

Making a Progress to Speed up the Nitrification and Denitrification Processes in Novel Biosorption Activated Media: Can Archaea be in Concert with Anammox?

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Nitrification and Denitrification Processes

The transition of nitrogen from one phase to another is commonly referred to as the nitrogen cycle. Nutrients, such as ammonia (NH₃), nitrite (NO₂⁻) and nitrate (NO₃⁻), are common contaminants in water bodies that affect public health and ecosystem integrity with acute and chronic harmful outcome directly or indirectly. For example, without proper treatment, ammonia in the wastewater effluents can stimulate phytoplankton growth, exhibit toxicity to aquatic biota, and exert an oxygen demand in surface waters [1]. Undissociated ammonia is extremely volatile. Ionized ammonia is very toxic for fish species [2]. Fish mortality, health and reproduction can be affected by the presence of a minute amount of ammonia-N [3]. Nitrate can cause human health problems such as liver damage and even cancers [4,5]. Nitrate can also bind with hemoglobin and create a situation of oxygen deficiency in an infant's body called methemoglobinemia [6]. Nitrite can react with amines chemically or enzymatically to form nitrosamines that are very potent carcinogens (Sawyer et al., 2003). Hence, how to appropriately manage the nitrogen cycle in both natural systems and the built environment has been deemed one of the fourteen's grand challenges as shown in the Grand Challenges for Engineering published by the National Academy of Engineering in 2009 (NAE, 2009) [56].

There are various players in the nitrogen cycle and the diversity and functions of the microorganisms involved in nitrification and denitrification is quite complex [7]. The general metabolic path of historical assumptions is pretty limited from ammonia to nitrite, to nitrate, and to nitrogen Gas (N₂), which has been widely acknowledged. In the presence of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), ammonium is converted to nitrite and further to nitrate. These two reactions are collectively called nitrification. Denitrification, conversely, performed by denitrifying community, is an anaerobic respiration process using nitrate as a final electron acceptor and result in stepwise microbiological reduction of nitrate, nitrite, nitric oxide (NO), nitrous oxide (N₂O) and nitrogen gas. On the top of this understanding, it is becoming clear that denitrifying fungi, nitrifying archaea, anammox bacteria, aerobic denitrifying bacteria and heterotrophic nitrifying microorganisms are all key players together in the nitrogen cycle [7].

Quantitative measurements of these key players could be another big challenge. Real-time Polymerase Chain Reaction (PCR) was oftentimes used to determine the presence of these common nitrifiers and denitrifiers in the nitrification and denitrification processes. These common nitrifiers and denitrifiers include but are not limited to Ammonium monooxygenase (*amoA*), 16S rRNA Nitrospira sp. (*NSR*), and Nitrite reductase (*nirK*). Such quantification skill can be used to amplify *amoA* gene (ammonia monooxygenase gene) from AOB, *NSR* gene (nitrite reductase gene) from NOB in nitrification, and *nirK* gene (nitrite reductase gene) from nitrite reductase denitrifiers. The oligonucleotide sequences of the primers are shown in Table 1.

The Occurrence of Ammonia Oxidizing Archaea

The oxidation of ammonia plays a significant role in the transforma-

tion of fixed nitrogen in the global nitrogen cycle [8] and autotrophic nitrifying bacteria are providing new insights into their ecology and functions in soils [7]. Autotrophic ammonia oxidation is known in three groups of microorganisms, which convert ammonia into nitrite during nitrification. Recently, there are two significant discoveries for nitrogen cycle: 1) the occurrence of ammonia oxidizing archaea (AOA). In some environments, AOA dominate the ammonia-oxidizing community in the nitrification process. 2) The occurrence of anaerobic ammonia oxidation (anammox) reaction. In this biological process, nitrite and ammonium can be converted directly into dinitrogen gas: NH₄⁺ + NO₂⁻ → N₂ + 2 H₂O. Such a shortcut of nitrogen cycle has been proven to exist and can be portrayed holistically in Figure 1 in which the biogeochemical nitrogen network associated with possible microbial species and reaction pathways may be connected [9].

Archaeon is a newly defined domain of life as prokaryotes (bacteria, viruses) and eukaryotes (protozoa, fungi, plants, animals) form the rest two domains of life at the same time [10,11]. Although archaea have no true nucleus, they have complex gene control system using histones and RNA, just like plants and animals, and their cell wall has glycerol. It has been shown that AOA may be important to the global nitrogen cycle [10] and that archaeal ammonia oxidizers are more abundant in soils than their well-known bacterial counterparts [11]. Following the discovery of the archaeal *amoA* gene to be ubiquitous in the ocean, the study on AOA in the terrestrial environment from soil [12-14] to aquatic macrophyte [15] has become more compelling than before.

Ammonium monooxygenase (<i>amoA</i>)	amoA-1F GGGGTTTC-TACTGGTGGT amoA-2R CCCCTCKG-SAAAGCCTTCTTC	Rothhauwe et al. 1997
16S rRNA Nitrospira sp. (<i>NSR</i>)	NSR 1113F CCT-GCTTTCAGTTGCTACCG NSR 1264R GTTTG-CAGCGCTTTGTACCG	Dionisi et al. 2002
Nitrite reductase (<i>nirK</i>)	nirK 876 ATYGCGGG-VAYGGCGA	Braker et al. 1998

Table 1: The oligonucleotide sequences of the primers.

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Comparisons between AOA and AOB challenge the traditional assumption of the dominant role of AOB in nitrification [12,16-26]. In these previous AOA studies, AOA were detected using the primer set of amoA-23F (50-ATG GTC TGG CTW AGA CG-30)/amoA-616R (50-GCC ATC CAT CTG TAT GTC CA30), which targets the amoA gene of AOA [17]. In addition, the primer set PARCH519F (50-CAG CCG CCG CGG TAA-30)/ARCH915R-GC (50-GCclamp-GTG CTC CCC CGC CAA TTC CT-30) were used to target the 16S rRNA gene of archaea [27], including those that might be involved in ammonia oxidation. In general, the influence of soil pH on the diversity, abundance and transcriptional activity of AOA and AOB was examined [28]. It is indicative that archaeal amoA gene and transcript abundance decreased with increasing soil pH, while bacterial amoA gene abundance was generally lower and transcripts increased with increasing pH [28].

The Occurrence of Anammox

The anaerobic ammonium-oxidizing (anammox) process plays an important role in the nitrogen cycle of the worldwide anoxic and mesophilic habitats, such as marine nitrogen cycle. The ubiquitous presence of anammox bacteria in marine ecosystems has actually changed our knowledge of the global nitrogen cycle [29]. Anammox bacteria belonging to the phylum *Planctomycetes* are responsible for N removal through coupled NH₄⁺ oxidation and NO₂⁻ reduction simultaneously while depleting dissolved oxygen. For example, up to 50% of N₂ production in marine sediments and oxygen depleted zones may be attributed to anammox bacteria [29]. Because eutrophication of coastal bays and lakes contributes significantly to the formation of hypoxia zones, monitoring of the anammox bacterial community offers a unique opportunity for assessment of anthropogenic perturbations in the natural environments on one hand, and provides a special avenue to confirm functional nitrogen removal in wastewater and stormwater treatment on the other hand.

Environmental factors that shape sediment anammox bacterial communities were thoroughly discussed [30]. [31] investigated nitrogen removal performance using anaerobic ammonium oxidation at low temperatures. Yet, high concentration (5.31–39.2 mg L⁻¹) of ammonium was detected in the production water from these oilfields with temperatures between 55°C and 75°C [32]. The potential role of anammox and denitrifying methanotrophic bacteria in natural and artificial wetlands can be

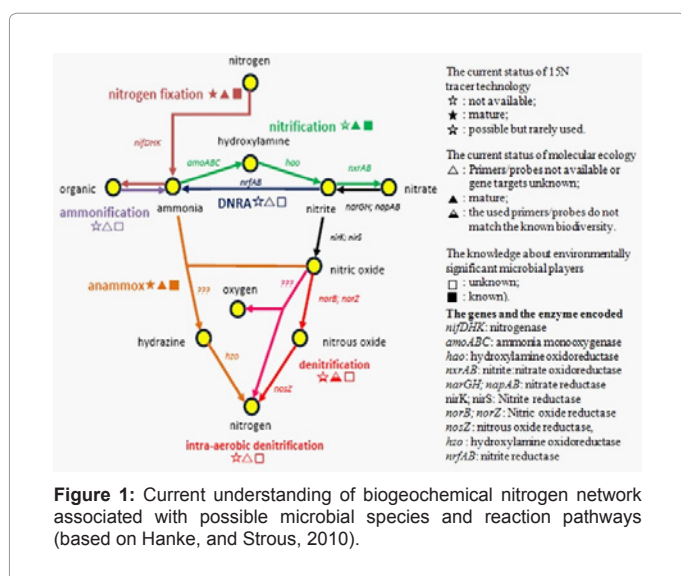
even linked together to account for global warming impact when both methane (CH₄) and nitrous oxides (N₂O) can be considered simultaneously [29].

It is known that the hydrazine oxidase gene was suggested as a proper genetic marker due to its high expression and ubiquitous presence in anammox bacteria [33]. The genus “*Ca. Scalindua*” comprised the apparent majority of active sediment anammox bacteria [30]. The previously recognized anammox bacterium, *Candidatus* Kuenenia stuttgartiensis, can be detected by using a Planctomycetes-specific 16S rRNA gene primer set [34]. In other words, the targeting of 16S rRNA genes may verify the presence of “*Candidatus Scalindua*,” albeit with a high microdiversity [30]. In laboratory-scale analysis, enrichment of anammox bacteria from marine environment can be carried out using a continuous culture system [35]. Tsushima, et al. (2007) [36] adopted a rotating disk reactor (RDR) biofilm in semi-batch cultures as a means to carry out such an enrichment of cultures and to quantify anammox by using real-time PCR. Phylogenetic analysis revealed that all the detected clones were related to the previously reported anammox bacteria, *Candidatus* Brocadia anammoxidans (AF375994), with 92% sequence similarity [36].

Bioenvironmental Engineering Applications

The presence of AOA appears to be dependent upon oxygen concentration and solids retention time (SRT) in a wastewater treatment process since all of the archaeal amoA positive sludge samples were collected from wastewater treatment processes operating at low DO concentrations for simultaneous nitrification and denitrification [37]. Besides, in aquaria and recirculating aquaculture systems, the accumulation of ammonia, an end product of protein metabolism in aquatic life, must be removed because of its toxicity to fish community. Urakawa et al. (2008) [18] started investigating the phylogenetic diversity of AOA and AOB in aquarium biofiltration systems to explore the nitrification of an engineered filtration media system. The findings imply that the phylogenetic diversity and species richness of AOA are greater than those of AOB and temperature is a key factor influencing the population structure and diversity of AOA and AOB in aquarium biofiltration systems. Even AOB can degrade halogenated compounds in the reclamation of wastewater [38]. Natural and artificial wetlands may be used as an ecological engineering unit to treat stormwater and wastewater. Anammox and anaerobic methane oxidation (ANME coupled to denitrification) with nitrite as electron acceptor are two of the most recent discoveries in the microbial nitrogen cycle of wetland ecosystem [29].

To improve the removal of ammonia and nitrite, development of high-rate anammox in biofilm reactors was established [36]. It is indicative that hydraulic retention time (HRT) and influent NH₄⁺ to NO₂⁻ molar ratio could be important determinant factors for efficient nitrogen removal [36]. A sequencing batch reactor (SBR) seeded with methanogenic granular sludge was started up to enrich anammox bacteria and to investigate the feasibility of granulation of anammox biomass [39]. NH₄⁺-N and NO₂⁻-N were removed simultaneously with higher speed and the maximum removal rates reached 14.6 g NH₄⁺-N / (m³ reactor -day) and 6.67 g NO₂⁻-N / (m³ reactor -day), respectively. Enrichment of anammox bacteria from marine environment was established to support the construction of a bioremediation reactor [35]. Quan et al. (2008) [40] examined the diversity of AOB in a granular sludge anammox reactor with high anaerobic ammonium oxidation activity (up to 0.8 kg NH₄⁺-N m⁻³ day⁻¹). The toxic effects of nitrite on these anammox bacteria were emphasized [41]. To characterize the role of anammox activity in sludge produced from wastewater treatment, ²⁹N₂ production in anaerobic batch reactor with N¹⁵-labeled inorganic nitrogen compounds was observed [42]. Nitrogen removal by co-occurring



methane oxidation, denitrification, aerobic ammonium oxidation, and anammox can be confirmed simultaneously [42]. Bae et al. (2010) [43] further investigated the distribution of anammox bacteria in five activated sludge samples collected in three types of domestic wastewater treatment plants including SBR, anoxic and oxic reactors of anaerobic–anoxic–oxic (A²O) process, and rotating biological contactor (RBC). The discovery of anammox bacteria in raw activated sludge samples provided a partial rationale for the utilization of activated sludge as a seeding source of the anammox process [43].

The bacterial diversity in an anammox reactor in different environmental conditions has received wide attention in research community since the late 2000s. Growth characteristic of anammox was fully investigated in an anaerobic biological filtrated reactor [44]. It was clearly demonstrated that nitrogen conversion rate was proportional to the population of anammox bacteria [44]. Pathak et al. (2007) [45] quantified anammox bacteria present in microbial communities in two laboratory-scale upflow anoxic reactors supplied with small amounts of ammonium (<3 mg L⁻¹) at low temperature. These reactors, operated at 20°C, were seeded with an immobilized microbial consortium (IMC) and anaerobic granules (AG) from an upflow anaerobic sludge blanket (UASB) treating brewery wastewater. However, the longer start-up period of the anammox process is due to the very low cellular yield and growth rates of anammox bacteria and nitrite inhibition is considered to be the key factor in the instability of the anammox process during the operation (Tang et al., 2009). The start-up and inhibition analysis with respect to the inhibitory effects of pH and free ammonia in the anammox process seeded with anaerobic granular sludge were further investigated (Tang et al., 2009). Besides, in a very recent testing, an anammox activity of the biomass in the reactor was operated for more than 500 days and the anammox activity of the biomass in the reactor reached 0.58 kg N_{total}/kg VSSd. The removal ratios of NO₂⁻-N to NH₄⁺-N in both reactor and activity tests were nearly 1 to 1 [34]. The AOB *Nitrosomonas europaea*–*eutropha* group, which is widely detected in oxygen-limited environments, was also found in this reactor.

More thorough review was also provided to illustrate the opportunities and challenges for managing nitrogen in the context of mechanisms and design enhancements for N uptake and denitrification through various stormwater control measures (SCMs) in urban stormwater management [46]. To nurture better engineering design for promoting various nitrification and denitrification processes in stormwater and wastewater treatment, the incorporation of Biosorption Activated Media (BAM) may promote the attraction of sorption surface between the nutrients in which the biofilm on the surface of BAM causes the nutrients to leave the aqueous solution and simply adhere to the sorption media [47–50]. From a bio-environmental systems point of view, “biosorption” is used generally to refer to sorption onto biomass or biofilm, i.e. an abiotic process. The media placed at the varying built environment or natural systems is functioning by combined sorption and biodegradation processes [51–54]. It is a worthwhile undertaking to further compare the abundances of AOA and AOB on BAM in which a mixed aerobic and anaerobic environment co-exist and co-vary, since AOA is less sensitive than that of AOB to the operation conditions. Given that AOA and AOB can be quantified by Real-time PCR analysis to track down the abundance and diversity of both species. This type of exploration is rewarding because BAM have been proven to have outstanding capacity to treat ammonia-rich wastewater in previous studies when anammox might already occur [36]. The extended efforts may be geared toward the examination as to whether there exists anammox in the BAM in an anaerobic condition that may be in concert with AOA and AOB.

Can AOA be in Concert with Anammox?

You et al. (2009) [55] summarized a suite of key research questions trying to clarify the role of AOA in N removal linked with wastewater treatment plants (WWTPs). They include: 1) What fraction of ammonia oxidation in WWTPs is due to AOA versus AOB? What are their activities? 2) Are there any nitrite-oxidizing archaea (NOA) similar to NOB involved in nitrification? 3) How do environmental factors change the AOA or AOB compositions of microbial communities and affect effluent water quality? 4) Are AOA also responsible for denitrification? 5) Will the bio-process models be modified to incorporate AOA in N removal?

However, the understanding of autotrophic ammonia oxidation must be elevated to a system level from microbial ecology point of view. AOB and AOA convert ammonia into nitrite during nitrification whereas anammox oxidize ammonia using nitrite as electron acceptor and producing atmospheric dinitrogen. It is believed that these two processes can be harmonized to some extent in order to gain the comparative advantage of nitrification and denitrification simultaneously through culture-independent approaches.

It is known that culture-independent approaches have contributed importantly to our understanding of the diversity and distribution of these microorganisms in the environment [8]. Yet, the isolation and cultivation of all three groups in the laboratory are quite problematic due to their slow growth rates, poor growth yields, unpredictable lag phases, and sensitivity to certain organic compounds [8]. Deepened molecular studies of ammonia oxidizers have to be focused for promoting their application in different environments. For instance, the study as to how to identify the optimal condition for anammox and track down the nitrogen removal through different pathways can be arranged as follows: 1) general metabolic path vs. AOA with a specific ratio of AOA and AOB, 2) general metabolic path vs. anammox, and 3) general metabolic path vs. AOA to be in concert with anammox. The comparison between general metabolic path and anammox in denitrification can be obtained by the measurements of the intermediate product (hydrazine vs. nitrous oxide) and bacteria quantification (anammox bacteria vs. denitrifying bacteria) using real time PCR.

These endeavour may be in connection to the use of BAM to investigate the following bioprocesses: 1) sorption properties retard movement of N; 2) physical and textural properties that may provide surfaces for biofilm formation and moisture retention, 3) the environments of saturated pores that become anoxic, providing conditions conducive for growth of denitrifiers, 4) the sustainability of BAM that is “self regenerating” as the sorbed and aqueous N is likely lost via combined nitrification/denitrification occurring as moisture content and O₂ levels vary naturally based on timing of infiltration, 5) the electron donor organic carbon (c) likely is supplied by infiltrating stormwater plus natural vegetative growth and C cycling in the shallow soil zone, or by wastewater inflow that carries the electron donor organic C, and 6) some measurable evidence of microbiological processes on the biofilm of the BAM may be monitored or probed with respect to these three groups of organisms, namely AOA, AOB, and anammox, which can co-exist and co-vary in a variety of engineered systems for N removal. Emphasis for engineering applications should be placed upon that engineering design for nitrogen removal relies on both biotic and abiotic processes associated with these three groups of organisms simultaneously.

References

1. Beutel MW (2006) Inhibition of ammonia release from anoxic profundal sediments in lakes using hypolimnetic oxygenation. *Ecological Engineering* 28: 271-279.

2. Tarazona JV, Munoz MJ, Ortiz JA, Nunez MO, Camargo JA (2008) Fish mortality due to acute ammonia exposure. *Aquaculture Research* 18: 167-172.
3. Servizi JA, Gordon RW (2005) Acute lethal toxicity of ammonia and suspended sediment mixtures to chinook salmon (*Oncorhynchus tshawytscha*). *Bulletin of Environmental Contamination and Toxicology* 44: 650-656.
4. Gabel B, Kozicki R, Lahl U, Podbielski A, Stachel B, et al. (1982) Pollution of drinking water with nitrate. *Chemosphere* 11: 1147-1154.
5. Huang CP, Wang HW, Chiu PC (1998) Nitrate reduction by metallic iron. *Water Research* 32: 2257-2264.
6. Kim-Shapiro DB, Gladwin MT, Patel RP, Hogg N (2005) The reaction between nitrite and hemoglobin: the role of nitrite in hemoglobin-mediated hypoxic vasodilation. *Journal of Inorganic Biochemistry* 99: 237-246.
7. Hayatsu M, Tago K, Saito M (2008) Various players in the nitrogen cycle: Diversity and functions of the microorganisms involved in nitrification and denitrification. *Soil Science and Plant Nutrition* 54: 33-45.
8. Junier P, Molina V, Dorador C, Hadas O, Kim OS (2010) Phylogenetic and functional marker genes to study ammonia-oxidizing microorganisms (AOM) in the environment. *Appl Microbiol Biotechnol* 85: 425-440.
9. Hanke A and Strous M (2010) Climate, fertilization, and the nitrogen cycle. *Journal of Cosmology* 8: 1838-1845.
10. Könneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, et al. (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* 437: 543-546.
11. Leininger S, Urlich T, Schloter M, Schwark L, Qi J, et al. (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* 442: 806-809.
12. He JZ, Shen JP, Zhang LM, Zhu YG, Zheng YM, et al. (2007) Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. *Environmental Microbiology* 9: 2364-2374.
13. Shen JP, Zhang LM, Zhu YG (2008) Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam. *Environmental Microbiology* 10: 1601-1611.
14. Chen XP, Zhu YG, Xia Y, Shen JP, He JZ (2008) Ammonia-oxidizing archaea: important players in paddy rhizosphere soil? *Environmental Microbiology* 10: 1978-1987.
15. Herrmann M, Saunders AM, Schramm A (2008) Archaea Dominate the Ammonia-Oxidizing Community in the Rhizosphere of the Freshwater Macrophyte *Littorella uniflora*. *Applied and Environmental Microbiology* 74: 3279-3283.
16. Boyle-Yarwood SA, Bottomley PJ, Myrold DD (2008) Community composition of ammonia-oxidizing bacteria and archaea in soils under stands of red alder and Douglas fir in Oregon. *Environmental Microbiology* 10: 2956-2965.
17. Sahan E, Muyzer G (2008) Diversity and spatio-temporal distribution of ammonia-oxidizing Archaea and Bacteria in sediments of the Westerschelde estuary. *Microbial Ecology* 64: 175-186.
18. Urakawa H, Tajima Y, Numata Y, Tsuneda S (2008) Low temperature decreases the phylogenetic diversity of ammonia-oxidizing archaea and bacteria in aquarium biofiltration systems. *Applied and Environmental Microbiology* 74: 894-900.
19. Moin NS, Nelson KA, Bush A, Bernhard AE (2009) Distribution and diversity of Archaeal and bacterial ammonia oxidizers in salt marsh sediments. *Applied and Environmental Microbiology* 75: 7461-7468.
20. Jiang H, Dong H, Yu B, Lv G, Deng S, et al. (2009) Diversity and Abundance of Ammonia-Oxidizing Archaea and Bacteria in Qinghai Lake, Northwestern China. *Geomicrobiology Journal* 26: 199-211.
21. Schauss K, Focks A, Leininger S, Kotzerke A, Heuer H, et al. (2009) Dynamics and functional relevance of ammonia-oxidizing archaea in two agricultural soils. *Environmental Microbiology* 11: 446-456.
22. Di H J, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, et al. (2010) Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. *Microbial Ecology* 72: 386-394.
23. Jin T, Zhang T, Yan QM (2010) Characterization and quantification of ammonia-oxidizing archaea (AOA) and bacteria (AOB) in a nitrogen-removing reactor using T-RFLP and qPCR. *Applied and Environmental Microbiology* 77: 1167-1176.
24. Morimoto S, Hayatsu M, Hoshino YT, Nagaoka K, Yamazaki M, et al. (2011) Quantitative analyses of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in fields with different soil types. *Microbes Environ.*
25. Li M, Cao H, Hong Y, Gu JD (2011) Spatial distribution and abundances of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in mangrove sediments. *Appl Microbiol Biotechnol* 89: 1243-1254.
26. Yapsakli K, Aliyazicioglu C, Mertoglu B (2011) Identification and quantitative evaluation of nitrogen-converting organisms in a full-scale leachate treatment. *Journal of Environmental Management* 92: 714-723.
27. Coolen MJL, Hopmans EC, Rijpstra WIC, Muyzer G, Schouten S, et al. (2004). Evolution of the methane cycle in Ace Lake (Antarctica) during the Holocene: response of methanogens and methanotrophs to environmental change. *Organic Geochemistry* 35: 1151-1167.
28. Nicol GW, Leininger S, Schleper C, Prosser JI (2008) The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environmental Microbiology* 10: 2966-2978.
29. Zhu G, Jettten MSM, Kusch P, Ettwig KF, Yin C (2010) Potential roles of anaerobic ammonium and methane oxidation in the nitrogen cycle of wetland ecosystems. *Appl Microbiol Biotechnol* 86: 1043-1055.
30. Dang H, Chen R, Wang L, Guo L, Chen P, et al. (2010) Environmental factors shape sediment anammox bacterial communities in hypereutrophic Jiaozhou Bay, China. *Applied and Environmental Microbiology* 76: 7036-7047.
31. Isaka K, Date Y, Kimura Y, Sumino T, Tsuneda S (2008) Nitrogen removal performance using anaerobic ammonium oxidation at low temperatures. *Microbiol Lett* 282: 32-38.
32. Li H, Chen S, Mu BZ, Gu JD (2010) Molecular detection of anaerobic ammonium-oxidizing (Anammox) bacteria in high-temperature petroleum reservoirs. *Microb Ecol* 60: 771-783.
33. Hirsch MD, Long ZT, Song B (2011) Anammox bacterial diversity in various aquatic ecosystems based on the detection of hydrazine oxidase genes (*hzxA/hzoB*). *Microb Ecol* 61: 264-276.
34. Li X R, Du B, Fu HX, Wang RF, Shi JH, et al. (2009) The bacterial diversity in an anaerobic ammonium-oxidizing (anammox) reactor community. *Systematic and Applied Microbiology* 32: 278-289.
35. Nakajima J, Sakka M, Kimura T, Furukawa K, Sakka K (2008) Enrichment of anammox bacteria from marine environment for the construction of a bioremediation reactor. *Applied Microbiol Biotechnol* 77: 1159-1166.
36. Tsushima I, Kandaichi T, Okabe S (2007) Quantification of anaerobic ammonium-oxidizing bacteria in enrichment cultures by real-time PCR. *Water Research* 41: 785-794.
37. Park HD, Wells GF, Bae H, Criddle CS, Francis CA (2006) Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. *Appl. Environ. Microbiol* 72: 5643-5647.
38. Sayavedra-Soto LA, Gvakharia B, Bottomley PJ, Arp DJ, Dolan ME (2010) Nitrification and degradation of halogenated hydrocarbons—a tenuous balance for ammonia-oxidizing bacteria. *Appl Microbiol Biotechnol* 86: 435-444.
39. Liao D, Li X, Yang Q, Zhao Z, Zeng G (2007) Enrichment and granulation of Anammox biomass started up with methanogenic granular sludge. *World Journal of Microbiology and Biotechnology* 23: 1015-1020.
40. Quan ZX, Rhee SK, Zuo JE, Yang Y, Bae JW, et al. (2008) Diversity of ammonium-oxidizing bacteria in a granular sludge anaerobic ammonium-oxidizing (anammox) reactor. *Environmental Microbiology* 10: 3130-3139.
41. Kimura Y, Isaka K, Kazama F, Sumino T (2010) Effects of nitrite inhibition on anaerobic ammonium oxidation. *Appl Microbiol Biotechnol* 86: 359-365.
42. Waki M, Yasuda T, Yokoyama H, Hanajima D, Ogino A, et al. (2009) Nitrogen removal by co-occurring methane oxidation, denitrification, aerobic ammonium oxidation, and anammox. *Applied Microbiol Biotechnol* 84: 977-985.
43. Bae H, Park KS, Chung YC, Jung JY (2010) Distribution of anammox bacteria in domestic WWTPs and their enrichments evaluated by real-time quantitative PCR. *Process Biochemistry* 45: 323-334.
44. Isaka K, Date Y, Sumino T, Yoshie S, Tsuneda S (2006) Growth characteristic of anaerobic ammonium-oxidizing bacteria in an anaerobic biological filtered reactor. *Appl Microbiol Biotechnol* 70: 47-52.

45. Pathak BK, Kazama F, Tanaka Y, Mori K, Sumino T (2007) Quantification of anammox populations enriched in an immobilized microbial consortium with low levels of ammonium nitrogen and at low temperature. Appl Microbiol Biotechnol 76: 1173-1179.
46. Collins KA, Lawrence TJ, Standerc EK, Jontoso RJ, Kaushale SS, et al. (2010) Opportunities and challenges for managing nitrogen in urban stormwater: a review and synthesis. Ecological Engineering 36: 1507-1519.
47. Xuan Z, Chang NB, Wanielista M, Hossain F (2010) Laboratory-scale characterization of the green sorption medium for on-site Sewage treatment and Disposal to improve Nutrient removal. Environmental Engineering Science 27: 301-312.
48. Hossain F, Chang NB, Wanielista M (2010) Modeling kinetics and isotherms of functionalized filter media for nutrient removal from stormwater dry ponds. Environmental Progress and Sustainable Energy 29: 319-333.
49. Chang NB, Hossain F, Wanielista M (2010) Filter media for nutrient removal in natural systems and built environments: (I)- previous trends and perspectives. Environmental Engineering Science 27: 689-706.
50. Chang NB, Wanielista M, Ammarin Daranpob (2010) Filter media for nutrient removal in natural systems and Built Environments: (II)-Design and application challenges. Environmental Engineering Science 27: 707-720.
51. Xuan ZM, Chang NB, Ammarin Daranpob, Wanielista M (2009) Initial test of a subsurface constructed wetland with green sorption media for nutrient removal in on-site wastewater treatment systems. Journal of Water Quality, Exposure and Health 1: 159-169.
52. Hossain F, Chang NB, Wanielista M, Xuan ZM, Makkeasorn A (2009) Nitrification and denitrification effect in a passive on-site wastewater treatment system with a recirculation filtration tank. Journal of Water Quality, Exposure and Health 1: 31-46.
53. Chang NB, Xuan ZM, Daranpob A, Wanielista M (2010) A subsurface upflow wetland system for nutrient and pathogen removal in on-site sewage treatment and disposal systems. Environmental Engineering Science 27: 707-720.
54. Chang NB, Wanielista M, Daranpob A, Hossain F, Xuan Z (2010) New performance-based passive septic tank underground drainfield for nutrient and pathogen removal using sorption medium. Environmental Engineering Science 27: 469-482.
55. You J, Das A, Dolan EM, Hu Z (2009) Ammonia-oxidizing archaea involved in nitrogen removal. Water Research 43: 1801-1809.
56. NationalAcademy of Engineering (NAE) (2009) Grand Challenges for Engineering.
57. Braker G, Fesefeldt A, Witzel KP (1998) Development of PCR primer systems for amplification of nitrite reductase genes (nirK and nirS) to detect denitrifying bacteria in environmental samples. Applied and Environmental Microbiology 64: 3769-3775.
58. Dionisi HM, Layton AC, Harms G, Gregory IR, Robinson KG, et al. (2002) Quantification of Nitrosomonas oligotropha-like ammonia-oxidizing bacteria and Nitrospira spp. from full-scale wastewater treatment plants by competitive PCR. Applied and Environmental Microbiology 68: 245-253.
59. Rotthauwe JH, Boer W, Liesack W (1997) The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine scale analyses of natural ammonia oxidizing populations. Applied and Environmental Microbiology 63: 4704-4712.
60. Ryan P, Wanielista M, Chang NB (2010) Nutrient reduction in storm water Pond Discharge using a chamber upflow filter and skimmer(CUFS). Water, Air and Soil Pollution 208: 385-399.