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Biological Removal of Nitrogen from Wastewater

Guibing Zhu, Yongzhen Peng, Baikun Li, Jianhua Guo, Qing Yang, and Shuying Wang

Contents

I. Introduction

The removal of ammonia from wastewater has become a worldwide emerging concern because ammonia is toxic to aquatic species and causes

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G. Zhu, Y. Peng (✉), J. Guo
School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150090, P.R. China

B. Li
Department of Civil and Environmental Engineering, University of Connecticut, 261 Glenbrook Rd., UNIT-2037, Storrs, CT 06269

Y. Peng, Q. Yang, S. Wang
Key Laboratory of Beijing Water Quality Science and Water Environment Recovery Engineering, Beijing University of Technology, Beijing 100022, P.R. China

G. Zhu
SKLEAC, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, P.R. China

eutrophication in natural water environments (Tchobanoglous et al. 2003). Nitrogen compounds in wastewater can only be effectively removed by biological approaches (EPA 1993; Zhu et al. 2007a,b). Based on the microbial nitrogen cycle and the metabolism of inorganic nitrogen compounds (Fig. 1), many biological technologies and processes have been developed and implemented for nitrogen removal from wastewater, such as predenitrification (Anoxic/Oxic), modified Bardenpho, Bio-denitro, sequencing batch reactor (SBR), oxidation ditch (OD), step feeding, anaerobic/anoxic/aerobic (A^2/O), and University of Cape Town (UCT) processes (Wentzel et al. 1992; Østgaard et al. 1997; Williams and Beresford 1998; Tchobanoglous et al. 2003; Pai et al. 2004). These processes have been widely employed in wastewater treatment plants for nitrification and denitrification (EPA 1993). However, with the effluent discharge standards having become more stringent (<10 mg total nitrogen/L), conventional processes cannot meet the new requirements (Khin and Annachhatre 2004).

Several novel nitrogen removal processes have been developed to enhance nitrification and denitrification. This review focuses on these novel processes, including simultaneous nitrification and denitrification (SND), shortcut nitrification and denitrification, anaerobic ammonium oxidation (ANAMMOX), aerobic deammonitrification, completely autotrophic nitrogen removal over nitrite (CANON), oxygen-limited autotrophic nitrification-denitrification (OLAND) processes (Muller et al. 1995; Strous et al. 1999; Fux et al. 2002; Third et al. 2001; Schmidt et al. 2003; Nielsen et al. 2005; Peng and Zhu 2006). Particularly, this review presents a critical comparison of various biological processes, discusses the key control parameters, and summarizes the current research status of functional microorganisms for nitrogen removal. Moreover, several challenging and unsolved problems of these processes are addressed.

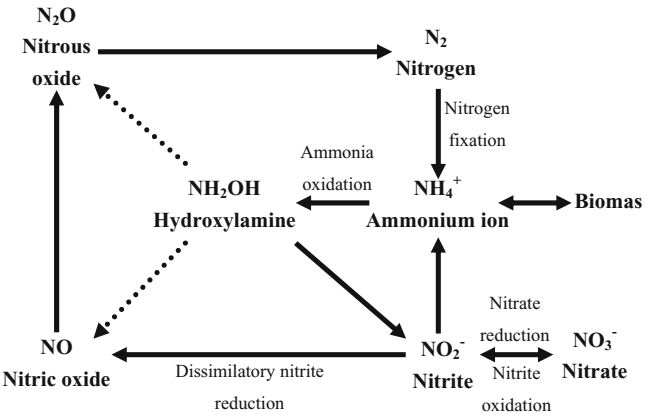

Fig. 1. Microbial nitrogen cycle. (From Rick and Stuart 2001.)

II. Conventional Biological Technologies for Nitrogen Removal

A. Mechanism and Principle

Conventional microbial nitrogen removal is based on autotrophic nitrification and heterotrophic denitrification. In the first step of nitrification, ammonia-oxidizing bacteria (AOB) oxidize ammonium (NH_4^+) to nitrite (NO_2^-) via hydroxylamine (NH_2OH) (reactions 1 and 2, below). Membrane-bound ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) are involved in these two reactions. In the second step, nitrite-oxidizing bacteria (NOB) oxidize nitrite to nitrate (NO_3^-) with the involvement of membrane-bound nitrite oxidoreductase (NOR) (reaction 3).

$$NH_3 + O_2 + 2[H] \xrightarrow{AMO} NH_2OH + H_2O \quad (1)$$

$$HN_2OH + 0.5O_2 \xrightarrow{HAO} NHO_2 + 2H^+ + 2e^- \quad (2)$$

$$NO_2^- + 0.5O_2 \xrightarrow{NOR} NO_3^- \quad (3)$$

In anoxic denitrification, NO_3^- and NO_2^- are reduced to gaseous nitrogen with a variety of electron donors, such as methanol, acetate, and organic substances in wastewater (reactions 4 and 5).

$$2NO_3^- + 10H^+ + 10e^- \rightarrow N_2 + 2OH^- + 4H_2O \quad (4)$$

$$2NO_2^- + 6H^+ + 6e^- \rightarrow N_2 + 2OH^- + 2H_2O \quad (5)$$

B. Typical Processes for Biological Removal of Nitrogen

Many biological nitrogen removal processes have been developed, including Bardenpho, predenitrification, postdenitrification, SBR, OD, and step feeding (Tchobanoglous et al. 2003; Dapena-Mora et al. 2004; Khin and Annachhatre 2004; Zhu et al. 2005, 2007a,b). The advantages and limitations of these processes are summarized in Table 1.

In most countries, especially in China, about 80% of wastewater treatment plants use the predenitrification [i.e., anoxic/oxic (A/O)] process for biological nitrogen removal (Zhu 2006). Predenitrification has distinct advantages for nitrogen removal. With influent first entering the anoxic denitrification zone, organic carbon sources serve as electron donors for denitrification and are biodegraded by denitrifying bacteria. This method can improve nitrogen removal efficiency and shorten the aerobic duration. However, because of the configuration of A/O processes, the NO_X-N concentration in effluent equals that of internally recycled wastewater, which results in an overall low nitrogen removal efficiency (Baeza et al. 2004; Rosso and Stenstrom 2005). For instance, based on the theory

Table 1. Advantages and Limitations of Conventional Biological Nitrogen Removal Processes.

Process	Advantages	Limitations
Bardenpho (4-stage)	Total N concentration less than 3 mg/L possible	Large reactor volume is required Second anoxic tank has low efficiency
Predenitrification	Very adaptable to existing activated sludge processes 5–8 mg/L total N is achievable Reparation of alkalinity because of denitrification	N-removal capability is a function of internal cycle DO control is required before recycle
Postdenitrification	Capable of achieving total N levels less than 3 mg/L	Higher operating cost due to additional carbon dosage Carbon dosage control is required
Bio-denitro	Large reactor volume is resistant to shock load 5–8 mg/L total N is achievable	More complex to operate Two reactors are required so as to increase construction cost
Sequencing batch reactor	Process is flexible and easy to design Quiescent settling provides low effluent TSS concentration Mixed liquid/solids cannot be washed out by hydraulic surges Secondary settling tank is not required	More complex to operate Effluent quality depends on reliable decanting facility Be not suitable for large plants
Oxidation ditch	Highly reliable process Simple operation Capable of treating shock/toxic loads without affecting effluent quality Economical process for small plants Well-stabilized sludge; low biosolids production	Large structure, greater space requirement Low F/M bulking is possible Some modifications are proprietary and license fees may be required Plant capacity expansion is more difficult Nitrogen removal capability is related to skills of operating staff and control methods
Step feeding	Distributes load to provide more uniform oxygen demand To minimize high clarifier solids loading in peak wet weather flows Adaptable to existing activated sludge processes With internal recycle in last stage, total N less than 5 mg/L possible.	N-removal capability is a function of flow distribution Flow split is not measured or known accurately DO control is required before recycle Flow split control is required to optimize operation

TSS, total suspended solids; DO, dissolved oxygen.

of predenitrification process (Chiou and Ouyang 2001), a 100% sludge recycle and 200% internal recycle can achieve only 75% nitrogen removal efficiency.

C. Disadvantages and Limitations of Conventional Processes

Because nitrification and denitrification are carried out by different microorganisms under different conditions, they should be designed and operated in separate time sequences or spaces (Lee et al. 2001). Consequently, a long retention time or a large volume is required to accomplish complete nitrogen removal. Moreover, a high level of oxygen, set as 4.2 g O_2/g NH_4^+-N, is required for nitrification (Bruce and Perry 2001), and a sufficient organic carbon source [2.86 g chemical oxygen demand (COD)/g NO_3^--N) is necessary for denitrification (Gradly and Lim 1980). A high level of external carbon sources (methanol, acetate, etc.) is normally added in the denitrification process when treating wastewater with high nitrogen concentration or low C/N ratio (Tam et al. 1992), which increases the operational cost for conventional biological processes. The limitations of low removal efficiency, high oxygen requirement, long retention time, and an external carbon source are the driving forces for developing new low-cost biological treatment processes for complete nitrogen removal (Jetten et al. 2002).

III. Novel Biological Processes for Nitrogen Removal

A. Simultaneous Nitrification and Denitrification (SND)

Mechanism and Advantages

Simultaneous nitrification and denitrification (SND) means that nitrification and denitrification occur concurrently in the same reactor (Keller et al. 1997; Helmer and Kunst 1998). There are two mechanisms for SND: physical and biological (Robertson and Kuenen 1984; Baumann et al. 1996; Hibiya et al. 2003). The conventional physical mechanism is that SND occurs as the consequence of dissolved oxygen (DO) concentration gradients within activated sludge flocs or biofilms due to diffusional limitation (Fig. 2). The nitrifiers exist in aerobic regions with DO higher than 1–2 mg/L, whereas the denitrifiers stay alive in anoxic zones with DO less than 0.5 mg/L. The presence of oxygen concentration gradients in activated sludge flocs and biofilm has been verified by microelectrode measurements (Snidaro et al. 1997; de Beer et al. 1998; Satoh et al. 2003; Li and Bishop 2004; Holman and Wareham 2005) and ^{15}N tracer techniques (Wyffels et al. 2003).

The biological mechanism for SND is more complicated than the physical ones and is contradictory to the traditional "engineering" conception of autotrophic aerobic nitrification and heterotrophic anoxic denitrification. Several species of heterotrophic nitrifiers and aerobic denitrifiers have been identified in wastewater and night soil treatment systems (Patureau et al.

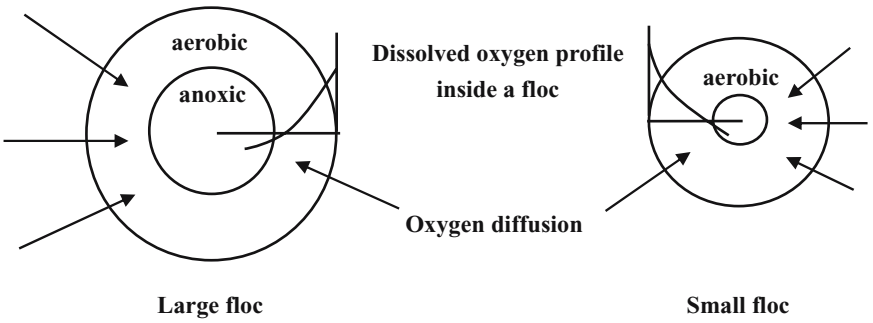

Fig. 2. Schematic of oxygen concentration profile within a microbial floc. Reprinted form Pochana and keller 1999, with permission from IWA Publishing.

1998; Hu and Kung 2000; Kim et al. 2005). *Alcaligenes* sp., *Corynebacterium* sp., *Acinetobacter* sp., *Xanthomonas* sp., and *Bacillus* strains were identified as heterotrophic nitrifiers (Castignetti and Gunner 1981; Castignetti and Hollocher 1982; Kshirsagar et al. 1995; Hu and Kung 2000; Kim et al. 2005). *Thiosphaera pantotropha* was identified as both heterotrophic nitrifier and aerobic denitrifier (Gupta 1997). *Paracoccus denitrificans,* an aerobic denitrifier isolated from activated sludge (Robertson et al. 1988, 1995; Baumann et al. 1996), reduced nitrate even under oxygen saturation. Other aerobic denitrifier strains, such as *Microvirgula aerodenitrificans* (Patureau et al. 1998) and *Thaurea mechernichensis* (Scholten et al. 1999), have been isolated. From a microbiological point of view, SND has been regarded as the consequence of the oxidization of ammonia by heterotrophic nitrifiers and the reduction of nitrate or nitrite by aerobic denitrifiers (Robertson et al. 1988, 1995; Wyffels et al. 2003).

SND has significant advantages over conventional processes (Pochana et al. 1999; Zhang et al. 2005). With denitrification taking place concurrently with nitrification in aeration tanks the SND process can save the costs for anoxic tanks, and simplify the overall process design. SND is of particular interest when treating wastewaters with a low C:N ratio (<5), because the cost of an extra carbon source will be saved (Guo et al. 2005).

Key Control Factors

On-line monitoring DO and redox potential (ORP) is an efficient approach in the SND process (Zhao et al. 1999; Fuerhacker et al. 2000). SND occurs at a DO of 0.5 mg/L, under which condition the nitrification and denitrification rates are the same (Munch et al. 1996; Peng et al. 2001). However, SBR occurs at a wide ORP range, from –60 to –198 mV (Hanaki et al. 1990; Fuerhacker et al. 2000; Hu et al. 2005).

Other operational parameters, such as sludge retention time (SRT), hydraulic retention time (HRT), and pH, have significant influence on the SND process. Because heterotrophic nitrifiers grow more rapidly and have

stronger tolerance to acidity than autotrophic nitrifiers, short SRT and an acidic environment would be favorable for their growth (Focht and Verstraete 1977; Killham 1986; van Niel 1991). A bench-scale SBR system achieved a complete removal of NH_4^+-N and COD with no NO_2^--N in the effluent at a C/N ratio of 11.1, SRT of 20 d, and HRT of 1 d. However, the nitrogen removal efficiency decreased gradually with increasing ammonium-loading rates and F/Ms (Chiu et al. 2007). Until now, the SND processes have been tested with consistent SRT and HRT. There is no research on the influence of SRT and HRT on the efficiency of SND. In addition, pH and free ammonia (FA) should be studied as critical parameters for SND via NO_2^- as they have significant effects on the competition between AOB and NOB.

Research Status and Unsolved Concerns

There are three major factors for the SND processes: carbon source, DO concentration, and floc size (Lee et al. 2001; Holman and Wareham 2005).

Organic carbon is critical for SND, because high biological oxygen demand (BOD) concentration causes the inhibition of autotrophic nitrifiers whereas low BOD leads to the deficiency of electron donors for denitrifiers (Tam et al. 1992). SND occurs well when treating municipal wastewater at a BOD of 100–150 mg/L (Castignetti and Gunner 1981; Kim et al. 2005).

DO concentration is also important for SND (Pochana et al. 1999): it has a twofold effect on SND performance (Pochana and Keller 1999; Hu et al. 2005; Zhang et al. 2005). Low DO concentration suppresses nitrification while high DO concentration inhibits denitrification. Nitrification and denitrification rates became the same at a DO concentration of 0.5 mg/L and achieved a complete SND (Munch et al. 1996). Zhao et al. (1999) found that an extended aeration duration in an intermittent aeration (IA) process favored sequential nitrification and denitrification (SQND). The optimal DO concentration for SND via nitrite was around 2.0 ± 2.5 mg/L at the end of the aeration period in the IA process (Yoo et al. 1999).

Some researchers attribute the occurrence of SND to the size of activated sludge floc, which is normally 80–100 μm (Li and Ganczarczyk 1990, 1993; Pochana and Keller 1999). SND is more likely to occur in the large-size floc (>125 μm) because of the oxygen diffusion limitation, but the occurrence of SND in activated sludge flocs smaller than 20 μm is unclear. If SND is detected in small floc sludge (Wilen and Balmer 1999), the current physical explanation of SND processes will be put in question.

B. Shortcut Nitrification and Denitrification

Mechanism and Advantages

Shortcut nitrification and denitrification, namely partial nitrification-denitrification, is the process in which nitrification and denitrification are

correlated by NO_2^- instead of NO_3^-. As an intermediate product, NO_2^- is produced in nitrification and reduced to N_2 in the following NO_2^- denitrification (Fdz-Polanco et al. 1996; van Dongen et al. 2001; Peng et al. 2006). Compared with traditional nitrification and denitrification via NO_3^-, shortcut nitrification and denitrification has the following advantages (Beccari et al. 1983; Turk and Mavinic 1989; Peng and Zhu 2006):

1. 25% lower oxygen consumption in the aerobic phase implies 60% energy saving in the entire process.
2. The requirement for electron donors is as much as 40% lower in the anoxic phase.
3. NO_2^- denitrification rate is 1.5 to 2 times higher than NO_3^- denitrification rate.

Partial nitrification via NO_2^- is reported to be technically feasible and economically favorable, especially when treating wastewater with high ammonia concentration or low C:N ratio (Turk and Mavinic 1989; Villaverde et al. 1997).

The Single reactor system for High Ammonia Removal Over Nitrite (SHARON) process, the first full-scale process with NO_2^- as the intermediate product, is a cost-effective treatment system for total nitrogen removal from wastewater with high nitrogen concentrations (>550 mg/L). The system has been used for treating wastewater generated from dewatered primary sludge, waste-activated sludge, sludge dryers, and incinerators (van Dongen et al. 2001).

Key Control Factors

The inhibition of nitrite-oxidizing bacteria (NOB) is critical for shortcut nitrification and denitrification because NOB oxidize NO_2^- to NO_3^- and convert partial nitrification to complete nitrification (Picioreanu et al. 1997; Hellinga et al. 1998; Hidaka et al. 2002; Peng and Zhu 2006). Several parameters, including DO concentration, temperature, SRT, substrate concentration, aeration pattern, and chemical inhibitor, have been found to selectively inhibit NOB.

Dissolved Oxygen Concentration. Compared with ammonia oxidizing bacteria (AOB), NOB require a high DO concentration. The DO half-saturation value for oxygen ($K_{s,O}$), representing the affinity for oxygen, is 62 μM for NOB whereas it is 16 μM for AOB (Picioreanu et al. 1997; Schramm et al. 1999, 2000). Therefore, AOB dominate NOB at low DO concentration, which results in the accumulation of NO_2^- and the occurrence of partial nitrification and denitrification via NO_2^-.

Although a low DO concentration (<1.5 mg/L) is favorable for partial nitrification, it reduces nitrification rates, lowers COD removal efficiencies, and causes sludge bulking. Different DO concentrations have been reported

for partial nitrification, ranging from 0.3 to 2.5 mg/L (Wyffels et al. 2004a,b). High DO concentrations (>2 mg/L) could convert partial nitrification to complete nitrification, whereas low concentrations (<0.5 mg/L) could reduce nitrification rat. A DO concentration of 1.0–1.5 mg/L has been found suitable for shortcut nitrification and denitrification in real municipal wastewater treatment, which has been verified (Hanaki et al. 1990; Hao et al. 2002a; Peng et al. 2003).

Temperature. Correlation of the maximum growth rate of nitrifying bacteria and temperature is described in the Arrhenius equation at temperatures of 5°–40°C (Anthonisen 1976):

$$\mu_{mt} = \mu_{m20} \exp\left[-\frac{E_a (20-t)}{293R (273+t)}\right] \tag{6}$$

in which μ_{mt} is the maximal specific growth rate (d^{-1}), μ_{m20} is the maximal specific growth rate at 20°C (d^{-1}), E_a is the activation energy (kJ/mol), and R is a constant of 8.314 (J/mol K).

Growth rates of AOB and NOB vary with temperature. AOB have a higher maximal specific growth rate (0.801 d^{-1}) than NOB (0.788 d^{-1}) at 20°C (Hellinga et al. 1998), while the specific growth rate of AOB (0.523 d^{-1}) was lower than that of NOB (0.642 d^{-1}) at 15°C. Therefore, NOB dominate AOB at temperatures below 15°C, and AOB outcompete NOB at temperatures above 20°C (Brouwer et al. 1996) (Fig. 3). A higher temperature not only promotes the growth of AOB but can also expand the growth rate differences between AOB and NOB (Balmelle 1992; Hunik 1993; Yoo et al. 1999).

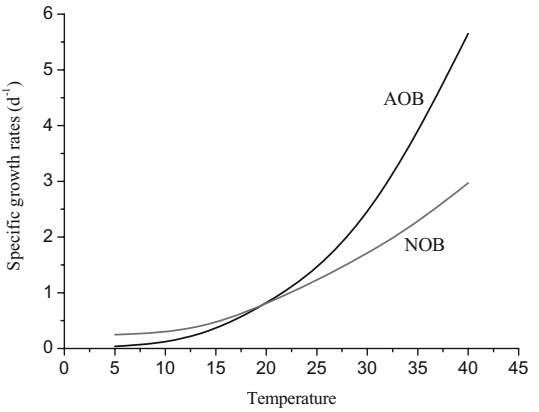

Fig. 3. Effect of temperature on growth rate of ammonia oxidizers and nitrite oxidizers.

The SHARON process has been successfully operated at 35°C, where AOB became dominant (Mulder et al. 2001). As a result of different bacterial growth rates in SHARON, a selection of microbial community should be made wherein NOB are washed out of the system while AOB are still retained along with denitrifying bacteria in the system. This operational mode allows a 25% reduction in oxygen consumption for nitrification and a 40% reduction in the external carbon source addition.

Sludge Age. AOB (e.g., *Nitrosomonas*) need a longer retention time than NOB (e.g., *Nitrobacter*) at temperatures below 15°C, while the trend was reversed at temperatures above 25°C (see Fig. 3). Thus, AOB and NOB can be selectively accumulated by appropriately adjusting SRT in a suspended-growth system (Hellinga et al. 1998).

SRT is equal to HRT in SHARON. Nitrogen was removed via nitrite in a SHARON process with an oxic HRT below 2 d. At an oxic HRT of approximately 1.5 days, the COD/N ratio clearly illustrates the metabolic pathways from ammonia to nitrogen via nitrite (Fig. 4).

Aeration Pattern. The aeration pattern has been proposed as an alternative to SRT for partial nitrification control (Hidaka et al. 2002). Aeration duration is inversely related to the extent of partial nitrification, because partial nitrification will be converted to complete nitrification at long

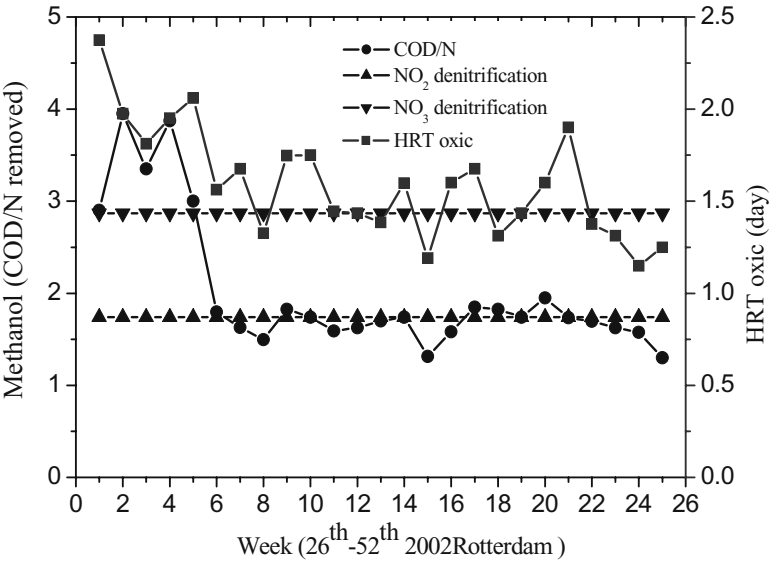

Fig. 4 Continuous operation chemical oxygen demand (COD)/N-removal nitrite pathway in Rotterdam *SHARON* system.

aeration periods (Turk and Mavinic 1989). Turk and Mavinic (1987) observed NO_2^- accumulated during a transition from anoxic to aerobic condition. This accumulation persisted 2–3 hr in the aerobic condition. Intermittent aeration favors partial nitrification (Yoo et al. 1999; Pollice et al. 2002). Peng et al. (2004a) reported that partial nitrification was successfully completed using the aeration control strategy, even though the temperature decreased from 32°C to 21°C.

Substrate Concentration and Load. AOB are divided into two groups according to cell growth rates: slow-growing and fast-growing (Zheng et al. 2004). Slow-growth bacteria, referred to as K strategists, have high affinity to substrate and are dominant at low substrate concentrations, whereas fast-growth bacteria, referred to as R strategists, have low affinity to substrate and thrive at high substrate concentrations. Because ammonia concentrations are normally below 5 mg/L in wastewater treatment processes to meet the discharge requirements, K strategists may be dominant. R strategists were found to become dominant in partial nitrification processes at high ammonia concentrations (>50 mg/L) (Surmacz-Gorska et al. 1997).

Inhibitors. Several inhibitors suppress NOB and lead to partial nitrification. Ag, Hg, Ni, Cr, Zn, Cu, and Pb, listed in increasing order of toxicity, inhibit nitrification (Camilla et al. 1998). Organic compounds such as aniline, ortho-cresol, and phenol exhibit stronger inhibitions on NOB than on AOB. Wastewater with these compounds might inhibit NOB and cause the accumulation of nitrite (Neufeld et al. 1986). Oxidants such as ClO_2^- and chlorate also inhibit NOB (Belser and Mays 1980). Seawater or saline wastewater containing a high level of ClO_2^- can achieve shortcut nitrification (Peng et al. 2004c).

High concentrations of free nitrous acid (HNO_2) and free ammonia (FA) also have adverse impacts on nitrification (Wouter et al. 1999; Villaverde et al. 2000). Anthonisen (1976) and Hellinga et al. (1998) reported HNO_2-N inhibited the nitrite oxidation at concentrations of 0.2–0.22 mg/L. Vadivelu et al. (2006) and Pratt et al. (2003) reported that free nitrous acid started inhibiting the anabolism of *Nitrobacter* at 0.011 mg HNO_2^-N/L (0.8 μM), and completely suppressed the biomass synthesis at 0.023 mg HNO_2^-N/L (1.6 μM).

Many lab-scale systems have achieved stable shortcut nitrification and denitrification through the inhibition of free ammonia (FA, NH_3-N). NOB are inhibited by NH_3-N in the range of 0.1–1.0 mg/L (Anthonisen 1976; Chang et al. 2002), while AOB can tolerate NH_3-N as high as 10–150 mg/L. However, FA only temporarily inhibits the activities of AOB and NOB (Anthonisen 1976; Peng et al. 2004b). The nitrite oxidation by NOB recovered when the FA concentration was lowered to 0.2 mg/L (Han et al. 2003). It should be noted that the FA concentration is affected by wastewater pH

and temperature (Anthonisen 1976), which further affects the stability of shortcut nitrification (Fdz-Polanco et al. 1994; Cecen 1996; Cecen et al. 1996; Surmacz-Gorska et al. 1997).

Research Status and Unsolved Matters

A stable partial nitrification can be achieved by regulating one of the factors described above. DO concentration is an economically feasible control parameter. Low DO concentration will save aeration cost but may reduce COD biodegradation rate and cause sludge bulking. Furthermore, idiographic and practical conditions should be considered. For example, because of the high specific heat of water, it is impractical to raise wastewater temperature to facilitate AOB. It is necessary to consider the economic feasibility when using DO, temperature, pH, and inhibitor as control parameters.

SHARON is the first full-scale process in which nitrification/denitrification can be achieved with nitrite as the intermediate product (Hellinga et al. 1998). It has been used for treating sludge digestion liquid in Rotterdam, Dokhaven, Utrecht, Zwolle, and Beverwijk (all in The Netherlands). However, SHARON needs to be operated at high temperatures (>35°C) and high ammonium concentrations, which limit its application (STOWA 1995). In contrast, SBR systems with long sludge ages (>30 d) have successfully achieved partial nitrification at low temperatures (<13°C) when treating municipal wastewater (Peng and Zhu 2006).

Until now the most successful operation of partial nitrification via nitrite has been achieved in sequencing batch processes (Cecen 1996; Verstraete and Philips 1998; Hidaka et al. 2002). The only study for partial nitrification in a continuous-flow process was conducted by Schmidt et al. (2003) with influent NH_4^+-N higher than 50 mg/L. The current challenge is how to implement stable partial nitrification in continuous-flow processes treating wastewater with low ammonia concentration (<60 mg/L).

C. Anaerobic Ammonium Oxidation (ANAMMOX)

Mechanism and Advantages

The Anaerobic Ammonia Oxidation (ANAMMOX) process, developed at Delft University of Technology in the 1990s, is a novel and low-cost approach to removing nitrogen from wastewater (van Graaf et al. 1995; Strous et al. 1999; Fux et al. 2002). In ANAMMOX, ammonia is oxidized to nitrogen by anaerobic AOB with nitrite as the electron acceptor. Hydrazine and hydroxylamine are the intermediate products (Schalk et al. 1998; Jetten et al. 1999). External carbon sources are not needed in ANAMMOX because carbon dioxide serves as the main carbon source for anaerobic AOB (van Graaf et al. 1996). Equation 7 is the ANAMMOX reaction (Jetten et al. 1999):

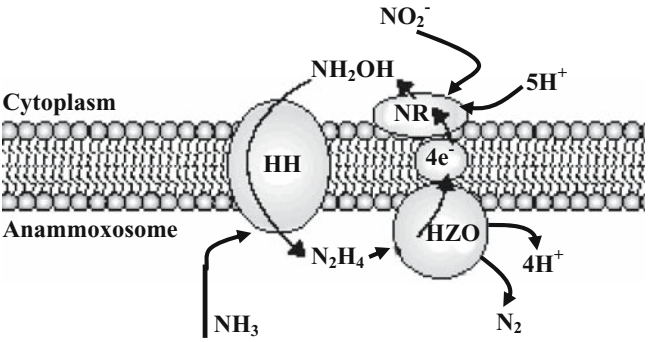

Fig. 5. Mechanism of anaerobic ammonium oxidation. NR is a nitrite-reducing enzyme (NH_2OH is the assumed product); HH (hydrazine hydrolase) condenses hydrazine from ammonia and hydroxylamine; HZO is a hydrazine-oxidizing enzyme (which may be equivalent to hydroxylamine oxidoreductase). (From Jetten et al. 2001.)

$$NH_4^+ + 1.31NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow$$
$$1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (7)$$

The possible mechanism for anaerobic ammonium oxidation is shown in Fig. 5. Nitrite-reducing enzyme (NR) is on the cytoplasm side of the cell membrane. It catalyzes the reduction of NO_2^- to hydroxylamine. Hydrazine hydrolase (HH) across the cell membrane condenses hydroxylamine and ammonia to hydrazine. Hydrazine-oxidizing enzyme (HZO) is on the anammoxosome side of the cell membrane and catalyzes hydrazine to nitrogen. The electrons generated from these reactions are transferred back to NR.

Two ANAMMOX bacteria, tentatively named *Brocadia anammoxidans* (Strous et al. 1997a) and *Kuenenia stuttgartiensis* (Schmid et al. 2000; Cirpus et al. 2005), were found to carry out anaerobic ammonium oxidation. *Brocadia anammoxidans* was detected in the Netherlands, whereas *Kuenenia stuttgartiensis* was detected in Germany and Switzerland. These two bacteria have similar structures and produce hydrazine from exogenously supplied hydroxylamine. Two new species of ANAMMOX bacteria, *Candidatus Scalindua brodae* and *Candidatus Scalindua wagneri*, have been recently discovered (Schmid et al. 2003).

Compared with conventional nitrification-denitrification processes, ANAMMOX has two major advantages. First, because ANAMMOX is carried out by autotrophic bacteria, there is no need for organic carbon sources, which saves chemical dosage costs. Second, the biomass yield of ANAMMOX is very low (0.11 g VSS/g NH_4^+-N, VSS—volatile suspended solids), which saves sludge treatment costs (Jetten et al. 1999; Fux et al. 2002; Cirpus et al. 2005).

Key control factors

Reactor configuration. As a result of the slow growth rate of ANAMMOX bacteria, these reactors should have long SRT to maintain high biomass concentrations, especially at the startup stage. Studies have revealed that biofilm systems (fixed-bed reactor, fluidized-bed reactor, gas-lift reactor, etc.) (Sliekers et al. 2003; Dapena-Mora et al. 2004) and SBR (Strous et al. 1997c, 1998, 1999; van Dongen et al. 2001) are feasible for ANAMMOX.

DO concentration. Strous et al. (1997a) demonstrated that the activity of ANAMMOX bacteria was temporarily inhibited at the DO concentration of 0.2 mg/L and later recovered under anoxic conditions. The activity of ANAMMOX bacteria was completely inhibited at DO concentration of 0.2–1.0 mg/L.

Substrate concentration. Ammonia (the substrate for ANAMMOX) and nitrate (the by-product) produce little inhibition on ANAMMOX bacteria when their concentrations are below 1000 mg/L (Jetten et al. 1999). However, nitrite (another substrate) exhibits an adverse impact on ANAMMOX bacteria at a concentration of 100 mg/L (Strous et al. 1999). It is critical to maintain nitrite concentration below 70 mg/L in this process (Schmidt et al. 2002a).

pH. pH affects this process in terms of substrate constituents. The percentage of ammonia and nitrite in wastewater is significantly influenced by pH (Anthonisen 1976; Abeling and Seyfried 1992) and can be expressed in the following equations (Eqs. 8 and 9):

$$NH_3/\% = \frac{100}{1+\frac{10^{-pH}}{[K_a]}} \tag{8}$$

$$NHO_2/\% = \frac{100}{1+\frac{[K_a]}{10^{-pH}}} \tag{9}$$

in which $[K_a]$ is the ionization constant. Appropriate pH range for ANAMMOX bacteria is 7.7–8.3 with the maximum reaction rates occurring at pH of 8.0 (Strous et al. 1997a). Reaction rates increased at pH of 6.0–7.5 but decreased at pH 8.0–9.5.

Temperature. Temperature is an important factor for cell growth and metabolic activity. Normally, cells grow faster at higher temperature. Because the growth rate of ANAMMOX bacteria is very slow, there has been no accurate correlation between their growth rates and temperatures. The activation energy of ANAMMOX bacteria is similar to that of aerobic AOB

(about 70 kJ/mol) (Strous et al. 1997b). ANAMMOX can take place at temperatures ranging from 6°C to 43 °C, whereas the optimal temperature for its bacteria is 26°–28°C (Fig. 6) (Thamdrup and Dalsgaard 2002). The reaction rate drops rapidly at temperatures lower than 15°C or higher than 40°C.

NO and NO_2. Both NO and NO_2 are the intermediate products of NOB. They affect not only ANAMMOX bacterial activities but also their growth rates. Schmidt et al. (2002b) found that consumption rates of NH_3 and NO_2^- and production rates of NO_3^- increased with addition of NO_2^-, and were highest at $[NO_2^-]$ of 50 mg/L, but dropped at $[NO_2^-]$ higher than 600 mg/L. The specific growth rate of *Brocadia anammoxidans* increased from 0.003 h^{-1} without the addition of NO_2^- to 0.004 h^{-1} at the $[NO_2^-]$ of 50 mg/L, but dropped to 0.0028 h^{-1} at $[NO_2^-]$ of 200 mg/L.

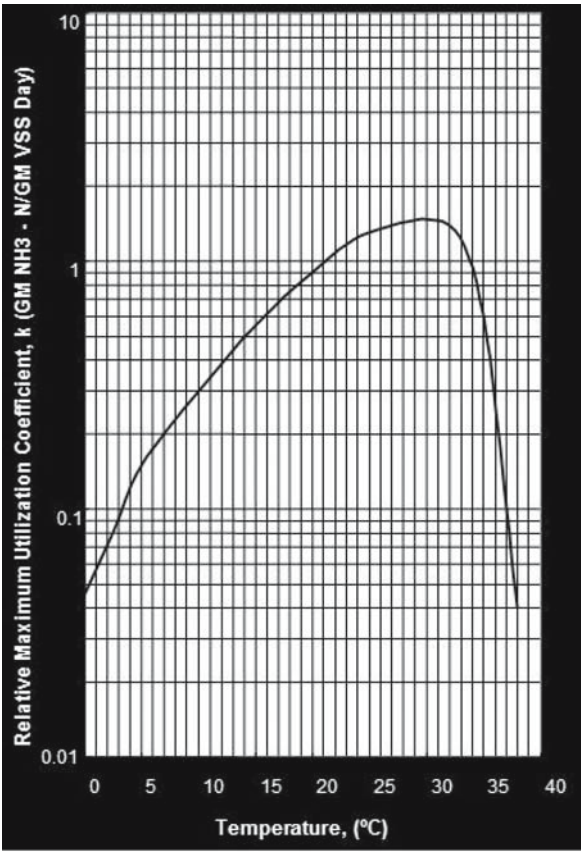

Fig. 6. Effect of temperature on growth rate of *ANAMMOX* bacteria.

Sludge age. Because of the slow growth rate and low biomass yield of ANAMMOX bacteria, a long sludge age is critical for this process. Although the theoretical doubling time of the bacteria is 11 d, longer SRT enhances ANAMMOX performance (Strous et al. 1999; Schmidt et al. 2002a).

Combined Partial Nitrification and ANAMMOX Process

The critical point for a successful process is to maintain a sufficient population of anaerobic AOB (Jetten et al. 1999). Because high [NO_2^-] inhibits anaerobic AOB, reducing NO_2^- accumulation will be a solution for this process. Partial nitrification can effectively convert NO_2^- to N_2 without significant accumulation; thereby, coupling with partial nitrification is expected to solve the NO_2^- problem in ANAMMOX (Fig. 7) (Fux et al. 2002). In this combined process, part of NH_4^+ is oxidized to NO_2^- by aerobic AOB, and NO_2^- is then reduced to N_2 by denitrifiers. The other part of NH_4^+ is oxidized to N_2 with NO_2^- as an electron acceptor by anaerobic AOB. The oxygen requirement of this combined process is 40% less than traditional nitrogen removal systems. The organic dosage for denitrification is also saved. In addition, sludge production is low because of the slow growth rate of anaerobic AOB, which reduces sludge treatment costs (Jetten et al. 1997). However, there are several problems for this combined system:

1. The residual DO in the effluent of partial nitrification might inhibit ANAMMOX bacterial activity because anaerobic AOB are sensitive to oxygen.
2. The optimal ratio of ammonia to nitrite should be 1.0:1.3 for the ANAMMOX process (see Eq. 5). This ratio might be difficult to maintain as a result of the involvement of complex biochemical reactions and diverse microorganisms in the process.
3. Because anaerobic AOB (cell yield, 0.11 g VSS/g NH_4^+-N) grow slower than aerobic AOB (cell yield, 0.13 g VSS/g NH_4^+-N), they will be outcompeted by the aerobic AOB present in the effluent of partial nitrification.

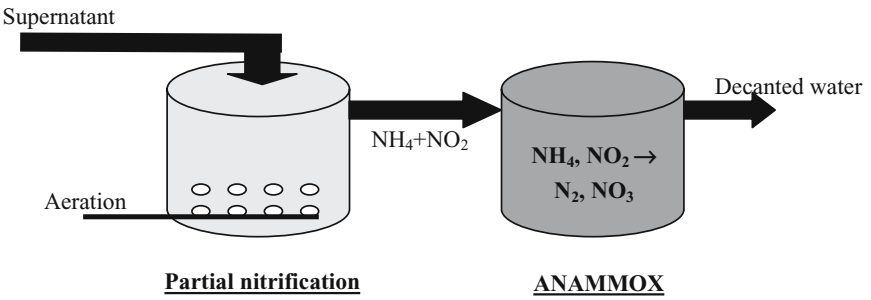

Fig. 7. The combined partial nitrification–*ANAMMOX* process.

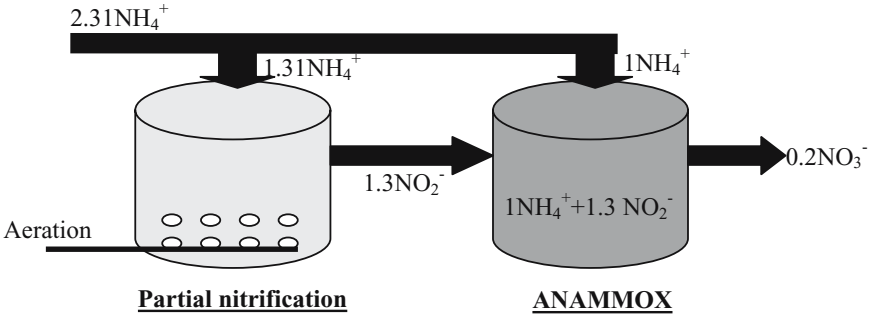

Fig. 8. Schematic graph of the combined partial nitrification–*ANAMMOX* process.

A step-feeding mode for partial nitrification and ANAMMOX can reduce the competition between anaerobic AOB and aerobic AOB (Fig. 8). Applying this operation mode can result in the following advantages:

1. Eliminating the competition of aerobic AOB and anaerobic AOB for ammonia, especially when ammonia concentration is low in raw wastewater.
2. Providing an optimal substrate ammonia and nitrite ratio (1.0:1.3) and thus enhancing total-N removal efficiency.
3. Ensuring an obligate anaerobic environment for ANAMMOX, because residual DO in the effluence of partial nitrification is depleted by COD in the influent.
4. Part of the nitrate produced in ANAMMOX can be removed by denitrification with the remaining COD as electron donors, which will improve total-N removal efficiency.
5. Because of the low growth rate of the bacteria, aerobic AOB in partial nitrification effluent might have a dilution or competition effect on ANAMMOX bacteria. The step-feeding strategy can reduce this dilution effect.

Research Status and Unsolved Matters

ANAMMOX has been operated in full-scale plants for treating sludge digestion supernatant in the Netherlands (Fux et al. 2002). Anammox effectively solves the nitrite inhibition problem in SHARON. The current challenge is how to efficiently accumulate anaerobic AOB. Strous et al. (1998) estimated that the cell yield value for anaerobic AOB was 0.066 mol cell/mol $(NH_4^+)_{reduced}$, ammonium consumption rate was 45 nmolNH_4^+/mgcell/min, and the maximum specific growth rate was 0.0027 hr^{-1}, which meant their doubling time was at least 11 d. Because the growth rate of these bacteria is slow, a long cell retention time is critical (Schmidt et al. 2003). When the level of cell loss is higher than cell growth, this process will become unstable

(Dapena-Mora et al. 2004). Moreover, due to the slow growth rate of ANAMMOX bacteria, it is difficult to analyze cell concentration. Until now there is no report on the quantitative relation between bacterial populations and nitrogen removal efficiency.

D. Aerobic Deammonitrification

Mechanism and Advantages

In aerobic deammonitrification, NH_4^+ is oxidized to N_2 in a single step (Poth and Focht 1985; Bock et al. 1995; Hippen et al. 1997; Siegrist et al. 1998b). Two models have been proposed for aerobic deammonitrification: the simultaneous nitrification and denitrification model (Fig. 9) and the separated nitrification and denitrification model (Fig. 10).

In the first model, aerobic deammonification is achieved by aerobic nitrifiers and anaerobic ANAMMOX AOB (Fig. 9). Alternate aerobic/anoxic biofilm reactors have been found to develop aerobic deammonification. Siegrist et al (1998a) observed that *Nitrosomomas* (aerobic AOB) on the surface layer of biofilm converted ammonia NH_4^+ to NO_2^- using oxygen from bulk wastewater in biological rotation contactors, and NH_4^+ and NO_2^- then transfused to the inner anoxic layer of biofilm and were removed by anaerobic AOB. This model has been verified stoichiometrically (Helmer-Madhok et al. 2002). *Paracoccus pantotropha* were found to carry out both anoxic and aerobic denitrification (Arts et al. 1995).

In the second model, aerobic deammonification is achieved by nitrifiers (mainly AOB, such as *Nitrosomomas*) (see Fig. 10). The AOB on the biofilm surface oxidize NH_4^+ to NO_2^- in the presence of oxygen. NO_2^- then transfuses

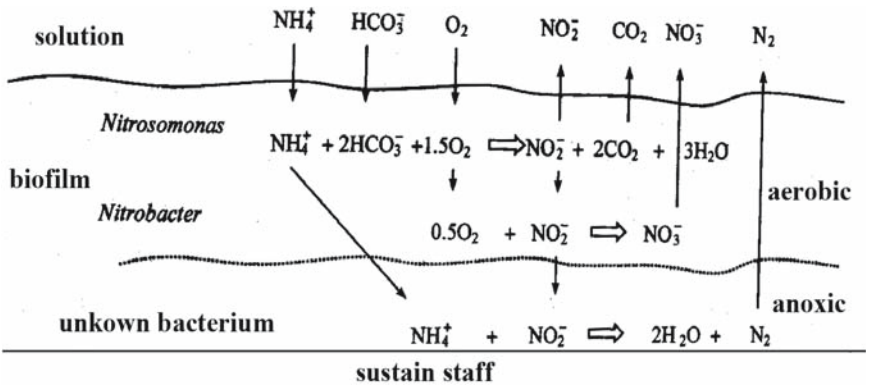

Fig. 9. The simultaneous nitrification and denitrification mode for aerobic deammonification. Reprinted from Stuven and Block 2001, with permission from Elsevier.

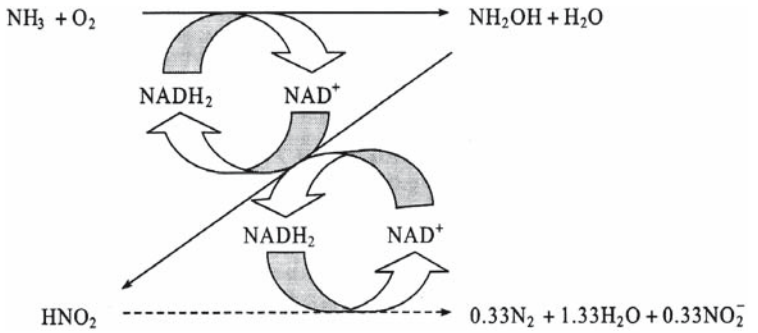

Fig. 10. Possible degradation of ammonia to dinitrogen and nitrite. Reprinted from Pochana and Keller 1999, with permission from IWA Publishing.

to the inner layer of the biofilm and is reduced to N_2 with $NADH_2$ as electron donor (Abeliovich 1987, 1992). In this model, hydroxylamine is the intermediate product of ammonium oxidation, and $NADH_2$ is the product of hydroxylamine oxidization. However, only 67% of NO_2^- can be converted to N_2 by AOB, based on this model (Siegrist et al. 1998b).

Research Status and Unsolved Matters

It has been found in pilot-scale and full-scale studies that NH_4^+ is oxidized to N_2 when treating municipal wastewater and landfill leachate (Siegrist et al. 1998a,b). Because aerobic deammonification normally occurs in the systems designed for conventional nitrification, the process design has not yet been optimized and nitrogen loading rates are low (90–250 g N/m^3 reactor d^{-1}) (Verstraete and Philips 1998). More studies need to be conducted to understand the mechanisms, characterize the microbial communities, and enhance process control.

E. Completely Autotrophic Nitrogen Removal over Nitrite (CANON)

Mechanism and Advantages

Because a significant amount of nitrogen is lost as nitrogen gas during the treatment of wastewater with high ammonia loadings but low organic loadings (Helmer and Kunst 1998; Koch et al. 2000; Helmer et al. 2001), a new process named Completely Autotrophic Nitrogen removal Over Nitrite (the CANON process) has been developed (Dijkman and Strous 1999). This process includes partial nitrification and anoxic oxidation of ammonia carried out by aerobic AOB and anaerobic AOB (Pynaert et al. 2002a,b; Third et al. 2001; Hao et al. 2002b; Nielsen et al. 2005). The interaction between these two types of nitrifiers under oxygen-limited conditions results in a complete conversion of ammonium to nitrogen gas in a single autotrophic reactor.

Under oxygen-limited condition, NH_4^+ is oxidized to NO_2^- by aerobic nitrifiers (Eq. 10) (Hanaki et al. 1990):

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad (10)$$

Anaerobic AOB subsequently convert NH_4^+ and NO_2^- to nitrogen gas and NO_3^-:

$$NH_4^+ + 1.3NO_2 \rightarrow 1.02N_2^- + 0.26NO_3^+ + 2H_2O \quad (11)$$

With NO_2^- serving as the electron donor for the formation of biomass from carbon dioxide, the oxidation of NO_2^- to NO_3^- is stoichiometrically coupled with cell growth. The combination of reactions 9 and 10 results in the following overall nitrogen removal reaction (Eq. 12) (Strous 2000):

$$NH_4^+ + 0.85O_2 \rightarrow 0.435N_2 + 0.13NO_3^- + 1.3H_2O + 1.4H^+ \quad (12)$$

Both CANON and ANAMMOX removed nitrogen through the reaction of NH_4^+ and NO_2^-. However, NO_2^- is the electron donor in CANON and is produced in shortcut nitrification by AOB, whereas NO_2^- is the electron acceptor in ANAMMOX and needs to be added from other sources. CANON is operated at low oxygen condition (DO < 0.5 mg/L) whereas ANAMMOX is operated at obligate anaerobic condition.

CANON is cost-effective for wastewater with high ammonia concentrations (Pynaert et al. 2004). No extra carbon source is required, because it is completely autotrophic. In addition, nitrogen removal can be achieved in a single reactor with low aeration intensity. CANON consumes 63% less oxygen than conventional nitrogen removal processes (Sliekers et al. 2002).

Key Control Factors

There are three key factors for CANON: dissolved oxygen concentration, ammonia concentration, and an AOB population. Oxygen has two inhibition impacts on this process. It is toxic to anaerobic AOB and suppresses anaerobic AOB with the excessive production of nitrite. A DO concentration of 0.5 ± 0.07 mg/L is recommended (Sliekers et al. 2003).

Ammonia concentration is critical for this process. Ammonia oxidation is limited only by oxygen concentration when ammonia is sufficient in CANON, while nitrite oxidation is limited by both oxygen and nitrite concentrations. If either dissolved oxygen or nitrite is maintained at a low level, NOB can be inhibited in the system and ensure a stable CANON performance (Pynaert et al. 2002b; Nielsen et al. 2005). It has been found that a deficiency of ammonia substantially lowered this process efficiency, with 31% of NO_2^- generated by AOB reacting in anaerobic ammonification and 69% of nitrite reacting in nitrification. When ammonia became sufficient,

100% of NO_2^- generated by AOB reacted in anaerobic ammonification (Third et al. 2001). The experimental results showed that an ammonia loading of 14 mg/L hr provided a sufficient ammonia source in CANON (Third et al. 2001).

The interaction between aerobic AOB and anaerobic AOB affects this process (Third et al. 2001, 2005). These two types of bacteria live on different substrates, with aerobic AOB requiring ammonia and oxygen and anaerobic AOB requiring ammonia and nitrite. Therefore, CANON will be disrupted by the presence of NOB because NOB use oxygen and nitrite as substrates and compete with aerobic AOB for oxygen and with anaerobic AOB for nitrite.

Research Status and Unsolved Matters

The CANON process has been tested in full-scale nitrification systems (Helmer et al. 2001; Boran et al. 2004). N-removal rates reached 1.5 kg $N\,m^{-3}\,d^{-1}$ in this process (Sliekers et al. 2003), 20 times higher than other biological nitrogen removal processes (Kuai and Verstraete 1998; Sliekers et al. 2002). More studies are needed to clarify microbial communities in CANON, broaden its application, and enhance its resistance to shocks.

F. Oxygen-Limited Autotrophic Nitrification-Denitrification (OLAND)

Mechanism and Advantages

Nitrogen removal can also be accomplished in another single-step process, named the Oxygen-Limited Autotrophic Nitrification-Denitrification (OLAND) process (Kuai et al. 1998), in which AOB oxidize a portion of NH_4^+ to NO_2^- with oxygen as the electron acceptor and then reduce NO_2^- to N_2 with the other portion of NH_4^+ as the electron donor. OLAND is supposed to take place via two steps (reactions 13 and 14) (Poth 1986; Muller et al. 1995):

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+ \tag{13}$$

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \tag{14}$$

Combining these two steps, we can get an overall reaction:

$$2NH_4^+ + 1.5O_2 \rightarrow N_2 + 3H_2O + 2H^+ \tag{15}$$

There is no clear distinction between OLAND and CANON. OLAND is achieved by aerobic AOB (*N. eutropha*) under oxygen-limited condition (Pynaert et al. 2004), while CANON is carried out by both aerobic AOB and anaerobic AOB under oxygen-limited condition. OLAND also exhibits a good tolerance of NH_4^+ and NO_2^- shocks (Windey et al. 2005). Compared

with conventional nitrogen removal processes, OLAND consumes 63% less oxygen and does not require alkalinity dosage (Bock et al. 1995; Hippen et al. 1997; Pynaert et al. 2004).

Key Control Factors

Oxygen concentration is critical for OLAND because the population of aerobic AOB drastically decreases at low oxygen concentration ($DO < 0.1\,mgL^{-1}$) (Kuai and Verstraete 1998; Philips et al. 2002; Zhang et al. 2004). Compared with CANON, OLAND has a shorter sludge retention time and a lower requirement for nitrite sources (Wyffels et al. 2004a; Windey et al. 2005).

Research Status and Unsolved Matters

Although OLAND is easier to operate than CANON, the application of this one-step process is severely limited by the low nitrogen removal efficiency (lower than 40%) and the uncertainty of the operational conditions (Schmidt and Bock 1997). To enhance OLAND performance, the oxidation rate of NH_4^+ to NO_2^- and the growth rate of aerobic AOB under oxygen-limited condition should be improved. In addition, the impacts of temperature and pH need to be studied, which is important for the growth of AOB and nitrogen removal efficiency.

G. Current Status of Nitrifying Bacteria

Understanding the population and function of nitrifying bacteria is critical to the design and operation of nitrogen removal processes. Because microorganisms are critical in nitrogen removal processes, many studies have investigated nitrifying bacterial species and activity. Based on gene sequence analysis, there are five genera of AOB: *Nitrosococcus, Nitrososmonas, Nitrosospira, Nitrosovibrio*, and *Nitrosolobus*; and four of NOB: *Nitrobacter, Nitrospira, Nitrococcus*, and *Nitrospira.* By using classical microbial screening techniques, and Painter (1986) found that *Nitrosomonas europa* and *Nitrobacter winogradskyi* were the main genera of AOB and NOB. In contrast, the studies using molecular biology-based techniques including 16S ribosomal RNA (rRNA)-targeted methods have shown a great diversity of nitrifiers in activated sludge. *Nitrosospira* and *Nitrospira* were found as the main genera of AOB and NOB in both bench-scale systems (Burrell et al. 1998; Schramm et al. 1998, 1999; Rittmann et al. 1999; Morgenroth et al. 2000; You et al. 2003; Gieseke et al. 2002) and wastewater treatment plants (Juretschko et al. 1998; Coskuner and Curtis 2002), whereas *Nitrosomonas* and *Nitrobacter* were still characterized as dominant nitrifying bacteria in some other studies using bench-scale systems (Gieseke et al. 2001; Chen et al. 2003: Tsuneda et al. 2003) and wastewater treatment plants (Wagner et al. 1996; Daims et al. 2001; Dionisi et al. 2002; Coskuner and

Curtis 2002; Hallin et al. 2005). In addition, although nitrifying bacterial populations (AOB + NOB) are generally supposed to be greater than 5%–8% in biomass for good nitrification (Randall et al. 1992; Koch et al. 2001), a wide variation in the percentage of nitrifying bacteria in the microbial community has been reported. It varied from 0.34% in activated sludge (Dionisi et al. 2002), through 6%–18% in a combined activated sludge and rotating biological contactor process (You et al. 2003) and a sewage plant (Wagner et al. 1995), and to more than 50% in a carbon-limited autotrophic nitrifying biofilm (Kindaichi et al. 2004) and an SBR system (Morgenroth et al. 2000). These studies reflect the differences between AOB and NOB populations in treatment facilities and raise two questions: (1) Does the dominance of specific nitrifying bacterial species vary with operational conditions and influent qualities? and (2) What is the correlation between microbial population and operational conditions in the treatment systems?

There are discrepancies in the dominance of AOB and NOB. It was generally accepted that AOB dominated over NOB with a ratio of 2.0–3.5 under conditions of good nitrification (Copp and Murphy 1995; You et al. 2003), possibly because of the inherent high growth rates of AOB (Schramm et al. 1999) and the higher energy generation of NH_4^+ oxidization by AOB than NO_2^- oxidization by NOB (You et al. 2003). Cell size could also contribute to the dominance of AOB, since AOB cells were larger than NOB (Altmann et al. 2003) and existed in larger colonies (Schramm et al. 1996; Juretschko et al. 1998). Another explanation was that AOB could maintain ribosome content under adverse conditions because inactive AOB could still be detected in high abundance by fluorescent *in situ* hybridization (FISH) targeting ribosomal RNA (rRNA) (Wagner et al. 1995; Okabe et al. 2004). However, several other studies revealed that the population of AOB was lower than that of NOB. Schramm et al. (1999) found that NOB (*Nitrospira*) was more than 30 times that of AOB (*Nitrosospira*) in a wastewater treatment plant, and Gieseke et al. (2001) found that NOB population (*Nitrospira*) was 85 times more than AOB (*Nitrosomonas*) in a bench-scale biofilm system. By using real-time quantitative PCR and assuming two copies of *amo*A gene per AOB cell and one copy of 16S rDNA gene per NOB cell, Dionisi et al. (2002) estimated the NOB population (*Nitrospira*) as 190 times greater than AOB (*Nitrosomonas*) in a wastewater treatment plant. This discrepancy between bacterial populations and their functions could be the result of different treatment processes and different types of genetic material targeted in these studies, and poses a requirement for information on gene expression or examination of the genetic material (e.g., mRNA) (Logan and Rittmann 1998).

To explain nitrifying bacterial communities in treatment systems, several studies assumed that *Nitrosomonas* and *Nitrobecter* were *r*-strategists (low affinity for substrates and high growth rates) and dominated at high substrate concentrations, whereas *Nitrosospira* and *Nitrospira* were

k-strategists (high affinity for substrates and low growth rate) and thrived at low substrate concentrations (Manz et al. 1996; Schramm et al. 1998, 2000; Noguera et al. 2002). In addition, *Nitrobecter* was found to have the unique ability to live heterotrophically, whereas most of *Nitrospina, Nitrococcus*, and *Nitrospira* were unable to grow heterotrophically (Ehrich et al. 1995; Burrell et al. 1998).

Unsolved Matters

The importance of elucidating nitrifying bacterial populations and function in wastewater treatment processes has been well recognized. The combination of microelectrode and molecular biology has revealed the variation of different nitrifying bacterial species in the microenvironment of biofilm and activated sludge. However, there is one major unsolved problem: the application of microbial community in engineering design and operation. Although diverse nitrifying bacterial groups have been identified, the current activated sludge/biofilm models still assume nitrifying bacteria as a single group with the same cell growth rate and use empirical kinetic parameters, which leads to malfunction of the nitrogen removal process and the uncertainty of seeking the real causes. To enhance the performance of nitrogen removal processes, it is critical to incorporate the microbial community findings into the design and operation of treatment processes.

IV. Critical Comparisons of the Various Technologies

The novel and conventional nitrogen removal processes are compared in terms of reactor complexity, treatment performance, and operational costs (Table 2) (Jetten et al. 2002; Schmidt et al. 2003). From the aspect of reactor numbers, conventional technologies require two reactors while the novel technologies require only one, thus saving construction costs. As for oxygen requirement, conventional technologies normally require high dissolved oxygen concentrations to carry out complete nitrification, while the novel technologies need only low or limited oxygen supply, thus saving aeration costs. In terms of the addition of carbon sources, the conventional technologies require high influent C:N ratios for denitrification, while novel technologies have a low carbon requirement and exhibit good adaptation to both high and low C:N ratios.

With the low cell growth rates of anaerobic nitrifiers, novel technologies produce less sludge than conventional technologies and thus reduce sludge treatment costs. All novel technologies except ANAMMOX can efficiently remove nitrogen from municipal wastewater. These advantages make the novel technologies promising. However, several features of these technologies need to be improved, such as operational stability, nitrogen removal efficiency, and growth of specific nitrifiers.

Shortcut nitrification-denitrification (partial nitrification) and ANAMMOX are the most developed among these novel technologies.

Table 2. Comparison of Various Biological Nitrogen Removal Technologies and Processes.

Technology or process	Conventional	SND	Short-cut (SHARON)	ANAMMOX	Aerobic deammonitrification	CANON	OLAND
Number of reactors	2	1	2	1	1	1	1
Feed	Wastewater	Wastewater	Wastewater	Ammonia + Nitrite	Wastewater	Wastewater	Wastewater
Discharge	NO_3^-, NO_2^-, N_2	N_2	NO_2^-, N_2	NO_3^-, N_2	N_2	NO_3^-, N_2	N_2
Operating conditions	Aerobic, anoxic	Aerobic	Aerobic, anoxic	Anaerobic	Aerobic	Oxygen limited	Oxygen limited
Oxygen requirements	High	Low	Low	None	Low	Low	Low
Biomass retention	None	None	None	Yes	Yes	Yes	Yes
COD requirements	Yes	No	No	No	No	No	No
Sludge production	High	Low	Low	Low	Low	Low	Low
Bacteria	Nitrifiers + heterotrophs	Heterotrophic Nitrifiers + aerobic denitrifier	Aerobic Ammonium Oxidizer	Planctomycetes	Aerobic nitrifiers + aerobic Denitrifier	Aerobic ammonium Oxidizer + planctomycetes	Aerobic ammonium Oxidizer + anaerobic Ammonium oxidizer
Max N loading (kg N m^{-3} reactor d^{-1})	2–8	1–3.5	0.5–1.5	10–20	1–2	2–3	0.1
Total-N removal efficiency	95%	100%	90%	87%	60%	75%	85%
Optimum temperature (°C)	12–35	20–30	Above 25	30–40	Unknown	30–40	30–40
Common reactor configuration	Activated sludge and biofilm	Oxidation ditch, SBR	Activated sludge and biofilm	Fixed and fluidized-bed reactor, gas-lift reactor, SBR	Biological rotating contactor, gas-lift reactor, fixed and fluidized-bed reactor	Fixed and fluidized-bed reactor, SBR	Fixed and fluidized-bed reactor, SBR
Application status	Established	Laboratory	Full-scale plants	Full-scale initiated	Laboratory	Laboratory	Laboratory
Electron donor	COD	Unknown	COD	Ammonium	Ammonium	Nitrite	Ammonia
Biofilms or suspension	Biofilms/ suspension	Biofilms/ suspension	Suspension	Biofilms/ suspension	Biofilms/suspension	Biofilms/suspension	Biofilms/suspension

SND, simultaneous nitrification and denitrification; COD, Chemical oxygen demand; SBR, sequencing batch reactor.

Shortcut nitrification-denitrification achieves nitrogen removal in a single tank at a low DO concentration. ANAMMOX has high efficiency when operated at higher total-N loadings and can be combined with partial nitrification through anaerobic AOB. The SHARON and CANON processes are derived from these two technologies. Aerobic dammonitrification is a combined process of partial nitrification and ANAMMOX reactions occurring at different layers of biofilm.

Summary

This comprehensive review discusses diverse conventional and novel technologies for nitrogen removal from wastewater. Novel technologies have distinct advantages in terms of saving configuration, aeration, and carbon sources. Each novel technology possesses promising features and potential problems. For instance, SND and OLAND processes can achieve 100% total nitrogen removal, but the low oxygen concentration required by these two processes substantially reduces the nitrification rate, which limits their application. On the other hand, denitrification can still be carried out by aerobic denitrifiers at high DO levels in activated sludge process, but it is difficult to cultivate this type of bacteria.

The SHARON process is most commonly used for shortcut nitrification and denitrification because of its low requirements for retention time, oxygen concentration, and carbon source. However, its high operational temperature (about 35°C) limits the application. Several real-time control strategies (DO, pH, and ORP) have been developed to achieve a stable nitrite accumulation in SHARON.

The ANAMMOX process can sustain at high total-N loadings and has been employed in full-scale treatment plants, but the problem of nitrite supply has not been solved, and the treated wastewater still contains nitrate. In addition, the inoculation and enrichment of ANAMMOX bacteria (i.e., anaerobic AOB) is difficult. The problem of nitrite supply has been solved by combining partial nitrification with ANAMMOX, which provides abundant nitrite for anaerobic AOB. ANAMMOX is currently used for treating sludge digestion supernatant.

Aerobic dammonitrification is a process combining partial nitrification and ANAMMOX at different layers of biofilm. Although the technology has been tested in pilot- and full-scale experiments, the mechanism is still unclear.

CANON and OLAND are one-step ammonium removal processes that possess distinct advantages of saving carbon sources and aeration costs. The major challenge is the enrichment of anaerobic microorganisms capable of oxidizing ammonia with nitrite as the electron acceptor.

Molecular biology and environmental biotechnology can help identify functional microorganisms, characterize microbial communities, and develop new nitrogen removal processes. Extensive research should be conducted

to apply and optimize these novel processes in wastewater treatment plants. More effort should be invested to combine these novel processes (e.g., partial nitrification, ANAMMOX) to enhance nitrogen removal efficiency.

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