literature studies. Additionally, applications of Anammox process in wastewater treatment in different types of bioreactors were discussed. Finally, three typical applications of Anammox process in nitrogen removal for landfill leachate, fertilizer residues from agricultural activities and petrochemical industrial wastewater treatment were analyzed.

Keywords: Anammox process, Denitrification, Wastewater treatment

1 Introduction

Water plays an extremely important role to maintain the life of all living organisms on Earth. In fact, water is definitely essential for human life and activities, such as agricultural and industrial productions. Practically, untreated industrial wastewater contains large amount of toxic materials, organic substances and hazardous microorganisms. From the beginning of 20th century until recent years, biological processes have played their major roles in industrial wastes and wastewater treatment worldwide. In general, various types of bioprocesses have been applied for wastewater treatment, for example the conventional activated

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sludge process and the membrane bioreactor [1].

One type of wastewater treatment process was developed in 1999, and it has been researched and developed for years before starting an actual application in industry, and its commercial name in global market is known as the Anammox [2]. Anammox process has been intensively conducted in several wastewater treatment plants and achieved positive results, particularly for nitrogen removal. In Netherlands, after the discovery of Anammox bacteria, the Rotterdam–Dokhaven plant was the first full scale municipal wastewater treatment plant (MWWTP) in the world, which applied Anammox process in 2002 [3].

Because of its high efficiency on nitrogen removal, nowadays Anammox has also been widely researched. Based on bibliometric analysis, there are 968 Anammox-related publications during 1995–2012, by which 374 articles were published in "Environmental science ecology" journal categories. And 71.54% of total numbers of articles were published by research groups from China, Netherlands, and USA [4]. In Southeast Asia, Thailand and Vietnam are well-known for scientific researches on the different aspects of the Anammox applications. In Thailand, research on the biochemical effects of Anammox bacteria in a sequencing batch reactor system for wastewater treatment was carried out [5]. In addition, inactivation factors of Anammox bacteria community were investigated to improve the process [6]. In Vietnam, several Anammox research projects were implemented by Ho Chi Minh University of Technology, especially for latex processing wastewater as well as for old landfill leachate treatment [7], [8].

2 The Natural Nitrogen Cycle and the Principle of Anammox Process

In 1887, the nitrogen cycle in nature was discovered [9]. In nature, the pathway of nitrogen contains three important steps: (1) the ammonification by nitrogen-fixing bacteria; (2) the nitrification by nitrifying bacteria; and (3) the denitrification by denitrifying bacteria to produce nitrogen gas. Over one hundred years after the first establishment of nitrogen cycle, the additional mechanism of nitrogen cycle was later discovered and being developed. This new concept of nitrogen pathway is well-known as the Anammox process (Figure 1).

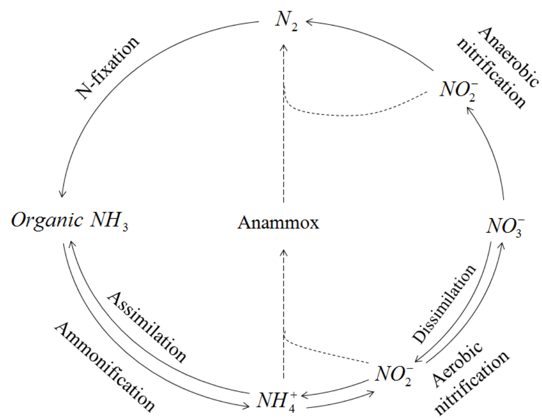

Figure 1: Nitrogen cycle in nature and in Anammox pathway [13].

The history and development of Anammox process can be seen back in 1990s, when the research group of Arnold Mulder and Astrid van der Graaf from Delft University of Technology (Netherlands) finally discovered the phenomena of an anaerobic ammonium oxidation in a denitrifying fluidized bed reactor. Nitrogen gas (N_2) was produced directly from ammonium (NH_4^+) and nitrite (NO_2^-) by new species of denitrifying bacteria, without other intermediate steps of nitrate (NO_3^-) formation and a denitrification process [10]. Therefore, this finding is the pioneer for researches on characteristics, and the applications of the new denitrifying bacteria, and became a scientific milestone in the field of wastewater treatment. In 1999, this process was published in Nature Journal [2], and finally it came up with a commercial name, which is known as the Anammox (ANAerobic AMMonium OXidation).

In the typical Anammox process, the formation of nitrate (NO_3^-) by nitrification is by-passed because nitrite (NO_2^-) and ammonium (NH_4^+) are converted directly into nitrogen gas (N_2) (Figure 1). Theoretically, the pathway of Anammox was suggested to be involved with two important biological processes: the catabolic (dissimilation) and anabolic (assimilation) reactions [11]:

(1) The process of catabolic reaction (dissimilation):
$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O$

(2) The process of anabolic reaction (assimilation):

$HCO_3^- + 0.2\ NH_4^+ + 2.1\ NO_2^- + 0.8\ H^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2} + 2.1\ NO_3^- + 0.4\ H_2O$

In general, the overall stoichiometry of the Anammox reaction to converse NH_4^+ and NO_2^- to N_2 without producing intermediate NO_3^- is [12]:

$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.0066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O$

However, nitrite has been proved to be toxic to Anammox at the concentration of 50–150 mg-N/L, which leads to the complete failure of the process [14].

By the nature of the Anammox pathway, which is an anaerobic ammonium oxidation process, various microbial species in the phylum of *Planctomycetes*, play their roles in the catabolic and anabolic reactions [15]. Moreover, they are easily found in the seawater, and freshwater resources of European, African, and Asian countries. Nowadays, five Anammox genera have been discovered, including *Brocadia*, *Kuenenia*, *Anammoxoglobus*, *Jettenia* and *Scalindua* [16].

The importance of study of Anammox bacteria is not only for ecological study but also for molecular biology and systemic biology. It can be observed that their cellular structures tightly associate to the biochemical mechanisms of the Anammox process. The sub-cellular structures of Anammox bacteria were compartmentalized to three important components by three membranes. The outermost membrane encloses the paryphoplasm compartment. The middle membrane encloses the riboplasm compartment, which contains the ribosomes and nucleoid. The innermost membrane encloses the largest compartment of the cell, the anammoxosome, which is the location of the energy metabolism (Figure 2) [17], [18].

Interestingly, the lipid bilayer membranes of Anammox bacteria contain ladderane lipids, which have been regarded as unique. Molecular modeling suggests that the ladderanes confer a low degree of rotational freedom to the membrane and limit its diffusion permeability of protons in ATP synthesis [19]. This character is hypothesized to be important for Anammox bacteria because their growth rate is very slow. So these lipids may help to reduce the loss of energy during diffusion of toxic compounds (such as nitric oxide and hydrazine) out of the cells to maintain the anammox process [20].

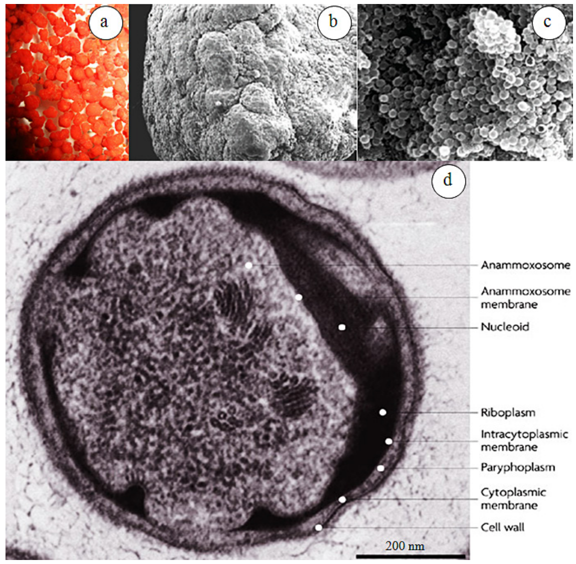

Figure 2: Microscopic morphology of Anammox bacteria. (a) Granular sludge, (b and c) Scanning electron microscopy picture of granular sludge and (d) Transmitting electron microscopy picture of *Kuenenia stuttgartiensis* [17], [27], [28].

In general, the operational conditions of Anammox process in nature have been widely varied in different environment. Typically, the Anammox process was found to be occurred at the temperature ranged from 20°C to 43°C [2]. In recent years, it was revealed that Anammox process also happened in hot springs at temperatures ranged from 36°C to 52°C [21], and in hydrothermal vents of the Mid-Atlantic Ridge at temperatures ranged from 60°C to 85°C [22].

Nowadays microbial structures and communities of Anammox bacteria were investigated by using simple molecular technique (sequencing of 16S rRNA [23]) or using high-throughput technology, such as 454-pyrosequencing [24], Denaturing Gradient Gel Electrophoresis (DGGE) and Fluorescence *in situ* Hybridization (FISH) [25]. Those Anammox bacteria were classified as *Proteobacteria*, *Bacteroidetes*, *Chloroflexi* and *Firmicutes*. It has been observed that microbial community structures changed depending on environmental parameters (particularly the ammonia concentration) or habitats [23].

Recently, microbial consortium within granules collected from a full-scale Anammox reactor located at the Dokhaven-Sluisjesdijk wastewater treatment

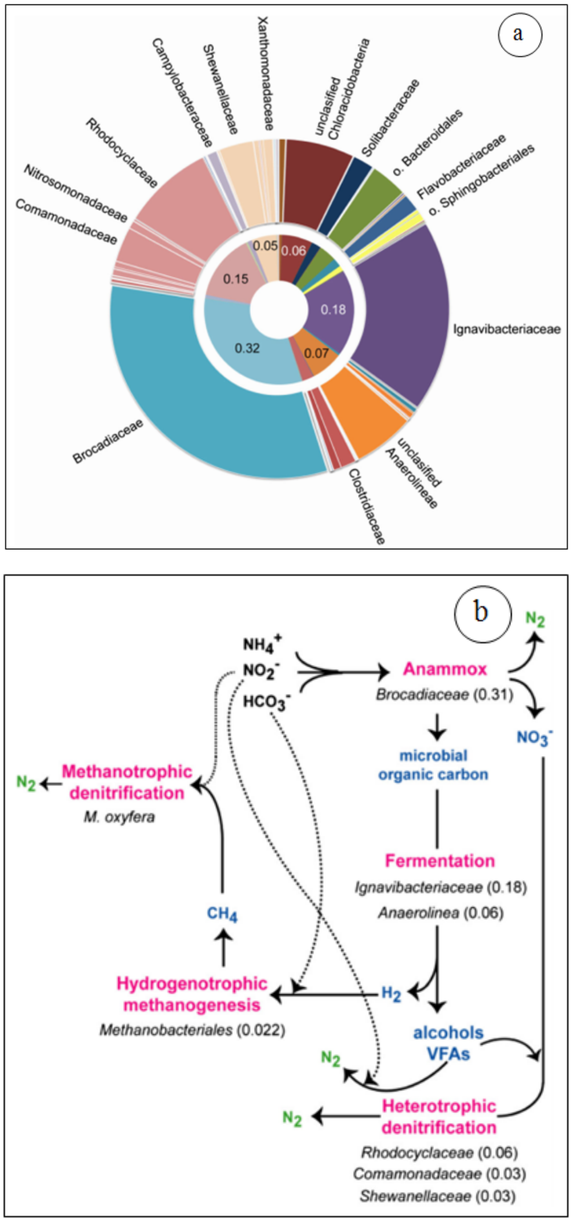

Figure 3: Microbial consortium analysis and systemic functional analysis of Anammox granular sludge. (a) Distribution of microbial consortium structures identified by pyrosequencing analysis of 16S rRNA gene fragments (The numbers are the fraction values of the most abundant classes) and (b) systemic functional analysis of microbial consortium found in granule (Dashed lines indicate paths of utilization of Anammox substrates by heterotrophic denitrifiers) [26].

plant in Rotterdam were identified and characterized using Pyrosequencing technique of 16S rRNA gene fragments. It was found that besides Anammox bacteria (*Brocadiacea*, 32%), substantial numbers of heterotrophic bacteria *Ignavibacteriacea* (18%) and *Anaerolinea* (7%) along with heterotrophic denitrifiers *Rhodocyclacea* (9%), *Comamonadacea* (3%), and *Shewanellacea* (3%) presented in the granules (Figure 3). The functional analysis suggested that these heterotrophic bacteria and the Anammox bacteria form a network in the same niche to achieve a well functioning denitrification system and heterotrophic bacteria may utilize the nitrate as nutrients produced by the Anammox bacteria [26].

3 Application of Anammox Systems in Wastewater Treatment

Nowadays, one of the most important applications of the Anammox process is for wastewater treatment, for example from the landfill leachate, monosodium glutamate wastewater, and pharmaceutical wastewater. These types of wastewater contain extremely high content of NH_4^+. In contrast to the domestic wastewater, it has only small amount of nitrogenous substances. In general, because of the special metabolism pathways which can easily shorten the conventional nitrogen cycle, Anammox process has received high attention among other biological treatment methods. For wastewater, which contains high amount of ammonium (NH_4^+) and nitrite (NO_2^-), the denitrification plays an important role because it can primarily determine the success of the treatment facilities by transformation of NH_4^+ to N_2 and finally release N_2 to the environment.

The conventional wastewater treatments usually use aerated basins which supply an adequate amount of oxygen for the oxidation and reduction of organic compounds in wastewater. However, if the conventional treatment are executed in case that wastewater contains high contents of nitrogenous compounds, the nitrification step to transform NH_4^+ to NO_3^- is unavoidable. Therefore, more energy is required to supply the aerated system to generate sufficient oxygen for the nitrification. Using Anammox process, the nitrification process can be easily eliminated and the NH_4^+ will be transformed directly to N_2. Consequently, by applying Anammox process for wastewater treatment, around 90% cost is reduced for the operation [23].

At the beginning of the Anammox process, seed sludge (available in the global market), which contains Anammox bacteria should be carefully prepared. The next step is the enrichment of Anammox bacteria, which usually takes around 100 days to obtain sufficient numbers and specific activities [23]. To enhance the efficiency of the Anammox process, specialized reactor systems, such as sequencing batch reactor (SBR) [29], rotating biological contactor (RBC), trickling filter, granular sludge bed reactor, upflow anaerobic sludge blanket (UASB) reactors [30] and membrane bioreactor have been applied in both laboratory and pilot plants [31].

Moreover, in recent years the hybrid biofilm-carrier reactor (also known as HBCR) is considered as a new design of reactor for the Anammox wastewater treatment, especially for petrochemical wastewater treatment process to remove the toxic and unwanted organic substances from effluent stream [32]. The general concept of biofilm reactors is the use of carriers (moving bed or fixed bed) to enhance the contact surface and the capacity of the system. The inlet stream of wastewater is moving upwards while the effluent stream is released from the top of biofilm reactor, which is operated in continuous flow conditions. During the operational process, biogas is generated as by-products and released through the port located at the top part of the reactor. The positive results were obtained with the average total nitrogen (TN) removal efficiency reached the high values from 62–67% and around 90% of total nitrogen can be removed [32].

The major pitfall of this Anammox process is the extremely low bacterial growth rate [14]. So, the Anammox reactors are often operated at a long retention time in order to accumulate the sufficient amounts of Anammox biomass in the system. The discovery of new Anammox bacterial species that have special properties draws attention from researchers worldwide. Recently, using novel Anammox bacteria, *Brocadia sinica*, with sulfate-dependent Anammox bacteria, *Anammoxoglobus sulfate* and *Bacillus benzoevorans*, as the inoculums in lab-scale UASB reactors can achieve the highest efficiency of nitrogen removal in the world (74.3–76.7 kg-N/m^3/d) [28].

By its own nature, leachate is considered as the streams of liquid waste generated from municipal solid waste (MSW) landfills, such as the run-off water over the surface of the landfill, or the deep intruding rainwater which can penetrates through the protection layers of landfills. In general, the leachate is widely regarded as a major problem for municipal solid waste landfills which can cause the severe contamination in surface and ground water resource and the destruction of the underground ecological systems [33]. Therefore, the treatment process of the landfill leachate is truly essential to protect the human health and also the biodiversity of the underground ecological systems.

In general, landfill leachate could be categorized, based on the amount of chemical oxygen demand (COD), biochemical oxygen demand (BOD) and the contents of nitrogenous products into two different groups: new landfill leachate and mature landfill leachate, which have the ages of less than 2 years and greater than 10 years, respectively (Table 1) [34]. Basically, the COD values of new landfill leachate usually vary between 3,000–60,000 mg/L, while the old landfill leachate has only the values of 100–500 mg/L. The BOD values of new landfill leachate are also higher than the mature landfill leachate, with 2,000–30,000 mg/L in comparison with only 100–200 mg/L in old leachate. Furthermore, the amount of organic nitrogen inside new landfill leachate is 10–800 mg/L, around 10 times higher than mature landfill leachate, which has the values of 80–120 mg/L. Additionally, the content of ammonia is also high in new landfill leachate in comparison with the mature landfill leachate, around 10–800 mg/L and 20–40 mg/L, respectively [34].

Table 1: Typical data on the composition of new and mature landfill leachate [34]

Constituent	Value (mg/L)		
	New landfill (< 2 years)		Mature landfill (> 10 years)
	Range	Typical	
BOD	2,000–30,000	10,000	100–200
TOC	1,500–20,000	6,000	80–160
COD	3,000–60,000	18,000	100–500
TSS	200–2,000	500	100–400
Organic nitrogen	10–800	200	80–120
Ammonia nitrogen	10–800	200	20–40
Nitrate	5–40	25	5–10

The conventional landfill leachate treatment process involves with the use of mechanical methods at the beginning by removing the large-size materials. Then, the chemical treatment process is applied by using the coagulation/flocculation technology to remove the amount of COD and Total Suspended Solid (TSS). Once this step is finished, the nitrogen removal step should be conducted. Afterwards, the next step is the anaerobic treatment by using SBR or UASB reactors. Finally, the wastewater should be delivered to stabilization ponds before being discharged to the environment [1].

Nowadays, the process of Anammox can be utilized to enhance the performance of nitrogen removal of landfill leachate treatment. In recent years, several researches have been carried out in different Anammox reactors. In India, the Single Reactor System for High Activity Ammonia Removal (SHARON) was established together with the use of Anammox bacteria in the initial sludge for treatment process of new landfill leachate [35]. In Vietnam, the recent Anammox process application in mature landfill leachate treatment with laboratory scale was demonstrated by using hybrid reactor (HAR), and this technique showed that 90% of the NH_4-N was converted into NO_2 or N_2 after 45 days [8].

Furthermore, another study of Anammox process involves with the removal of fertilizers in rice paddy fields in Southern China [36]. Because of the use of fertilizers, high amount of ammonium was accumulated in the paddy fields, with has values of 6.2–178.8 mg/kg-soil. This study illustrated that the applications of Anammox process are not limited for wastewater treatment, and Anammox process can also be applied for the solid waste treatment, especially for fertilizer degradation in the paddy fields. In the near future, more specific studies on the appearance of Anammox bacteria in different kinds of the fertilized soil should be carried out.

4 Conclusions

Comparing to conventional nitrification, Anammox process has significant advantages in wastewater treatment. Anammox process does not require the addition of external carbon nutrient to the system. Also, this process produces less sludge, and requires less energy and less oxygen [37]. However, there are remaining problems that still needed to be improved before implementing the Anammox system into full scale wastewater treatment. For example, Anammox system is sensitive to high C/N ratio containing stream feed and low temperature condition. In typical wastewater that normally contains high C/N ratio (in the form of COD/N between 10–20), Anammox bacteria are prone to be over-grown by heterotrophic denitrifying bacteria, which inactivates the Anammox activity for nitrogen removal. Another challenge for Anammox process is the longer start-up period and loss of Anammox biomass in the reactor. In this case, immobilization of Anammox biomass to suitable media, such as polyethylene glycol, can help to reduce the start-up period. In addition, finding method to reduce the energy consumption by Anammox process should be inquired, such as integration of Microbial Fuel Cell (MFC) system to the Anammox system.

Nowadays, the Anammox process has became an important pathway for industrial wastewater treatment plants, especially for denitrification processes to remove large amount of nitrogen and also other nitrogenous products from different kinds of wastewater. From the year of 1995 to 2015, Anammox process for wastewater treatment has been widely studied in many countries all over the world. In fact, the Anammox process has contributed greatly to the waste treatment technologies, especially for various kinds of "hard-to-do" wastewater such as landfill leachate. However, the main difficulties should be the problem of laboratory scale experiments vs. in-situ Anammox treatment plants, mostly because of the changes in operational parameters and other factors during the scaling up process. In conclusion, the Anammox technologies have lots of opportunities to becomes the typical wastewater treatment process in this 21st century.

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