

The Anammox process for nitrogen removal from wastewater – achievements and future challenges

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Sammendrag

Anaerob ammonium oksidasjon (Anammox) for fjerning av nitrogen fra ulike avløpsstrømmer er en relativt ny prosess som er blitt utviklet i løpet av de siste ca 20 år. Det gjenstår imidlertid mange uavklarte spørsmål og tilhørende FoU-oppgaver knyttet bl.a. til Anammox-bakterienes metabolisme og hvordan disse bakteriene kan utnyttes på best mulig måte i fullskala renseanlegg for fjerning av nitrogen. Dette gjelder ikke minst hvordan prosessen kan utvikles til å fjerne nitrogen fra vanlig kommunalt avløpsvann, og ikke bare fra «sidestrømmer» med høyt ammoniuminnhold og høy temperatur.

Sammenlignet med konvensjonelle metoder for fjerning av nitrogen fra kommunalt avløpsvann (nitrifikasjon/denitrifikasjon) er Anammox-prosessen svært energibesparende, og det er ingen behov for en karbonkilde, slik denitrifikasjonsprosessen krever. Dette vil gi muligheter for å ta ut så mye som mulig av det organiske materialet i avløpsvannet som primærslam og produsere fornybar energi av dette i form av biogass. Dersom man lykkes med å implementere Anammox-prosessen for fjerning av nitrogen fra kommunalt avløpsvann, vil dette innebære en kraftig økning av bærekraften til avløpsrenseanlegg, og det er ikke lenger utopisk å se på avløpsrenseanlegg som en netto energiproducent.

Summary

Although the study of the Anammox process has been ongoing for about 20 years and knowledge about Anammox bacteria has significantly increased, there are still many questions regarding bacteria behaviour and process performance involving these bacteria. Therefore, Anammox bacteria still require further research.

Conventional wastewater nitrogen removal systems require a lot of energy for nitrification, and often an external organic carbon source for denitrification. The application of the Anammox process (which significantly reduces the need for energy and does not need organic matter to convert nitrogen to gas) in the main stream of a wastewater treatment plant would be a great alternative to save energy costs for aeration. Moreover, the organic matter present in wastewater could be regarded as a source of additional energy. Such a sustainable approach is now intensely studied around the world. Its implementation seems to be a revolution in the field of sustainable wastewater treatment. This brings a vision that soon the pollutants in the wastewater will no longer be seen as a problem, but as a source of renewable energy.

Introduction

Great efforts are made today in the field of nitrogen removal from municipal wastewater, since

nitrogen is one of the compounds that are regulated in the discharge permit of Wastewater Treatment Plants (WWTPs) in many countries. There are in the nature groups of bacteria which are well known and have been widely applied in biological wastewater treatment systems. One of them, the nitrifiers, has the ability to oxidise ammonium with oxygen into nitrite which is next oxidised into nitrates (nitrification). The second group of bacteria, the denitrifiers, reduces oxidized forms of nitrogen in response to the oxidation of an electron donor such as organic matter (denitrification). Both processes involve large expenditures: nitrification requires aeration, and denitrification needs supply of organic matter if not sufficient in incoming wastewater.

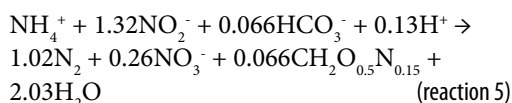
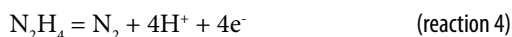
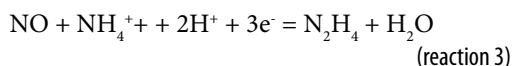
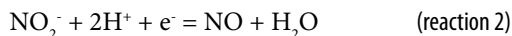
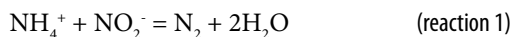
However, in the last few decades our understanding of the nitrogen cycle on the earth has changed drastically, and therefore the concept of biological wastewater treatment has evolved rapidly. New and more sustainable solutions for wastewater treatment have appeared, since more effective and autotrophic bacteria responsible for the anaerobic ammonium oxidation (Anammox) reaction has been discovered. Before these bacteria were identified by micro-biologists, the existence of Anammox bacteria was predicted in 1977 by Engelbert Broda, who pointed out anaerobic ammonium oxidisers as “missing in nature”, based on thermodynamic considerations.

The first evidence of anaerobic ammonium oxidation to dinitrogen gas was obtained from a denitrifying fluidized-bed reactor system (Mulder et al., 1995). It was discovered that these organisms related to Planctomycetales were capable of oxidizing ammonium using nitrite instead of O_2 as the electron acceptor (Strous et al., 1999a). Moreover, it occurred that these microorganisms have a number of unique features, including the use of hydrazine (N_2H_4 , i.e., rocket fuel and normally poisonous to living organisms) as a free catabolic intermediate.

The discovery of the Anammox bacteria changed the view of the nitrogen cycle and resulted in a remarkably active wastewater treatment research around the world.

The Anammox reaction

The overall reaction 1, half reactions 2, 3 and 4 (Kartal et al., 2011) and reaction 5 with cell synthesis proposed by Strous et al. (1998), are presented below:



The first step involves the reduction of nitrite to nitric oxide by nitrate reductase (reaction 2). Then ammonium is combined with nitric oxide by hydrazine hydrolase to the form of hydrazine (reaction 3). In the final step (reaction 4) hydrazine is oxidised to dinitrogen gas via hydrazine/hydroxylamine oxidoreductase (Kartal et al., 2011). The Anammox reaction is always associated with nitrate production. These reactions occur within the anammoxosome, a specialized pseudo-organelle within the bacterium, and create a proton gradient across the anammoxosome membrane (van Niftrik et al., 2008). Researchers propose different stoichiometrical quotients for individual components of the Anammox reactions but always the same and important for the process is nitrite to ammonium ratio (NAR) which is equal to 1.3. Moreover, similarities are visible in released nitrogen: per 2.3 moles of ammonium and nitrite, about 1 mole of nitrogen gas (N_2) is generated. From reaction 5 it can be seen that 0.066 mole of carbon is fixed per 1 mole of oxidized ammonium, which equals to 1 carbon per 15 catabolic cycles. Anammox bacteria are obligate anaerobic chemolithoautotrophs.

Microbial characteristics

Anammox bacteria have complicated and not yet well understood microbiology (Kartal et al.,

2012). Bacteria share numerous properties with both eukaryotes and archaea. Particularly noteworthy is the exceptional construction of a cell which is divided into three separate compartments by bilayer membranes and consists of the cell wall, paryphoplasm, riboplasm, and anammoxosome. The knowledge about the composition or function of both the cell wall and the paryphoplasm compartment is limited. The Anammox reaction takes place in the anammoxosome which occupies most of the cell volume and is a so-called “prokaryotic organelle” (Lindsay et al., 2001). The current knowledge about Anammox cell biology is presented by Niftrik and Jetten (2012).

So far 10 Anammox species have been identified. Known species are divided into 5 genera: (1) *Kuenenia*, represented by *Kuenenia stuttgartiensis*; (2) *Brocadia* including 3 species: *B. anammoxidans*, *B. fulgida*, and *B. sinica*; (3) *Anammoxoglobus*, 1 species: *A. propionicus*; (4) *Jettenia* 1 species: *J. asiatica*; and (5) *Scalindua* having 4 species: *S. brodae*, *S. sorokinii*, *S. wagneri*, and *S. profunda* (Kartal et al., 2013). Phylogenetic analysis places them all within the phylum *Planctomycete*.

All the five currently recognized genera of Anammox bacteria share unique physiological and morphological features with the key one being the presence of the anammoxosome. Nearly two thousands of these gene sequences of 16S rRNA affiliated with Anammox bacteria have been deposited in the GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) (Kartal et al., 2012; 2013).

Anammox in the natural ecosystems

After the first identification of the Anammox bacteria, its occurrence was affirmed in different reactor configurations (Jetten et al., 1999; Helmer et al., 1999; Strous et al., 1999b) and followed by detection in the natural environments such as marine sediments (Dalsgaard et al., 2002, 2003; Engström, 2004; Kuypers et al., 2003). There the Anammox bacteria are estimated to be, in particular in oxygen minimum zones (OMZs), the

major source of nitrogen release into the atmosphere from the oceans (Kartal et al., 2010). The Anammox bacterial population was even found in deep marine hypersaline gradient systems (Borin et al., 2013). Moreover, it was detected in terrestrial ecosystems as marshes, lakeshores, a contaminated porous aquifer, permafrost soil, agricultural soil and in samples associated with nitrophilic or nitrogen-fixing plants (Humbert et al., 2010). It has even been estimated that about 50% of the annual fixed nitrogen loss on the earth could be attributed to Anammox activity (Lam & Kuypers, 2011). In the natural environment the Anammox process has been reported to occur at temperatures as low as -2.5°C in sea ice (Dalsgaard and Thamdrup, 2002; Rysgaard and Glud, 2004) and as high as 70°C in hot springs and hydrothermal vent areas (Byrne et al., 2009, Jaeschke et al., 2009).

Metabolism inhibition

As Anammox bacteria favour anaerobic conditions, the Anammox process is reversibly inhibited by the dissolved oxygen (DO) concentration. The effect of oxygen on process performance is described in the following sub-chapter. The effect of oxygen on Anammox bacteria has been examined in marine ecosystems (OMZs). The results showed that oxygen is a major controlling factor for Anammox activity in OMZ waters (Kavelage et al., 2011).

Despite the fact that nitrite is a substrate for Anammox bacteria, its concentration can slow down, or at higher concentrations, completely (but reversibly) stop cellular metabolism. It is clear that nitrite has a negative effect on the Anammox process albeit given inhibitory nitrite concentration levels and process recovery times vary (Strous et al., 1999a; Dapena-Mora et al., 2007; Szatkowska et al., 2007a; Bettazzia et al., 2010; Lotti et al., 2012). Strous et al. (1999a) reported that addition of catalytic amounts of hydrazine or hydroxylamine to the culture medium may bring back inactive Anammox bacteria. However, the research conducted by Schalk et al. (1998) showed that presence of hydrazine in a bioreactor reduces the viability of

Anammox bacteria despite the fact that hydrazine is the key process intermediate.

Based on studies performed with Anammox enrichment cultures from wastewater, methanol has been suggested as a next specific inhibitor of the Anammox process (Güven et al., 2005). It caused complete and irreversible loss of activity at concentrations of ≥ 0.5 mM. Tang et al. (2009) indicated the inhibitory effects of high pH and free ammonia on Anammox bacteria. It was suggested that free ammonia particularly contributes to the destabilization of the Anammox bioreactor seeded with anaerobic granular sludge during the first days of the process start-up.

The inhibitory effect of unknown compounds alleged to be associated with soluble inert COD (chemical oxygen demand) in sludge liquor from dewatering of digested sludge preceded by thermal hydrolysis was reported by Figdore et al. (2011). The Anammox process is successfully implemented for sludge liquor treatment in full-scale operation. However, the impact of any process prior to the anaerobic digestion, as installation of thermal hydrolysis (associated with feed solids characteristics and temperature of the thermal hydrolysis) or change in performance of the digestion process (e.g. thermophilic temperatures) is not fully known.

Process performance

Over the years, the Anammox process has been tested for treatment of different types of highly concentrated ammonium streams, such as landfill leachate (Wyffels et al., 2004), piggery manure (Hwang et al., 2005), digested fish canning effluents (Dapena-Mora et al., 2006) and tannery wastewater (Hulshof, 2007). However, the Anammox process has been most successfully implemented as a sidestream process for treating centrate and filtrate (reject water) streams from dewatering anaerobically digested biosolids. High ammonia concentrations and relatively high temperature typically found in these reject water streams make them ideal candidates for this process. The Anammox bacteria use CO_2 as their carbon source for growth and hence do not require organic carbon. The nitrite required for their growth may be provided by aerobic ammonium oxidizing bacteria or archaea (Francis et al., 2007).

As mentioned before, the Anammox process needs anoxic conditions, and the DO is one of the key factors influencing this process as its excess inhibits the process. Particularly, oxygen is significant in single stage reactor systems where the Anammox process occurs simultaneously with nitrite production (from ammonium oxidation), figure 1. Here the dual properties of a biofilm are used. Such system configuration

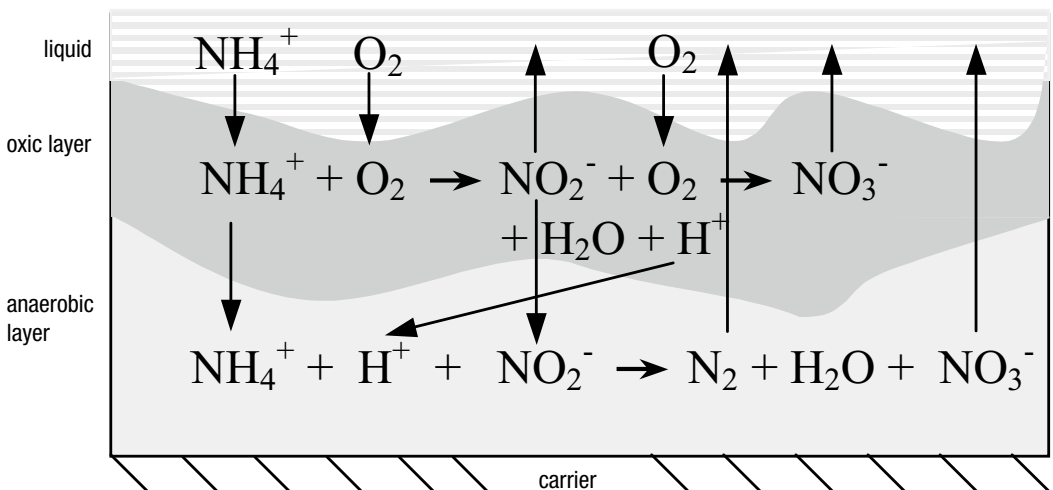


Figure 1. Partial nitrification and Anammox in biofilm single stage reactor system.

where Anammox bacteria in the inner layer are protected from oxygen by nitrifiers in the outer layer seems to be most commonly used in the world. Proper adjustment of the oxygen concentration results in the optimum nitrite production sufficient for the Anammox process (Cema et al., 2011). It was proved that the nitrite production rate is the rate-limiting step for the Anammox process and the overall reaction in a single stage system (Szatkowska et al., 2007b).

Currently, processes which involve Anammox bacteria are designed to treat wastewaters with high ammonium concentration at temperatures 25 – 40 °C. The implementation of this technology for nitrogen removal at lower temperatures would lead to a more sustainable treatment solution with energy, cost and carbon emission savings. Therefore, a laboratory study on process performance with possible high nitrogen removal at lower temperatures was conducted and proved the Anammox adaptability to lower temperatures (Szatkowska and Płaza, 2006). Experiments ran by Hu et al. (2013) showed a possibility of 90% nitrogen removal by the Anammox process at temperature of 12°C.

The combined partial nitrification/Anammox process can save up to 60 % of the oxygen demand (energy for aeration), reduce biomass production by 80%, and there is no alkalinity requirements compared to the nitrification/denitrification processes. With this combined process, less CO₂ emission is recorded as the Anammox process itself consumes CO₂. Table 1 presents a comparison of the Anammox process in combination with the SHARON process (Single reactor system for High activity Ammo-

nium Removal Over Nitrite) and conventional nitrification/denitrification.

Reported nitrogen removal rate for the partial nitrification/Anammox process in full scale operation varies in the range of 0.3-0.6 kgN m⁻³·d⁻¹ for different process configurations (Gustavsson, 2010). For the Anammox process itself, in full scale application, the removal rate was as much as 9.5 kgN m⁻³·d⁻¹ (van der Star et al., 2007).

Due to the fact that the Anammox bacteria are characterized by a very low maximum growth rate of 0.0027 h⁻¹ and a doubling time of at least 11 days (Strous et al., 1998), a system where an Anammox culture is cultivated requires long biomass retention time. The Anammox organisms are extremely slow growing, and the process is slow to start without seed organisms from an operating facility. Currently, the seeding strategy is one of the most effective methods to shorten start-up of the process. The development of the process results in a growing number of full-scale plants what facilitates the access to already working bacterial cultures. The enrichment time of Anammox bacteria is very long and varies between 60 – 150 days for laboratory scale (Zhang et al., 2008) and lasted up to 1250 day for the first full-scale application in the Netherlands (van der Star et al., 2007). Long start-up time of the process is one of the few, but important, process drawbacks and therefore still a field of research.

Process configurations

Many systems configurations applying Anammox bacteria have been tested by different researchers in recent years. The major difference

	Nitrification/Denitrification	SHARON-Anammox	unit
Methanol dosing	3	0	kg/kg N
Power consumption	2.8	1	kWh/kg N
Production of excess sludge	0.5-1.0	0.1	kgVSS/kgN
CO ₂ emission	> 4.7	0.7	kg/kg N
Costs (opex & capex)	3-5	1-2	€/kg N

Table 1. Advantages of the combined SHARON-Anammox process over conventional nitrification/denitrification (after van Loosdrecht, 2008).

between them is whether the complete process is run in two separate steps – partial nitrification in reactor one followed by Anammox in reactor two, or the processes are accomplished in one single reactor. The second difference is the bacteria growth type (granular sludge, activated sludge, biofilm) and the reactor type, see table 2.

Very often combined processes of partial nitrification and Anammox are called deammonification, independently of whether the process is run in one single or in two separate steps. Currently, the most commonly used and already applied in several full-scale systems are: DEMON, SHARON-Anammox, ANITA-Mox and DeAmmon.

DEMON is based on a suspended growth activated sludge process, SBR (sequencing batch reactor) where the nitrification and Anammox processes occur simultaneously (one step process). The aeration is controlled intermittently out of a narrow pH range and low oxygen content (Wett et al. 2007). Due to the fact that the Anammox bacteria tend to grow as relatively heavy granules, which allows for the possibility of separating them from other ammonia and nitrite oxidizing bacteria, the process uses a hydrocyclone which separates Anammox granules in the excess sludge and recirculates granules back to the SBR (Wett et al., 2010). The DEMON process was developed and patented by the University of Innsbruck.

The SHARON-Anammox process is a two-stage suspended growth process implementing a SHARON reactor (proper adjustment of temperature, pH, and retention time allows to select nitrifying bacteria and to prevent nitrate forma-

tion) (Mulder et al., 2001), followed by an anoxic Anammox reactor. The SHARON is operated in completely mixed reactors without sludge retention, while the Anammox reactor uses an upflow solids granulation process to generate biomass that will be retained despite of the low growth rate of Anammox bacteria (van der Star et al., 2007).

The Anita-MOX and DeAmmon processes use carrier media allowing for biofilm growth on the protected surface area, allowing the Anammox organisms to retain in the system. In these attached growth systems, nitrification takes place in the outer biofilm layer while the Anammox bacteria are found in the inner biomass.

Anammox in the main wastewater stream

Due to the fact that Anammox is a process which enables significant cost savings, researchers have started to investigate its implementation for the main wastewater stream of WWTPs. Many questions are addressed to researchers regarding this issue (Gustavsson et al., 2012), and the main challenges are: the inhibition of nitrite oxidizing bacteria (NOB) growth for the partial nitrification, the relatively lower temperature and ammonia concentrations in municipal wastewater compared to the sludge liquor streams, and the need for selective retention of Anammox bacteria. However, the first full-scale plants with the DEMON process have been tested at the Strass WWTP in Austria and at Glarnerland WWTP in Switzerland, where a sidestream process provides seed for inoculation of the main stream with DEMON-sludge (Wett et al., 2010, 2013). This

	Reactor type	Reference
Granular sludge	Air-lift reactor, ALR Up-flow anaerobic sludge bed, UASB Sequencing batch reactor, SBR	Sliekers et al., 2003; Dapena-Mora et al., 2004; Ahn et al., 2004; Schmidt et al., 2004 Arrojo et al., 2005
Activated sludge	Sequencing batch reactor, SBR Membrane bioreactor, MBR	Strous et al., 1998; Third et al., 2005; Trigo et al., 2006
Biofilm	Rotating biofilm contactor, RBC Moving bed biofilm reactor, MBBR	Siegrist et al., 1998; Hippen et al., 2001; Gut et al., 2006; Jaroszynski et al., 2012

Table 2. Different concepts of bacteria growth systems and different reactor types.

solution is named EssDe, which stands for Energy self-sufficient by DEMON. Research on implementation of Anammox in the main wastewater stream takes also place in the Netherlands and in Sweden, where the Manammox (mainstream Anammox) concept is tested.

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