Journal of Hazardous Materials 178 (2010) 1–9

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Review

Co-existence of anammox and denitrification for simultaneous nitrogen and carbon removal—Strategies and issues

Mathava Kumar, Jih-Gaw Lin*

Institute of Environmental Engineering, National Chiao Tung University, 1001, University Road, Hsinchu City 30010, Taiwan

ARTICLE INFO

Article history:
Received 20 August 2009
Received in revised form 13 January 2010
Accepted 13 January 2010
Available online 21 January 2010

Keywords:
Anammox
Denitrification
Organic carbon removal
Nitrogen removal
Wastewater treatment

ABSTRACT

The discovery of anaerobic ammonium oxidation (anammox) has greatly improved the understanding of the nitrogen cycle. Anammox provides great promise for the removal of nitrogen from wastewater, containing high concentration of ammonium. However, the presence of organic carbon is considered as unfavorable to this autotrophic process, i.e. anammox. Most of the real wastewaters contain both organic carbon and nitrogen. Under this circumstance, several processes have been established primarily for the complete removal of organic carbon. Subsequently, the wastewater containing no or low organic carbon and nitrogen is treated via a variety of nitrogen removal processes. The co-existence of anammox and denitrification could be useful for the simultaneous removal of nitrogen and organic carbon in a single system rather than a sequential chain of treatment. This review addresses the microbiology, strategies, consequences and the future research challenges in the co-existence of anammox and denitrification.

Contents

1. Introduction

The global nitrogen cycle, most complex of the mineral cycles, has been studied with great interest because nitrogen is the mineral nutrient most in demand by microorganisms and plants. Nitrogen is the fourth most common element found in cells and includes the microbially catalyzed processes of nitrogen fixation, ammonium oxidation, assimilatory and dissimilatory nitrate reduction, ammonification and ammonium assimilation. Over the last four decades, anthropogenic processes have substantially altered the global nitrogen cycle by increasing both the availability and mobility of nitrogenous compounds in the environment including water systems. The wastewater discharges from human activities containing excessive nitrogen compounds in the form of NH_4^+–N, organic bound N, NO_2^- and NO_3^- can be toxic to aquatic life, deplete dissolved oxygen (DO) levels, cause eutrophication in receiving water bodies, and affect the suitability of wastewater for reuse [1]. Nitrogen compounds present in the wastewater can be removed by a variety of processes, out of which biological nitrogen removal has been widely adopted [2–4].

Conventionally, biological nitrogen removal is achieved by nitrification followed by a denitrification process, i.e. (i) aerobic nitrification of NH_4^+ by chemolithoautotrophic bacteria to NO_2^- or NO_3^- with O_2 as the electron acceptor, and (ii) anoxic denitrification of NO_2^- or NO_3^- to gaseous N_2 by heterotrophic microorganisms using organic matter as carbon and energy source. The short-cut nitrification–denitrification (SND) and anaerobic

* Corresponding author. Tel.: +886 3 5722681; fax: +886 3 5725958.
E-mail addresses: mathavakumar@gmail.com (M. Kumar), jglin@mail.nctu.edu.tw (J.-G. Lin).

0304-3894/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2010.01.077

ammonium oxidation (anammox) [5] are the recent inventions for nitrogen removal. Based on SND, a technology called single reactor system for high-activity ammonium removal over nitrite (SHARON) has been developed for nitrogen removal [6]. Among the nitrogen removal processes, anammox offers a novel, energy saving and cost-effective biological nitrogen removal technique. Following the identification of anammox, several researchers accelerated the application of anammox via different reactor configurations and coupling anammox with several other processes. At the same time, significant attention has been given on autotrophic nitrogen removal in one single unit. As a result, different technologies including completely autotrophic nitrogen removal over nitrite (CANON) [7], oxygen-limited autotrophic nitrification–denitrification (OLAND) [8], single-stage nitrogen removal using anammox and partial nitritation (SNAP) [9] and deammonification [10] have been developed following the concepts of autotrophic nitrogen removal in one single unit. Recently, simultaneous partial nitrification, anammox and denitrification (SNAD) [11] have been developed following the concepts of anammox and SND.

Anammox removes only 90% of the incoming nitrogen as ammonium/nitrite and leaves 10% of nitrogen as nitrate in the effluent. The presence of oxygen and/or organic carbon can completely inhibit the anammox activity [12–14]. Most of the real wastewaters contain both organic carbon and nitrogen. Several wastewater treatment processes have been developed for the complete removal of organic carbon in the presence of nitrogen. Subsequently, the wastewater containing no or low organic carbon and nitrogen is treated via a variety of nitrogen removal processes. The direct application of anammox for wastewaters containing both organic carbon and nitrogen is questionable or else it requires an organic carbon removal process ahead. Alternatively, the development of anammox and denitrification in a single reactor can facilitate the simultaneous nitrogen and carbon removal.

In the past, various conventional treatment techniques available for nitrogen removal have been reviewed [3,4,15]. In addition, nitrogen removal and anammox in the marine environments, and the future research challenges have been addressed [16,17]. These reviews emphasize separately the state of art of denitrification and anammox for nitrogen removal. Although, anammox was first identified in a denitrification reactor, the interaction of anammox organisms with denitrifiers and the role of organic compounds in anammox process are still unclear [18]. Therefore, this review is focused to address the issues related with co-existence of anammox and denitrification for the simultaneous removal of nitrogen and carbon including: (1) the microbiology of anammox and denitrification, (2) issues in their co-existence, (3) coupling of anammox and denitrification in laboratory and full-scale systems, and (4) the effect of various environmental factors in the coupling of anammox and denitrification.

2. Stoichiometry and microbiology of denitrification

Wastewater denitrification describes the use of NO_3^- or NO_2^- ions by denitrifiers to degrade carbonaceous biological oxygen demand (cBOD). Most denitrifiers are facultative anaerobic-heterotrophs that transfer redox equivalents from the oxidation of a carbon source to an N-oxide under anaerobic conditions [19]. The modular organization of denitrification respiratory systems utilizing NO_3^-, NO_2^-, NO and N_2O is shown in Fig. 1. In addition, the overall energy yielding (catabolism or dissimilation) and cell synthesis (anabolism or assimilation) reactions of denitrification in the presence of acetic acid are shown as Eqs. (1) and (2), respectively [20]. The hydroxyl ion (OH^-) and some of the carbon dioxide (CO_2) produced during denitrification are returned in the system

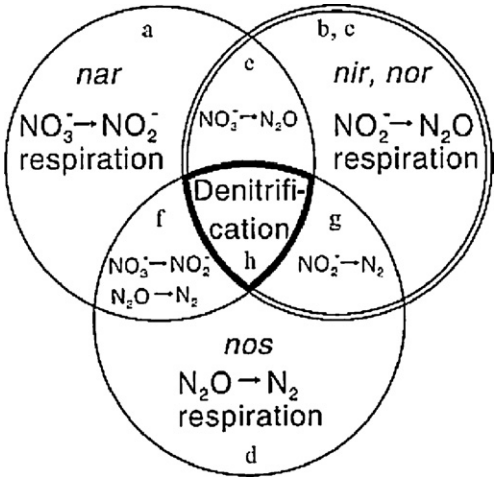

Fig. 1. Modular organization of denitrification (four modules representing the respiratory systems utilizing (a) NO_3^-, (b) NO_2^-, (c) NO and (d) N_2O constitute the overall process. Complete denitrification (h) is achieved only when all four modules are activated. Pair wise overlaps (e–g) of the individual respiratory modules occur naturally in denitrifying or other N oxide-utilizing bacteria [19].

as alkalinity (Eq. (2)).

$$1.6NO_3^- + CH_3COOH \rightarrow 0.8N_2 + 2CO_2 + 1.2H_2O + 1.6OH^- \quad (\Delta G^\circ = -843\,\text{kJ/M}) \tag{1}$$

$$1.2NO_3^- + CH_3COOH + 0.1NH_4^+ \rightarrow 0.1C_5H_7O_2N + 0.6N_2 + 1.5CO_2 + 1.1OH^- + 1.3H_2O \tag{2}$$

Denitrifying bacteria degrade cBOD in the absence of free molecular oxygen to obtain energy for cellular activity and carbon for cellular synthesis under a redox potential range from +50 to −50 mV. Most denitrifiers reduce NO_3^- via NO_2^- to molecular nitrogen without accumulation of intermediates. Four enzymes are involved in a complete denitrification system, i.e. reduction of NO_3^- to N_2. The reduction of NO_3^- to NO_2^- is catalyzed by the enzyme nitrate reductase (*Nar*). This is a membrane-bound molybdenum–iron–sulphur protein that is found in denitrifiers as well as in other dissimilatory nitrate reducing organisms. Both the synthesis and activity of nitrtate reductase are inhibited by oxygen. The second enzyme in this pathway is nitrite reductase (*Nir*), which catalyzes the conversion of NO_2^- to N_2O. Nitrite reductase is unique to denitrifying organisms, which is found in the periplasm. Nitric oxide reductase (*Nor*), a membrane-bound protein, is the third enzyme in the pathway, catalyzing the conversion of N_2O to NO. Nitrous oxide reductase (*Nos*), a periplasmic copper-containing protein, is the last enzyme in the pathway and converts NO to N_2. Both the synthesis and activity of all four denitrification enzymes are controlled by oxygen. Nitrous oxide reductase is the most sensitive denitrification enzyme and it is inhibited by DO concentrations less than 0.2 mg/L. However, some denitrifiers lack key enzyme systems to denitrify completely, and the lack of these enzyme systems can allow the production and accumulation of free intermediates. Organisms with the capability of denitrification belong to a variety of groups and encompass a wide range of physiological traits. Table 1 lists genera of denitrifying species grouped according to their principle growth mode or dominant physiological feature. Many genera of denitrifying bacteria can use NO_3^- and NO_2^- to degrade cBOD, some genera such as *Enterobacter* and *Escherichia* can use only NO_3^- [20].

Table 1
The metabolic diversity of archaeal and bacterial genera harboring denitrifying species [19].

Archaea	**Bacteria (gram-negative)**
Organotrophic	Diazotrophic
Halophilic	*Aquaspirillum*
Haloarcula,	*Azospirillum*
Halobacterium	*Azoarcus*
Haloferax	*Bacillus*
Hyperthermophilic	*Bradyrhizobium*
Pyrobaculum	*Pseudomonas*
	Rhodobacter
Bacteria (gram-positive)	*Rhodopseudomonas*
Organotrophic	*Sinorhizobium*
Spore forming	Thermophilic
Bacillus	*Aquifex*
Nonspore forming	*Bacillus*
Jonesis	*Thermothrix*
	Psychrophilic
Bacteria (gram-negative)	*Aquaspirillum*
Phototrophic	*Halomonas*
Rhodobacter	Halophilic
Rhodopseudomonas	*Halomonas*
Rhodoplanes	*Bacillus*
Lithotrophic	Pigment-forming
S oxidizing	*Chromobacterium*
Beggiatoa	*Flavobacterium*
Thibacillus	*Pseudomonas*
Thioploca	Budding
H_2 oxidizing	*Blastobacter*
Ralstonia	*Hyphomicrobium*
Paracoccus	Gliding
Pseudomonas	*Cytophaga*
NO_2^- or NH_4^+ oxidizing	*Flexibacter*
Nitrobacter	Magnetotactic
Nitrosomonas	*Magnetospirillum*
Organotrophic	Pathogenic
Carboxidotrophic	*Achromobacter*
Pseudomonas	*Alcaligenes*
Zavarzinia	*Agrobacterium*
Oligocarbophilic	*Campylobacter*
Aquaspirillum	*Eikenella*
Hyphomicrobium	*Flavobacterium*
Fermentative	*Kingella*
Empedobacter	*Moraxella*
Azospirillum	*Morococcus*
Facultative anaerobic	*Neisseria*
Alteromonas	*Ochrobacterum*
Pseudomonas	*Oligella*
Aerobic	*Pseudomonas*
Paracoccus	*Sphingobacterium*
Alcaligenes	*Tsukamurella*

On the other hand, some genera of denitrifying bacteria including *Thiosphaera pantotropha* [21] or *Paracoccus denitrificans* [22], *Magnetospirillum magnetotacticum* [23] and *Pseudomonas stutzeri SU2* [24] can denitrify under aerobic or microaerophilic conditions. In addition, *Nitrosomonas*-like microorganisms including *Bacillus cereus*, *Bacillus subtilis* and *Bacillus licheniformis* [25,26], nitrify and denitrify simultaneously even under fully oxic or anoxic condition

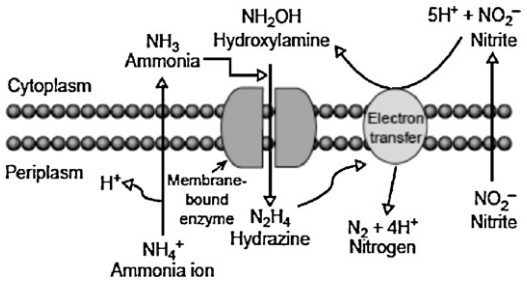

Fig. 2. Schematic demonstration of anammox by the planctomycetales [29].

with N_2 as main final product, which has been reviewed previously [3,4]. *Nitrosomonas eutropha* is an obligate lithoautotrophic nitrifying bacterium and also a denitrifying organism that uses hydrogen as the electron donor and nitrite as the electron acceptor. The denitrification activity of *N. eutropha* could be stimulated by adding gaseous nitrogen oxide under anaerobic conditions. Mostly, the denitrifying nitrifiers are detected in a bioreactor operating with the coupling of aerobic and anaerobic ammonia oxidation, for example, SNAP.

3. Stoichiometry and microbiology of anammox

In anammox, ammonium is oxidized to N_2 strictly under anoxic condition using nitrite as the electron acceptor (Eq. (3)) [5,12,27,28]. Anammox organisms utilize CO_2 as the source of carbon and NO_2^- as the electron acceptor for ammonium oxidation. Concurrently, NO_2^- is used as the electron donor for the reduction of CO_2. Hydroxylamine (NH_2OH) and hydrazine (N_2H_4) were identified as the metabolites of anammox process [28]. The overall anammox reaction is specified in Eq. (4) and the schematic demonstration of the anammox process is shown in Fig. 2. The ^{15}N-studies have shown that one N-atom of the produced N_2 originate from NO_2^- and the other from ammonium. However, the growth of anammox organism is reversibly inhibited even by oxygen concentrations below 0.5% air saturation [18].

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \qquad (\Delta G^\circ = -297\,\text{kJ/M}) \tag{3}$$

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2.03H_2O + 0.066CH_2O_{0.5}N_{0.15} \tag{4}$$

Anammox species express various unusual lipids that contain ladderanes, i.e. lipids build from concatenated cyclobutane rings, which form a molecular ladder [30]. These ladderane lipids surround the anammoxosome, a special compartment of the anammox cell, in which the anaerobic oxidation of ammonium to N_2 is taking place [31]. Thus far, ladderanes have been found only in association with anammox bacteria [18] that could be used to positively iden-

Table 2
Various microbial species claimed for anammox.

Species	Origin	Reference
Candidatus Scalindua sorokinii	Marine species, originated from Black Sea	[31]
Candidatus Brocadia *anammoxidans*	Denitrifying pilot-plant	[34]
Candidatus Kuenenia *stuttgartiensis*	Nitrifying wastewater treatment plant	[35]
Candidatus Scalindua brodae	Wastewater treatment plant treating landfill leachate in Pitsea, UK	[36]
Candidatus Scalindua wagneri	Wastewater treatment plant treating landfill leachate in Pitsea, UK	[36]
Candidatus Brocadia *fulgida*	Laboratory scale anammox reactor	[37]
Candidatus Anammoxoglobus propionicus	Activated sludge sample from secondary stage of Dokhaven municipal wastewater treatment plant, Rotterdam, The Netherlands	[38]
Candidatus Jettenia *asiatica*	Granular sludge anammox reactor	[39]
Candidatus Scalindua *arabica*	Suboxic zones of Black sea and in three major oxygen minimum zones of Namibia, Peru and Arabian sea	[40]

tify anammox organisms [32,33]. Table 2 categorizes the anammox species according to their genera. The involvements of bacteria in anammox process [18] and ammonia-oxidizing archaea in nitrogen removal have been reviewed [12]. The application, eco-physiology and biodiversity of anammox bacteria have been reviewed in detail [37]. In addition, a comprehensive review on the application of various biomarkers for in situ detetction of anammox species has been done [41].

4. Issues in the co-existence of anammox and denitrification

First and foremost, the coupling of denitrification and anammox in a single reactor system could induce a competition between autotrophic anammox species and heterotrophic denitrifiers. Comparing Eqs. (1) and (3), the standard free energy (ΔG°) of denitrification reaction is higher than the anammox reaction; therefore, the denitrifcation reaction is thermodynamically more feasible compared to anammox reaction [3,42]. Anammox is less competitive with denitrification [43,44] because denitrifiers have higher growth yield (yield coefficient of heterotrophs; $Y = 0.3$ g VSS/g NH_4^+–N) compared to nitrifiers/anammox bacteria ($Y = 0.066 \pm 0.01$ g VSS/g NH_4^+–N) [45,46]. Comparing the maximum growth rates of denitrifiers (around 0.35/h for fast growing *Rhizobium* spp. under anaerobic conditions) [47,48] and anammoxidans (0.003/h), fast growing denitrifiers have nearly one hundred times faster growth rate than the *Candidatus* Brocadia *anammoxidans*.

The next issue in the co-existence of anammox and denitrification is the availability of NO_2^-. In NO_3^- rich environments, anammox process relies on other processes to reduce NO_3^- to NO_2^-. Under anoxic conditions, NO_3^- can be reduced by denitrifiers or other species promoting dissimilatory reduction of NO_3^- to NH_4^+ (well known as dissimilatory nitrate reduction to ammonia, DNRA) and release NO_2^- as a free intermediate [16]. Subsequently, NO_2^- can be utilized by anammox bacteria for the oxidation of NH_4^+ as per the stoichiometric equation (3). However, the reduction of organic matter is not possible in case of DNRA [49,50]. A similar coupling between anammox and denitrification was observed in the marine environments [27]. The experimental observations of the various physicochemical data and the theoretical calculations using the stoichiometric relationship of anammox (i.e. 1 NH_4^+ requires 1.32 NO_2^-) and denitrification (i.e. quantity of organic carbon required for the conversion of NO_3^- to NO_2^-) can demonstrate the contributions of denitrification and anammox in nitrogen removal [51]. Alternatively, $^{15}N_2$ labelling experiments can be used to demonstrate the N_2 production associated with the above two processes [52–54].

In addition, the environmental conditions including pH, temperature, dissolved oxygen concentration and presence of different substrates could cause severe impacts in the coupling of anammox and denitrification. The decrease in the pH of a denitrifying system (pH < 5) can affect the activities of *Nir* and *Nor* enzymes of the denitrifying pathway due to the formation of NO. Whereas, the change is pH (highly alkaline or acidic) of the anammox system provoke the instability in the reactor performance. The temperature of the system has an effect on the consumption rate of nitrate and growth rate of denitrifying organism. In anammox systems, temperature has major influence on the maximum specific anammox activity. Moreover, both denitrifying and anammox activity is inhibited reversibly under aerobic conditions.

The C/N ratio in the wastewater is the most critical factor determining the direction of a dissimilative pathway to either denitrification or DNRA. At high C/N ratio, nitrate will probably be reduced to ammonia due to excessive reducing power. Moreover, changes in Gibbs free energy of denitrification reaction are less favorable under high C/N ratios. On the other hand, the presence of organic carbon is not suitable for anammox. The addition of alternative electron sources can influence the Gibbs free energy of denitrifying reaction. For example, using xylene as an electron source instead of acetic acid can increase the Gibbs free energy from −843 to −4136 kJ/M. However, the effect of alternative electron source on the performance of anammox should be identified before its application.

Finally, the overall characteristics of the anammox process are quite remarkable, especially considering the high toxicity of the intermediate N_2H_4 [17], which is used as a rocket fuel and constitute an intermediate in the production of explosives and pesticides [55]. Although anammox catabolism takes place within the ladderanes [56], in some instances, N_2H_4 and NH_2OH are added to speed up the anammox process [34]. Under such circumstances, the tolerance of denitrifiers to N_2H_4 is another issue in the success of simultaneous carbon and nitrogen removal.

5. Coupling of anammox and denitrification

The coupling of anammox and denitrification will be successful only when the denitrification reaction is not competing with anammox for NO_2^- (i.e. only partial denitrification is preferable). The first two steps in the denitrification pathway (NO_3^- to NO_2^- and NO_2^- to N_2O) are uncoupled; therefore, there is a potential for NO_2^- accumulation [17]. The optimum additions of acetylene, allylthiourea and N_2H_4 as inhibitors could be useful to control denitrification and anammox at lab-scale investigations but are not feasible at a field-scale application. Alternatively, denitrification rates can be controlled by the regulated additions of cBOD [1,44,50]. A recent study indicated that anammox bacteria were successful in the oxidation of propionate and the presence of glucose, formate and alanine had no effect on the anammox process [38]. Moreover, anammox bacteria can be competitive with heterotrophic denitrifiers for the utilization of organic matter, i.e. propionate [57]. The rate of propionate utilization by anammox bacteria was 0.6 mM/mg of protein/d, which is far less than the utilization rate by denitrifiers in real-time wastewater systems. On the other hand, the presence of methanol is found to have irreversible inhibition at concentration as low as 0.5 mM [54,57].

Although the co-existence of anammox and denitrification is complicated, several researchers reported the successful linkage between denitrification and anammox in natural environments [16,17,31,52,58] and laboratory/full-scale systems. This review is focused mainly to address the coupling of denitrification and anammox in laboratory and full-scale systems. Table 3 shows the list of studies reported the co-existence of anammox and denitrification. While only limited studies have reported the co-existence of anammox and denitrification, to clearly understand the co-existence of anammox and denitrification the detailed operating conditions, experimental outcomes and important remarks are summarized in Table 3. It can be seen in Table 3 that the performance of anammox bacteria was low at high C/N ratio of the system. At high C/N ratios, denitrifers and DNRA organisms are greatly competitive with anammox bacteria. Therefore, the COD of the system should be maintained as per the stoichiometric requirement or lesser than the stoichiometric requirement for denitrification of NO_3^-–N to NO_2^-–N to facilitate a strong coupling between denitrifiers and anammox bacteria. The attached growth systems are adopted in most of the studies listed in Table 3. Because, these systems are effective for anammox development even at slightly higher DO levels. The excess DO in the system could be consumed by ammonia oxidizers in the outer layers of the biofilm, and simultaneously, the anammox bacteria could develop in the anoxic layers.

Table 3
Co-existence of anammox and denitrification in lab-scale and full-scale reactors.

Type and nature of investigation	Operating conditions	Key experimental outcomes	Remarks	Reference
Lab-scale non-woven rotating biological contactor (NRBC) for simultaneous partial nitrification, anammox and denitrification (SNAD).	NH_4^+–N—210 mg/L; COD—160 and 110 mg/L; pH—8.0–8.2; *T*—35 °C; DO—0.5–0.7 mg/L. Source of seed sludge—partial nitrification biomass from oxygen-limited nitrifying chemostat and the anammox biomass from anammox upflow column reactor.	(1) NH_4^+–N and COD removals were 52% and 70%, respectively, at a C/N ratio of 3:4. (2) At C/N ratio of 1:2, the NH_4^+–N and COD removals were increased to 79% and 94%, respectively. (3) Aerobic heterotrophic bacteria were also responsible for COD consumption.	Keeping the COD of the system as per the stoichiometric requirement or lesser than the stoichiometric requirement for denitrification of NO_3^-–N to NO_2^-–N could facilitate the strong coupling between heterotrophic denitrification and anammox.	[11]
Lab-scale (1 L upflow anaerobic sludge bed reactor (UASB) with a 0.5 L settling tank)—anaerobic nitrogen removal from piggery waste.	NH_4^+–N—0.43 kg/m^3; NH_4^+–N/NO_2^-–N—0.83–1.02 kg/m^3 day; TCOD—5.55 kg/m^3; pH—8.2–8.5; *T*—35 °C; HRT—5 days, recycle ratio—0.5; alkalinity—5150–12400 mg $CaCO_3$/L. Source of seed sludge—full-scale UASB reactor treating brewery wastewater.	(1) The average TN removal was 0.59–0.66 kg TN/m^3, and the average COD removal was 4.7–5.2 kg COD/kg NH_4^+–N. (2) NO_2^-–N removal was 100%. (3) The removal ratio of NH_4^+–N and NO_2^-–N was 1:1.13–1:1.65. (4) The composition of gas produced in the system was 95% N_2 and 5% CO_2.	Analyzing the gas produced in the system is an alternative approach for the preliminary evaluation of other heterotrophs in the system, i.e. acidogens and sulphate reducing bacteria. Attached growth systems are much more effective in the quick start-up of anammox reaction.	[43]
Lab-scale (500 mL serum bottles)—evaluation of anammox during anaerobic digestion.	NH_4^+–N—250 mg/L; NO_3^-–N—200 mg/L; NO_2^-–N—250 mg/L; COD—5400 mg/L; pH—7.55; *T*—37 °C. Source of seed sludge—returned activated sludge from wastewater treatment plant (Will Hunter Rd, Athens, GA).	(1) NH_4^+–N and NO_2^-–N removals were 22.5% and 100%, respectively. (2) The ratio of NH_4^+–N and NO_2^-–N consumption was greater than 1:1.32. (3) More nitrite is consumed than that required for anammox. Reduction of nitrite is mainly through denitrification, using VFAs or reduced inorganic compounds as electron donors.	The C/N ratio of the wastewater is high. At this condition, denitrifers and DNRA organisms are greatly competitive with anammox bacteria. Therefore, controlling C/N ratio is an important step in establishing a strong coupling between denitrifiers and anammox bacteria.	[44]
Lab-scale batch reactor (500 mL serum bottles)—evaluation of anammox and heterotrophic denitrification in presence of organic matter.	NH_4^+—150 mg/L; NO_3^-—782 mg/L; COD—564 mg/L (sucrose as electron acceptor); pH—7.5; *T*—30–32 °C. Source of seed sludge—adopted biomass of cow dung and tannery sludge.	(1) NH_4^+, NO_3^- and COD removals were 44%, 93% and 82%, respectively. (2) The presence of sucrose helped the heterotrophic denitrification. (3) Nitrate is the preferred anammox oxidation product in presence of organic matter at an ORP of −248 mV.	The C/N ratio of the wastewater is so high. It could have resulted in the poor performance of anammox process. At the same time, the ammonia removal (44%) was also be due to ammonia oxidation through biochemical routes at pH < 7.	[46]
Full-scale (192 m^3)—SNAD in a real-time wastewater treatment plant treating landfill leachate, Taiwan.	NH_4^+–N—634 mg/L; NO_3^-–N—3 mg/L; NO_2^-–N—0 mg/L; COD—554 mg/L; pH—7.9; *T*—30–33 °C; SRT—12–18 days. Source of seed sludge—real-time wastewater treatment plant treating landfill leachate.	(1) NH_4^+–N and COD removal were 80% and 28%, respectively. (2) TN removal by partial nitrification and anammox is 68%; and, TN removal by denitrification is 8%. (3) Anammox bacteria were confirmed by FISH and PCR.	Mass balance of the system using stoichiometric equation of nitrification, anammox and denitrification is a best approach for the evaluation of SNAD process. Different FISH and PCR probes could be used for the identification of various species involved in SNAD system.	[51]
Full-scale (500 m^3) pH controlled rejection water deammonification, i.e. partial nitritation and anammox system (DEMON) at the WWTP Strass, Austria.	Flow rate—119 m^3/day; NH_4^+–N—1.83 kg/m^3; nitritation was used to convert NH_4^+–N into NO_2^-–N by means of limiting the DO close to 0.3 mg/L; pH—8.2–8.5; *T*—30 °C. Source of seed sludge—4 L of inoculum from pilot-plant operated by the EAWAG in Zurich.	(1) NH_4^+–N and SCOD removals were 89.3% and 38%, respectively. (2) TN removal was around 83.9%. (3) Energy saving in the form of aeration in deammonification process was 73%. (4) Molecular tools were not used to characterize the microbial activity but the mass balance was used to analyze the performance of the system.	Mass balance of the system is a best approach for the evaluation of anammox and nitritation processes. On the other hand, molecular tools and tracer studies with labeled nitrogen are useful approaches for the evaluation of anammox.	[59]
Lab-scale (100 mL upflow bioreactors inoculated with biopellets and anaerobic granules)—investigation of the activity of anammox and denitrification in low ammonium-fed bioreactors.	Flow rate—200 mL/day; NH_4^+–N—2.3 mg/L; NO_3^-–N–1.47 mg/L; NO_2^-–N–2.1 mg/L; pH—6.7; HRT—12 days; *T*—20 °C; DO—0.5–4 mg/L; 20 g of caprolactone as an additional carbon source. Source of seed sludge—biomass from activated sludge process and the anaerobic granules were from UASB reactors treating brewery wastewater in Ibaraki, Japan.	(1) NH_4^+–N, NO_3^-–N and NO_2^-–N removals were 75%, 67% and >97%, respectively. (2) TN removal rate was 9.3 g/m^3/day. (3) The removal ratio of NO_2^-–N and NH_4^+–N is 1:1.29. (4) Anammox bacteria grew at the central part of the biopellets and the large size aggregates of biopellets promoted the anammox activity.	Denitrification is more favorable along with anammox when the C/N of the system is maintained around 0.6. Mostly attached growth systems are effective for anammox development even at slightly more DO levels. Under this situation (DO > 0.5 mg/L), ammonia oxidizers could consume oxygen in the outer layers of the biofilm/aggregate and anammox could develop in the anoxic layers.	[60]

Table 3 (*Continued*)

Type and nature of investigation	Operating conditions	Key experimental outcomes	Remarks	Reference
Pilot-scale (400 L single-stage SBR)—investigation of the adaptation and stable operation of the nitritation/anammox process.	NH_4^+–N—600–700 g/m^3; 50–60% of nitrogen removal by partial oxidation of ammonia to nitrite with continuous aeration (DO < 0.5 g of O_2/m^3); pH—6.5–8.0; *T*—25 and 20 °C; total operation cycle of SBR—8 h. Source of seed sludge: anammox sludge and activated sludge from the sludge liquid treatment plant of the WWTP, Bulach.	(1) NH_4^+–N and NO_3^-–N removals were nearly 82% and 100%, respectively. (2) Anammox bacterial growth rate was 0.023/d. (3) Anammox activity decreases with about 0.07/1 °C.	Anammox activity is not affected by higher initial concentration of NH_4^+–N even after partial nitritation, i.e. around 300 g/m^3. As the anammox activity has good correlation with temperature, it could be modeled using Arrhenius equation.	[61]

For establishing a coupling between denitrification and anammox: (1) suitable reactor system with much needed hydraulic and sludge retention times, i.e. reactor configuration with suitable HRT and SRT, and (2) suitable environmental conditions including DO, pH, temperature, alkalinity and limiting substrate concentrations (carbon, NH_4^+, NO_3^- and NO_2^-) are essential. UASB reactors [62] and SBR [63] are the two most suitable reactor configurations for the stable establishment of anammox and denitrification process, which can also be noticed in Table 3. The main limitation in the applicability of anammox process is the longer doubling time of anammox bacteria. Therefore, the reactor carrying out anammox must be capable of holding the biomass efficiently. On the other hand, the application and performance of anammox reactor relies upon its stability with respect to the fluctuations in substrate concentration and flow rate. However, it is reported that anammox process is more tolerant to flow rate shock than substrate shock loading [62]. For quick start-up of anammox process in SBRs, initial sludge wash out is considered as an important step; and moreover, higher total suspended solids (TSS) concentration with high cell retention time are not suitable for improved nitrogen removal [64]. In addition to the selection of reactor configurations, it is essential to know the optimum/favorable growth conditions of anammox and denitrifying microorganisms. Both these organisms can survive at extreme conditions, which can be evidenced from their identification in deep-sea regions [31,65]. A recent study reported the adaptability of a freshwater anammox biomass, i.e. *Candidatus* Kuenenia *stuttgartiensis*, to salt concentrations as high as 30 g/L in a lab-scale investigation [66].

Table 4
Favorable environmental conditions/parameters for anammox and heterotrophic denitrifying microorganisms.

Parameter	Anammox		Heterotrophic denitrification	
	[a]Suitable range	Effect at out of range	[a]Suitable range	Effect at out of range
DO (mg/L)	[b]0.5–0.7	(1) Reversible inhibition of anammox activity. (2) Under excess oxygen conditions, NH_4^+ could be oxidized excess and undesired nitrite oxidation can take place.	Near zero	(1) Sensibility to DO is related to microbial genus; best suitable ORP is between +50 and −50 mV. (2) Inhibit nitrite reduction because of competence of electrons between the oxidase and nitrite reductase.
pH	6.7–9.5	(1) At very high or very low pH values, microbial activity is suppressed by high acidity/alkalinity.	6–9	(1) Mainly, the acidic pH decreases the denitrifying rate due to nitrous oxide formation (at pH < 5). (2) Affects *Nir* and *Nor* enzymes of the denitrifying pathway.
Temperature (°C)	20–40 °C	(1) Below 10 °C, anammox activity is completely ceased. (2) Specific anammox activity is directly proportional to temperature, which is higher between 35 and 40 °C.	20–35 °C	(1) High and low temperatures could cause physicochemical change in the cell membrane structure, either for lipids or proteins.
NH_4^+:NO_2^-/C:N	NH_4^+:NO_2^- between 1:1 and 1:5	(1) The best ratio other than the stoichiometric value (1:1.32) could decrease the TN removal efficiency. (2) According to a simulation data, when the biomass is immobilized and the biofilm is thick enough, anammox activity can be obtained up to a COD/N ratio of 10 [67]. If the biofilm is sensitive, anammox activity can be lost even at a COD/N ratio of 2 [68,69].	C:N between 0.8 and 1.6	(1) At high C/N ratios, methanogenesis might compete with denitrification. (2) At high C/N ratios, Gibbs free energy are less favorable for denitrification.
Substrate	N_2H_4 and NH_2OH	(1) Methanol is highly toxic and can produce irreversible inhibition.	Methanol is the most preferred	(1) Activity of *Nar* is inhibited by azide, thiocyanate, cyanide, dinitrophenol. (2) Activity of *Nir* could strongly inhibited by nitric oxide (NO).

Note:
[a] Sometimes vary based on species involved.
[b] Only for single-stage autotrophic nitrogen removal.

Table 4 shows the effect of environmental conditions on anammox and denitrifying microorganisms. Denitrification can be performed under a wide range of pH between 6 and 9. Under acidic condition (pH < 5), decreases in the yield, denitrifying rate and efficiency are observed, which could be associated to the formation of NO. On the other hand, the optimum pH for anammox activity is around 8. However, the optimum pH value could vary between the anammox species, i.e. optimum pH for *Candidatus* Kuenenia *stuttgartiensis* is around 9. The anammox activity could also be related to temperature by using Arrhenius law when the system is operated between 20 and 37 °C. Moreover, the maximum specific anammox activity is exponentially related with the temperature of the anammox system from 10 to 40 °C [70]. The unfavorable temperature can induce changes in the genetic expression of the denitrifying process and could affect the consumption efficiency of substrates and the yield of the denitrifiers. On the other hand, the aerobic conditions can reversibly inhibit the activities of anammox and denitrifying organisms. The inhibition of denitrifying activity could be due to the competence of electrons between oxidase and nitrite reductase. However, the sensitivity to oxygen is related to the microbial genus. The favorable environmental conditions for both anammox and denitrifying microorganisms vary a lot based on the microbial genus and their metabolic activity; thus, a wide range of environmental conditions can be seen in the literature.

In summary, coupling of anammox and denitrification could be established in a single reactor system by adopting the following steps: (1) initially, start the anammox process under stoichiometric quantities of NH_4^+ and NO_2^- (1:1.32) with anammox seed sludge, (2) maintain the DO concentration around 0.5 mg/L until the NH_4^+ removal reaches steady state, (3) estimate the quantity of NO_3^- at the steady state, (4) initiate heterotrophic denitrification by adding COD as per the stoichiometric requirement (1 mole of NO_3^- per mole of COD) for the conversion of NO_3^- to NO_2^-, and (5) analyze the activities of anammox bacteria and denitrifiers by using reactor mass balance and biotechnological techniques such as fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR).

A comprehensive analysis of influent wastewater characteristics is very important before developing a coupling between anammox and denitrification. Because the ratio of NH_4^+:NO_2^- and C:N have greater influence on anammox and denitrification, respectively. On the other hand, controlling the coupling between denitrification and anammox is relatively simpler by means of reactor mass balance than by biotechnological operations, i.e. controlling enzymatic activity. But, the quantity of nitrogen species, i.e., NH_4^+, NO_2^-, NO_3^-, NO and N_2O, utilized by anammox species, heterotrophic denitrifiers and/or autotrophic denitrifiers is difficult to estimate where a strong coupling between anammox and denitrification exists. Most of the researchers, utilized mass balance and simple stoichiometric modeling to estimate the coupling of anammox and denitrification [11,43,59,61,71]. Alternatively, anammox bacteria have lipids with unique properties that can be used as biomarkers to analyze their presence in the reactor system. Moreover, the isotope pairing technique could be applied for estimating the co-existence of anammox and denitrification [72].

6. Outlook for future research

The development of simultaneous anammox and denitrification could be useful especially for treating landfill leachate, digester effluents, deammonification and recirculating aquaculture systems [73,74] with lower energy demand and cost-effectiveness. However, majority of wastewaters including seafood processing, leather tanning, oil refining and alcohol fermentation, not only contain organic carbon and nitrogen but also sulphur compounds. Recently, anoxic ammonia removal with sulphidogenesis [75] and simultaneous removal of ammonium and sulphate by anammox process have been reported [76]. Moreover, sulphide and nitrate could be removed under anaerobic conditions [77]. Therefore, combining anammox, denitrification and sulphidogenesis in a single reactor can be useful for removing nitrogen, organic carbon and sulphate simultaneously instead of removing each pollutant in a sequential chain of treatment units. The establishment of anammox, denitrification and sulphidogenesis in a single reactor is more complicated considering the difficulty in the determination of favorable operating conditions including the ORP of the system and the ratios of NH_4^+:NO_2^-, COD:NO_3^- and COD:SO_4^{2-}. Additionally, the competition for nitrite by anammox, denitrification and sulphidogenesis could create intricacy in the coupling of these processes. However, further research on the optimization of biochemical routes of these processes can advance the wastewater treatment process ahead.

7. Conclusion

The co-existence of biological denitrification and anammox is technically feasible and economically favorable when the wastewater contains both ammonium and organic carbon. Anammox species and denitrifiers encompass a wide range of genera; therefore, the application of reactor mass balance is considered as a suitable approach for establishing the coupling between anammox and denitrification. DO and nitrite concentrations are the two most important and economically feasible control parameters. Furthermore, combining anammox, denitrification and sulphidogenesis in a single reactor for the simultaneous removal of nitrogen, organic carbon and sulphate could be a future research perspective in advanced wastewater treatment.

Acknowledgement

We thank National Science Council (NSC), Taiwan, ROC, for the financial support (NSC 98-2221-E-009-022-MY3).

References

[1] Metcalf and Eddy, Wastewater Engineering-Treatment and Reuses, 4th ed., McGraw-Hill, 2003.
[2] US EPA, Process Design Manual of Nitrogen Control, EPA 625/r-93/010, Cincinnati, Ohio, 1993.
[3] Y.-H. Ahn, Sustainable nitrogen elimination biotechnologies: a review, Process Biochem. 41 (2006) 1709–1721.
[4] Y. Peng, G. Zhu, Biological nitrogen removal with nitrification and denitrification via nitrite pathway, Appl. Microbiol. Biotechnol. 73 (2006) 15–26.
[5] A. Mulder, Anoxic ammonia oxidation, US Patent 5,078,884 (1992).
[6] C. Hellinga, A.A.J.C. Schellen, J.W. Mulder, M.C.M. van Loosdrecht, J.J. Heijnen, The SHARON process: an innovative method for nitrogen removal from ammonium-rich wastewater, Water Sci. Technol. 37 (9) (1998) 135–142.
[7] A.O. Sliekers, N. Derwort, J.L.C. Gomez, M. Strous, J.G. Kuenen, M.S.M. Jetten, Completely autotrophic nitrogen removal over nitrite in one single reactor, Water Res. 36 (2002) 2475–2482.
[8] K. Pynaert, B.F. Smets, D. Beheydt, W. Verstraete, Start-up of autotrophic nitrogen removal reactors via sequential biocatalyst addition, Environ. Sci. Technol. 38 (2004) 1228–1235.
[9] K. Furukawa, P.K. Lieu, H. Tokitoh, T. Fujii, Development of single-stage nitrogen removal using anammox and partial nitritation (SNAP) and its treatment performances, Water Sci. Technol. 53 (6) (2006) 83–90.
[10] A. Hippen, K.H. Rosenwinkel, G. Baumgarten, C.F. Seyfried, Aerobic deammonification: a new experience in the treatment of wastewaters, Water Sci. Technol. 35 (1997) 111–120.
[11] H. Chen, S. Liu, F. Yang, Y. Xue, T. Wang, The development of simultaneous partial nitrification, ANAMMOX and denitrification (SNAD) process in a single reactor for nitrogen removal, Bioresour. Technol 100 (2009) 1548–1554.
[12] J. You, A. Das, E.M. Dolan, Z. Hu, Review: ammonia-oxidizing archaea involved in nitrogen removal, Water Res. 43 (2009) 1801–1809.
[13] M. Strous, E. van Gerven, J.G. Kuenen, M. Jetten, Effects of anaerobic and microaerobic condition on anaerobic ammonium-oxidizing (anammox) sludge, Appl. Environ. Microbiol. 63 (1997) 2446–2448.
[14] M. Strous, J.J. Heijnen, J.G. Kuenen, The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms, Appl. Microbiol. Biotechnol. 50 (1998) 589–596.

[15] T. Khin, A.P. Annachhatre, Research review paper: novel microbial nitrogen removal processes, Biotechnol. Adv. 22 (2004) 519–532.
[16] T. Dalsgaard, B. Thamdrup, D.E. Canfield, Mini-review: anaerobic ammonium oxidation (anammox) in the marine environment, Res. Microbiol. 156 (2005) 457–464.
[17] S. Hulth, R.C. Aller, D.E. Canfield, T. Dalsgaard, P. Engstrom, F. Gilbert, K. Sundback, B. Thamdrup, Nitrogen removal in marine sediments: recent findings and future research challenges, Mar. Chem. 94 (2005) 125–145.
[18] J.G. Kuenen, Timeline:, Anammox bacteria: from discovery to application, Nat. Rev. 6 (2008) 320–326.
[19] W.G. Zumft, Cell biology and molecular basis of denitrification, Microbiol. Mol. Biol. Rev. 61 (4) (1997) 533–616.
[20] M.H. Gerardi, Nitrification and Denitrification in the Activated Sludge Process, Wiley-Interscience, 2002.
[21] L.A. Robertson, G. Kuenen, Aerobic denitrification: a controversy revived, Arch. Microbiol. 139 (1984) 351–354.
[22] W. Ludwig, G. Mittenhuber, C.G. Friedrich, Transfer of *Thiosphaera pantotropha* to *Paracoccus denitrificans*, Int. J. Syst. Bacteriol. 43 (1993) 363–367.
[23] D.A. Bazylinski, R.P. Blakemore, Denitrification and assimilatory nitrate reduction in *Aquaspirillum magnetotacticum*, Appl. Environ. Microbiol. 46 (1983) 1118–1124.
[24] J.J. Su, B.Y. Liu, C.Y. Liu, Comparison of aerobic denitrification under high oxygen atmosphere by *Thiosphaera pantotropha* ATCC35512 and *Pseudomonas stutzeri SU2* newly isolated from the activated sludge of piggery wastewater treatment system, J. Appl. Microbiol. 90 (2001) 457–462.
[25] B. Brycki, K. Seifert, K. Szymanska, F. Domka, The effect of oxidizing biocides on desulfurication and denitrification processes, Pol. J. Environ. Stud. 9 (5) (2000) 363–367.
[26] J.K. Kim, K.J. Park, K.S. Cho, S.-W. Nam, T.-J. Park, R. Bajpai, Aerobic nitrification–denitrification by heterotrophic *Bacillus* strains, Bioresour. Technol. 96 (2005) 1897–1906.
[27] A.A. van de Graaf, P. deBruijn, L.A. Robertson, M.S.M. Jetten, J.G. Kuenen, Anaerobic oxidation of ammonium is a biologically mediated process, Appl. Environ. Microbiol. 61 (1995) 1246–1251.
[28] A.A. van de Graaf, P. deBruijn, L.A. Robertson, M.S.M. Jetten, J.G. Kuenen, Metabolic pathway of anaerobic ammonium oxidation on the basis of N-15 studies in a fluidized bed reactor, Microbiology-UK 143 (1997) 2415–2421.
[29] R.W. Ye, S.M. Thomas, Microbial nitrogen cycles: physiology, genomics and applications, Curr. Opin. Microbiol. 4 (2001) 307–312.
[30] V. Mascitti, E.J. Corey, Total synthesis of [±]-pentacycloanammoxic acid, J. Am. Chem. Soc. 126 (2004) 15664–15665.
[31] M.M.M. Kuypers, A.O. Sliekers, G. Lavik, M. Schmid, B.B. Jorgensen, J.G. Kuenen, J.S.S. Damste, M. Strous, M.S.M. Jetten, Anaerobic ammonium oxidation by anammox bacteria in the Black Sea, Nature 422 (2003) 608–611.
[32] J.S.S. Damste, W.I.C. Rijpstra, J.A.J. Geenevasen, M. Strous, M.S.M. Jetten, Structural identification of ladderane and other membrane lipids of planctomycetes capable of anaerobic ammonium oxidation (anammox), FEBS J. 272 (2005), 4720–4283.
[33] H.A. Boumann, E.C. Hopmans, I. van de Leemput, H.J.M. Op den Camp, J. van de Vossenberg, M. Strous, M.S.M. Jetten, J.S.S. Damste, S. Schouten, Ladderane phospholipids in anammox bacteria comprise phosphocholine and phosphoethanolamine headgroups, FEMS Microbiol. Lett. 258 (2006) 297–304.
[34] M. Strous, J.A. Fuerst, E.H.M. Kramer, S. Logemann, G. Muyzer, K.T. Van de Pas-Schoonen, R. Webb, J.G. Kuenen, M.S.M. Jetten, Missing lithotroph identified as new planctomycete, Nature 400 (1999) 446–449.
[35] M. Schmid, U. Twachtmann, M. Klein, M. Strous, S. Juretschko, M. Jetten, J.W. Metzger, K.H. Schleifer, M. Wagner, Molecular evidence for genus level diversity of bacteria capable of catalyzing anaerobic ammonium oxidation, Syst. Appl. Microbiol. 23 (2000) 93–106.
[36] M. Schmid, K. Walsh, R. Webb, W.I.C. Rijpstra, K. van de Pas-Schoonen, M.J. Verbruggen, T. Hill, B. Moffett, J. Fuerst, S. Schouten, J.S.S. Damste, J. Harris, P. Shaw, M. Jetten, M. Strous, *Candidatus* "*Scalindua brodae*", sp. nov., *Candidatus* "*Scalindua wagneri*", sp. nov., two new species of anaerobic ammonium oxidizing bacteria, Syst. Appl. Microbiol. 26 (2003) 529–538.
[37] B. Kartal, L. van Niftrik, O. Sliekers, M.C. Schmid, I. Schmidt, K. van de Pas-Schoonen, I. Cirpus, W. van de Star, M. van Loosdrecht, W. Abma, J.G. Kuenen, J. Mulder, M.S.M. Jetten, H. op den Camp, M. Strous, J. van de Vossenberg, Application, eco-physiology and biodiversity of anaerobic ammonium-oxidizing bacteria, Rev. Environ. Sci. Biotechnol. 3 (2004) 255–264.
[38] B. Kartal, J. Rattray, L.A. van Niftrik, J. van de Vossenberg, M.C. Schmid, R.I. Webb, S. Schouten, J.A. Fuerst, J.S. Damste, M.S.M. Jetten, M. Strous, *Candidatus* "Anammoxoglobus propionicus" a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria, Syst. Appl. Microbiol. 30 (2007) 39–49.
[39] Z. Quan, S. Rhee, J. Zuo, Y. Yang, J. Bae, J.R. Park, S. Lee, Y. Park, Diversity of ammonium-oxidizing bacteria in a granular sludge anaerobic ammonium-oxidizing (anammox) reactor, Environ. Microbiol. 10 (2008) 3130–3139.
[40] D. Woebken, P. Lam, M.M.M. Kuypers, S.W.A. Naqvi, B. Kartal, M. Strous, M.S.M. Jetten, B.M. Fuchs, R. Amann, A microdiversity study of anammox bacteria reveals a novel *Candidatus* Scalindua phylotype in marine oxygen minimum zones, Environ. Microbiol. 10 (11) (2008) 3106–3119.
[41] M.C. Schmid, B. Maas, A. Dapena, K. van de Pas-Schoonen, J. van de Vossenberg, B. Kartal, L. van Niftrik, I. Schmidt, I. Cirpus, J.G. Kuenen, M. Wagner, J.S.S. Damsté, M. Kuypers, N.P. Revsbech, R. Mendez, M.S.M. Jetten, M. Strous, Mini review—biomarker for in situ detection of anaerobic ammonium-oxidizing (Anammox) bacteria, Appl. Environ. Microbiol. 71 (2005) 1677–1684.
[42] M.S.M. Jetten, M. Strous, K.T. van de Pas-Schoonen, J. Schalk, U.G.J.M. van Dongan, A.A. van de Graff, S. Logemann, G. Muyzer, M.C.M. van Loosdrecht, J.G. Kuenen, The anaerobic oxidation of ammonium, FEMS Microbiol. Rev. 22 (1999) 421–437.
[43] Y.-H. Ahn, I.-S. Hwang, K.-S. Min, ANAMMOX and partial denitrification in anaerobic nitrogen removal from piggery waste, Water Sci. Technol. 49 (5–6) (2004) 145–153.
[44] X. Dong, E.W. Tollner, Evaluation of anammox and denitrification during anaerobic digestion of poultry manure, Bioresour. Technol. 86 (2003) 139–145.
[45] M. Strous, J.J. Heijnen, J.G. Kuenen, M.S.M. Jetten, The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium oxidizing microorganisms, Appl. Microbiol. Biotechnol. 50 (1998) 589–596.
[46] P.C. Sabumon, Anaerobic ammonia removal in presence of organic matter: a novel route, J. Hazard. Mater. 149 (2007) 49–59.
[47] G.A. El Hassan, R.M. Zablotowicz, D.D. Focht, Kinetics of denitrifying growth by fast-growing cowpea rhizobia, Appl. Environ. Microbiol. 49 (3) (1985) 517–521.
[48] R.E. Murray, L.L. Parsons, M.S. Smith, Aerobic and anaerobic growth of rifampin-resistant denitrifying bacteria in soil, Appl. Environ. Microbiol. 56 (2) (1990) 323–328.
[49] J.M. Tiedje, Ecology of denitrification and dissimilatory nitrate reduction to ammonia, in: A.J.B. Zehnder (Ed.), Biology of Anaerobic Microorganisms, Wiley, N.Y., 1990, pp. 179–244.
[50] J. van Rijn, Y. Tal, H.J. Schreier, Denitrification in recirculating systems: theory and applications, Aquaculture Eng. 34 (2006) 364–376.
[51] C.C. Wang, P.H. Lee, M. Kumar, Y.T. Huang, S. Sung, J.G. Lin, Simultaneous partial nitrification, anaerobic ammonium oxidation and denitrification (SNAD) in a full-scale landfill-leachate treatment plant, J. Hazard. Mater. (2009), doi:10.1016/j.jhazmat.2009.10.052.
[52] T. Dalsgaard, D.E. Canfield, J. Petersen, B. Thamdrup, J. Acuna-Gonzalez, N_2 production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica, Nature 422 (2003) 606–608.
[53] M. Trimmer, J.C. Nicholls, B. Deflandre, Anaerobic ammonim oxidation measured in sediments along the Thames estuary, United Kingdom, Appl. Environ. Microbiol. 69 (2003) 6447–6454.
[54] M.M. Jensen, B. Thamdrup, T. Dalsgaard, Effects of specific inhibitors on anammox and denitrification in marine sediments, Appl. Environ. Microbiol. 73 (1) (2007) 3151–3158.
[55] A.M. Molinder, J.J. Street, Decomposition of hydrazine in aqueous solutions, J. Environ. Qual. 18 (1989) 483–487.
[56] J.S.S. Damste, M. Strous, W.I. Rijpstra, E.C. Hopmans, J.A.J. Geenevasan, A.C.T. van Duin, L.A. van Niftrik, M.S.M. Jetten, Linearly concatenated cyclobutane lipids form a dense bacterial membrane, Nature 419 (2002) 708–712.
[57] D. Guven, A. Dapena, B. Kartal, M.C. Schmid, B. Maas, K. van de Pas-Schoonen, S. Sozen, R. mendez, H.J.H. Op den Camp, M.S.M. Jetten, M. Strous, I. Schmidt, Propionate oxidation by and methanol inhibition of anaerobic ammonium-oxidizing bacteria, Appl. Environ. Microbiol. 71 (2) (2005) 1066–1071.
[58] T. Dalsgaard, B. Thamdrup, Factors controlling anaerobic ammonium oxidation with nitrite in marine sediments, Appl. Environ. Microbiol. 68 (2002) 3802–3808.
[59] B. Wett, Solved upscaling problems for implementing deammonification of rejection water, Water Sci. Technol. 53 (12) (2006) 121–128.
[60] B.K. Pathak, F. Kazama, Y. Saiki, T. Sumino, Presence and activity of anammox and denitrification process in low ammonium-fed bioreactors, Bioresour. Technol. 98 (2007) 2201–2206.
[61] H. Siegrist, D. Salzgeber, J. Eugster, A. Joss, Anammox brings WWTP closer to energy autarky due to increased biogas production and reduced aeration energy for N-removal, Water Sci. Technol. 57 (3) (2008) 383–388.
[62] R.-C. Jin, B.-L. Hu, P. Zheng, M. Qaisar, A.-H. Hu, E. Islam, Quantitative comparison of stability of anammox process in different reactor configurations, Bioresour. Technol. 99 (2008) 1603–1609.
[63] N. Chamchoi, S. Nitisoravut, Technical note: anammox enrichment from different conventional sludges, Chemosphere 66 (2007) 2225–2232.
[64] D.D. Kieling, V. Reginatto, W. Schmidell, D. Travers, R.J. Menes, H.M. Soares, Sludge wash-out as strategy for anammox process start-up, Process Biochem. 42 (2007) 1579–1585.
[65] M.C. Schwartz, C. Woulds, G.L. Cowie, Sedimentary denitrification rates across the Arabian sea oxygen minimum zone, Deep-Sea Res. II 56 (2009) 324–332.
[66] B. Kartal, M. Koleva, R. Arsov, W. van der Star, M.S.M. Jetten, M. Strous, Adaptation of a freshwater anammox population to high salinity wastewater, J. Biotechnol. 126 (2006) 546–553.
[67] F.J. Cervantes, Environmental Technologies to Treat Nitrogen Pollution: Principles and Engineering, IWA Publishing, London, U.K., 2009.
[68] J.R. Vázquez-Padin, I. Fernández, M. Figueroa, A. Mosquera-Corral, J.L. Campos, R. Méndez, Applications of anammox based processes to treat anaerobic digester supernatant at room temperature, Bioresour. Technol. 100 (2009) 2988–2994.
[69] X. Hao, M.C.M. van Loosdrecht, Model-based evaluation of the influence of COD on a partial nitrification-Anammox biofilm (CANON) process, Water Sci. Technol. 49 (11–12) (2004) 83–90.
[70] J. Dosta, I. Fernández, J.R. Vázquez-Padin, A. Mosquera-Corral, J.L. Campos, J. Mata-Alvarez, R. Méndez, Short- and long-term effects of temperature on the anammox process, J. Hazard. Mater. 154 (2008) 688–693.

[71] G. Sun, D. Austin, Completely autotrophic nitrogen-removal over nitrite in lab-scale constructed wetlands: evidence from a mass balance study, Chemosphere 68 (2007) 1120–1128.
[72] N. Risgaard-Petersen, L.P. Nielsen, S. Rysgaard, T. Dalsgaard, R.L. Meyer, Application of the isotope pairing technique in sediments where anammox and denitrification coexist, Limnol. Oceanogr.: Methods 1 (2003) 63–73.
[73] B. Wett, Development and implementation of a robust deammonification process, Water Sci. Technol. 56 (7) (2007) 81–88.
[74] O. Lahav, I.B. Massada, D. Yackoubov, R. Zelikson, N. Mozes, Y. Tal, S. Tarre, Quantification of anammox activity in a denitrification reactor for a recirculating aquaculture system, Aquaculture 288 (2009) 76–82.
[75] P.C. Sabumon, Development of a novel process for anoxic ammonia removal with sulphidogenesis, Process Biochem. 43 (2008) 984–991.
[76] Z. Yang, S. Zhou, Y. Sun, Start-up of simultaneous removal of ammonium and sulfate from an anaerobic ammonium oxidation (anammox) process in an anaerobic up-flow bioreactor, J. Hazard. Mater. 169 (1–3) (2009) 113–118.
[77] C. Jing, Z. Ping, Q. Mahmood, Simultaneous sulfide and nitrate removal in anaerobic reactor under shock loading, Bioresour. Technol. 100 (2009) 3010–3014.