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Review:

Anaerobic ammonium oxidation for treatment of ammonium-rich wastewaters*

Lei ZHANG†, Ping ZHENG†‡, Chong-jian TANG, Ren-cun JIN

(*Department of Environmental Engineering, Zhejiang University, Hangzhou 310029, China*)

†E-mail: angellina2006@hotmail.com; pzheng@zju.edu.cn

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Abstract: The concept of anaerobic ammonium oxidation (ANAMMOX) is presently of great interest. The functional bacteria belonging to the Planctomycete phylum and their metabolism are investigated by microbiologists. Meanwhile, the ANAMMOX is equally valuable in treatment of ammonium-rich wastewaters. Related processes including partial nitritation-ANAMMOX and completely autotrophic nitrogen removal over nitrite (CANON) have been developed, and lab-scale experiments proved that both processes were quite feasible in engineering with appropriate control. Successful full-scale practice in the Netherlands will accelerate application of the process in future. This review introduces the microbiology and more focuses on application of the ANAMMOX process.

Key words: Anaerobic ammonium oxidation (ANAMMOX) bacteria, Metabolism, Partial nitritation-ANAMMOX, Completely autotrophic nitrogen removal over nitrite (CANON), Application

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INTRODUCTION

Ammonia in wastewaters if discharged inappropriately has adverse environmental effects to aquatic systems. It is toxic to living organisms and causes eutrophication in water bodies (Wu *et al*., 2008). Thus, both physico-chemical and biological technologies have been applied in elimination of ammonium from wastewaters for a long period (Bonmatí and Flotats, 2002; Sugiyama *et al*., 2005). However, with the ever stringent discharge standards on ammonium, they are not powerful enough, and more effective technologies are called out. Anaerobic ammonium oxidation (ANAMMOX) is one of the novel biotechnologies for nitrogen removal. In the process, two pollutants of ammonium and nitrite are removed simultaneously, which was first discovered in a denitrifying-fluidized bed reactor in 1994 (Mulder *et al*., 1995). During the past decade, lots of efforts have been made to investigate the mechanism of microorganisms responsible for the ANAMMOX. Additionally, its application in wastewater treatment was pushed forward dramatically.

ANAMMOX BACTERIA

Microorganisms capable of ANAMMOX involve basically two categories. *Nitrosomonas eutropha*, a classic aerobic ammonium oxidizer, belongs to one group which oxidizes ammonium with NO_2 as electron acceptor under anoxic conditions (Schmidt and Bock, 1997). The so-called ANAMMOX bacteria actually are specialized into the other group of deep-branching planctomycetes (Table 1). They have been found in both wastewater treatment plants and natural systems (Strous *et al*., 1999a; Kuypers *et al*., 2003; Dalsgaard *et al*., 2003; Engström *et al*., 2005). Almost 30%~70% gaseous nitrogen production is

‡ Corresponding author

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Table 1 ANAMMOX bacteria discovered up-to-date

Genus	Species	Source
Brocadia	*Candidatus Brocadia anammoxidans* (Strous *et al*., 1999a)	Wastewater
	Candidatus Brocadia fulgida (Kartal *et al*., 2004)	Wastewater
Kuenenia	*Candidatus Kuenenia stuttgartiensis* (Penton *et al*., 2006)	Wastewater
Scalindua	*Candidatus Scalindua brodae* (Schmid *et al*., 2003)	Wastewater
	Candidatus Scalindua wagneri (Schmid *et al*., 2003)	Wastewater
	Candidatus Scalindua sorokinii (Schmid *et al*., 2003)	Seawater
Others	*Candidatus Jettenia asiatica* (Tsushima *et al*., 2007)	Not reported
	Candidatus Anammoxoglobus propionicus (Kartal *et al*., 2007)	Synthetic water

attributed to the ANAMMOX process in the nitrogen cycle (Thamdrup and Dalsgaard, 2002; Dalsgaard and Thamdrup, 2002). The unculturable Gram-negative ANAMMOX bacteria were first isolated through density gradient centrifugation (van de Graaf *et al*., 1996; Strous *et al*., 2002). Their distinct phenotypic characteristics involve red colour, budding production, crateriform structure on the cell surface, intracellular compartment "anammoxosome", and intracytoplasmic membrane containing ladderane lipid (van de Graaf *et al*., 1996; Lindsay *et al*., 2001; Sinninghe Damsté *et al*., 2002). As a special organelle in the cell, anammoxosome was considered to have three functions: (1) providing a place for catabolism; (2) generating energy for ATP synthesis through proton motive force across the anammoxsome membrane; (3) protecting the bacteria from the proton diffusion and intermediate toxicity due to their impermeable membranes (Lindsay *et al*., 2001).

The ANAMMOX bacteria are characterized by slow growth rate and low biomass yield (Strous *et al*., 1998). They have a relatively high affinity for the substrates. Nitrite is more toxic than ammonium to them in a pH range of 6.7~8.3 (Strous *et al*., 1999b; Dapena-Mora *et al*., 2007). The ANAMMOX bacteria can sustain partial oxygen pressure lower than 0.5% air saturation. However, the biological reaction is irreversibly inhibited by high dissolved oxygen (>18% oxygen saturation) (Strous *et al*., 1997a; Egli *et al*., 2001).

METABOLISM

Ammonium is converted to dinitrogen gas with nitrite as the electron acceptor in a ratio of 1:1.32 anoxically (Eq.(1)) (Strous *et al*., 1998).

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow$$
$$1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O. \quad (1)$$

The metabolic pathway was first proposed by van de Graaf *et al*.(1997) based on ^{15}N studies in a fluidized bed reactor with the dominant species of *Candidatus Brocadia anammoxidans*. Hydroxylamine was considered as an intermediate of nitrite reduction (Fig.1) (van de Graaf *et al*., 1997). A slightly different and complementary metabolism mechanism, brought forward through environmental genomics analysis of another species *Candidatus Kuenenia stuttgartiensis*, postulated NO to be the intermediate instead of hydroxylamine (Fig.2) (Strous *et al*., 2006).

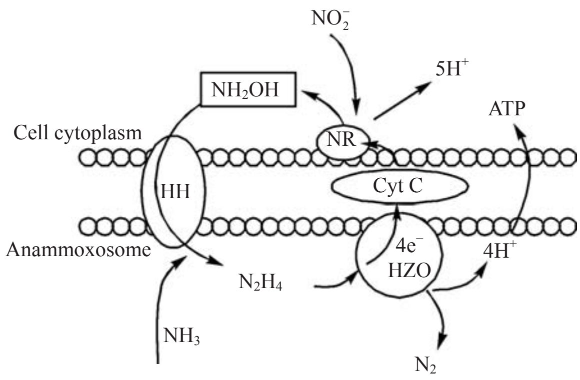

Fig.1 Metabolic pathway of *Candidatus Brocadia anammoxidans* by ^{15}N study

Both hypotheses agreed that hydrazine was an important intermediate in the process. It was formed from oxidation of ammonia with the help of hydrazine hydrolase, and then converted to N_2 through hydrazine-oxidizing enzyme or hydroxylamine oxidoreductase (van de Graaf *et al*., 1997; Strous *et al*., 2006). The two enzymes were proposed to be different in their specificities (Shimamura *et al*., 2007). Cytochromes were important electron transporters between enzymes (Huston *et al*., 2007). Their abundance was closely related to bacterial activity. Experiments had shown a colour alteration from khaki to brownish and red with the increment of nitrogen removal rate (Trigo *et al*., 2006).

The ANAMMOX bacteria are known to anabolize CO_2 taking advantage of energy generated from oxidation of nitrite to nitrate (Fig.2). Both stable carbon isotopic fractionation and genome detection proved that inorganic carbon was fixed through the acetyl-CoA pathway (Schouten *et al*., 2004; Strous *et al*., 2006).

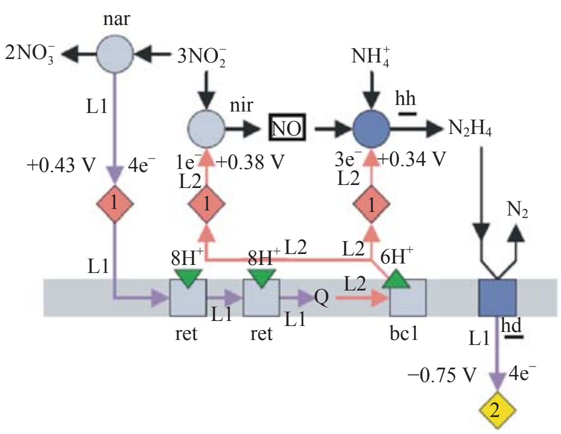

Fig.2 Metabolic pathway of *Candidatus Kuenenia stuttgartiensis* by environmental genomics analysis
Diamond 1: Cytochrome; Diamond 2: Ferredoxin; Line L1: Reduction; Line L2: Oxidation

ANAMMOX-INVOLVED PROCESSES

The ANAMMOX process was carried out with NH_4^+/NO_2^- close to 1. Wastewaters, however, do not always fulfill this characteristic. Thus, several novel processes were developed to ensure the optimal substrate ratio.

Partial nitritation-ANAMMOX

Partial nitritation-ANAMMOX process is based on two biotechnologies. Firstly, ammonium is partly nitrified to nitrite in the partial nitritation stage, and then the produced nitrite is denitrified with the residual ammonium in the ANAMMOX. The nitritation of ammonium is conducted by aerobic ammonium-oxidizing bacteria (AOB) that differentiate greatly from nitrite-oxidizing bacteria (NOB) in physiology. Therefore, selective retention of AOB is important in the partial nitritation stage. In practice, the following five factors are considered as the priorities.

1. Temperature

The two groups of bacteria are quite sensitive to temperature (Hellinga *et al*., 1998). Increased temperature facilitates AOB to outcompete NOB (Fig.3). The optimal temperatures for pure cultural AOB and NOB are 38 and 35 °C, respectively (Grunditz and Dlhammar, 2001). Hellinga *et al*.(1998) have obtained a stable nitritation process at temperature over 35 °C based on a two years' experiment. This value is not fixed. Recently, the nitritation process was also successfully started up and maintained between 15 and 30 °C (Yamamoto *et al*., 2006). However, the system performance deteriorated dramatically below 15 °C, which agreed well with the theoretical value in Fig.3.

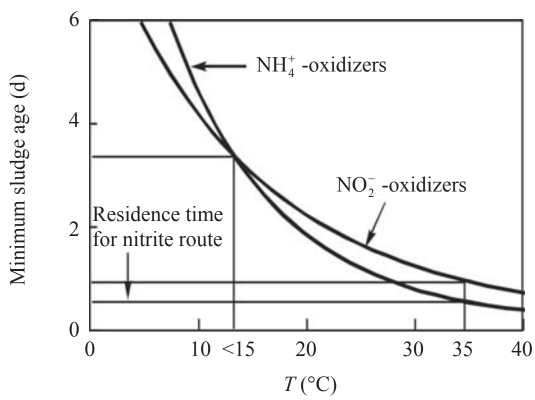

Fig.3 Minimum residence time for ammonium and nitrite oxidizers at different temperatures (Hellinga *et al*., 1998)

2. Sludge residence time (SRT)

At higher temperature stated above, doubling time of AOB is shorter than that of NOB. Thus, SRT should be properly mediated in a limited range that enables retention of AOB but washing out of NOB. Full scale experience in Utrecht and Rotterdam wastewater treatment plants suggested that SRT between 1 and 2.5 d was acceptable (van Kempen *et al*., 2001). Nevertheless, SRT as long as 5 d in an SBR (sequential batch reactor) also created a favourable environment for AOB to outgrow NOB (Galí *et al*., 2007).

3. Dissolved oxygen (DO)

DO strategy for nitritation process control is based on different affinities of AOB and NOB. The K_s (half saturation constant) value of AOB (0.3 mg/L) is lower than that of NOB (1.1 mg/L), which means that AOB will outcompete under DO limitation condition (Wang and Yang, 2004). Some researchers have taken DO concentration around 1.0 mg/L as suitable concentration for nitritation (Ciudad *et al*., 2005; Ruiz *et al*., 2006). This value can be quite a specific case due to variation of the oxygen mass transfer efficiency in reactors (Ciudad *et al*., 2005).

4. Influent alkalinity/ammonium

Ammonium oxidation is an alkali-consuming reaction. In order to assure a proper NH_4^+/NO_2^-, 1 mol alkali per mol ammonium is used (Eq.(2)). Both lab experiments and engineering practices have proved that an alkalinity/ammonium ratio around 1 was suitable for the partial nitritation-ANAMMOX (van Dongen *et al*., 2001; Fux *et al*., 2002).

$$NH_4^+ + HCO_3^- + 0.75O_2 \rightarrow 0.5NH_4^+ + 0.5NO_2^- + CO_2 + 1.5H_2O. \quad (2)$$

5. pH

pH plays triple roles in the partial nitritation process prior to the ANAMMOX. Firstly, it directly influences the growth rates of the two groups of bacteria. The growth rate of NOB at pH 7 is eight times that at pH 8, whereas the variation of AOB is negligible (Hellinga *et al*., 1998). Secondly, pH is closely related to the available substrate forms. Actually, NH_3 and HNO_2 are true substrates for AOB and NOB, respectively. Wastewaters with pH around 8 created an environment containing more NH_3 and less HNO_2, which obviously promotes AOB but suppresses NOB (Hellinga *et al*., 1998). Therefore, the partial nitritation process is recommended to operate in a weak alkaline condition. Thirdly, pH is a simple indicator for automatic control of HNO_2/NH_3 in a fixed alkalinity/ammonium ratio (Fux *et al*., 2002).

Orthogonal experiment suggested that pH, temperature and DO concentration influence the nitritation performance significantly (Lu *et al*., 2006). Furthermore, other factors such as operation mode, aeration pattern, reactor configurations and operating costs all should be considered comprehensively. For example, it was feasible to run the nitritation process at lower temperature (around 15 °C) with an extended SRT (Yamamoto *et al*., 2006). Besides, if the DO concentration was limited, the process would remain stable with SRT as long as 24 d (Pollice *et al*., 2002). Both the SBR and the chemostat with the nitrogen conversion rates of 1.1 kg N/(d·m^3) and 0.35 kg N/(d·m^3) respectively were reliable reactors for the nitritation phase, whereas the chemostat was more stable (Galí *et al*., 2007). In engineering, cascade O_2 control plus pH control was superior to other strategies based on the economic cost analysis (Volcke *et al*., 2006). Table 2 lists several SHARON practices with different control strategies.

Completely autotrophic nitrogen removal over nitrite (CANON)

The prototype of the CANON process was derived from conversion of ammonium to dinitrogen gas in a microaerobic or a combined aerobic-anoxic environment. For the lack of knowledge about the microbial reactions in the reactors, the process was named as "aerobic/anoxic deammonification" (Hippen *et al*., 1997). It was not until 2000 that the concept of CANON was proposed for treatment of low C/N wastewaters (Strous, 2000). Two groups of autotrophic bacteria, i.e., the AOB and the ANAMMOX bacteria, are involved. The AOB take advantage of

Table 2 Control strategies for the partial nitritation phase in the partial nitritation-ANAMMOX process

Reactor type	pH	*T* (°C)	*DO* (mg/L)	*SRT* (d)	NH_4^+-N / NO_2^--N *	References
CSTR		30~40		1.0	1.09	van Dongen *et al*., 2001
		30		1.2	1.10	Fux *et al*., 2002
	7.23		Cascade control		NR	Volcke *et al*., 2006
SBR			3~4	1	0.60	Hwang *et al*., 2005
Swim-bed		15~30		0.5~0.25	0.61	Yamamoto *et al*., 2006

CSTR: Continuous stirred tank reactor; SBR: Sequential batch reactor; NR: Not reported; * NH_4^+-N / NO_2^--N after the partial nitritation phase oxygen to oxidize ammonium to nitrite. After the depletion of oxygen, nitrite with the remaining ammonium is converted to gaseous nitrogen. The overall reaction is as follows:

$$NH_4^+ + 0.85O_2 \rightarrow 0.435N_2 + 0.13NO_3^- + 1.4H^+ + 1.3H_2O. \quad (3)$$

As a result, the CANON process saves 63% oxygen and 100% carbon sources in comparison with the traditional nitrification-denitrification process (Third *et al*., 2005).

An early process named "OLAND" (oxygen limited autotrophic nitrification denitrification) can be taken as a variation of CANON. Unlike the co-existence of AOB and the ANAMMOX bacteria, nitrifying bacteria such as *Nitrosomonas eutropha* conduct aerobic ammonium oxidation and anaerobic ammonium oxidation simultaneously. Impressively, molecular biological experiments discovered that typical ANAMMOX species *Kuenenia stuttgartiensis* was active in the OLAND system as well (Pynaert *et al*., 2002).

Virtually, the CANON process is an integration of partial nitritation-ANAMMOX into one single reactor. Although the mentioned factors in partial nitritation-ANAMMOX control are applicable to the CANON system theoretically, most attention has been paid to the manipulation of influent ammonium loading rate and DO concentration, which greatly influence the microbial composition in the CANON system. Appropriate ammonium and DO concentration enable the consumption of oxygen by AOB to an extent in which DO concentration is not over the threshold toxic to the ANAMMOX bacteria and inadequate for the growth of NOB. Subsequently, the produced nitrite, an inhibitor to AOB, is used as electron acceptor by the ANAMMOX bacteria. Thus, a symbiosis between AOB and the ANAMMOX bacteria is finely established as indicated by fluorescence in situ hybridization (Sliekers *et al*., 2002; Nielsen *et al*., 2005). For ammonium-limited or DO-excess influent, ammonium is exhausted by the AOB rapidly. The accumulated nitrite and relatively excess oxygen in bulk liquid stimulate the outgrowth of NOB over the ANAMMOX bacteria. The perfect balance among the three bacteria is destroyed, and inefficient performance of the CANON process is not unreasonable. Lower limit of ammonium loading rate of 0.12 kg N/(d·m^3) for steady nitrogen removal was reported by Third *et al*.(2001). The nitrogen elimination efficiency resulted from limited ammonium decreased by 35% in both SBR and chemostat when the DO concentration was kept around 0.24 mg/L. Model simulation indicated that the maximum nitrogen removal rate was achieved only when the DO concentration kept pace with the ammonium surface load (Hao *et al*., 2001). Thus, the stoichiometry of ammonium and oxygen is the key control parameter in the CANON process. For fluctuating ammonium loading rates in engineering, DO can be regulated through on-line feed-back control (Nielsen *et al*., 2005).

Presently, lab-scale CANON has been conducted in biofilm reactors, SBRs and gas-lift reactors (Table 3). The efficient retention of biomass in SBR enables itself a powerful tool for slowly-growing bacteria, but its performance in the CANON process is not outstanding. The highest nitrogen removal rate of 1.5 kg N/(d·m^3) was obtained in a gas-lift reactor (Sliekers *et al*., 2003). For one thing, AOB and the ANAMMOX bacteria are active in the aerobic and the anaerobic phases alternately in the SBR system. In a continuous oxygen-limited reactor, AOB and the ANAMMOX bacteria cooperate simultaneously and cause maximum conversion rates. For another thing, it seems that oxygen mass transfer is the rate-limiting

Table 3 CANON process in different reactors

Reactor type	NRR (kg N/(m^3·d))	Sludge form	Aeration type	References
SBR	0.160	Suspended	Continuous	Sliekers *et al*., 2002
	0.120	Suspended	Continuous	Third *et al*., 2001
	0.080	Suspended	Intermittent	Third *et al*., 2005
Gas-lift reactor	1.500	Suspended	Continuous	Sliekers *et al*., 2003
RBC	7.390	Biofilm	Continuous	Pynaert *et al*., 2003
GSBR	0.057	Granular	External aeration	Ahn and Choi, 2006

NRR: Nitrogen removal rate; SBR: Sequential batch reactor; RBC: Rotating biological contactor; GSBR: Granular sludge bed reactor step. The transference of oxygen to bulk liquid is dramatically elevated in the gas-lift reactor due to its suspended flocs. The compact granular sludge bed reactor and the rotating biological contactor (RBC) with biofilm are unfavourable for the oxygen to approach the active microbes (Pynaert *et al*., 2003; Ahn and Choi, 2006). The thicker the biofilm, the more DO is needed to get the maximum nitrogen elimination (Hao *et al*., 2001). Thickness of the biofilm or size of the granular sludge influences bacteria composition as well. Large aggregates (> 500 μm) account for 68% of the ANAMMOX bacteria, while small aggregates (<500 μm) only account for 35% (Nielsen *et al*., 2005). The reason is obvious. Large granular sludge is not so permeable to oxygen as small one, and a relatively large anoxic sphere is created to facilitate growth of the anaerobic bacteria.

The CANON process has a great advantage over the partial nitritation-ANAMMOX in investment. However, difficulties in DO regulation in large reactors and incomplete nitrogen removal of high nitrogen loads prevent them from application to wastewaters with high ammonium concentration. For wastewaters with higher ammonium, it is worth sacrificing relatively high investment for a partial nitritation-ANAMMOX practice. The expenses will be compensated by lower operational costs and efficient nitrogen removal performance (Hao *et al*., 2001; Nielsen *et al*., 2005).

APPLICATION

The ANAMMOX and relevant processes mentioned above with a merit of low operational costs have attracted much attention since their births. A number of researches have been conducted on various ammonium-rich wastewaters (Table 4). Sludge reject water was the first candidate due to its characteristics including low organics and proper NH_4^+/alkalinity for the partial nitritation-ANAMMOX process (van Dongen *et al*., 2001). The 10 L-lab experimentation was directly scaled up to an engineering practice. This full-scale ANAMMOX was initiated in Rotterdam on July 1st, 2002. The start-up phase took nearly 3.5 years, and the quantitative-PCR (polymerase chain reaction) depicted exponential growth of the ANAMMOX bacteria. Now stable operation reached a nitrogen removal rate of 9.5 kg N/(m^3·d) (van der Star *et al*., 2007).

Additionally, wastewaters from anaerobic treatment of animal waste were also tested (Ahn and Kim, 2004; Hwang *et al*., 2005; Waki *et al*., 2007). These wastewaters are known for high organic nitrogen content. During anaerobic digestion, ammonium is elevated considerably due to protein decomposition. The ANAMMOX is hereby emerging with the denitrification (Dong and Tollner, 2003). Most investigations described that denitrifiers contributed more than the ANAMMOX bacteria. NO_2^--N to

Table 4 ANAMMOX application to different wastewaters

Wastewater	Process	NRR (kg N/(m^3·d))	Start-up (d)	Scale	References
Sludge liquor	Partial nitritation-ANAMMOX	0.710	110	10 L	van Dongen *et al*., 2001
Sludge supernatant	Partial nitritation-ANAMMOX	2.400	150	2.5 m^3	Fux *et al*., 2002
Partially nitrified sludge digestate	ANAMMOX	3.500		3.5 L	Fux *et al*., 2004
Sludge digestate	Partial nitritation-ANAMMOX	9.500	1250	70 m^3	van der Star *et al*., 2007
Slaughterhouse wastewater	Nitrification-denitrification	0.031		790 ml+745 ml	Reginatto *et al*., 2005
Piggery wastewater	ANAMMOX	0.600		1.5 L	Ahn and Kim, 2004
	Partial nitritation-ANAMMOX	1.360	~60	1 L	Hwang *et al*., 2005
Synthetic coke-oven wastewater	ANAMMOX	0.062	~465	1 L	Toh and Ashbolt, 2002
Monosodium glutamate wastewater	ANAMMOX	0.460	71	5 L	Chen *et al*., 2007

NRR: Nitrogen removal rate

NH_4^+-N ratios ranged between 1.48 and 1.79 (Ahn and Kim, 2004). This was closely related to the relatively higher COD (chemical oxygen demand) of 600~25700 mg/L (Ahn and Kim 2004; Hwang *et al.*, 2005). The COD was 800~1800 mg/L in sludge digest liquids (Hellinga *et al.*, 1998; Ahn and Choi, 2006). It has been reported that if COD was reduced to less than 135 mg/L, the NO_2^--N / NH_4^+-N ratio was close to 1:1 (Waki *et al.*, 2007).

Application in coke-oven wastewater was first proposed by Toh *et al.*(2002). The assumption was considered as a big breakthrough for the ANAMMOX concept, because the coke-oven wastewater contains not only high concentration of organics (COD: 2000~2500 mg/L), but also some toxic chemicals such as phenol (300~800 mg/L), cyanides (10~90 mg/L) and thiocyanates (300~500 mg/L) (Toh and Ashbolt, 2002; Toh *et al.*, 2002). Though the initial attempt to enrich the bacteria from industrial coke-oven wastewater sludge failed, the acclimation of the ANAMMOX consortium (from municipal wastewater sludge) to synthetic coke-oven wastewater was successfully established. Phenol was added to the influent from (50±10) to (500±10) mg/L stepwise. After a culture of 15 months, the ammonium removal rate peaked to 0.062 kg N/(m^3·d).

Monosodium glutamate is a popular flavor in most Asian countries. It is produced through fermentation of rice, starch and molasses. Wastewater from this process contains high SS (suspended solids) (200~10000 mg/L), COD (1500~60000 mg/L), NH_4^+-N (200~15000 mg/L) and sulfate (3000~70000 mg/L). Traditional treatment usually involves physico-chemical and biological methods in succession. After the physico-chemical step, SS, COD and NH_4^+-N are reduced to 200~270, 1000~1400 and 250~350 mg/L, respectively. The ANAMMOX process was tried in this water with preliminary nitrition by our research group and a total nitrogen removal rate of 0.46 kg N/(m^3·d) was obtained (Chen *et al.*, 2007). The performance of ANAMMOX was better than the conventional nitrification-denitrification.

The highest nitrogen removal rate in lab has reached 26 kg N/(m^3·d) with synthetic wastewater hitherto (Tsushima *et al.*, 2007). In contrast, the nitrogen removal load is not so high in engineering. The slow growth turns out to be the biggest challenge. Therefore, principles of biomass enrichment and growth stimulation of the microorganisms are widely accepted by scientists and engineers.

Inoculum

The planctomycete-like ANAMMOX bacteria exist in many natural and artificial environmental niches. They have been found in different water treatment installations based on PCR analysis, involving membrane bioreactors, denitrifying basins, nitrifying RBCs, oxidation ditches, anaerobic digesters and aeration tanks (Tsushima *et al.*, 2007). Accordingly, it is not strange to start up the process with various aerobic and anaerobic seeding sludges (Table 5). Tsushima *et al.*(2007) reported that seeding sludge containing more ANAMMOX bacteria from a denitrifying basin kicked off the reaction fastest and an effective nitrogen removal was obtained. Sequential addition of the pre-enriched sludge with ANAMMOX activity was also selected as a strategy for the engineering practice in the Netherlands (van der Star *et al.*, 2007).

Table 5 Start-up of the ANAMMOX with different seeding sludges

Inocula	Start-up (d)	NRR (kg N/(m^3·d))	References
Nitrification sludge	105	2.090	Zheng *et al.*, 2004
Activated sludge	150	0.090	Chamchoi and Nitisoravut, 2007
Denitrification sludge	100	0.609	Zhang *et al.*, 2004
	392	6.200	Tsushima *et al.*, 2007
Anaerobic digestion sludge	150	0.090	Chamchoi and Nitisoravut, 2007
Upflow anaerobic sludge blanket	150	0.090	Chamchoi and Nitisoravut, 2007
Nitrification sludge+upflow anaerobic sludge blanket	250	1.800	Pynaert *et al.*, 2004

NRR: Nitrogen removal rate

Reactor configurations

Slow growth of the ANAMMOX bacteria demands that the reactor carrying out the process has an effective retention of the biomass. Herein biofilm systems such as fix-bed and fluidized-bed reactors with different carriers, SBRs and gas-lift reactors, were selected to improve the reactor performance. The SBR has been considered to be a powerful reactor configuration for slowly growing bacteria by Strous *et al*.(1998). The SBR had been stably operated for one year and reached a biomass retention of 90% which was 1.4 times that in a fluidized-bed reactor (Strous *et al*., 1998). Otherwise, Membrane SBR which is a combination of a SBR and a biofilm system was applied. After cultivation of 350 d, a maximum nitrogen removal rate of 0.7 kg $N/(m^3 \cdot d)$ was achieved (Trigo *et al*., 2006).

Strous *et al*.(1997b) started up the ANAMMOX in the fixed-bed and fluidized-bed reactors with glass beads and sand particles as carriers. However, the biomass loss was not prevented, especially in the fixed-bed reactor due to sludge floating. The same situation appeared in the gas-lift reactor at an increased nitrogen removal rate as well (Dapena-Mora *et al*., 2004). Gas bubbles entrapped within the sludge made the sludge density decrease, which enabled the sludge to float and escape from the reactors. The problem was solved by converting the carriers to nonwoven fiber. Complete biomass retention was observed and the measured doubling time of the ANAMMOX bacteria was shorten to 1.8 d (Isaka *et al*., 2006). Experiments with nonwoven fiber were characterized by short start-up time (34~247 d) and high nitrogen removal rate (0.77~26 kg $N/(m^3 \cdot d)$) (Furukawa *et al*., 2003; Isaka *et al*., 2006; Tsushima *et al*., 2007; Gong *et al*., 2007). Plus, the quantitative stability analysis backed up that membrane systems were more resistant to substrate concentration shock than SBRs (Jin *et al*., 2008). But for all membrane systems, fouling is a big problem. The operation costs due to backwashing or external cleaning with chemicals are inevitable in engineering practice.

Nutrients

Nutrient balance is important in enrichment of microorganisms, and the ANAMMOX bacteria are not exceptional. Both macroelements (C, N, P) and microelements (Fe, Ni, Co, Cu, Zn, Mn, Mo) should be kept at an appropriate ratio. Wastewaters for the ANAMMOX treatment may not lack of nitrogen, but carbon source and microelements especially Fe can be limited. Besides, availability of the nutrients to the microbes should also be considered. Precipitation of phosphate has been observed in experiments with synthetic wastewaters, which disabled the use of phosphate and resulted in phosphorus shortage (Trigo *et al*., 2006).

CONCLUSION

The ANAMMOX process as a cost-effective and energy-saving biotechnology has a great potential in treatment of ammonium-rich wastewaters, especially after its successful case in treatment of sludge digest liquids. Long start-up time of this process severely limits its application, but this process can be improved through inoculation with pre-cultivated ANAMMOX sludge, selection of reactors with efficient biomass retention, adjustment of nutrient balance and environmental conditions. At present, the ANAMMOX process is still confined to a few types of wastewaters (sludge digestate and animal wastewaters). Wide application is possible if the biomass can be largely enriched and more adapted to the organic matters. Some species from seawaters, which is psychrophilic can also be investigated for the application to cold regions.

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