กลุ่มประชากรแอมโมเนียออกซิไดซ์ซึ่งแบคทีเรียและอาเคียในระบบบำบัดน้ำเสียในประเทศไทย

นางสาวพันธุ์ทิพา สนธิพันธ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาการจัดการสิ่งแวดล้อม (สหสาขาวิชา)
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2551

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

COMMUNNITIES OF AMMONIA-OXIDIZING BACTERIA AND ARCHAEA IN FULL-SCALE WASTEWATER TREATMENT PLANTS IN THAILAND

Miss Puntipar Sonthiphand

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Environmental Management
(Interdisciplinary Program)
Graduate School
Chulalongkorn University
Academic Year 2008
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Thesis Title	COMMUNITIES OF AMMONIA-OXIDIZING BACTERIA AND ARCHAEA IN FULL-SCALE WASTEWATER TREATMENT PLANTS IN THAILAND
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Field of Study	Environmental Management
Advisor	Tawan Limpiyakorn, Ph.D.

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พันธุ์ทิพา สนธิพันธ์: กลุ่มประชากรแอมโมเนียออกซิไดซ์ซิ่งแบคทีเรียและอาเคียในระบบบำบัดน้ำเสียในประเทศไทย. (COMMUNITIES OF AMMONIA-OXIDIZING BACTERIA AND ARCHAEA IN FULL-SCALE WASTEWATER TREATMENT PLANTS IN THAILAND) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : อ. ดร.ตะวัน ลิมปิยากร, 105 หน้า.

จากความเชื่อในอดีตที่ว่าแอมโมเนียออกซิไดซ์ซิ่งแบคทีเรีย (AOB) เป็นจุลชีพที่มีบทบาทหลักในการออกซิไดซ์แอมโมเนียไปเป็นไนไตรท์ ในปัจจุบันมีการค้นพบว่านอกจากแบคทีเรียแล้วจุลชีพกลุ่มอาเคีย (Archaea) ก็สามารถออกซิไดซ์แอมโมเนียไปเป็นไนไตรท์ได้ จากหลักฐานการมีอยู่ของยีน *amo*A ของแอมโมเนียออกซิไดซ์ซิ่งอาเคีย (AOA) ในทะเลและดิน นอกจากนี้ยังมีการแยกอาเคียจากทะเลได้เป็นครั้งแรกชื่อ *Nitrosopumilus maritimus* และจากเทคนิค real-time PCR พบว่า มีปริมาณยีน *amo*A ของอาเคียมากกว่าแบคทีเรีย จึงเกิดคำถามว่า AOA เป็นจุลชีพหลักในการออกซิไดซ์แอมโมเนียไปเป็นไนไตรท์หรือไม่ งานวิจัยนี้มุ่งศึกษากลุ่มประชากร AOB และ AOA ในระบบบำบัดน้ำเสีย 10 แห่ง (โรงบำบัดน้ำเสียอุตสาหกรรม 4 แห่ง และโรงบำบัดน้ำเสียชุมชน 6 แห่ง) ที่มีลักษณะน้ำเสีย, องค์ประกอบของระบบ, และการควบคุมระบบที่แตกต่างกัน นอกจากนี้ยังศึกษาผลกระทบของปริมาณแอมโมเนียต่อกลุ่มประชากร AOB และ AOA ในไนตริไฟอิงแอกทิเวเต็ดสลัดจ์ (NAS) โดยนำเชื้อจากระบบบำบัดน้ำเสียชุมชนมาเลี้ยงในถังปฏิกรณ์ 3 ถัง โดยอาหารอนินทรีย์ที่มีปริมาณแอมโมเนียต่างๆกัน 2mM, 10mM, และ 30 mM NH^{+}_{4}-N (NAS2, NAS10, และ NAS30 ตามลำดับ) ทำการศึกษากลุ่มประชากร AOB โดยใช้เทคนิค specific PCR amplification ตามด้วย DGGE และอ่านลำดับรหัสพันธุกรรมของ AOB 16S rRNA ยีน ส่วนกลุ่มประชากร AOA ทำการศึกษาโดยใช้เทคนิค specific PCR amplification ตามด้วย clone libraries และอ่านลำดับรหัสพันธุกรรมของ AOA *amoA* ยีน จากผลการทดลองพบว่ากลุ่มประชากร AOB ในระบบบำบัดน้ำเสียอุตสาหกรรมมีความหลากหลายมากกว่าในระบบบำบัดน้ำเสียชุมชน AOB ที่พบในระบบบำบัดน้ำเสียอุตสาหกรรมตกอยู่ใน 4 คลัสเตอร์ ดังนี้ unknown *Nitrosomonas* cluster, *N. europaea-Nc. mobilis* cluster, *N. communis* cluster, และ *N. oligotropha* cluster ส่วน AOB ที่พบในระบบบำบัดน้ำเสียชุมชนตกอยู่ใน *N. communis* cluster และ *N. oligotropha* cluster จะเห็นว่าความแตกต่างของลักษณะน้ำเสีย (ความเข้มข้นของแอมโมเนีย) อาจเป็นปัจจัยหลักที่ทำให้เกิดรูปแบบการกระจายของกลุ่มประชากร AOB ในระบบบำบัดน้ำเสียทั้ง 2 แบบ ระบบบำบัดน้ำเสียชุมชนเกือบทั้งหมดเป็นแอคติเวเต็ดสลัดจ์ (activated sludge process) ยกเว้นเพียงหนึ่งโรงบำบัดเป็นบ่อเติมอากาศ (aerated lagoon system) พบว่ากลุ่มประชากร AOB จากระบบบำบัดน้ำเสียชุมชนทุกแห่งเหมือนกัน จะเห็นว่าองค์ประกอบและการควบคุมระบบมีความสำคัญน้อยกว่าลักษณะน้ำเสีย ในส่วนของ AOA สามารถแยกได้ 72 โคลน จากแปดในสิบระบบบำบัดน้ำเสีย และสามารถแบ่งได้เป็น 38 OTUs กระจายใน 12 คลัสเตอร์ สิ่งที่ต่างจาก AOB คือ กลุ่มประชากร AOA ในระบบบำบัดน้ำเสียอุตสาหกรรมมีความหลากหลายน้อยกว่าในระบบบำบัดน้ำเสียชุมชน จากระบบบำบัดน้ำเสียอุตสาหกรรมพบ AOA จาก 2 แห่ง เท่านั้น คือ จากระบบบำบัดน้ำเสียอุตสาหกรรมที่รับภาระแอมโมเนียปานกลาง (40 -70 mg-N/l) พบ AOA อยู่ใน คลัสเตอร์ G และ K แต่ไม่พบ AOA ในระบบบำบัดน้ำเสียอุตสาหกรรมที่รับภาระแอมโมเนียต่ำ (13 mg-N/l) และสูง (422 mg-N/l) ในทางตรงกันข้ามพบ AOA จากระบบบำบัดน้ำเสียชุมชนทั้งหกแห่งซึ่งรับภาระแอมโมเนียต่ำ (5 – 13 mg-N/l) พบ AOA อยู่ใน คลัสเตอร์ A, B, C, E, F, G, I, J, K, L, M, และ N นอกจากนี้ยังพบว่าจากตัวอย่างที่มาจากระบบบำบัดแบบแอคติเวเต็ดสลัดจ์ AOA จะกระจายอยู่ใน 1 – 3 คลัสเตอร์ แต่ตัวอย่างที่มาจากบ่อเติมอากาศพบว่า AOA จะกระจายอยู่ใน 6 คลัสเตอร์ ดังนั้นในกรณีของ AOA องค์ประกอบและการควบคุมระบบมีผลอย่างมีนัยสำคัญต่อการกระจายของกลุ่มประชากร AOA จากการนำสลัดจ์จากระบบบำบัดน้ำเสียชุมชนมาเพาะเลี้ยงใน NAS พบว่า กลุ่มประชากร AOB มีการเปลี่ยนแปลงอย่างมีนัยสำคัญ กลุ่มประชากร AOB จากสลัดจ์ตกอยู่ในกลุ่ม *N. communis* cluster และ *N. oligotropha* cluster หลังจากเลี้ยงในหลายความเข้มข้นแอมโมเนีย *N. communis* cluster หายไปจากทุก NAS พบ AOB ที่มี affinity ต่อแอมโมเนียสูงใน NAS 2 (*N. oligotropha* cluster), พบ AOB ที่มี affinity ต่อแอมโมเนียต่ำใน NAS 30 (*N. europaea* cluster), และพบ AOB ทั้งสองชนิดใน NAS 10 (unknown *Nitrosomonas* cluster and *N. europaea* cluster) ตรงข้ามกับ AOB เกือบทุกโคลนของ AOA จาก NAS 2 (19 จาก 21), NAS10 (23 จาก 26), และ NAS 30 (30 จาก 30) ตกในคลัสเตอร์ K อีกทั้งกลุ่มประชากร AOA ใน NAS 2 และ NAS10 มีความหลากหลายมากกว่าใน NAS 30 ชี้ให้เห็นว่าปริมาณแอมโมเนียมีผลอย่างมีนัยสำคัญต่อกลุ่มประชากร AOB แต่ไม่มีผลอย่างมีนัยสำคัญต่อกลุ่มประชากร AOA ในทุก NAS

สาขาวิชา การจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต พันธุ์ทิพา สนธิพันธ์

ปีการศึกษา 2551 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก ตะวัน

#508 75295 20: MAJOR ENRONMENTAL MANAGEMENT
KEYWORDS : AMMONIA-OXIDIZING BACTERIA/ AMMONIA-OXIDIZING ARCHAEA/ *amoA* GENE/ 16S rRNA GENE/ MICROBIAL COMMUNITY/ WASTEWATER TREATMENT PLANT

PUNTIPAR SONTHIPHAND: COMMUNITIES OF AMONIA-OXIDIZING BACTERIA AND ARCHAEA IN FULL-SCALE WASTEWATER TREATMENT PLANTS IN THAILAND. ADVISOR: TAWAN LIMPIYAKORN, PH.D , 105 PP.

For several decades, it had been believed that ammonia-oxidizing bacteria (AOB) is the only microorganisms that are responsible for the oxidation of ammonia to nitrite in the global nitrogen cycle. Recently, a few evidences have shown that autotrophic ammonia oxidation is not only restricted to the domain *Bacteria*, but also the domain *Archaea*. The presence of an ammonia monooxygenase (*amo*A) genes of ammonia-oxidizing archaea (AOA) has been revealed in a few marine and soil environments; in addition, the first AOA, *Nitrosopumilus maritimus,* has been isolated from sea water. Abundances of *amoA* genes of AOA over those of AOB have been found in few studies using quantitative real-time PCR technique. As results, AOA is questionable of the major environmental microorganisms that oxidize ammonia to nitrite. In this study, communities of AOB and AOA in ten full-scale wastewater treatment plants (WWTPs) (four industrial WWTPs and six municipal WWTPs) that are different in influent characteristics, system configuration and system operation were observed. In addition, effect of ammonium loads on AOB and AOA communities was investigated by enriching sludge taken from a municipal WWTP with inorganic medium containing different ammonium concentrations of 2, 10, and 30 mM NH^{+}_{4}-N (NAS2, NAS10, and NAS30, respectively). The communities of AOB and AOA were analyzed using specific PCR amplification followed by DGGE and sequencing of AOB 16S rRNA genes and specific PCR simplification followed by clone libraries and sequencing of AOA *amoA* genes, respectively. Results from full-scale WWTPs showed that AOB communities in four industrial WWTPs were more diverse than those in the six municipal WWTPs. AOB found in industrial WWTPs fell into 4 clusters that are unknown *Nitrosomonas* cluster, *N. europaea-Nc. mobilis* cluster, *N. communis* cluster, and *N. oligotropha* cluster, while AOB found in municipal WWTPs were restricted to only *N. communis* cluster and *N. oligotropha* cluster. The difference of influent wastewater characteristic (ammonium concentration) might be the key factor causing distinct distribution patterns of AOB communities in both types of WWTPs. Almost all municipal WWTPs in this study were activated sludge processes with exception for one plant that was aerated lagoon system. The communities of AOB in all municipal WWTPs were similar. As results, system configuration and system operation were less significant than influent wastewater characteristics. A total 72 AOA *amo*A sequences, recovered from eight out of ten WWTPs, could be categorized into 38 OTUs, and distributed in 12 clusters. Unlike AOB, communities of AOA in the industrial WWTPs were less diverse than those in the municipal WWTPs. Only 2 industrial WWTPs with moderate ammonium loads (40 -70 mg-N/l), contained AOA (clusters G and K). Negative PCR amplification occurred with another two industrial WWTPs with low (13 mg-N/l) and high (422 mg-N/l) ammonium loads. In contrast, all six municipal WWTPs, that received low ammonium loads of 5 – 13 mg-N/l, possessed AOA (cluster A, B, C, E, F, G, I, J, K, L, M, and N). Five out of the six municipal WWTPs were activated sludge processes, whereas another was an aerated lagoon system. For each sample of activated sludge processes, only 1 – 3 clusters of AOA were observed, while in the sample from an aerated lagoon system much more AOA clusters of 6 were found. Therefore, system configuration and system operation might be significant for the distribution of AOA in WWTPs. Results from enriched NASs suggested that AOB communities obviously shifted from seed sludge to enriched NASs and in each enriched NAS, communities of AOB varied particularly. Sludge taken from a municipal WWTP used as a seed for all reactors contained members of *N. communis* cluster and *N. oligotropha* cluster. After it was enriched under various ammonium loads, members of *N. communis* cluster disappeared from all enriched NASs. AOB with high affinity to ammonia presented in NAS 2 (*N. oligotropha* cluster), AOB with low affinity to ammonia presented in NAS 30 (*N. europaea* cluster), and both types of AOB survived in NAS 10 (unknown *Nitrosomonas* cluster and *N. europaea* cluster). In contrast to AOB, almost all AOA clones from NAS2 (19 out of 21), NAS10 (23 out of 26), and NAS30 (30 out of 30) fell in the same cluster (cluster K). AOA communities in NAS2 and NAS10 were more diverse than those of NAS30. These demonstrated that ammonium load significantly influenced AOB communities, but not AOA communities in enriched NASs.

Field of Study : Environmental Management Student's Signature : พันธุ์ทิพา สนธิพันธ์

Academic Year : 2008 Advisor's Signature :

ACKNOWLDGEMENTS

I do wish to express my graceful appreciation to Dr. Tawan Limpiyakorn who is my thesis advisor for his guidance and support during the entire of my study and for his time, patience and suggestions and comments, I am deeply indebted.

Moreover, I would like to express my appreciation and sincere gratitude to Dr. Manaskorn Rachakornkij who is a chairman of my thesis committee, Assistant Professor Ekawan Luepromchai, Associate Professor Alissara Reungsang, and Dr. Sorawit Powtongsook who are committee members for their suggestions and recommendations throughout my research work.

In addition, I would like to thank the National Center of Excellence for Environmental and Hazardous Waste Management (NCE-EHWM) for the full scholarship, funding and supporting facilities for my study.

I also would like to thank the Bangkok Metropolitan Administration (BMA) for providing municipal wastewater samples and their information for this study.

Special thanks to Miss Chantana Intim who is a Laboratory supervisor and all staffs at NCE-EHWM program for their helps especially in the analytical works.

I thank to my fellow all students at NCE-EHWM for making the time enjoyable in the lab.

Last but definite not least, I would like to express my deep appreciation to my family. They always support and encourage in any time.

CONTENTS

Page

Page

Page

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

CHAPTER I

INTRODUCTION

1.1 Background and motivation

Discharge of improper treated wastewater into natural receiving waters can cause negative impacts on humans, aquatic lives or even natural microorganisms. Nitrogens at sufficiently high levels in water can be toxic to fish and other aquatic life, as well as contribute to eutrophication, creating large amounts of oxygen demand depleting oxygen level in natural receiving waters (Hall, 1986; Painter, 1986). Other than the common pollutants of concerns (organic, nitrogen, and phosphorous), discharging micropollutants from industrial and municipal wastewater treatment plants (WWTPs) into the water bodies is responsible for severe direct adverse effects such as genetic and developmental abnormalities in humans, aquatic life, and natural microorganisms (Taewoo Yi and Willie F., 2007). Micropollutants are the chemical or biochemical pollutants which are present in very small amounts in water but likely enough to impact human, aquatic life, and natural microorganisms. Examples of micropollutants are chlorinated organic compounds (tricholoethylene (TCE), polychlorobiphenyls (PCB)), and endocrine disrupting compounds (estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethynylestradiol (EE2)). A multitude of substances have been shown to endocrine disrupt disturbing the hormonal systems of human and aquatic organisms. Among these, natural estrogens, i.e., E1, E2, and E3 as well as synthetic estrogens, i.e., EE2 are effective at nanograms levels (Routledge *et al*.,1998 and Purdom *et al*., 1994). A laboratory study on the endocrine disrupting potency of EE2 demonstrated that EE2 at low concentrations of 1–10 ngL^{-1} caused estrogenic response in caged fish (Purdom *et al*., 1994). Although the amounts of estrogens detected in treated wastewater is at nanogram levels, studies revealed that the presence of estrogens in treated wastewater is responsible for the feminization of male fish and sexual disruption in many aquatic wildlife (IUPAC, 2003).

Nitrification is a key process in the removal of nitrogens from WWTPs. It is the microbiological activity by which ammonia (NH_3) is oxidized to nitrite (NO_2^-) by ammonia-oxidizing bacteria (AOB) and nitrite is subsequently oxidized to nitrate (NO_3^-) by nitrite-oxidizing bacteria (NOB).

Beside ammonia, AOB probably play roles in removing a few persistant micropollutants in WWTPs. AOB is capable of degrading organic pollutants via cometabolism during ammonia oxidation. There are some evidences indicating that efficiencies of estrogen removal related to operational parameter of WWTPs (Carballa *et al*., 2004; Clara *et al*., 2005). The positive influence of long sludge retention times (SRTs) on nitrification in activated sludge systems has been also associated with increasing in the estrogen removals. Kreuzinger *et al*. (2004) have observed that with the increase in SRT, the biodegradation of estrogens increased. Clara *et al*. (2005) showed that WWTPs with nitrogen removal also effectively removed micropollutants (including estrogens). Shi *et al*. (2004) has showed that *Nitrosomonas europaea* is capable of oxidizing E1, E2, E3 and EE2 at 200 mg/L of estrogens added in the presence of ammonia. On the other hand, it has been discovered that chemicals present in low concentrations (lower than mg/L range) may show quite different biodegradation behavior than they typically do at high concentrations (mg/L range). Moreover, it appears that biodegradation often is favored at low concentrations, and in such cases, co-metabolic degradation mechanisms may dominate (Alexander, 1985). In fact, clear evidence of co-metabolism is still needed to come up with the conclusion.

Activated sludge is the most common process in full-scale WWTPs in Thailand due to its low operational cost with high performance. However, nitrification failure in activated sludge occurs so frequently. This is because AOB are very sensitive to environment factors; for instance, inappropriate HRT or SRT results in washing out of nitrifying bacteria from WWTPs. When this circumstance occurs, It takes very long period to recovery them in the system owing to their slow growth rates, their inability to compete heterotrophs. Nevertheless, almost all of the troubles in function or performance are related to the changes of microbial community structures in WWTPs (Liu Xin-chun *et al*., 2007). Consequently, a thorough knowledge of the ecology and microbiology communities of AOB is required to link

between system configuration, system operation and stability of nitrification process in system performance.

The ecology and microbiology of AOB have been suggested to differ among distinct species. The distribution patterns of distinct AOB in the environments reflect the physiological properties of AOB isolates observed in the laboratory (Koops and Pommerening-Roser, 2001). Among several factors, ammonia, the essential energy source, is the most important factor affecting the AOB communities in the environments. AOB have been reported on their presence in several environments such as freshwater, salt lake, acidic soil, and wastewater treatment plant, etc. The capability in adapting themselves to different ammonia concentrations in the diverse environments, results from the difference in affinity constants for ammonia in distinct AOB species (Suwa *et al*., 1994, 1997; Stehr *et al*., 1995a; Koops and Pommerening-Röser, 2001). Members of the *Nitrosomonas oligotropha* cluster, *Nitrosomonas communis* cluster, and the *Nitrosospira* cluster are the most common AOB found in the systems with low ammonium loads (Schramm *et al*., 1998; Dionisi *et al*., 2002; Harms *et al*., 2003; Limpiyakorn *et al*., 2005, 2006b), whereas members of the *Nitrosomonas europaea–Nitrosococcus mobilis* cluster are found in the systems with high ammonium loads (Juretschko *et al*., 1998; Wagner *et al*., 1998).

In the year 2004, it was first time revealed that autotrophic ammonia oxidation is not only restricted to the domain *Bacteria*, but also the domain *Archaea*. Venter *et al.* (2004) found the presence of an ammonia monooxygenase gene (*amo*A)–like gene on an archaeal-associated scaffold and indicated the potential role of archaea in nitrification process in the ocean. Then Treusch *et al.* (2005) discovered gene that potentially encode ammonia monooxygenase (AMO), a key enzyme in ammonia oxidation. The ultimate confirmation of ammonia-oxidizing archaea (AOA) activity was achieved by cultivation of a mesophilic crenarchaeote (Konneke *et al*., 2005). Like AOB, AOA grows chemolithoautotrophically by oxidizing ammonia to nitrite under mesophilic conditions. In addition, it contains putative genes for all three subunits (*amoA*, *amoB*, and *amoC*) of AMO (Konneke *et al*., 2005). In 2006, Park *et al*. clearly demonstrated the presence of molecular markers of AOA, suggesting an archaeal *amoA* cluster D that might be widespread in activated sludge bioreactors. However, this study was not designed to rigorously identify factors controlling AOA

diversity and community composition. So far, physiological properties information on AOA is not yet discovered. Only rough information could be obtained from few molecular evidences from natural environments. It has been reported that bioavailability of nitrogen and carbon, oxygen, salinity, pH, and especially ammonia concentration affect distribution pattern and communities of AOA in the environments (Konneke *et al*., 2005; Francis *et al*., 2005; Leininger *et al*., 2006; Coolen *et al*., 2007). The maximum growth rate of Crenarchaeota in culture, containing 500 μM ammonium, was 0.78 d^{-1}. While ammonium typically reaches concentrations of < 0.03–1μM in the open ocean and < 0.03–100 μM in coastal waters. The maximum growth rate of Crenarchaeota in nature was vary between 0.05 and 0.3 d^{-1}(Konneke *et al*., 2005). Moreover, the archaeal amoA libraries from the site, which is highly enriched in ammonium ($[NH_4^+]$ >150 μM), showed the most diverse strain of AOA (Francis *et al*., 2005).

To enhance the efficiency and stability in removing ammonia and recalcitant micropollutants in WWTPs, study on the ecology and microbiology of AOB and AOA in WWTPs is required. There is no information of AOB and AOA communities in full-scale WWTPs in Thailand, including their ability in degrading recalcitant micropollutants for now. To link between their communities and their ability in degrading persistant micropollutants in full-scale WWTPs, This work focuses on studying communities of AOB and AOA in full-scale WWTPs and another work done by Napasawan Khongkham on the topic of effects of AOB communities in nitrifying activated sludge on degradation 17α- ethynylestradiol via co-metabolism focuses on the ability of AOB in full-scale WWTPs in degrading recalcitant micropollutants. The main goal of this part is to analyze effects of influent characteristics, system configuration, and system operation on their communities.

1.2 Objectives

1. To study communities of AOB in full-scale wastewater treatment plants
2. To analyze effects of influent characteristics, system configuration, and system operation on communities of AOB in full-scale wastewater treatment plants

3. To study communities of AOA in full-scale wastewater treatment plants
4. To analyze effects of influent characteristics, system configuration, and system operation on communities of AOA in full-scale wastewater treatment plants
5. To investigate effect of ammonium concentrations on communities of AOB and AOA in enriched nitrifying activated sludge

1.3 Hypotheses

1. Wastewater treatment plants harbor different AOB communities.
2. Communities of AOB in wastewater treatment plants affect ammonia removal efficiency and stability of nitrification process in each plant.
3. Wastewater treatment plants harbor different AOA communities.
4. Communities of AOA in wastewater treatment plants affect ammonia removal efficiency and stability of nitrification process in each plant.
5. Ammonium concentrations affect significantly communities of AOB and AOA

1.4 Scope of the study

1. Communities of AOB and AOA in species level were analyzed using molecular tools.
2. Primers specific to groups of AOB and AOA were use for polymerase chain reaction (PCR) amplification of 16S rRNA gene and *amoA* gene respectively.
3. Effect of ammonium concentrations on communities of AOB and AOA were studied in enriched nitrifying activated sludge receiving inorganic medium containing different ammonium concentrations.

CHAPTER II

LITERATURE REVIEW

2.1 Microbial nitrification

Nitrification is central to the global nitrogen cycle and involves the oxidation of ammonia to nitrate (through nitrite) by two physiologically distinct groups of organisms: autotrophic ammonia- and nitrite-oxidizers. Ammonia-oxidizing organisms convert ammonia to nitrite through hydroxylamine using ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO). Autotrophic nitrite oxidizers subsequently use the enzyme nitrite oxidoreductase (NOR) to convert nitrite to nitrate, which can be assimilated or subjected to denitrification processes. In anaerobic environments, ammonia can be converted to molecular nitrogen by the anammox process by several enzymatic steps (represented by dashed arrows).

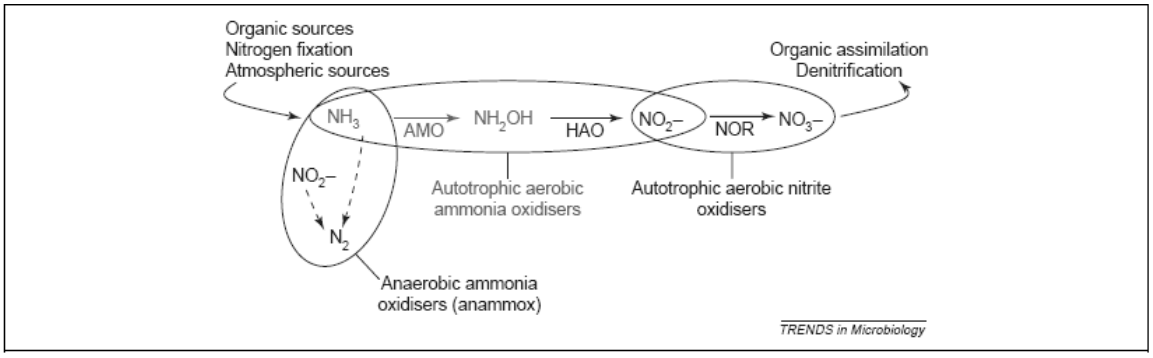


Figure 2.1 Autotrophic ammonia oxidation during nitrification (Nicol *et al.*, 2006).

The ammonia oxidation is the rate-limiting step of nitrification. In the soil environment, it can lead to substantial amounts of net nitrogen loss through subsequent denitrification or leaching of nitrate. In the marine environment, ammonia oxidation is an important component of nitrogen mineralization from organic sources and the removal of anthropogenic nitrogen inputs in coastal waters. Ammonia oxidation is also a key step in the removal of nitrogen during wastewater treatment (Prosser *et al*., 2002 and Kowalchuk *et al*., 2001).

Ammonia oxidation is considered to be carried out largely by autotrophic ammonia-oxidising bacteria (AOB) that form two distinct monophyletic groups within the α- and β- proteobacteria. To date, most cultured strains belong to the β-subgroup (Kowalchuk *et al*., 2001) and β-proteobacterial AOB are believed to be the dominant ammonia oxidizers in most environments. The same as AOB, Cultivation-independent molecular methods show that members of the kingdom Crenarchaeota within the domain Archaea represent a substantial component of microbial communities in aquatic and terrestrial environments. In 2004, metagenomic studies have revealed that such Crenarchaeota contain ammonia monooxygenases genes which related to those of bacterial. Furthermore, the first marine chemolithoautotrophic strain was isolated that uses ammonia as a sole energy source (Konneke *et al*., 2005).

It is questionable from these recent discoveries that who is the important organisms playing the key role in nitrification process. In most environments, autotrophic AOB are considered to be the most important organisms that responsible for ammonia oxidation. However, in most mesophilic environments, Crenarchaeota are more abundant than AOB populations. Wuchter *et al*. (2006) and Leininger *et al*. (2006) have found that most mesophilic Crenarchaeota are AOA, and that these organisms are the numerically dominant ammonia oxidizers in the ocean and in soils respectively.

2.2 Ammonia-oxidizing bacteria (AOB)

The lithoautotrophic ammonia-oxidizing bacteria (AOB) are well defined by their fundamental metabolism. Ammonia serves as the sole energy source and carbon dioxide is used as the carbon need. Together with the lithotrophic nitrite-oxidizing bacteria (NOB), the AOB catalyze the nitrification process ($NH_3 \rightarrow NO_2 \rightarrow NO_3$), which has a key step in natural nitrogen cycling.

2.2.1 Phylogeny of ammonia-oxidizing bacteria

2.2.1.1 16S rRNA-based phylogenetic tree

The first phylogenetic analyses of AOB were carried out by Woese and co-workers in the 1980s (Woese *et al*., 1984; Woese *et al*., 1985) and demonstrated

that two phylogenetically distinct groups of AOB exist. The major group, containing the genera *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio* and *Nitrosolobus*, belongs to the class Betaproteobacteria, while the second group of AOB, represented by two species of the genus *Nitrosococcus*, is affiliated with the class Gammaproteobacteria.

The genera *Nitrosospira*, *Nitrosolobus* and *Nitrosovibrio* are closely related, and 16S rRNA phylogeny provides no convincing support for subdivision of this lineage into three genera, although morphological and ecophysiological differences exist between the different genera. It has therefore been suggested these genera be lumped into the single genus *Nitrosospira* (Head *et al*., 1993). In contrast, the cultured nitrosomonads can be subdivided into six lineages which are consistently retrieved using different treeing methods and which have parsimony bootstrap support of above 90%. This phylogenetic substructure is also retrieved if all betaproteobacterial AOB isolates (56 nitrosomonads and 48 nitrosospiras), for which a 16S rRNA sequence longer than 1000 bases has been determined, are included in the treeing analysis (Figure 2.2 and 2.3).

2.2.1.2 AmoA-based phylogenetic tree

Recently, the *amoA* gene coding for the active site polypeptide of the ammonia monooxygenase has been used as an additional phylogenetic marker molecule for AOB (McTavish *et al*., 1993; Klotz and Norton, 1995; Rotthauwe *et al*., 1995; Suwa *et al*., 1997; Hommes *et al*., 1998; Alzerreca *et al*., 1999; Yamagata *et al*., 1999; Horz *et al*., 2000; Purkhold *et al*., 2000; Aakra *et al*., 2001a; Casciotti and Ward, 2001; Purkhold *et al*., 2003). PCR primers that allow amplification of a 453-bp fragment of this gene are generally used in these studies (Rotthauwe *et al*., 1997; modified by Stephen *et al*., 1999). Phylogeny inference based on the deduced amino acid sequence of the *amoA* gene fragment is overall consistent with the 16S rRNA phylogeny of AOB. Members of the genera *Nitrosospira*, *Nitrosolobus* and *Nitrosovibrio* form a tight monophyletic grouping with no obvious substructure. Within the nitrosomonads the *N. europaea*/*Nc. mobilis* lineage and the *N. marina* lineage are also found with all treeing methods, while the *N. communis* and the *N. oligotropha* lineage are not always monophyletic (Figure 2.4). If *amoA* nucleic acid sequences are used for phylogenetic analysis, basically the same picture emerges with the exception that the *N. europaea*/*eutropha* lineage is no longer monophyletic.

If compared to the 16S rRNA-based phylogeny of AOB, AmoA analysis does provide less resolution, reflecting that a relatively short (151 positions) and highly conserved (93 positions have an identical amino acid in at least 98% of the betaproteobacterial AOB) amino acid sequence stretch is used as marker. The information content of AmoA sequences could be significantly extended in future studies by the development of primers that allow the amplification of more complete *amoA* gene fragments. First attempts in this direction were recently published by Norton *et al*., 2002.

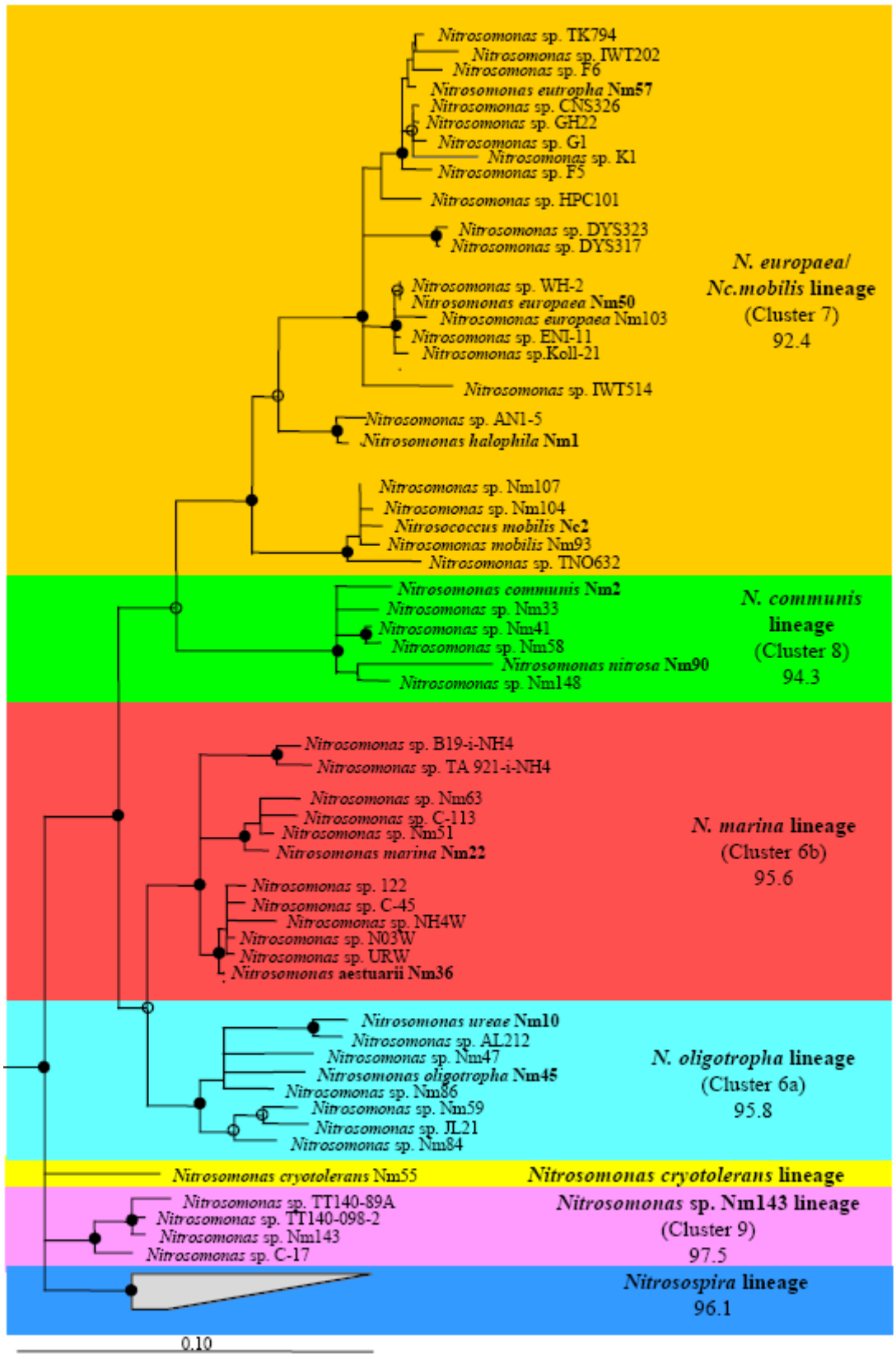


Figure 2.2 16S rRNA based phylogenetic tree of the nitrosomonads. (Koops *et al.*, 2003)

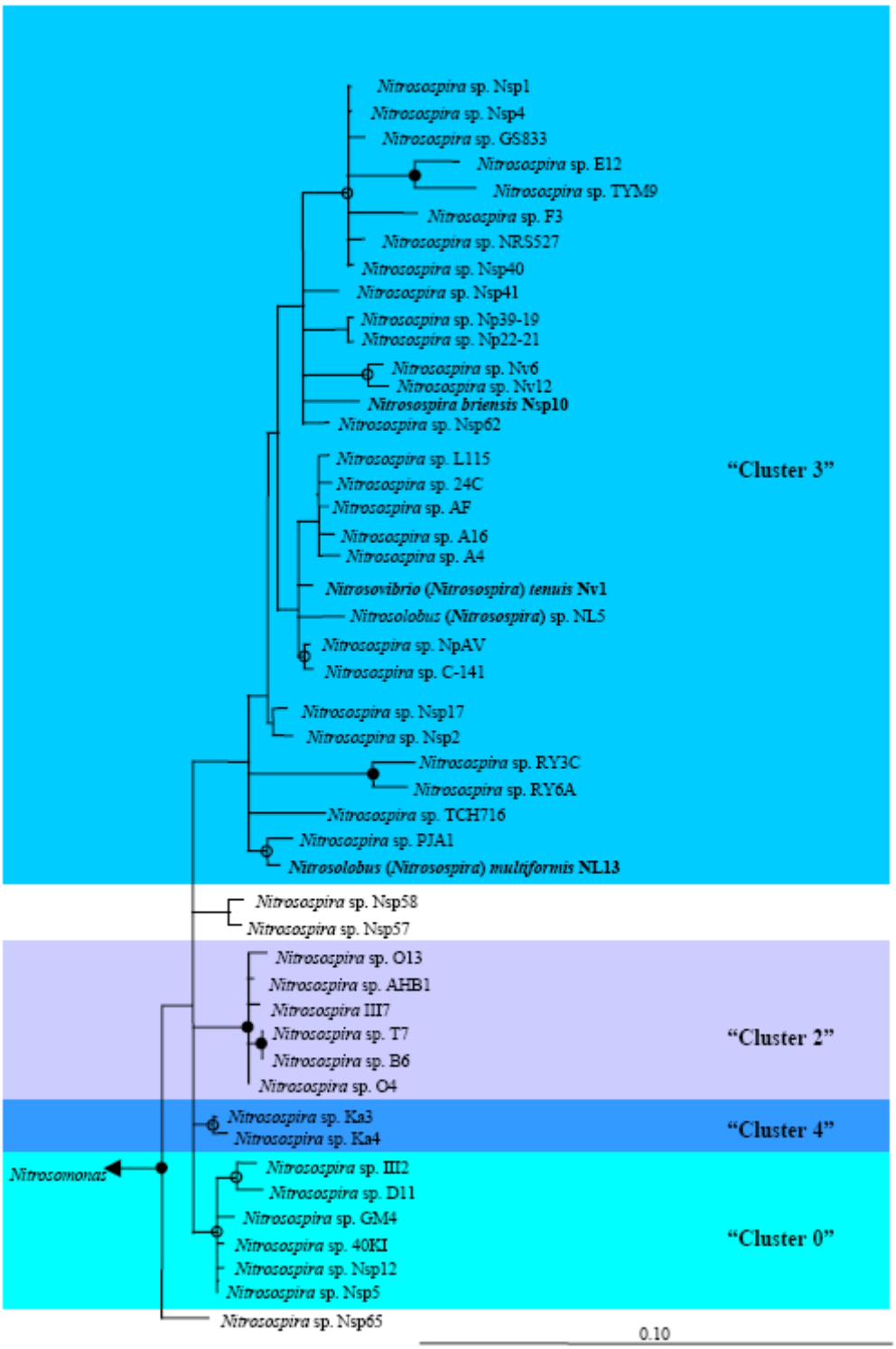


Figure 2.3 16S rRNA-based phylogenetic tree of the highly related genera *Nitrosospira*, *Nitrosolobus*, and *Nitrosovibrio*. (Koops *et al.*, 2003)

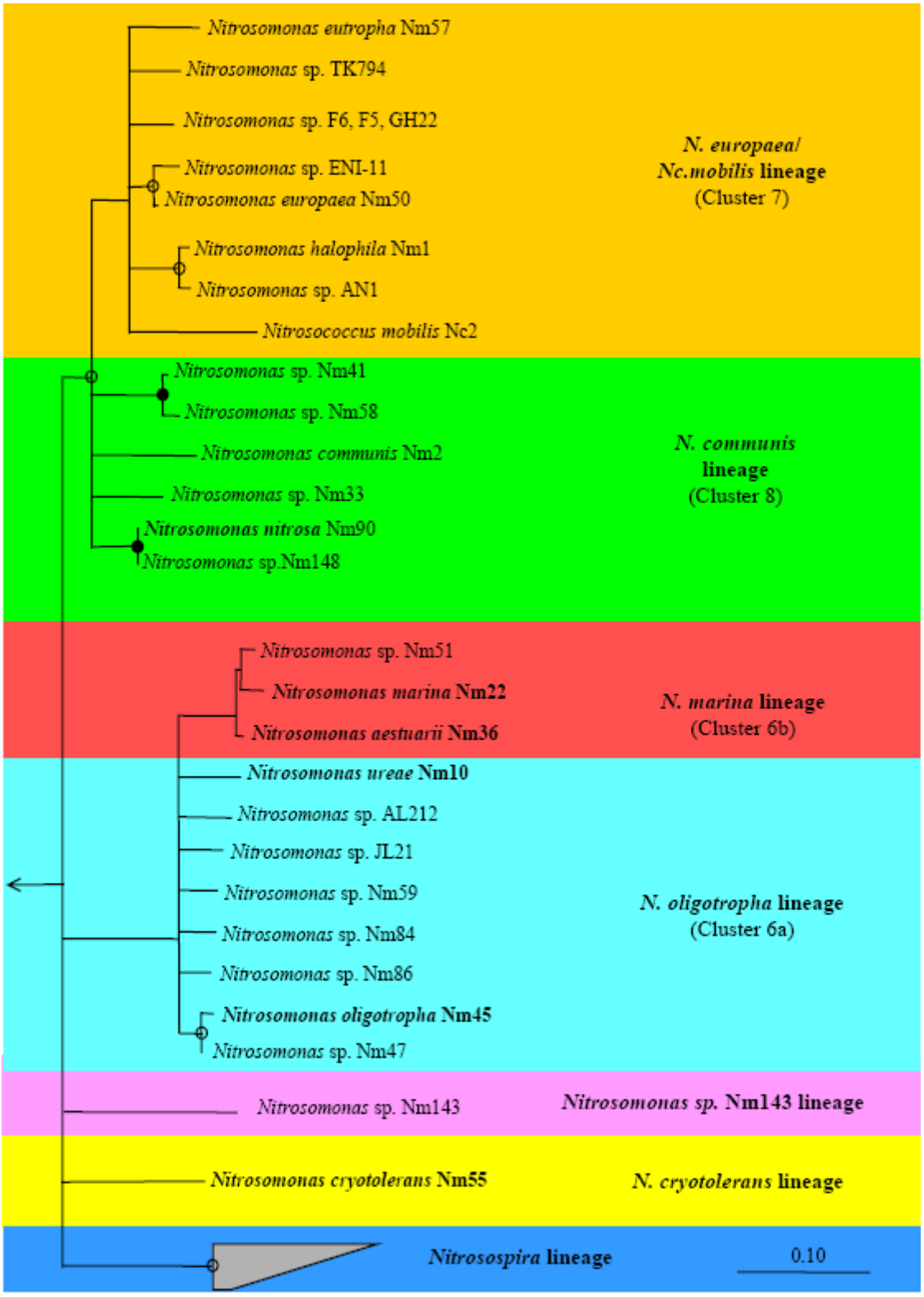


Figure 2.4 AmoA-based phylogenetic tree of the betaproteobacterial AOB. (Koops *et al.*, 2003)

2.2.2 Factors influencing communities of ammonia-oxidizing bacteria

2.2.2.1 Ammonia concentration

The distribution pattern of AOB in nature is coupled to geological, biological and anthropogenic sources of reduced nitrogen because ammonia is the essential energy source for these organisms. Consequently, they have adapted to a broad range of different ammonia concentrations in the diverse environments, reflected by different affinity constants for ammonia. This is one of the most important factors influencing the distribution patterns of AOB in nature (Suwa *et al*., 1994; Suwa *et al*., 1997; Stehr *et al*., 1995a; Koops and Pommerening-Roser, 2001).

2.2.2.2 The tolerance of increasing of ammonia concentrations

Due to ammonia is a toxic compound. The tolerance of increasing ammonia concentrations (Table 2.1) is another aspect affecting the distribution patterns of AOB (Bollmann and Laanbroek, 2001).

2.2.2.3 Urease activity

The presence or absence of urease activity was observed to be of ecophysiological relevance for AOB. This property is of special importance in acidic environments, where free ammonia is missing as substrate because it is nearly quantitatively ionized to ammonium. Under such conditions, only those AOB species that can use urea as an alternative ammonia source can survive (De Boer and Laanbroek, 1989; De Boer *et al*., 1991; Jiang and Bakken, 1999; Burton and Prosser, 2001). Possession of urease seems also to be an essential property for AOB that successfully colonize oligotrophic soils or aquatic environments.

2.2.2.4 Different salt requirements, salt tolerances and salt sensitivities

Distribution of AOB in nature also is affected by different salt requirements, salt tolerances and salt sensitivities of the distinct species (Table 2.1). This is of special importance for their distribution patterns in aquatic systems (such as rivers, lakes, estuaries, marine environments and salt lakes) that significantly differ in salinity (Koops *et al*., 1990; Koops *et al*., 1991).

Table 2.1 Characteristics and preferred habitats of described species of the ammonia-oxidizing bacteria (Koops *et al*., 2003).

Species	G+C (mol%)	Carboxy-somes	Urease activity	Substrate (NH_3) affinity (K_s in μM)	Maximum ammonia tolerance NH_4Cl (in mM; pH 8.0)	Salt requirement	Maximum salt tolerance (in mM)	Preferred habitats
Nitrosomonas europaea	50.6–51.4	–	–	30–61	400	–	400	Sewage disposal plants, eutrophic freshwater and brackish water
Nitrosomonas eutropha	47.9–48.5	+	–		600	–	400	
Nitrosomonas halophila	53.8	+	–		400	+	900	
Nitrosococcus mobilis	49.3	–	–		250	+	500	
Nitrosomonas communis	45.6–46.0	–	–	14–43	250	–	250	Soils (not acid) and eutrophic freshwater
Nitrosomonas nitrosa	47.9	+	+	19–46	100	–	300	
Nitrosomonas ureae	45.6–46.0	–	+	1.9–4.2	200	–	200	Oligotrophic freshwater and natural soils
Nitrosomonas oligotropha	49.4–50.0	–	+		50	–	150	
Nitrosomonas marina	47.4–48.0	–	+	50–52	200	+	800	Marine environments
Nitrosomonas aestuarii	45.7–46.3	–	+		400	+	600	
Nitrosomonas cryotolerans	45.5–46.1	–	+	42–59	400	+	550	Marine environments
Nitrosolobus multiformis	53.5	ND	+/–	ND	50	–	200	Soils (not acid)
Nitrosovibrio tenuis	53.9	ND	+/–	ND	100	–	100	Soils, rocks and freshwater
Nitrosospira briensis	54	ND	+/–	ND	200	–	250	Soils, rocks and freshwater
Nitrosococcus oceani	50–51	ND	+	ND	1000	+	1100	Marine environments
Nitrosococcus halophilus	50–51	ND	–	ND	500	+	1800	Marine environments and salt lakes

Symbols and Abbreviations: +, present; –, not present; +/–, present in some strains; and ND, no data.

2.2.2.5 Different oxygen concentration

The AOB are aerobes. Although their oxygen affinity constants are relatively high (Painter, 1986), AOB can also survive at extremely low oxygen concentrations (Goreau *et al*., 1980). Even ammonia oxidation under anaerobic conditions is being discussed (Schmidt and Bock, 1997; Schmidt and Bock, 1998; Zart *et al*., 2000). However, specific selection of distinct AOB species by different oxygen concentrations has not yet been reported in the literature.

2.2.2.6 Temperature

Temperature also may be of importance for distribution patterns of AOB in nature. This has been revealed in some publications (Golovacheva *et al*., 1976; Jones *et al*., 1988; Jiang and Bakken, 1999). Beside environments characterized by constant high or low temperatures, such as hot springs or permafrost soils, environments showing pronounced temperature changes, such as rock surfaces, might harbor interesting AOB. However, it is not enough information for now to allow general conclusions on their AOB diversity.

2.2.3 Dominant populations of ammonia-oxidizing bacteria in wastewater treatment plant

Urea and ammonia are the most frequently found nitrogen compounds in sewage. In wastewater treatment plants, AOB oxidize ammonia to nitrite, which is subsequently converted to nitrate by the NOB. Nitrate is then removed from the sewage by denitrifying bacteria via anaerobic respiration. The slow growth rate of AOB and their susceptibility to pH and temperature swings as well as to several sewage compounds is responsible for frequent failure of the nitrification in municipal and industrial WWTPs.

2.2.3.1 Municipal wastewater treatment plant

Isolation techniques indicate that in standard municipal WWTPs, *N. eutropha* seems to be the dominant representative (Watson and Mandel, 1971b; Koops and Harms, 1985), but *N. europaea* and *Nc. mobilis* have also been repeatedly cultivated (Juretschko *et al*., 1998; Koops and Pommerening- Roser, 2001). All these species belong to the same *Nitrosomonas* lineage (Figure 2.2)

2.2.3.2 Industrial wastewater treatment plant

In industrial WWTPs, the cultivation of representatives of members of the *N. oligotropha* lineage as well as *N. nitrosa* has been regularly reported (Koops and Harms, 1985; Suwa *et al*., 1997). In laboratory experiments, a remarkable high tolerance of members of the *N. oligotropha* lineage to heavy metals was observed, and the production of significant amounts of exopolymeric materials by these species was suggested to be the major reason for this tolerance (Stehr *et al*., 1995b). This resistance to heavy metals may be responsible for the presence of members of this lineage in special WWTPs.

2.2.4 Co-metabolism of organic compounds by ammonia-oxidizing bacteria

AOB, which is obligate chemolithotrophic aerobe using ammonia as a sole energy source, is widely for the oxidation of hydrocarbon substrates through the action of ammonia monooxgenase (AMO) (Arciero *et* al., 1989). During oxidation of ammonia to nitrite, AMO catalyzes the oxidation of ammonia to hydroxylamine. Subsequently, hydroxylamine is oxidized to nitrite by hydroxylamine oxidoreductase

(HAO). During the last process four electrons are released. Two of four electrons transfer to AMO in order to activate oxygen and maintain steady-state rate of ammonia oxidation. The rest two electrons are used in another oxidation reaction which is called co-metabolism (Arciero *et al*., 1989; William and Daniel, 1993). Currently, many hydrocarbons and halogenated hydrocarbons which are able to be degraded by co-metabolism of AOB such as in Figure 2.5 show ethylene is degraded by co-metabolism of AOB.

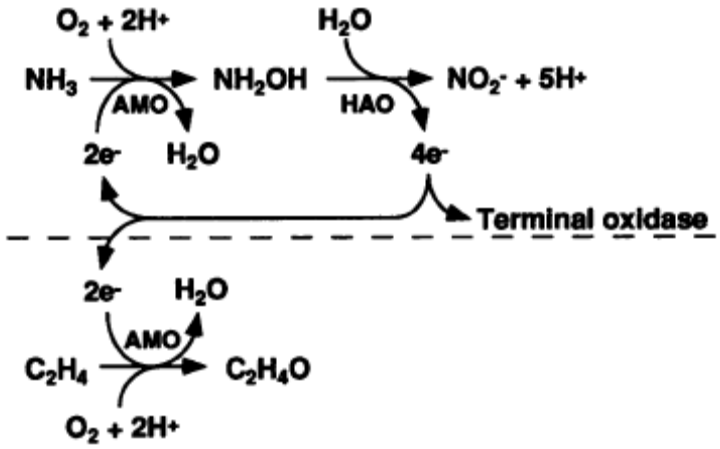


Figure 2.5 Co-metabolism of ethylene by AOB
(William and Daniel, 1993)

In mixed culture, in batch experiments with nitrifying activated sludge (NAS), 0.050 mg/l of EE2 was degraded completely within 6 days by oxidizing ammonium at rate of 50 mg NH_4^+/gDW/ hr and degrading EE2 at maximum rate of 1 μg/gDW/hr (Vader *et al*., 2000). Furthermore, in initial concentration of 1 mgL^{-1} of estrogen were degraded with NAS by the degradation rate of 0.056 hr^{-1} for E1, 1.3 hr^{-1} for E2, 0.030 hr^{-1} for E3, and 0.035 hr^{-1} for EE2. By using inhibitor for ammonia monooxygenase, the key enzyme for ammonia oxidation by AOB confirmed that NAS significantly degrade E1, E2, E3 and EE2. In NAS, E1, E2 and E3 were degraded by heterotrophic bacteria whereas EE2 was degraded by AOB (Shi *et al*., 2004). As for pure culture, ammonia-oxidizing bacteria (AOB), *Nitrosomonas europaea*, degraded 0.4 mg/l estrogens with constant biodegradation rates of 0.0022 mg/l/hr for E1, 0.0020 mg/l/hr for E2, 0.0016 mg/l/hr for E3 and 0.0019 mg/l/hr for EE2. Corresponding ammonia

consumption rates were 1.5 mg$NH4^+$-N/l/hr for E1, 1.45 mg$NH4^+$-N/l/hr for E2, 1.35 mg$NH4^+$-N/l/hr for E3 and 1.55 mg$NH4^+$-N/l/hr for EE2 (Shi *et al*., 2004).

A variety of biological treatment technologies for the destruction of chlorinated hydrocarbons, such as TCE, are now under development. Microorganisms that can grow on TCE as a sole carbon or energy source have not yet been isolated. However, several physiologically diverse types of bacteria can cometabolically dechlorinate and then partially or fully degrade TCE. For instance, Vogel and McCarty (1985) found that TCE could be cometabolically transformed when used as a nongrowth-supporting electron acceptor by methanogens under anaerobic conditions, while Wilson and Wilson (1985) found that methanotrophic bacteria could cometabolize TCE aerobically by means of the enzyme methane monooxygenase (MMO) when methane was supplied as a primary substrate. Recently, several studies have indicated that chlorinated aliphatic compounds, including TCE, can be cometabolized by nitrifying bacteria (*Nitrosomonas europaea*) (Ely *et al*., 1995; Arciero *et al*., 1989; Hyman *et al*., 1995). It was found that the enzyme ammonia monooxygenase (AMO) wasinvolved in these reactions. Usually, aerobic cometabolic processes are preferred overanaerobic ones because TCE can be mineralized to CO_2 , H_2O and Cl^- without the accumulation of stable and carcinogenic intermediates such as vinyl chloride (Vogel *et al*., 1985). However, due to the low solubility of methane gas, it is difficult to handle this primary substrate for methanotrophs in cometabolism of TCE. Thus, using the more soluble of ammoniarammonium instead of methane as a primary substrate for nitrifying bacteria may increase the operation efficiencies in in situ bioremediation of TCE.

2.3 Ammonia-oxidizing archaea (AOA)

Cultivation-independent molecular surveys show that members of the kingdom Crenarchaeota within the domain Archaea represent a substantial component of microbial communities in aquatic and terrestrial environments. In 2004, metagenomic study of the Sargasso Sea by shotgun DNA sequencing, Venter *et al*. found the presence of an ammonia monooxygenase gene (*amo*A–like gene) on an archaeal-associated scaffold and indicated the potential role of archaea in nitrification processes of the ocean. After that a search for homologues of the *amoA* and *amo*B-

like genes of the soil clone revealed several highly similar genes in the Sargasso Sea dataset, which enabled these marine genes to be assigned also to Crenarchaeota. This is the first link between *amo*-like genes and mesophilic Crenarchaeota. This evidence also led to the hypothesis that non-thermophilic Crenarchaeota of soil could be ammonia oxidizers (Schleper *et al*., 2005). Furthermore, Treusch *et al*. (2005) demonstrated that incubation of soil in the presence of ammonia resulted in a significant increase of crenarchaeal amoA expression compared with controls incubated without ammonia. These experiments suggested that the identified genes encoded an ammonia monooxygenase. The ultimate confirmation of AOA activity was achieved by cultivation of a mesophilic crenarchaeote from a marine aquarium in Seattle, USA (Konneke *et al*., 2005). *Nitrosopumilus maritimus* is phylogenetically placed within the 'marine' group 1.1a lineage (Figure 2.6). It grows chemolithoautotrophically, using ammonia as a sole energy source, and seems to grow at similar rates and densities as cultured AOB with near-stoichiometric conversion of ammonia to nitrite (Konneke *et al*., 2005). With primers designed from the *amo* gene sequences obtained in the metagenomic studies of Treusch *et al*. (2005) and Venter *et al*. (2004), the authors amplified highly similar *amo*ABC-like genes from *N. maritimus*, which again suggested that these genes encode the key metabolic enzyme of AOA. The first molecular evidence demonstrate the archaeal *amo*A gene to be pervasive in areas of the ocean that are critical for the global nitrogen cycle – including the base of the euphotic zone, suboxic water columns and coastal/estuarine sediments (Francis *et al*., 2005). They use specific PCR primers specifically targeting the archael *amo*A to investigate the distribution and diversity of AOA in water columns and sediments of the ocean. For the first time, these data indicated that many marine Crenarchaeota might be capable of ammonia oxidation. The archaeal amoA sequences revealed diverse and distinct AOA communities associated with different habitats and sampling sites, with little overlap between water columns and sediments. There are two evidences, showing that AOA are more abundant than AOB in marine and soil environments. In the North Sea, the archaeal amoA abundance was 1–2 orders of magnitude higher than those of bacterial nitrifiers; and , in the North Atlantic, crenarchaeotal amoA copy numbers are also 1–3 orders of magnitude higher than those of bacterial amoA (Wuchter *et al*., 2006). Leininger *et al*. (2006)

demonstrated that *amo*A gene copies of Crenarchaeota were up to 3,000-fold more abundant than bacterial *amo*A genes. High amounts of crenarchaeota-specific lipids, including crenarchaeol, correlated with the abundance of archaeal *amo*A gene copies. Reverse transcription quantitative PCR studies and complementary DNA analysis using novel cloning-independent pyrosequencing technology demonstrated the activity of the archaea in situ and supported the numerical dominance of archaeal over bacterial ammonia oxidizers. They assume that Crenarchaeota may be the most abundant ammonia-oxidizing organisms in soil ecosystems on earth. Moreover, AOA have also recently been detected in nitrifying wastewater treatment bioreactors used to remove ammonia from wastewater by using PCR primers targeting archaeal *amo*A gene (Park *et al*., 2006). All of these sequences showed similarity to sequences previously found in soil and sediments. AOA were distributed primarily in four major phylogenetic clusters (A, B, C, and D) This study clearly demonstrates the presence of molecular markers for AOA, including an archaeal amoA cluster (cluster D) that may be widespread in activated sludge bioreactors.

2.3.1 Phylogeny of AOA

Phylogenetic tree showed major non-thermophilic and cultivated (hyper)thermophilic lineages within the kingdom Crenarchaeota. Lineage descriptions follow those of Schleper *et al*. (Schleper *et al*., 2005). Two lineages with representatives that have crenarchaeal AMO genes are highlighted from marine and soil environments. Pairwise distances (with LogDet–Paralinear correction) of unambiguously aligned positions were calculated using variable sites only (estimated from a maximum-likelihood model). Bootstrap support was calculated using maximum likelihood, distance and parsimony methods (100, 1000 and 1000 replicates, respectively) with values at major nodes representing the most conservative value from all three methods (expressed as a percentage). Multifurcation indicates where the relative branching order of major lineages could not be determined in the majority of bootstrap replicates with all methods. The scale bar represents an estimated 0.05 changes per nucleotide position (Nicol *et al.,* 2006).

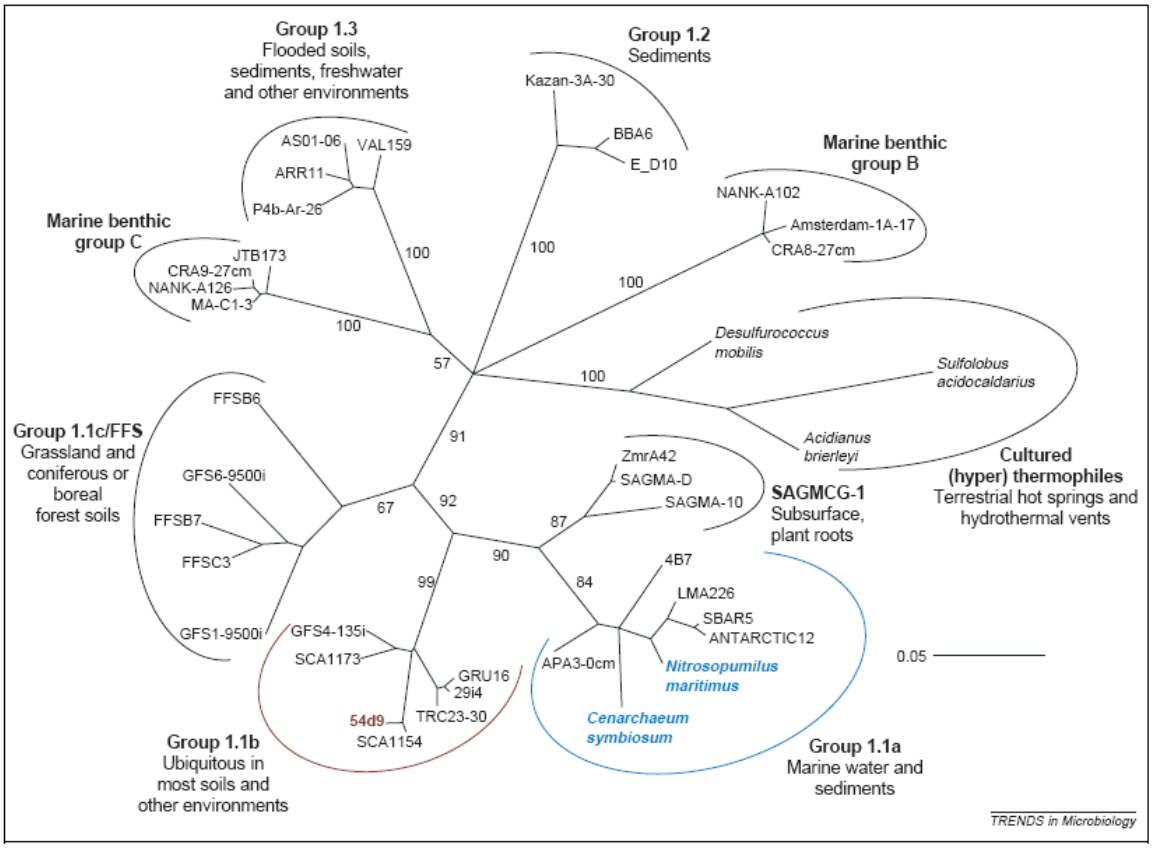


Figure 2.6 Phylogenetic tree of AOA (Nicol *et al.*, 2006)

2.3.2 Factors influencing communities of ammonia-oxidizing archaea

2.3.2.1 Ammonium concentration

A pure culture of Crenarchaeota grew to a maximal density of 1.4 x10^7 cells/ml at 28 ˚C in defined medium containing 500 µM ammonium, with a minimum generation time of 21 h. This cell density is approximately three orders of magnitude greater than that observed for marine Crenarchaeota in natural bacterioplankton samples. Ammonium typically reaches concentrations of < 0.03–1µM in the open ocean and < 0.03–100 µM in coastal waters (Konneke *et al*., 2005). The maximum growth rate of Crenarchaeota in culture (0.78 d^{-1}) was higher than the range of rates estimated for natural bacterioplankton communities, which vary between 0.05 and 0.3 d^{-1}. Moreover, Francis *et al*. found that NH_4^+ might also be expected to influence AOA community structure. Coastal permeable sediments from Huntington Beach are characterized by consistently elevated NH_4^+ concentrations caused by hydrologic

connection with groundwater, which is highly enriched in NH_4^+ (groundwater [NH_4^+] >150 μM). The archaeal *amoA* library from this site was the most diverse in his study.

2.3.2.2 Organic material

The addition of organic compounds, even in very low concentrations, appeared to inhibit the growth of Crenarchaeota in culture (Konneke *et al*., 2005). Thus, organic material excreted by other organisms (for example, phototrophic primary producers) and a low concentration of ammonium may limit the abundance of marine Crenarchaeota in the environment.

2.3.2.3 Habitat/geographic location

Using PCR primers designed to specifically target archaeal *amoA*, Francis *et al*. find AOA to be pervasive in areas of the ocean that are critical for the global nitrogen cycle, including the base of the euphotic zone, suboxic water columns, and estuarine and coastal sediments. Diverse and distinct AOA communities are associated with each of these habitats, with little overlap between water columns and sediments. Within marine sediments, most AOA sequences are unique to individual sampling locations, whereas a small number of sequences are evidently cosmopolitan in distribution (Francis *et al*., 2005).

2.3.2.4 Salinity

Differences in AOA community composition in San Francisco Bay, the largest estuary on the west coast of the United States, are likely associated with salinity (Francis *et al*., 2005). Sequences from the 30.5-practical salinity units (psu) Central San Francisco Bay site were distributed throughout a number of different regions of the tree. While all of the low-salinity (0.5 psu) North San Francisco Bay sequences fell exclusively into one distinct phylogenetic cluster. From these result, they assumed that more salinity were responsible for more diversity of AOA.

2.3.2.5 Season

PCR amplification of archaeal 16S rRNA gene by using a general archaeal primer followed by phylogenetic analysis of sequenced denaturing gradient gel electrophoresis (DGGE) fragments revealed that Crenarchaeota dominated the archaeal community from late fall to early spring (Wuchter *et al*., 2006). In December, abundances of Crenarchaeota decreased considerably, for reasons presently unclear, whereas ammonium levels remained relatively constant at ~9μM.

While in early January, crenarchaeotal abundance again increased substantially by 1 order of magnitude, coinciding with a decrease in ammonia levels from 10 to 3 μM.

2.3.2.6 Depth of soil

To analyse the distribution of AOA and AOB at different depths, Leininger *et al*. investigated an agricultural soil, which has been treated with different amounts and qualities of fertilizers for more than 100 years, and a natural, pristine calcareous grassland soil site. Bacterial amoA genes in the former declined significantly with depth in the unfertilized and inorganically fertilized sites while archaeal amoA genes stayed high, resulting in a maximal AOA to AOB ratio of 3,000 (Leininger *et al*., 2006).

2.3.2.7 Bioavailability of nitrogen and carbon

Both archaeal and bacterial amoA copy numbers varied little with depth at the site treated additionally with manure and these higher levels of archaeal and bacterial amoA were associated with the highest bioavailability of nitrogen and carbon (Leininger *et al*., 2006).

2.3.2.8 Operational system and retention time of wastewater treatment system

All of the PCR-positive samples were collected from WWTPs operating with aerated-anoxic processes (i.e., the Orbal and VLRprocesses), in which extremely low DO concentrations are maintained, enabling simultaneous nitrification and denitrification. Additionally, AOA-positive samples were collected from WWTPs operating with long retention times (>15 days of solids retention time, >24 h of hydraulic retention time). Thus, it is possible that either or both of these features (low DO levels and long retention times) facilitate the growth of AOA (Park *et al*., 2006)

2.3.2.9 Oxygen level

Most of the marine Crenarchaeota located within the suboxic layer are putative nitrifiers and live at oxygen levels ≤1 mM (Coolen *et al*., 2007). Moreover, the presence of crenarchaeol in the oxygen minimum zone of the Arabian Sea, where oxygen levels were less than 5 μM, provided indirect evidence that marine Crenarchaeota are capable of thriving at low oxygen levels (Sinninghe Damsté *et al*., 2002b).

2.3.2.10 Sulfide

Different phylotypes of marine Crenarchaeota (both 16S rDNA and *amoA*) were found in the top of the sulfidic zone, and those phylotypes were not detected in the suboxic zone. Living marine Crenarchaeota were present even in the sulfidic waters with up to a few tens of mM sulfide (Coolen *et al*., 2007).

2.4 Molecular technique

Developed molecular tools, incorporating sequence analysis of the 16S rRNA and *amoA* genes, allow investigating ammonia oxidizer in the environments without concerning to culture-dependent techniques. In combination with clone libraries or denaturing gradient gel electrophoresis (DGGE), the application of specific polymerase chain reaction (PCR) amplification (Kowalchuk *et al*., 1997; Rotthauwe *et al*., 1997; Nicolaisen and Ramsing, 2002) provides clarification of the ammonia oxidizer community at the species level. The implementation of fluorescence in situ hybridization (FISH) (Wagner *et al*., 1995; Mobarry *et al*., 1996; Juretschko *et al*., 1998; Wagner *et al*., 1998; Okabe *et al*., 1999; Gieseke *et al*., 2001) makes it possible to analyze in situ complex community structure of AOB and estimate their numbers. The use of PCR-based quantification techniques, such as competitive PCR and real-time PCR, supports enumeration of AOB populations in the environments (Hermansson and Lindren, 2000; Dionisi *et al*., 2002; Harms *et al*., 2003). Recently, the gene encoding for the active site of ammonia monooxygenase (*amoA*) has been established as molecular marker for AOB diversity research in natural ecosystems and in engineered systems (Gieseke *et al*., 2001; Horz *et al*., 2000; Rotthauwe *et al*., 1997). In addition, a putative *amoA* gene has been detected in an autotrophic marine ammonia-oxidizing archaea (Konneke *et al*., 2005), but the amino acid sequence are of low similarity to bacterial AMO-encoding genes. Consequently, we can use specific primer (Table 2.2) targeting both 16S rRNA and *amoA* gene of AOB and AOA

Table 2.2 Specific primer targeting 16S rRNA and *amoA* gene of AOB and AOA

	Targeting gene	Primer	Nucleotide sequence (5′–3′)	Reference
AOB	16S rRNA	CTO189A/Bf-GC	CGCCCGCCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGAGRAAAGCAGGGGATCG	Kowalchuck *et al*., 1997)
		CTO 189Cf-GC	CGCCCGCCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGAGGAAAGTAGGGGATCG	Kowalchuck *et al*., 1997)
		CTO 654r	CTAGCYTTGTAGTTTCAAACGC	Kowalchuck *et al*., 1997)
	amoA gene	amoA 1F	GGGGTTTCTACTGGTGGT	Rotthauwe *et al*., 1997
		amoA 1F-GC	CGCCGCGCGGCGGGCGGGGCGGGGGCGGGTTTCTACTGGTGGT	Rotthauwe *et al*., 1997
		amoA 2R	CCCCTCTGCAAAGCCTTCTTC	Rotthauwe *et al*., 1997
AOA	16S rRNA	A21F	TTCCGGTTGATCC[CT]GCCGGA	Delong, 1992
		A958R	[C/T]CCGGCGTTGA[A/C]TCCAATT	Delong, 1992
	amoA gene	Arch-amoAF	STAATGGTCTGGCTTAGACG	Francis *et al.*, 2005
		Arch-amoAR	GCGGCCATCCATCTGTATGT	Francis *et al.*, 2005

CHAPTER III

METHODOLOGY AND MATERIALS

3.1 Experimental framework

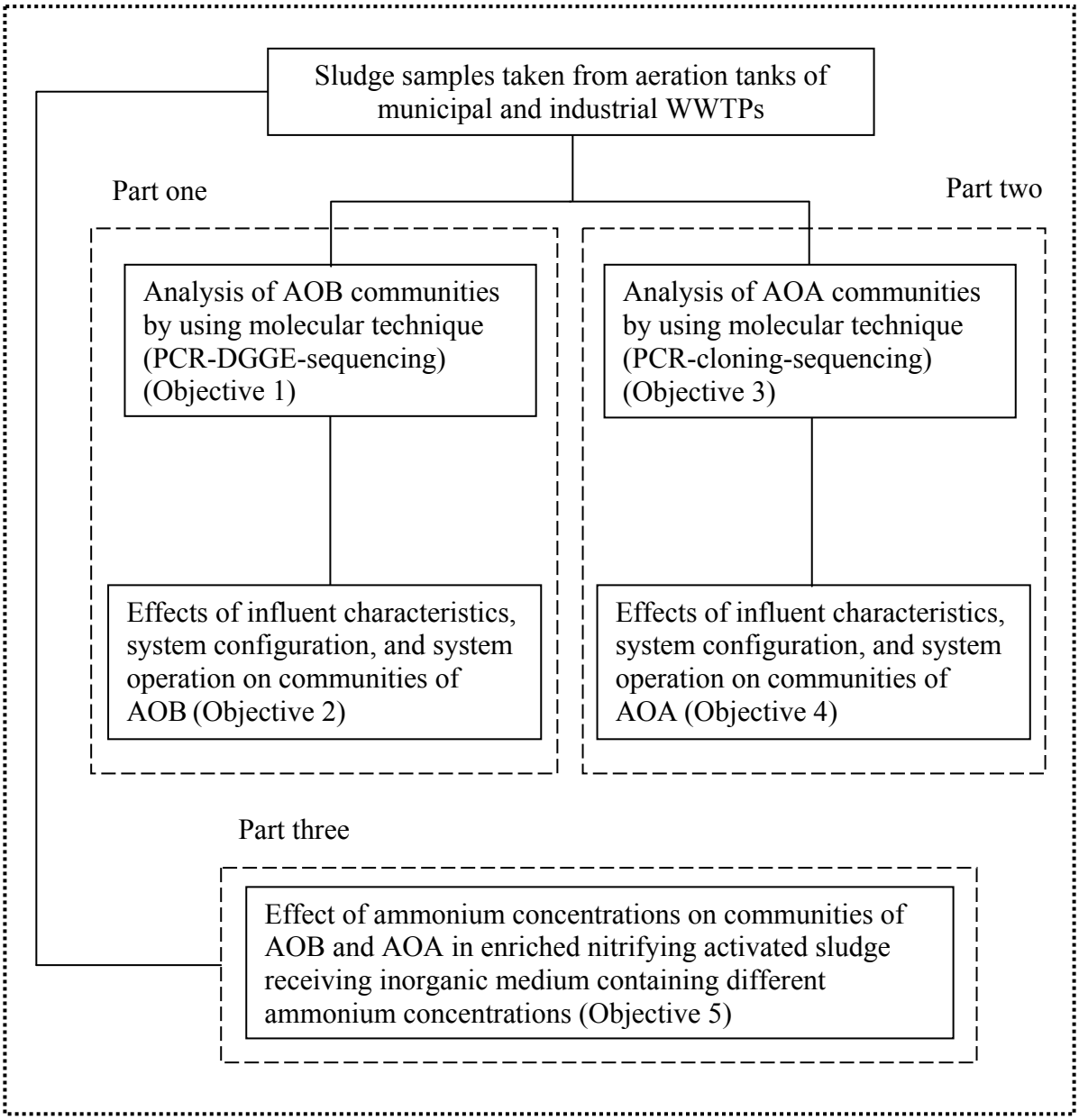


Figure 3.1 Experimental framework

This work was divided into three parts. The first part of this study focused on the study of communities of AOB in species level by using molecular technique (PCR-DGGE-sequencing) and analysis of influent characteristics, system configuration, and system operation on communities of AOB. The second part focused on the study of communities of AOA in species level by using molecular technique (PCR-cloning-sequencing) and analysis of influent characteristics, system configuration, and system operation on communities of AOA. The last part of this work was to investigate the effect of ammonium concentrations on communities of AOB and AOA in enriched nitrifying activated sludge (NAS) receiving inorganic medium containing different ammonium concentrations.

3.2 Measurement ammonia, nitrite, and nitrate

3.2.1 Measurement of ammonium

Sample was diluted with deionized water. 5 ml of dilution sample and 0.2 mL of phenol solution (Mix 11.1 mL liquefied phenol ($\geq$89%) with 95% v/v ethyl alcohol to a final volume of 100 mL) were added and then mixed. 0.2 mL of sodium nitroprusside solution (0.5% w/v: dissolve 0.5 g of sodium nitropusside in 100 mL of deionized water), and 0.5 mL of oxidizing solution (Mix 100 mL alkaline citrate solution: dissolve 200 g of trisodium citrate and 10 g of sodium hydroxide in 1000 mL of deionized water with 25 mL of sodium hypochloride) were added into the tube. Sample was covered with plastic wrap or paraffin wrapper film and kept at room temperature in subdued light for at least 1 hr to develop color. Sample was measured for absorbance at 640 nm with UV visible spectrophotometers (Thermo Electron Corporation, Hexious α, Cambridge, UK) (Phenate method, Standard Method for the Examination of Water and Wastewater 20th edition).

3.2.2 Measurement of nitrite

Sample was diluted with deionized water. 5ml of diluted sample and 0.1mL of Sulphanilamide solution (dissolve 5 g of Sulphanilamide and 50 mL of hydrochloric in 500 mL) was added, and allowed to react 5 min, then 0.1 mL of NNED solution (dissolve 1 g of (N-(1-Naphthyl)-Ethylenediamine Dihydrochloride in

1000mL of de-ionized water) was added and allowed at room temperature in subdued light for at least 1 hr to develop color. Sample was measured for absorbance at 543 nm with UV visible spectrophotometers (Thermo Electron Corporation, Hexious α, Cambridge, UK) (Colorimetric method, Standard Method for the Examination of Water and Wastewater 20th edition).

3.2.3 Measurement of nitrate

Sample was diluted with deionized water. 5 mL of diluted sample was filtered and measured for absorbance at 220 nm to obtain NO_3^- reading and absorbance at 275 nm to determine interference due to dissolved organic matter with UV visible spectrophotometers (Thermo Electron Corporation, Hexious α, Cambridge, UK) (Ultraviolet Spectrophotometric Screening Method, Standard Method for the Examination of Water and Wastewater 20th edition).

3.3 Analysis of communities of ammonia-oxidizing bacteria and archaea

3.3.1 Sample preparation and DNA extraction

Sludge of approximately 2 mg of MLSS was transferred into a 1.7 ml eppendorf tube and centrifuged at 14,000 rpm for 10 min. The supernatant was removed, and the pellet was kept at -20 ^{0}C until analysis. DNA was extracted from samples using Fast-DNA SPIN kits for soil (QBiogene, Solon, Ohio, USA) according to the manufacturer's instructions. The product from DNA extraction was verified by electrophoresis in 2% agarose (Bio-Rad, Spain).

3.3.2 Analysis of ammonia-oxidizing bacteria communities

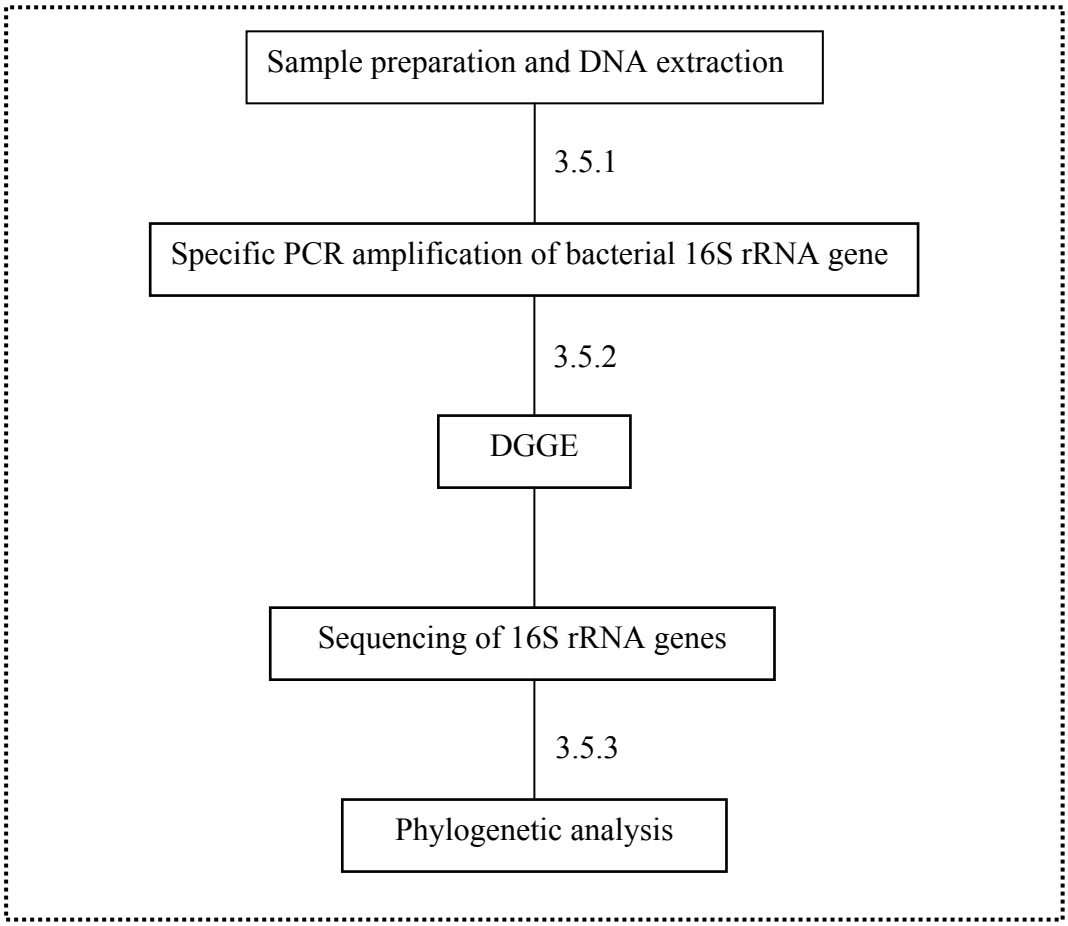


Figure 3.2 Experimental framework for analysis of ammonia-oxidizing bacteria communities

3.3.2.1 Polymerase chain reaction (PCR)

Primers CTO189f and CTO654r (Kowalchuck *et al*., 1997) were used to amplify 465-bp of 16S rRNA gene fragment of *Betaproteobacteria*. The oligonucleotide sequences of the primers are shown in Table 3.1. Extracted DNA was PCR-amplified using the primer sets (the forward primer had a GC clamp) for 35 cycles in a 25 µl reaction volume. DNA eluted from bands excised from DGGE gels were amplified for 20–25 cycles using the primer set lacking the GC clamp in a 25 µl reaction volume. The PCR mixture was prepared using Takara DNA polymerase (TAKARA Inc, Tokyo, Japan) following the manufacturer's instructions. PCR amplification was performed in an Authorized thermal cycler (Biorad, USA) under

the conditions of 3 min at 94 ^{0}C followed by 35 cycles of 1 min at 94 ^{0}C, 1 min at 57 ^{0}C, and 1 min at 72 ^{0}C, followed by 10 mins final extension at 72 ^{0}C.

Table 3.1 Specific primers for AOB used in this study

Primer	Nucleotide sequence (5'–3')
CTO189A/Bf	GGAGRAAAGCAGGGGATCG
CTO 189Cf	GGAGGAAAGTAGGGGATCG
CTO189A/Bf-GC	CGCCCGCCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGAGRAAA GCAGGGGATCG
CTO 189Cf-GC	CGCCCGCCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGAGGAAA GTAGGGGATCG
CTO 654r	CTAGCYTTGTAGTTTCAAACGC

3.3.2.2 Denaturing gradient gel electrophoreses (DGGE)

Denaturing gradient gel electrophoreses was performed according to the modification of a described method (Kurisu *et al*., 2002). We use 8% polyacrylamide gels, and the urea–formamide denaturant gradient was 30–70%. Gels were run on the D Code system (Bio-Rad Laboratories, Hercules, CA, USA) for 16 h at 60 ^{0}C and 75 V. After electrophoresis, the gels are stained with Ethidium bromide (Amersham Biosciences, Munich, Germany) and analyzed by gel documentation (Dolphin-DOC Plus, NV, USA). Prominent bands were excised and dissolved in 30µl sterilized water. DNA was recovered from the gel by freeze–thawing three times.

3.3.2.3 Phylogenetic analysis

The completed 400-bp from 465-bp analyzed sequences were aligned with sequences from the SSU rRNA database (Antwerm, Belgium) using the ARB program package (Department of Microbiology, Technische Universitat Munchen, Munich, Germany; [http://www.arb-home.de]). Phylogenetic tree was constructed using the ARB program package. We added our 400-bp sequences into the distance tree, which was previously constructed based on comparison of 1000-bp sequences of all AOB, which are available in the SSU rRNA database, and some related non-AOB, which were used as outgroup sequences. Additionally, our 400-bp sequences and 400-bp sequences of described AOB species (Koops *et al*., 2003) and some related non-AOB were calculated based on maximum parsimony, maximum likelihood, and

distance analyses using the external software provided in the ARB program package (Phylip DNAPARS, AxML, and Phylip Distance Method, respectively).

3.3.3 Analysis of ammonia-oxidizing archaea communities

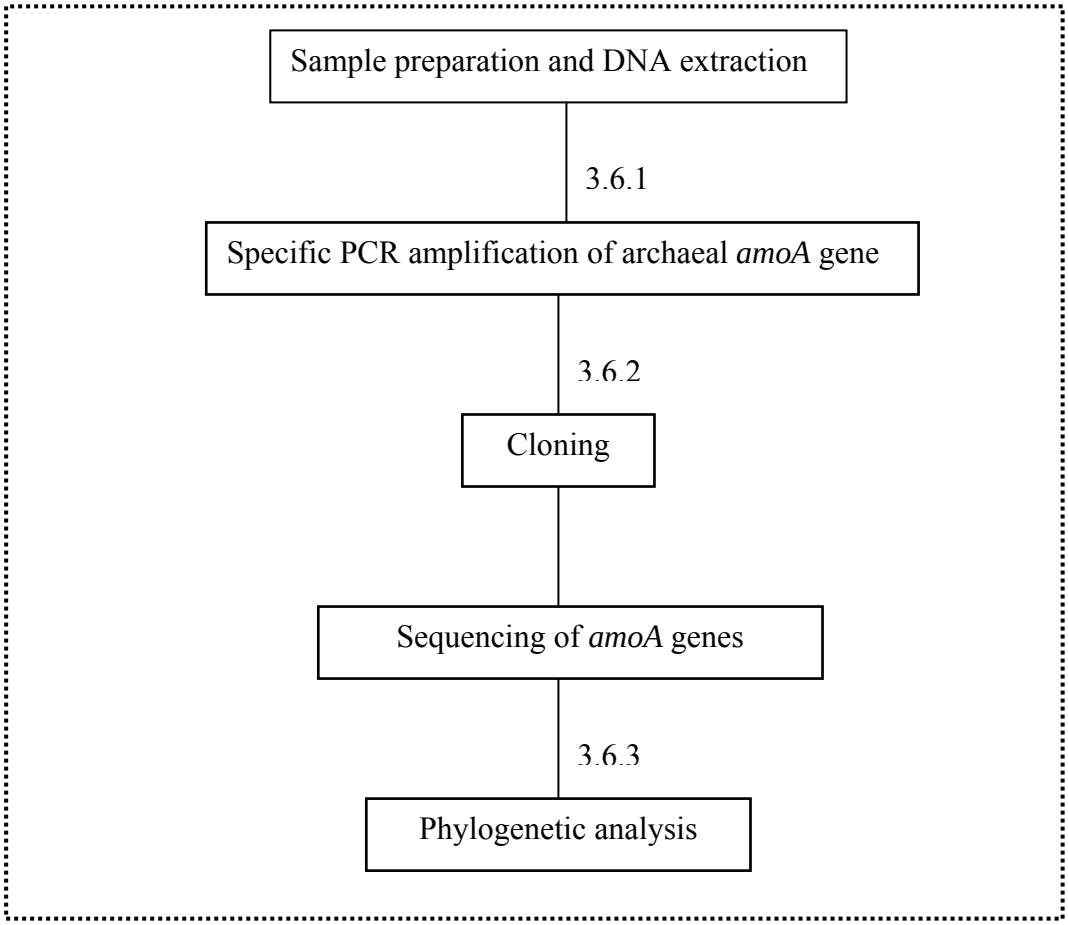


Figure 3.3 Experimental framework for analysis of ammonia oxidizing archaea communities

3.3.3.1 Polymerase chain reaction (PCR)

Primers Arch-amoAF and Arch-amoAR (Francis *et al*., 2005) were used to amplify 635-bp of Archaeal *amoA* gene fragments. The PCR mixture was prepared using Takara DNA polymerase (TAKARA Inc, Tokyo, Japan) following the manufacturer's instructions. PCR amplification was performed in an Authorized thermal cycler (Biorad, USA) under the conditions of 3 min at 94 ^{0}C followed by 35

cycles of 1 min at 94 ^{0}C, 1 min at 57 ^{0}C, and 1 min at 72 ^{0}C, followed by 10 mins final extension at 72 ^{0}C.

Table 3.2 Specific primers of AOA used in this study

Primer	Nucleotide sequence (5'–3')
Arch-amoAF	STAATGGTCTGGCTTAGACG
Arch-amoAR	GCGGCCATCCATCTGTATGT

3.3.3.2 Cloning

Duplicate PCR products were pooled and purified by gel electrophoresis using a QIAEXII gel extraction kit (QIAGEN Inc., Valencia, CA). The purified PCR products were cloned using the pGEM-T Easy vector system (Promega, Madison, WI) following the manufacturer's instructions. For each sample, 10-20 clones were randomly selected for sequencing.

3.3.3.3 Phylogenetic analysis

Phylogenetic trees for AOA was constructed by tree different methods comprising of distance matrix, maximum parsimony, and maximum likelihood to confirm the grouping of AOA analyzed in this study and all major AOA reported in previous studies so far. Cluster was defined based on operational taxonomic units (OTUs) using the DOTUR program (Scholoss *et al*., 2005). AOA clusters were defined based on OTUs using the DOTUR program. Any AOA sequences, showing >86% identity, were identified as the same AOA cluster.

3.4 Experiment Part I and II: Analysis of communities of ammonia-oxidizing bacteria and archaea in full-scale wastewater treatment plants (WWTPs)

3.4.1 Sample collection and description

Sludge sample were taken from 10 full-scale WWTPs, including 4 industrial WWTPs (I1-I4), 4 large municipal WWTPs (LM1-LM4), and 2 small municipal WWTPs (SM1 and SM4). All municipal WWTPs belong to Bangkok metropolitan administration (BMA). The WWTPs were selected basing on the difference in influent wastewater characteristics, system configuration, and system operation. The details were shown in Table 3.3.

3.4.2 Analysis of communities of ammonia-oxidizing bacteria in full-scale WWTPs

See section 3.3.2

3.4.3 Analysis of communities of ammonia-oxidizing archaea in full-scale WWTPs

See section 3.3.3

Table 3.3 Influent and effluent characteristics, system configuration, and system operation of full-scale WWTPs

WWTP	Treatment Process	Design Flow (m^3/d)	Actual flow (m^3/d)	BOD in influent (mg/l)	BOD in effluent (mg/l)	NH_3 in influent (mg/l)	NH_3 in effluent (mg/l)	% NH_3 removal	DO conc. in effluent (mg/l)
I1[a]	AS	6,000	4,500	400	7	NA	NA	NA	NA
I2[a]	AS	6,000	5,000	3,150	11	443	14	96.84	3
I3[a]	AS	4,000	1,300	NA	NA	30	4	86.67	2
I4[a]	AS	8,000	6,500	299	4	199	15	92.46	4.44
LM1[b]	AS	200,000	124,282	35.20	6.6	8.75	1.41	83.89	6.33
LM2[b]	AS	40,000	28,107	57.99	13.76	6.53	2.90	55.59	5.94
LM3[b]	AS	157,000	124,423	32.43	3.84	7.08	0.43	93.93	6.24
LM4[b]	AS	30,000	20,961	54.22	5.35	NA	NA	NA	3.27
SM1[b]	AS	800	600	116.82	5.50	NA	NA	NA	3.50
SM4[b]	Aerated lagoon	1,500	1,188	69.82	10.64	NA	NA	NA	1.35

AS, activated sludge process; NA, data not available

[a] The values were the averages of the three months in before the sludge was collected.

[b] The values were the averages of the year 2007, providing on the website (http://dds.bma.go.th)

3.5 Experiment Part III: Effect of ammonium concentrations on communities of ammonia-oxidizing bacteria and archaea in enriched nitrifying activated sludge (NAS)

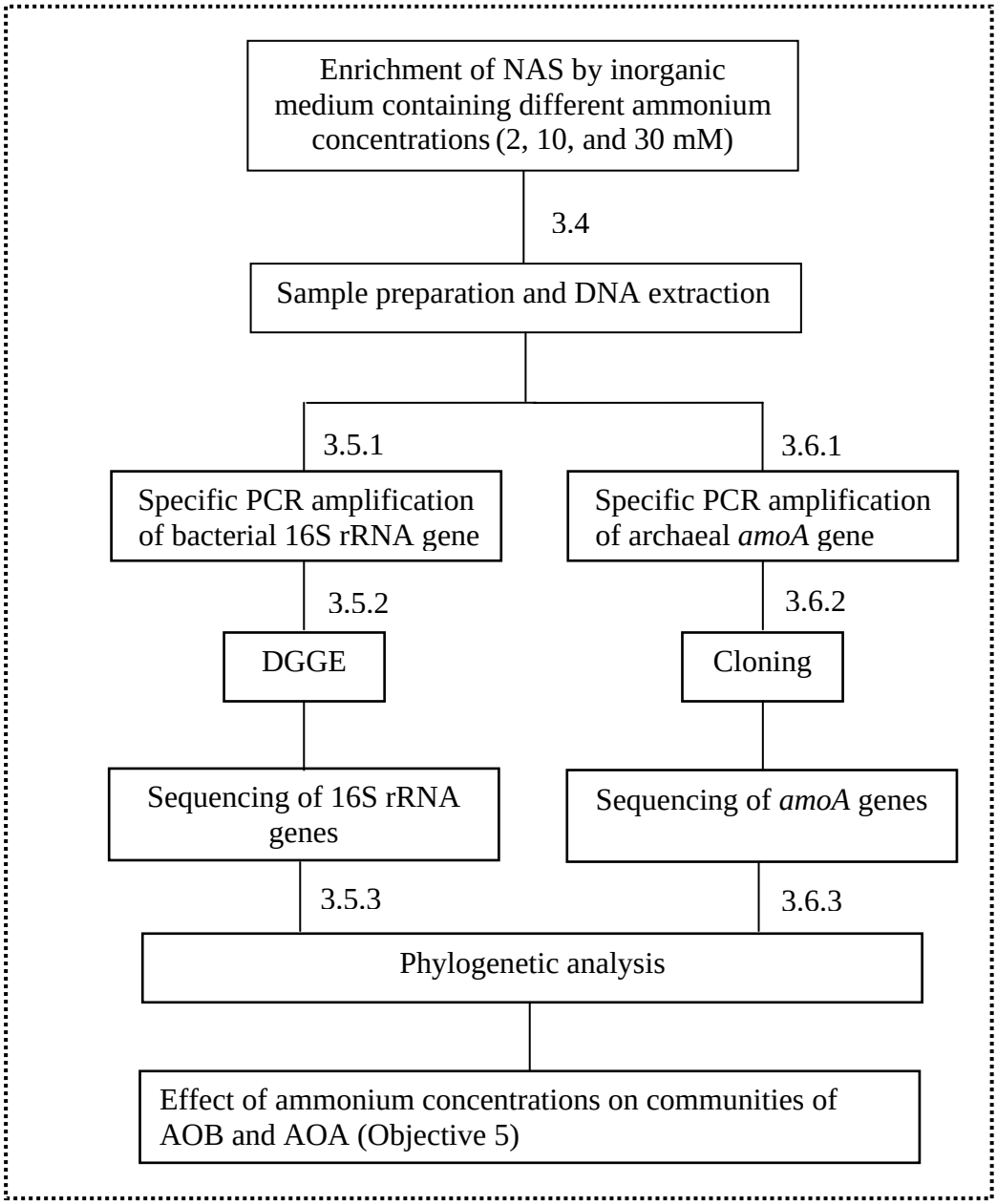


Figure 3.4 Experimental framework for analyzing the effect of ammonium concentrations on communities of AOB and AOA in enriched NAS

3.5.1 Seed sludge

Seed sludge was taken from a sludge buffer tank of a Chong Nonsi municipal wastewater treatment plant. This system is Cyclic Activated Sludge System (CASS) which is modified from Sequencing Batch Reactor (SBR) and can receive up to 200,000 m^3/day. On the day of sampling, biological oxygen demand (BOD) in the influent was 40 mg/ l, whereas ammonium concentration was 13 mg N/l. BOD and ammonium removal efficiencies of this system were 92.5 % and 84.6 %, respectively. Nitrite concentration in the aeration tank was 0.01 mg N/l, and pH was controlled around 6-7. Mixed-liquor suspended solids (MLSS) concentration on the day of sampling was 9385 mg/l.

3.5.2 Medium for enriching nitrifying activated sludge

The inorganic medium for enriching NAS contained $(NH_4)_2SO_4$, 40 mg of $MgSO_4 \cdot 7H_2O$, 40 mg of $CaCl_2 \cdot 2H_2O$, 200 mg of KH_2PO_4, 1 mg of $FeSO_4 \cdot 7H_2O$, 0.1 mg of $Na_2Mo_4O_4 \cdot 2H_2O$, 0.2 mg of $MnCl_2 \cdot 4H_2O$, 0.02 mg of $CuSO_4 \cdot 5H_2O$, 0.1 mg of $ZnSO_4 \cdot 7H_2O$, and 0.002 mg of $CoCl_2 \cdot 6H_2O$ per liter (Limpiyakorn *et al*., 2007). $NaHCO_3$ was added to achieve 2 mg bicarbonate (HCO_3^-) per 1 mg of ammonium added. pH was adjusted to around 7.5 - 8.0 using 40 g/l $NaHCO_3$.

3.5.3 Enrichment of nitrifying activated sludge by inorganic medium containing different ammonium concentrations (2, 10, and 30 mM)

Sludge taken from the municipal wastewater treatment system was enriched in three laboratory-scale continuous flow reactors without sludge recycling introduced with inorganic medium containing three different ammonium concentrations: 2, 10 and 30 mM NH_4^+-N (28, 140, and 420 mg N/l, respectively). Total volume of each reactor was 4 l, with an effective volume of 2 l. To obtain the optimum condition for AOB growth, temperature was kept at 30 ^{0}C, DO concentration was controlled at around 2 mg/l, pH was maintained in a range of 7.5 - 8.0 using 1 N HCl and 1 N NaOH, and mixing was provided at rotating speed of 300 rpm. Inorganic medium was introduced into all reactors at a fixed dilution rate of 0.01 hr^{-1} (Limpiyakorn *et al.*, 2007).

3.5.4 Analysis of communities of ammonia-oxidizing bacteria in seed sludge and enriched NAS

See section 3.3.2

3.5.5 Analysis of communities of ammonia-oxidizing archaea in seed sludge and enriched NAS

See section 3.3.3

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Communities of ammonia-oxidizing bacteria and archaea in full-scale wastewater treatment plants (WWTPs)

4.1.1 Description of full-scale WWTPs

Sludge sample were taken from 10 full-scale WWTPs, including 4 industrial WWTPs (I1-I4), 4 large municipal WTTPs (LM1-LM4), and 2 small municipal WWTPs (SM1 and SM4). The WTTPs were selected based on the difference in influent wastewater characteristics, system configuration, and system operation (Table 4.1). Four industrial WWTPs observed in this work were categorized into 3 types, depending on ammonia concentrations in influent wastewaters. Plant I2 represented the industrial WWTP with high ammonium concentration, Plants I3 and I4 represent the industrial WWTPs with moderate ammonium concentrations, and Plant I1 represented the industrial WWTP with low ammonium concentration. All industrial WWTPs were operated with activated sludge processes. All municipal WWTPs belong to Bangkok Metropolitan Administration (BMA). All of them were not different in influent characteristics. All municipal WWTPs received low ammonium concentration (5-13 mg-N/l), but different in system configuration and operation. All large and one small municipal WWTPs were operated with activated sludge processes, another small WWTPs was aerated lagoon system. The detail of each plants were shown in Table 4.1

Table 4.1 Description of wastewater treatment plants

WWTP	Treatment process	Flow (m^3/d)	ammonia (mg-N/l)		nitrite (mg-N/l)		nitrate (mg-N/l)		BOD (mg/l)		COD (mg/l)		DO (mg/l) Eff.	MLSS (mg/l)	HRT (hr)	SRT (day)
			Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.				
I1	AS	4,500	12.90	3.27	0.03	ND	22.56	35.24	NA	NA	NA	NA	NA	NA	1.20	NA
I2	AS	5,000	422.26	29.19	0.04	0.21	3.24	31.05	1,400	9	NA	NA	NA	4,000	24	14
I3	AS	1,300	72.50	3.32	0.04	0.02	3.76	46.87	NA	NA	NA	NA	NA	4,000	117.6	10
I4	AS	6,740	36.05	13.25	ND	0.15	1.43	0.96	192	3	505	26	3.68	5,530	118.5	10
LM1	AS	102,132.61	8.12	4.22	0.02	0.33	0.81	3.95	27.31	7.04	63.1	21.68	6.5	12,067	4	10
LM2	AS	27,634	5.54	5.05	0.03	0.18	0.79	1.80	49.16	12.47	85.63	45.38	5.74	NA	2.17	22
LM3	AS	133,986	5.74	7.30×10^{-3}	ND	0.03	0.78	7.23	27.07	3.21	NA	NA	6.12	3,933	4.27	11.04
LM4	AS	16,596	0.03	0.06	0.08	ND	3.56	12.32	57.42	5.45	106.85	19.42	NA	5,006	63.60	30
SM1	AS	600	13.87	2.35×10^{-3}	0.01	0.02	2.27	18.15	102	2	433	20	NA	6,790	43.20	21
SM4	Aerated lagoon	1,442	5.68	4.53	ND	0.23	0.94	1.33	75	5	185	15	1.58	105	67.24	> 1year

ND, not detected; NA, data not available
AS, activated sludge process; HRT, hydraulic retention time; SRT, solid retention time; MLSS, mixed liquor suspended solid;
[a] The values were the averages of the month in which sludge was collected.
[b] The values were analyzed from one-day grab samples collected on the day close to the day of sludge collection.;

4.1.2 Communities of ammonia-oxidizing bacteria in full-scale wastewater treatment plants

Communities of AOB in samples from full-scale WWTPs were investigated using specific PCR amplification, followed by DGGE, and sequencing of 16S rRNA gene of AOB belonging to beteproteobacteria. All bands recovered from DGGE were cut, reamplified, and run on new gels until they were purified before selecting for sequencing. In total, 14 bands of AOB 16S rRNA gene sequences were tested for sequence similarity using blast program (Table 4.2). All of the analyzed sequences showed 95 -100% identity at nucleotide level to the previous reported sequences in the database.

Table 4.2 Closely related sequences of AOB 16S rRNA gene fragments

Sample	Band	Score	Gap	Percent Identity	Accession No. of closely related sequence	closely related sequence
I1	AOB-I1-1	619	2/383 (0%)	367/383 (95%)	AY123811	*Nitrosomonas* sp. Nm59
I2	AOB-I2-1	667	0/364 (0%)	363/364 (99%)	AL954747	*Nitrosomonas europaea* ATCC 19718
I3	AOB-I3-1	662	0/364 (0%)	362/364 (99%)	AB176858	DGGE A-W-3
	AOB-I3-2	699	0/378 (0%)	378/378 (100%)	FM997803	Clone LEQUIA_R0CTO43
I4	AOB-I4-1	397	2/215 (0%)	212/215 (98%)	AJ297415	Clone GaN50304
LM1	AOB-LM1-1	787	1/443 (0%)	436/443 (98%)	AB222811	DGGE 0NO2c-3
	AOB-LM1-2	806	0/444 (0%)	441/444 (99%)	AJ297415	Clone GaN50304
	AOB-LM1-3	811	0/442 (0%)	441/442 (99%)	AJ297415	Clone GaN50304
LM2	AOB-LM2-1	623	3/357 (0%)	351/357 (98%)	EU224365	Clone 9R-27
LM3	AOB-LM3-1	577	2/349 (0%)	337/349 (96%)	EF016119	*Nitrosomonas oligotropha*
	AOB-LM3-2	401	2/227 (0%)	224/227 (98%)	AJ297415	Clone GaN50304
LM4	AOB-LM4-1	577	2/349 (0%)	337/349 (96%),	AB176858	DGGE A-W-3
SM1	AOB-SM1-1	630	0/347 (0%)	345/347 (99%)	AB176858	DGGE A-W-3
SM4	AOB-SM2-1	462	1/263 (0%)	259/263 (98%)	FM997808	Clone LEQUIA_R0CTO49

Phylogenetic trees were constructed by using three different methods comprising of distance matrix, maximum parsimony, and maximum likelihood. All methods exhibited the same grouping of AOB sequences in the tree (data not shown).

For phylogenetic presented in Figure 4.1, we add our partial 400-bp AOB 16S rRNA sequences using parsimony method into the phylogenetic tree prior constructed by neighbor joining (distance matrix) methods using 1000-bp sequences of all reference AOB species to avoid changing in the tree topology when shorter sequences than 1000-bp were used to constructed the tree. AOB found in each sample were summarized in Table 4.3.

Table 4.3 Summary of AOB found in full-scale WWTPs

AOB Cluster	I1	I2	I3	I4	LM1	LM2	LM3	LM4	SM1	SM4
Nitrosospira cluster										
unknown *Nitrosomonas* cluster			✓							
Nitrosomonas cryototerans cluster										
Nitrosomonas europaea-Nitrosococcus mobilis cluster		✓								
Nitrosomonas communis cluster			✓	✓	✓✓	✓	✓	✓	✓	
Nitrosomonas marina cluster										
Nitrosomonas oligotropha cluster	✓				✓		✓			✓

✓, present (amount of symbols represents numbers of band found)

AOB communities in industrial WWTPs (I1-I4) were more diverse than those in the municipal WWTPs (LM1-LM4 and SM1, SM4). AOB found in industrial WWTPs fell in 4 clusters that are unknown *Nitrosomonas* cluster, *N. europaea-Nc. mobilis* cluster, *N. communis* cluster, and *N. oligotropha* cluster. While AOB found in municipal WWTPs were restricted to only *N. communis* cluster and *N. oligotropha* cluster. The difference of wastewater characteristic might be the key factor causing distinct distribution patterns of AOB communities in both types of WWTPs. In the case of industrial WWTPs, characteristics of wastewater were varied considerably (Table 4.1). BOD and ammonia concentrations were in a range of 192 – 1,400 mg/l and 13 – 420 mg/l respectively. In contrast, influent wastewater of municipal WWTPs were similar in their characteristics. BOD and ammonia concentration were in a narrow ranges between 27 -102 mg/l and 5 – 14 mg/l respectively. It has been reported that ammonia concentrations is the important factor influencing the presence of AOB in different environments (Suwa *et al.*, 1994; Stehr *et al.*, 1995a; Koops and

Pommerening-Roser, 2001. Study on physiological properties of isolated AOB cultures suggested the difference in affinity constant for ammonia among the distinct AOB species. It is believed that this reflects the preference of AOB existing in the habitats (Koops *et al.,* 2003).

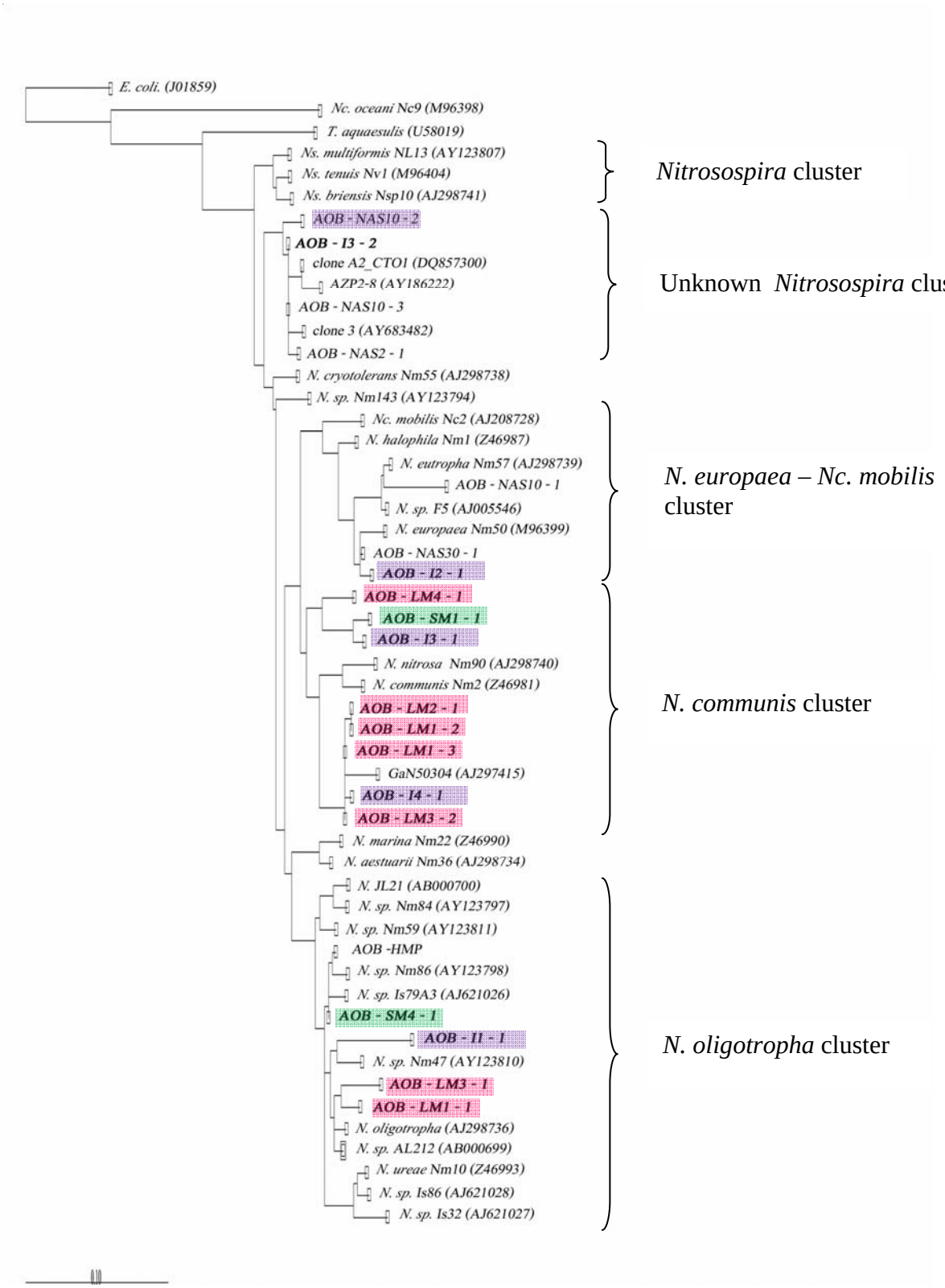


Figure 4.1 Phylogenetic tree showing 16S rRNA genes of AOB belonging to Betaproteobacterial with addition of 400-bp sequences from full-scale WWTPs into the distance tree that was previously constructed based on comparison of 1000-bp sequences of described AOB (Koops *et al*., 2003)

Ammonium concentration in all 4 industrial WWTPs were varied significantly Plant I2 represented a WWTP receiving high ammonium concentration (400 mg-N/l). Phylogenetic analysis suggested that sequences recovered from plant I2, related closely to AOB in *N. europaea-Nc. mobilis* cluster. All members of *N. europaea-Nc. mobilis* cluster have relatively high affinity constants for ammonia (50 -100 μM). This make AOB in this cluster prefer eutrophic environments such as fertilized soil (Koops *et al.,* 2003). In addition *N. europaea* are common AOB found in WWTPs receiving high ammonium loads (Limpiyakorn *et al.,* 2007). Plants I3 and I4 represented WWTPs receiving mid-low ammonium concentrations (30 – 70 mg-N/l). Bands retrieved from these two plants fell in unknown *Nitrosomonas* cluster and *N. communis* cluster. So far, no isolate culture has been obtained for the unknown *Nitrosomonas* cluster. Therefore the physiological properties of AOB in this cluster have not been revealed. The only one way to make a discussion on the AOB cluster is using information obtained by direct molecular study. Previously, clone A2_CTO1 and clone 3 which are members of this cluster were recovered from activated sludge and aquarium biofilter which are mid-low ammonia concentrations. This implied that this AOB clusters are a group that have moderate affinity to ammonia. *N. communis* cluster have been classified as AOB with moderate affinity constants for ammonia (14 - 43 μM). From this information, they should restrict to moderate eutrophic habitats; however, this group of AOB is occasionally observed in freshwater which is one of oligotrophic environments (Koops *et al.,* 2003). In addition, they have been detected in soil, activated sludge, and biofilm system (Purkhold *et al.,* 2000; Gieseke *et al*., 2001). Plant I1 represented a WWTP with low ammonium loads (10 mg-N/l), AOB found in this plant was the member of *N. oligotropha* cluster. *N. oligotropha* cluster are low in affinity constants for ammonia (1.9 – 4.2 μM). They frequently found in oligotrophic environments such as freshwater, unfertilized soil, WWTPs with relatively low ammonium loads (Koops and Pommerening-Roser 2001; Limpiyakorn *et al.,* 2005). Only members of *N. communis* cluster and *N.oligotropha* cluster were found in all 7 minicipal WWTPs. All municipal WWTPs received low ammonium strength wastewater (5 – 13 mg-N/l). If ammonium is the major factor, it is not surprising to find only 2 restrict AOB groups in all WWTPs. It has been reported extensively that *N. communis* cluster and *N.oligotropha* cluster were common AOB in

municipal WWTPs (Limpiyakorn *et al.*, 2007; Siripong and Rittmann 2007). Five out of the six plants contained AOB belonging to *N. communis* cluster. SM4 harbored only AOB of *N.oligotropha* cluster. It seemed that *N. communis* cluster might be more important than *N.oligotropha* cluster. From the study of Limpiyakorn 2007 by using real-time PCR to quantify each specific group of AOB in municipal WWTPs in Tokyo, *N.oligotropha* cluster were more abundant than *N. communis* cluster in all plants studied.

Other than ammonium loads or ammonium concentrations in the influent wastewater, system configuration and operation can be the other factors, influencing AOB communities in WWTPs. In this case only municipal WWTPs will be considered as it is possible to avoid the effect of influent characteristics. Almost all municipal WWTPs in this study were activated sludge process with the only exception for plant SM4 that was aerated lagoon system. In general, the SRT of aerated lagoon are relatively longer than the activated sludge process. SRT was firstly considerable as one of a major factor for system configuration and operation. However the results suggested that the AOB communities in all municipal WWTPs are similar. Therefore, system configuration and operation did not influence in this case.

4.1.3 Communities of ammonia-oxidizing archaea in full-scale wastewater treatment plants

Communities of AOA in samples from full-scale WWTPs were analyzed using specific PCR amplification, followed by clone libraries, and sequencing of AOA *amo*A gene fragments. For each library, 10 clones were randomly selected for sequencing. In total 72 clones, analyzed for sequencing, were tested for sequence similarity using blast program (Table 4.4). Results suggested that all analyzed showed 93 – 99% identity at nucleotide level to previously reported AOA *amo*A gene sequences. All analyzed sequences were calculated by DOTUR program to arrange for operational taxonomic units (OTUs) (Scholoss *et al*., 2005). Any sequences from the same library that showed 100% identity OTUs were assembled as one OTU. All 72 AOA *amo*A sequences were categorized into 38 OTUs. The best quality AOA *amo*A sequence of each OTU was selected to be the representative one. The amounts

of the sequences of each OTU were displayed by the number in parentheses of Table 4.4.

Table 4.4 Closely related sequences of AOA *amo*A gene fragments

Sample	Clone	Score	Percent Identity	Gap	Accession No. of closely related sequence	closely related sequence	Source
I3	AOA-I3-1 (6)	1070	585/588 (99%)	0/588 (0%)	EU590198	Clone BGA-661	Soil
	AOA-I3-2 (1)	1050	579/584 (99%)	1/584 (0%)	EU590198	Clone BGA-661	Soil
	AOA-I3-3 (1)	1046	579/585 (98%)	2/585 (0%)	EU590198	Clone BGA-661	Soil
	AOA-I3-4 (1)	1048	580/586 (98%)	1/586 (0%)	EU590198	Clone BGA-661	Soil
	AOA-I3-5 (1)	1053	579/583 (99%)	1/583 (0%)	EU590198	Clone BGA-661	Soil
I4	AOA-I4-1 (1)	979	553/564 (98%)	2/564 (0%)	EU239976	Clone MamSp.H08	Mammoth Hot Spring sediment
LM1	AOA-LM1-1 (2)	1029	566/570 (99%)	1/570 (0%)	EU651295	Clone SF05-BG30-E01	Estuary sediments
	AOA-LM1-2 (3)	924	543/564 (96%)	1/564 (0%)	DQ278527	Clone DI-20	WWTP operated with low dissolved oxygen levels and long retention times
	AOA-LM1-3 (1)	985	558/570 (97%)	1/570 (0%)	EU239976	Clone MamSp.H08	Mammoth Hot Spring sediment
	AOA-LM1-4 (2)	996	560/570 (98%)	1/570 (0%)	EU239976	Clone MamSp.H08	Mammoth Hot Spring sediment
	AOA-LM1-5 (1)	1003	560/568 (98%)	1/568 (0%)	EU239976	Clone MamSp.H08	Mammoth Hot Spring sediment
	AOA-LM1-6 (1)	981	540/544 (99%)	1/544 (0%)	EU651295	Clone SF05-BG30-E01	Estuary sediments
LM2	AOA-LM2-1 (7)	1064	587/592 (99%)	1/592 (0%)	DQ304863	Clone 2	Trrestrial archaea
	AOA-LM2-2 (1)	1046	581/588 (98%)	2/588 (0%)	DQ304863	Clone 2	Terrestrial archaea
	AOA-LM2-3 (1)	1035	578/586 (98%)	3/586 (0%)	DQ304863	Clone 2	Terrestrial archaea
	AOA-LM2-4 (1)	1042	581/589 (98%)	3/589 (0%)	DQ304863	Clone 2	Terrestrial archaea
LM3	AOA-LM3-1 (2)	963	546/558 (97%)	2/558 (0%)	EU239976	Clone MamSp.H08	Mammoth Hot Spring sediment
	AOA-LM3-2 (1)	977	553/564 (98%)	3/564 (0%)	EU239976	Clone MamSp.H08	Mammoth Hot Spring sediment
	AOA-LM3-3 (1)	826	526/565 (93%)	2/565 (0%)	FJ227760	Clone WBM050405_45P2A1	Sediment
	AOA-LM3-4 (2)	841	530/567 (93%)	1/567 (0%)	FJ227760	Clone WBM050405_45P2A1	Sediment
	AOA-LM3-5 (2)	854	535/571 (93%)	1/571 (0%)	FJ227760	Clone WBM050405_45P2A1	Sediment
	AOA-LM3-6 (1)	990	557/567 (98%)	1/567 (0%)	EU239976	Clone MamSp.H08	Mammoth Hot Spring sediment
	AOA-LM3-7 (1)	965	567/589 (96%)	1/589 (0%)	EU852665	Clone PLANTC AR RSF-I OTU1	Water sampled

Sample	Clone	Score	Percent Identity	Gap	Accession No. of closely related sequence	closely related sequence	Source
LM3	AOA-LM3-8 (1)	979	555/567 (97%)	1/567 (0%)	EU239976	Clone MamSp.H08	Mammoth Hot Spring sediment
LM4	AOA-LM4-1 (9)	1013	573/585 (97%)	1/585 (0%)	EU885673	Clone 3063-A-04	Deep-sea sediments
	AOA-LM4-2 (1)	1009	568/579 (98%)	0/579 (0%)	EU885673	Clone 3063-A-04	Deep-sea sediments
SM1	AOA-SM1-1 (9)	981	554/565 (98%)	3/565 (0%)	FJ755701	Clone AOA-10	Sediment
	AOA-SM1-2 (1)	904	546/571 (95%)	15/571 (2%)	FJ755701	Clone AOA-10	Sediment
SM4	AOA-SM4-1 (1)	1057	586/592 (98%)	4/592 (0%)	EU885647	Clone 3057-A-16	Deep-sea sediments
	AOA-SM4-2 (1)	994	557/566 (98%)	1/566 (0%)	EU239976	Clone MamSp.H08	Mammoth Hot Spring sediment
	AOA-SM4-3 (1)	1018	576/588 (97%)	2/588 (0%)	EU885647	Clone 3057-A-16	Deep-sea sediments
	AOA-SM4-4 (1)	771	502/544 (92%)	2/544 (0%)	EU022958	Clone HB_C_0604__C02	Coastal sediments
	AOA-SM4-5 (1)	828	527/566 (93%)	1/566 (0%)	FJ227760	Clone WBM050405_45P2A1	Sediment
	AOA-SM4-6 (1)	924	553/579 (95%)	2/579 (0%)	FJ601561	Clone MS_26B4	Tropical marine estuary sediment
	AOA-SM-7 (1)	948	550/567 (97%)	5/567 (0%)	EF382617	Clone PA6-23	Coral colony
	AOA-SM4-8 (1)	1044	576/581 (99%)	1/581 (0%)	EU590435	Clone SGX-123	Soil
	AOA-SM4-9 (1)	1018	563/569 (98%)	0/569 (0%)	DQ501096	Clone MX_5_JAN_6	Estuarine sediments
	AOA-SM4-10 (1)	922	542/562 (96%)	5/562 (0%)	EF382617	Clone PA6-23	Coral colony

Number in parenthesis indicated amounts of AOA *amo*A clones showing 100% identity

Due to the limited information on AOA phylogenetic taxonomy, phylogenetic trees for AOA was constructed by tree different methods comprising of distance matrix, maximum parsimony, and maximum likelihood to confirm the grouping of AOA analyzed in this study and all major AOA reported in previous studies so far (Figure 4.3, 4.4, 4.5). It must be noted that Figure 4.3, 4.4, 4.5 have been used to confirm AOA clusters only. No information of species was provided in these three trees. Figure 4.2 showed phylogenetic tree constructed based on distance matrix (neighbor joining) with the complete sequence detail that will be used for further discussion. AOA clusters were defined based on OTUs using the DOTUR program (Scholoss *et al*., 2005). Any AOA sequences, showing >86% identity, were

identified as the same AOA cluster. AOA communities in seed sludge, and enriched NAS were summarized in Table 4.5.

Table 4.5 Summary of AOA communities in full-scale WWTPs

Cluster	I1	I2	I3	I4	LM1	LM2	LM3	LM4	SM1	SM4
B					✓					
E										✓
A					✓✓					
F										✓
C								✓✓		✓✓✓
G			✓✓✓✓✓							
N										✓
D										
I							✓✓✓		✓✓	✓
J						✓✓✓✓				
K				✓	✓✓✓		✓✓✓✓			✓
L							✓			
M										✓✓

✓, present (amount of symbols represents numbers of OTUs)

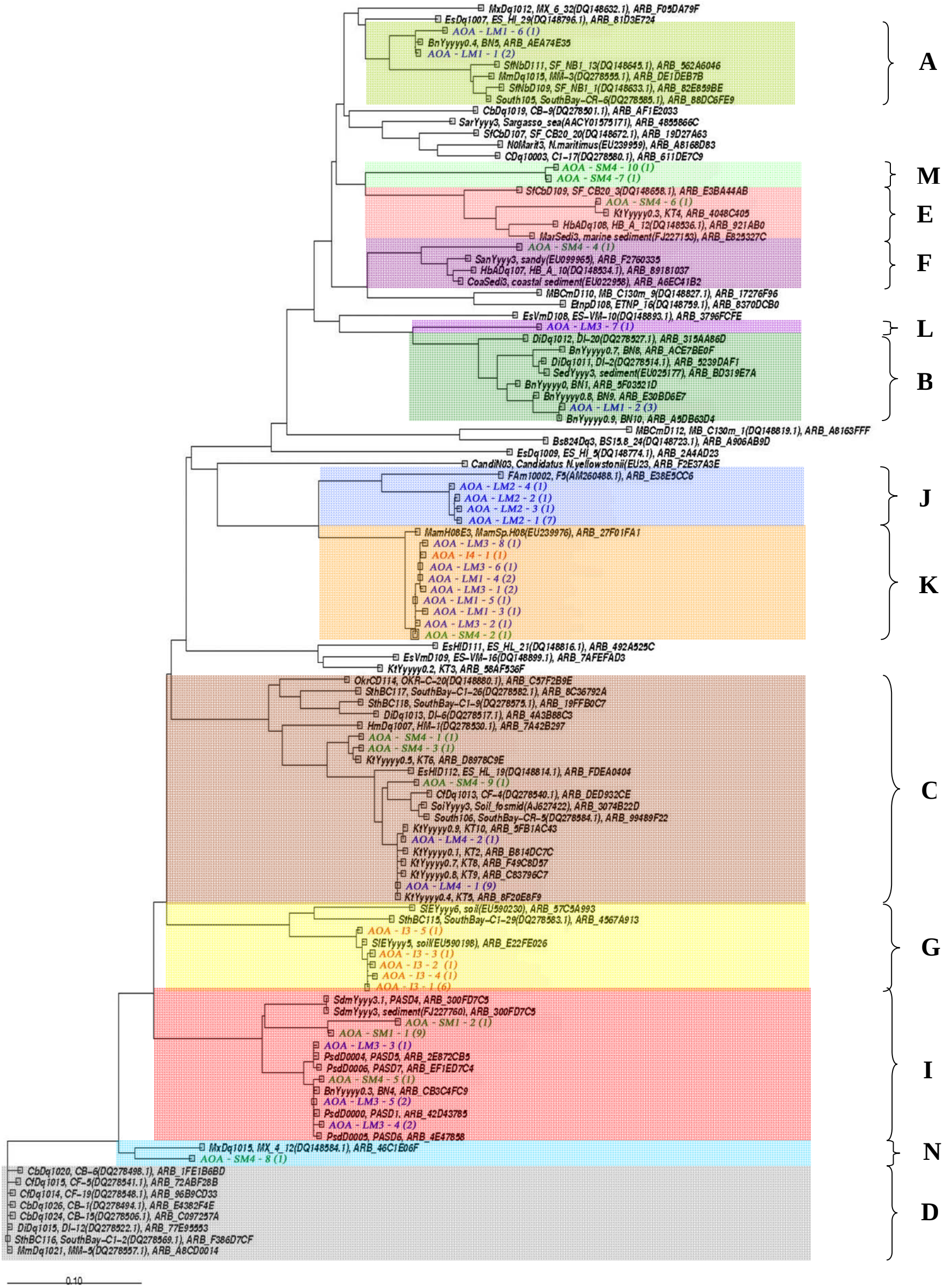


Figure 4.2 Neighbor joining tree of AOA *amo*A sequences from full-scale WWTPs (Details information is provided in this tree and this tree will be used for discussion)

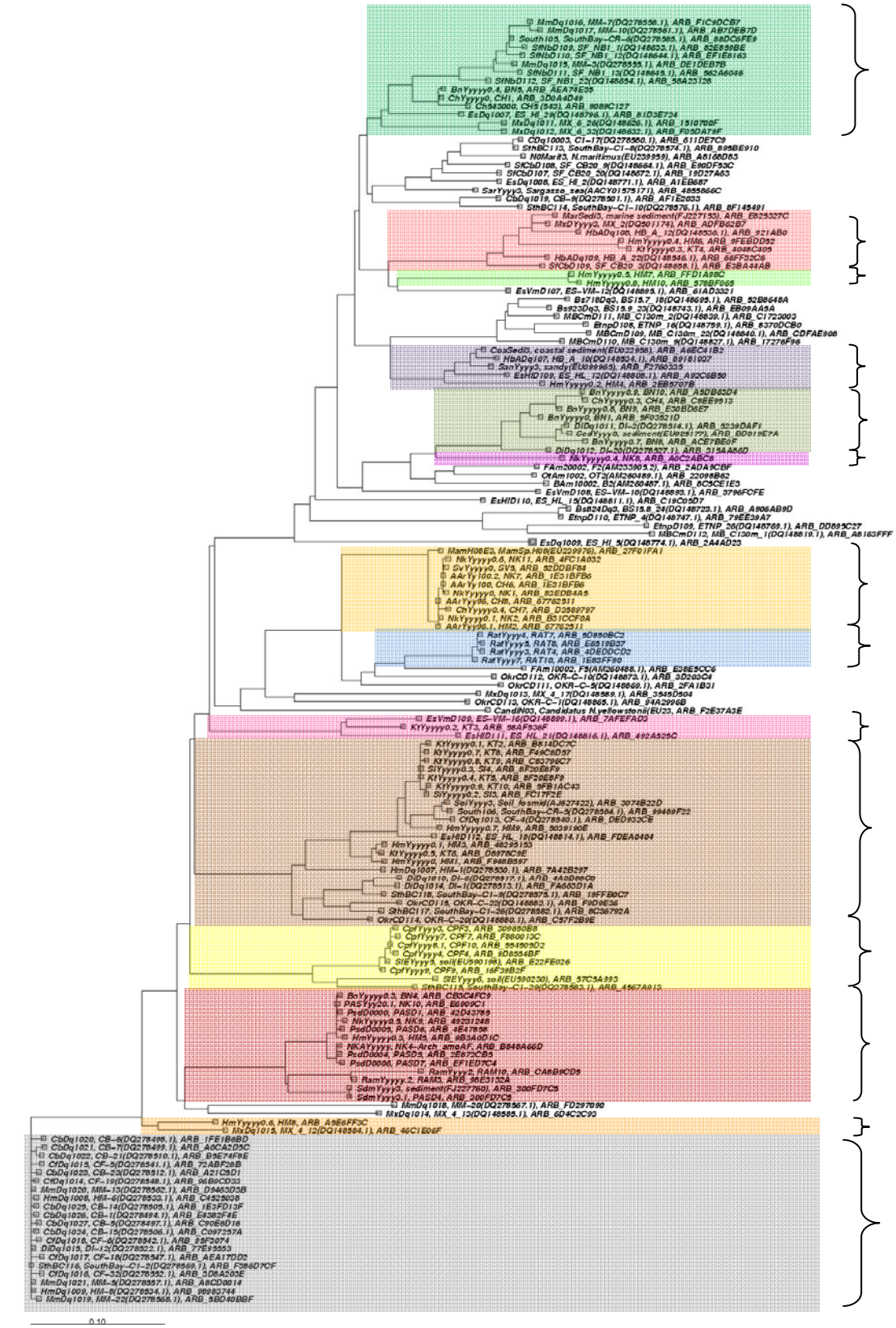


Figure 4.3 Distance matrix tree of AOA *amo*A sequences from full-scale WWTPs. (It must be noted that details information of species is not provided in this tree); Clusters A, B, C, and D were indicated by Park *et al*., 2006, while other than those were found in this study.

Figure 4.4 Maximum parsimony tree of AOA *amo*A sequences from full-scale WWTPs. (It must be noted that details information of species is not provided in this tree) Clusters A, B, C, and D were indicated by Park *et al.*, 2006, while other than those were found in this study.

Figure 4.5 Maximum likelihood tree of AOA *amo*A sequences from full-scale WWTPs. (It must be noted that details information of species is not provided in this tree); Clusters A, B, C, and D were indicated by Park *et al*., 2006, while other than those were found in this study.

So far, the only one study on AOA communities in WWTPs has been done by Park *et al.*, 2006. Results demonstrated that AOA communities in all of activated sludge of WWTPs were observed using the same technique used in this study. AOA *amo*A sequences, retrieved from activated sludge, fell in 4 clusters (A, B, C, and D) only. Cluster D contained the largest AOA *amo*A sequences, so this cluster was proposed to be the dominant cluster of AOA found in activated sludge. All AOA *amo*A sequences retrieved from full-scale WWTPs examined in this study distributed in 12 clusters (Table 4.5). However, in this study more AOA clusters have been observed. In addition, AOA sequences found were not restricted to the cluster D as well as other clusters indicated by Park *et al.*, 2006. These suggested that it was found more AOA diversity in our WWTP samples. Most clones obtained from this study fell in cluster K. AOA *amo*A clones from WWTPs in this study closely related to those recovered from various habitats including soil, sediment, estuary sediment, marine sediment, deep sea sediment, hot spring sediment, and subsurface thermal spring. Interestingly, the environmental conditions in those sources were far different from those in our WWTPs (such as salinity, temperature, and ammonia concentration). These results suggested that those AOA clusters are not restricted to certain environmental factors. They are more flexible to adapt themselves to survive in more variety of environments.

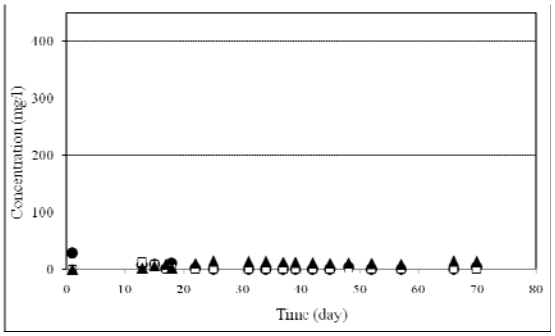
Communities of AOA in the industrial WWTPs were less diverse than those in the municipal WWTPs. Not all samples of industrial WWTPs showed positive PCR amplification of AOA *amo*A fragments. Only 2 industrial WWTPs for plants I3 and I4, which represented the industrial WWTPs with moderate ammonium loads (40 -70 mg-N/l), contained AOA *amo*A gene fragments. Furthermore, only one AOA cluster was found in each sample (cluster G in plant I3 and cluster K in plant I4). Negative PCR amplification occurred with samples I1 and I2 that represented the industrial WWTPs with low (13 mg-N/l) and high (422 mg-N/l) ammonium loads respectively. The reason that AOA were absent from samples I2 might be because of high ammonium loads for this plant. However, the reason for the case of plant I1 was unclear. In the case of the municipal WWTPs, the influent ammonium concentration comes were in a range of 5 – 13 mg-N/l. AOA *amo*A gene fragments were detected in all samples and they distributed in various clusters (Table 4.5). And in most

samples, more than one AOA clusters was found suggesting more variety of AOA clusters in the municipal WWTPs than in the industrial WWTPs.

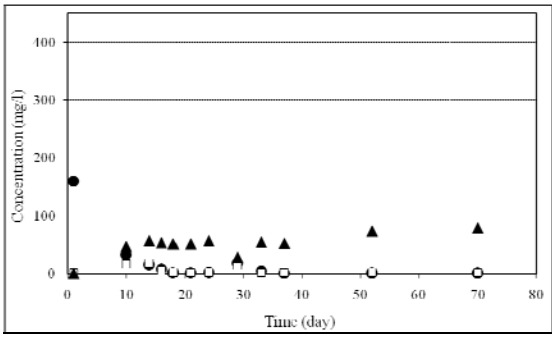
Other than influent characteristics, system configuration and operation might be considered as the factors that affected AOA communities. All industrial WWTPs (I3 and I4) and 5 out of 6 municipal WWTPs (LM1, LM2, LM3, LM4, and SM1) were activated sludge processes, whereas another one (SM4) was aerated lagoon system (Table 4.1). For each sample of activated sludge processes, only 1 – 3 clusters were observed, while in the sample of aerated lagoon much more AOA clusters of 6 were found. It was implied that the longer SRT might be a result of more AOA diversity in the WWTPs. It has been reported that 5 out of 9 WWTPs operating with long retention times (>15 days of SRT, >24 h of HRT) contained AOA communities in their system, whereas 4 out of 9 operating with shorter retention times, no AOA was observed (Park *et al.*, 2006). System configuration and operation can be important factors, influencing AOA communities in WWTPs.

4.2 Effect of ammonium concentrations on communities of ammonia-oxidizing bacteria and archaea in enriched nitrifying activated sludge

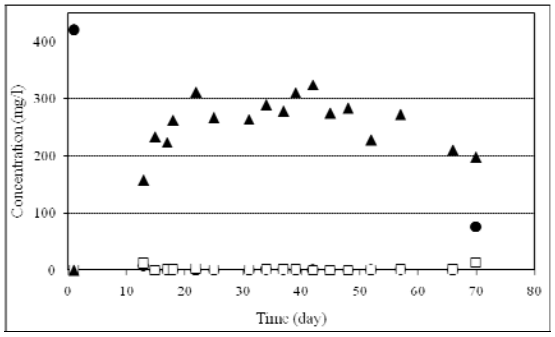
4.2.1 Enrichment of nitrifying activated sludge by inorganic medium containing different ammonium concentrations of 2, 10, and 30 mM NH_4^+-N (NAS 2, NAS 10, and NAS 30)



(a)



(b)



(c)

Figure 4.6 Concentrations of ammonium (●), nitrite (□), and nitrate (▲) during enrichment of nitrifying activated sludge by inorganic medium containing different ammonium concentrations (a) NAS 2, (b) NAS 10, and (c) NAS 30

This experiment was conducted to observe effect of ammonium concentrations on communities of AOB and AOA. Three enriched NAS feeding with inorganic medium containing different ammonium concentrations (2, 10, and 30 mM NH_4^+-N) were operated for 80 days. During operation, ammonium, nitrite, and nitrate concentrations were monitored (Figure 4.6). Ammonium concentrations in all three reactors reached the steady-state conditions after certain periods of operation (NAS 2 after 22 days, NAS 10 after 37, and NAS 30 after 15 days of operation). In all cases, ammonium was completely oxidized. Nitrite was detected shortly after starting the operation of all reactors (NAS 2 after 13 days, NAS 10 after 10 days, and NAS 30 after 10 days of operation). Then nitrite gradually decreased nearly zero (NAS 2 after 18 days, NAS 10 after 15 days, and NAS 30 after 18 days of operation). Nitrate was detected in all reactors and temporarily increased until reaching the steady-state condition (NAS 2 after 22 days, NAS 10 after 14 days, and NAS 30 after 18 days of operation). Total nitrogen concentrations in all reactors were slightly lost. It was probably due to denitrification reducing nitrate to nitrogen gas. DO concentration in each reactor was controlled to be above 2 mg/l to ensure absolute aerobic conditions. After 60 days of operation, sludge samples were collected to analyze for communities of AOB and AOA.

4.2.2 Communities of ammonia-oxidizing bacteria in seed sludge and enriched NAS

Communities of AOB in samples (seed sludge, NAS 2, NAS 10, and NAS 30) were investigated by using specific PCR amplification, followed by DGGE, and sequencing of 16S rRNA gene of AOB belonging to beteproteobacteria . All bands recovered from DGGE were cut, reamplified, and run on new gels until they were purified before selecting for sequencing. In total 8 bands, analyzed for sequencing, were tested for sequence similarity using blast program (Table 4.6). Results suggested that all analyzed sequences showed 96 -99% identity at nucleotide level to the previous reported sequences in the database.

Table 4.6 Closely related sequences of AOB 16S rRNA gene fragments

Sample	Band	Score	Percent Identity	Gap	Accession No. of closely related sequence	closely related sequence
Seed sludge	AOB-S-1	787	436/443 (98%)	1/443 (0%)	AB222811	DGGE 0NO2c-3
	AOB-S-2	806	441/444 (99%)	0/444 (0%)	AJ297415	Clone GaN50304
	AOB-S-3	811	441/442 (99%)	0/442 (0%)	AJ297415	Clone GaN50304
NAS 2	AOB - NAS2-1	712	403/411 (98%)	3/411 (0%)	AM295532	Clone Nm 271104_1
NAS 10	AOB – NAS10-1	813	452/457 (98%)	3/457 (0%)	AY123795	*Nitrosomonas eutropha*
	AOB - NAS10-2	739	431/446 (96%)	6/446 (1%)	AM295532	Clone Nm 271104_1
	AOB - NAS10-3	728	405/410 (98%)	1/410 (0%)	AM295532	Clone Nm 271104_1
NAS 30	AOB – NAS30-1	830	455/458 (99%)	1/458 (0%)	AL954747	*Nitrosomonas europaea* ATCC 19718

Phylogenetic trees were constructed by using three different methods comprising of distance matrix, maximum parsimony, and maximum likelihood. All methods exhibited the same grouping of AOB sequences in the tree (data not shown). For phylogenetic presented in Figure 4.7, we add our partial 400-bp AOB 16S rRNA sequences using parsimony method into the phylogenetic tree prior constructed by neighbor joining (distance matrix) methods using 1000-bp sequences of all reference AOB species to avoid changing in the tree topology when shorter sequences than 1000-bp were used to constructed the tree. AOB found in each sample were summarized in Table 4.7.

Table 4.7 Summary of AOB found in seed sludge and enriched NAS

AOB Cluster	Seed sludge	NAS 2	NAS 10	NAS 30
Nitrosospira cluster				
unknown *Nitrosomonas* cluster			✓✓	
Nitrosomonas cryototerans cluster				
Nitrosomonas europaea-Nitrosococcus mobilis cluster			✓	✓
Nitrosomonas communis cluster	✓✓			
Nitrosomonas marina cluster				
Nitrosomonas oligotropha cluster	✓	✓		

✓, present (amount of symbols represents numbers of band found)

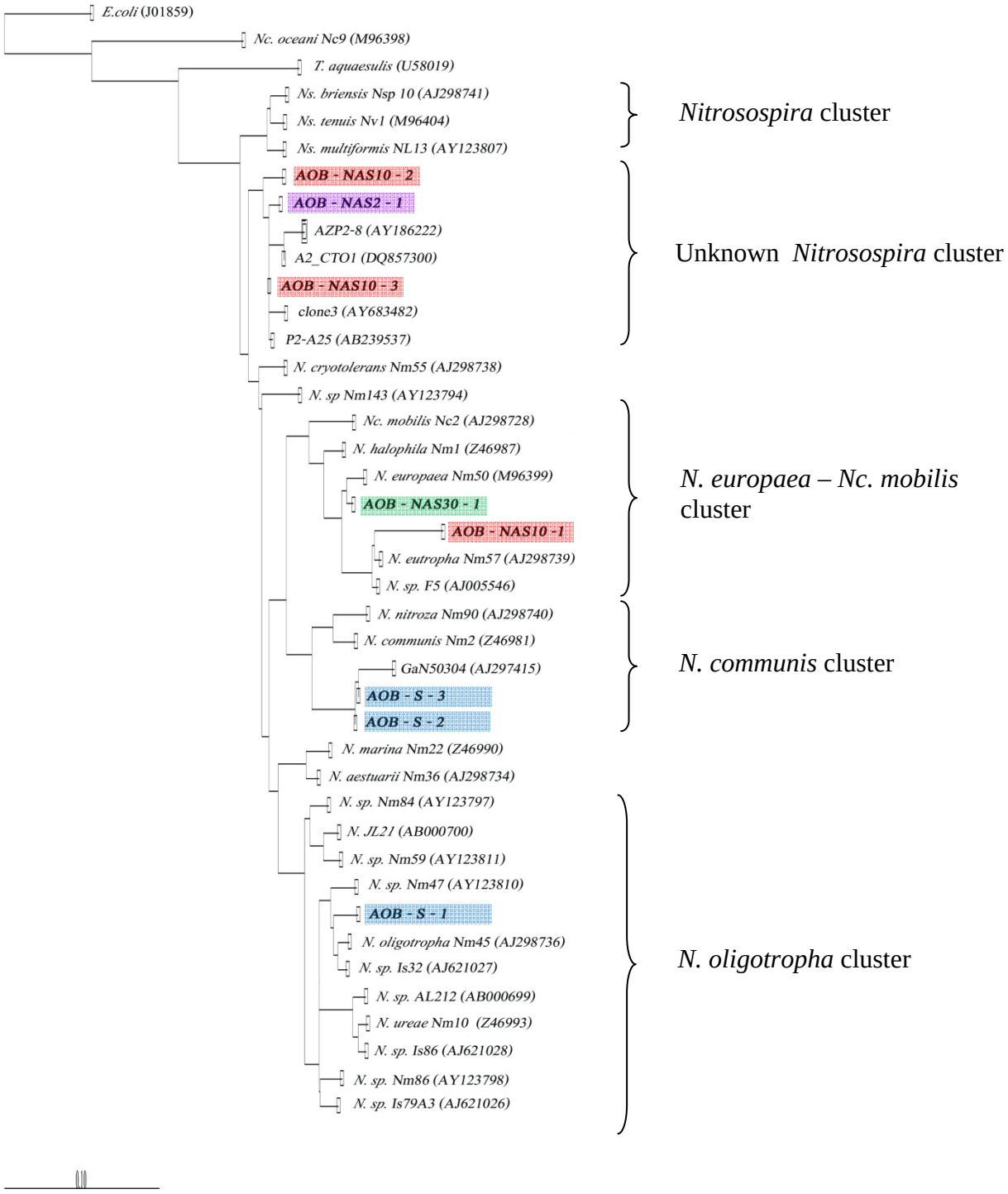


Figure 4.7 Phylogenetic tree showing 16S rRNA genes of AOB belonging to Betaproteobacterial with addition of 400-bp sequences from seed sludge and enriched NAS into the distance tree that was previously constructed based on comparison of 1000-bp sequences of described AOB (Koops *et al*., 2003)

Sludge taken from a municipal WWTP used as a seed for all reactors contained members of *N. communis* cluster and *N. oligotropha* cluster. After it was enriched under various ammonia concentrations, members of *N. communis* cluster disappeared from all enriched NASs. In NAS 2, only AOB closely related to *N. oligotropha* cluster were found. Sequence types of unknown *Nitrosomonas* cluster and *N. europaea* cluster were recovered from NAS 10. Only AOB related to *N. europaea* cluster were observed in NAS 30.

Results revealed the shift in AOB communities from a seed sludge to enriched NAS, and the communities of AOB in each NAS varied significantly. AOB in a seed sludge related closely to DGGE 0NO2c-3 and clone GaN50304 which were recovered from a sequencing batch biofilm reactor (Gieseke *et al*., 2001) and laboratory-scale continuous-flow reactor (Limpiyakorn *et al*., 2006). And fell in the clusters *N. communis* and *N. oligotropha* cluster respectively. Although members of *N. communis* cluster were reported for their moderate affinity to free ammonia (K_s = 14 to 43 µM; Koops *et al*., 2003), they were often recovered from wastewater treatment systems receiving low ammonium loads (Gieseke *et al*., 2001, Koops *et al*., 2003, Limpiyakorn *et al.*, 2005). *N. oligotropha* cluster are well known to be common AOB in municipal WWTPs (Limpiyakorn *et al.*, 2005) as they exhibited high affinity to ammonia (K_s = 1.9 to 4.2 µM; Koops *et al*., 2003), meaning that they prefer low ammonia habitats. It is not surprised to find these two AOB cluster in the seed sludge, as the seed sludge was taken from a municipal WWTP receiving low ammonium load.

Band analyzed for NAS 2 closely related to the clone Nm 271104_1 that was recovered from marine aquaculture (Foesel *et al*., 2007), and fell in *N. oligotropha* cluster. This cluster was AOB with high affinity to ammonia, being the range of a few µM. Member of *N. oligotropha* cluster are absolute majority strains originating from oligotropic freshwaters and generally the dominant AOB representatives in natural freshwater environments. Moreover, *N.oligotropha* are the most common AOB found in WWTPs with low ammonium loads (Limpiyakorn *et al*., 2005, 2006b).

Bands analyzed from NAS 10 were found to be related to Clone Nm 271104_1, that was obtained from marine aquaculture (Foesel *et al*., 2007), and fell in unknown *Nitrosomonas* cluster. While, another band of this sample was closely

related to *N. eutropha* C91 (Purkhold *et al*., 2003), and fell in *N. europaea* cluster. Surprisingly, groups of AOB exhibiting low and high affinity to ammonia was found in this NAS that received the moderate ammonium load.

Band from NAS 30 was affiliated to *N. europaea* ATCC19718 that fell in *N. europaea* cluster. These AOB are low in affinity to ammonia (K_s > 30 µM; Koops *et al*., 2003). They are often found in wastewater with high ammonium loads, eutrophic freshwaters, or fertilized soil (Koops *et al*., 2003).

AOB can be divided into two groups which are AOB with high and low affinity to free ammonia. AOB with high affinity to ammonia could be retrieved from NAS 2 and NAS 10. Whereas AOB with low affinity to ammonia could be recovered from NAS 10 and NAS 30. AOB communities in NAS 10 were the mixture of AOB of high and low affinity to ammonia. This may be because of the moderate ammonium load supplied to the NAS 10. This demonstrated that ammonium load is the important factor, selecting the communities of AOB in the enriched NAS and the selection is based on the physiological properties (ammonia affinity) reported for the isolated AOB cultures in the previous studies.

4.2.3 Communities of ammonia-oxidizing archaea in seed sludge and enriched NAS

Communities of AOA in samples (seed sludge, NAS 2, NAS 10, and NAS 30) were analyzed using specific PCR amplification, followed by clone libraries, and sequencing of *amo*A gene of AOA. For each library, 10-30 clones were randomly selected for sequencing. In total 88 clones, analyzed for sequencing, were tested for sequence similarity using blast program (Table 4.8). Results suggested that all analyzed showed 88 – 98% identity at nucleotide level to previously reported AOA *amo*A gene sequences. All analyzed sequences were calculated by DOTUR program to arrange for operational taxonomic units (OTUs) (Scholoss *et al*., 2005). Any sequences from the same library that showed 100% identity OTUs were assembled as one OTU. All 88 AOA *amo*A sequences were categorized into 30 OTUs. The amounts of the sequences of each OTU were displayed by the number in parentheses of Table 4.8.

Table 4.8 Closely related sequences of AOA *amo*A gene fragments

Sample	Clone	Score	Percent Identity	Gap	Accession No. of closely related sequence	closely related sequence
Seed Sludge	AOA-S-1 (5)	957	541/552 (98%)	2/552 (0%)	EU239976	Clone MamSp.H08
	AOA-S-2 (1)	946	539/552 (97%)	2/552 (0%)	EU239976	Clone MamSp.H08
	AOA-S-3 (1)	953	548/563 (97%)	4/563 (0%)	EU239976	Clone MamSp.H08
	AOA-S-4 (1)	833	527/564 (93%)	3/564 (0%)	FJ227760	Clone WBM050405_45P2A1
	AOA-S-5 (1)	693	473/537 (88%)	2/537 (0%)	FJ227760	Clone WBM050405_45P2A1
	AOA-S-6 (1)	819	518/555 (93%)	1/555 (0%)	FJ227760	Clone WBM050405_45P2A1
NAS 2	AOA-NAS2-1 (5)	976	552/563 (98%)	3/563 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS2-2 (9)	981	553/563 (98%)	3/563 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS2-3 (2)	977	553/564 (98%)	3/564 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS2-4 (1)	955	549/564 (97%)	4/564 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS2-5 (1)	941	546/563 (96%)	5/563 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS2-6 (1)	815	537/583 (92%)	7/583 (1%)	FJ227153	Clone 3_15
	AOA-NAS2-7 (1)	992	557/566 (98%)	3/566 (0%)	EU022958	Clone HB_C_0604__C02
	AOA-NAS2-8 (1)	673	497/561 (88%)	10/561 (1%)	EU239976	Clone MamSp.H08
NAS 10	AOA-NAS10-1 (14)	979	553/564 (98%)	2/564 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS10-2 (6)	983	554/564 (98%)	3/564 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS10-3 (1)	955	549/564 (97%)	3/564 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS10-4 (1)	966	551/564 (97%)	3/564 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS10-5 (1)	966	557/573 (97%)	3/573 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS10-6 (1)	963	556/573 (97%)	2/573 (0%)	EU590230	Clone BGA-781
	AOA-NAS10-7 (1)	896	539/564 (95%)	7/564 (1%)	DQ501174	Clone MX_2_OCT_29
	AOA-NAS10-8 (1)	965	566/587 (96%)	4/587 (0%)	EU025177	Clone S18-A-16
NAS 30	AOA-NAS30-1 (19)	990	559/570 (98%)	1/570 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS30-2 (5)	992	558/569 (98%)	1/569 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS30-3 (1)	970	552/565 (97%)	2/565 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS30-4 (1)	972	553/565 (97%)	5/565 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS30-5 (1)	963	551/565 (97%)	3/565 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS30-6 (1)	970	551/563 (97%)	3/563 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS30-7 (1)	987	560/572 (97%)	3/572 (0%)	EU239976	Clone MamSp.H08
	AOA-NAS30-8 (1)	961	551/566 (97%)	2/566 (0%)	EU239976	Clone MamSp.H08

Number in parenthesis indicated amounts of AOA *amo*A sequences showing 100% identity

Due to the limited information on AOA phylogenetic taxonomy, phylogenetic trees for AOA was constructed by tree different methods comprising of distance matrix, maximum parsimony, and maximum likelihood to confirm the grouping of AOA analyzed in this study and all major AOA reported in previous studies so far (Figure 4.9, 4.10, 4.11). It must be noted that Figure 4.9, 4.10, 4.11 have been used to confirm AOA clusters only. No information of species was provided in these three trees. Figure 4.8 showed phylogenetic tree constructed based on distance matrix (neighbor joining) with the complete sequence detail that will be used for further discussion. AOA clusters were defined based on OTUs using the DOTUR program (Scholoss *et al*., 2005). Any AOA sequences, showing >86% identity, were identified as the same AOA cluster. AOA communities in seed sludge, and enriched NAS were summarized in Table 4.9.

Table 4.9 Summary of AOA communities in seed sludge and enriched NAS

Cluster	Seed sludge	NAS 2	NAS 10	NAS 30
B			✓	
E		✓	✓	
F		✓		
G			✓	
I	✓✓✓			
K	✓✓✓✓✓✓✓	✓✓✓✓✓✓✓✓✓ ✓✓✓✓✓✓✓✓✓✓ ✓✓✓	✓✓✓✓✓✓✓✓✓ ✓✓✓✓✓✓✓✓✓✓ ✓✓✓✓✓	✓✓✓✓✓✓✓✓✓ ✓✓✓✓✓✓✓✓✓ ✓✓✓✓✓✓✓✓✓✓ ✓✓✓

✓, present (amount of symbols represents numbers of clones)

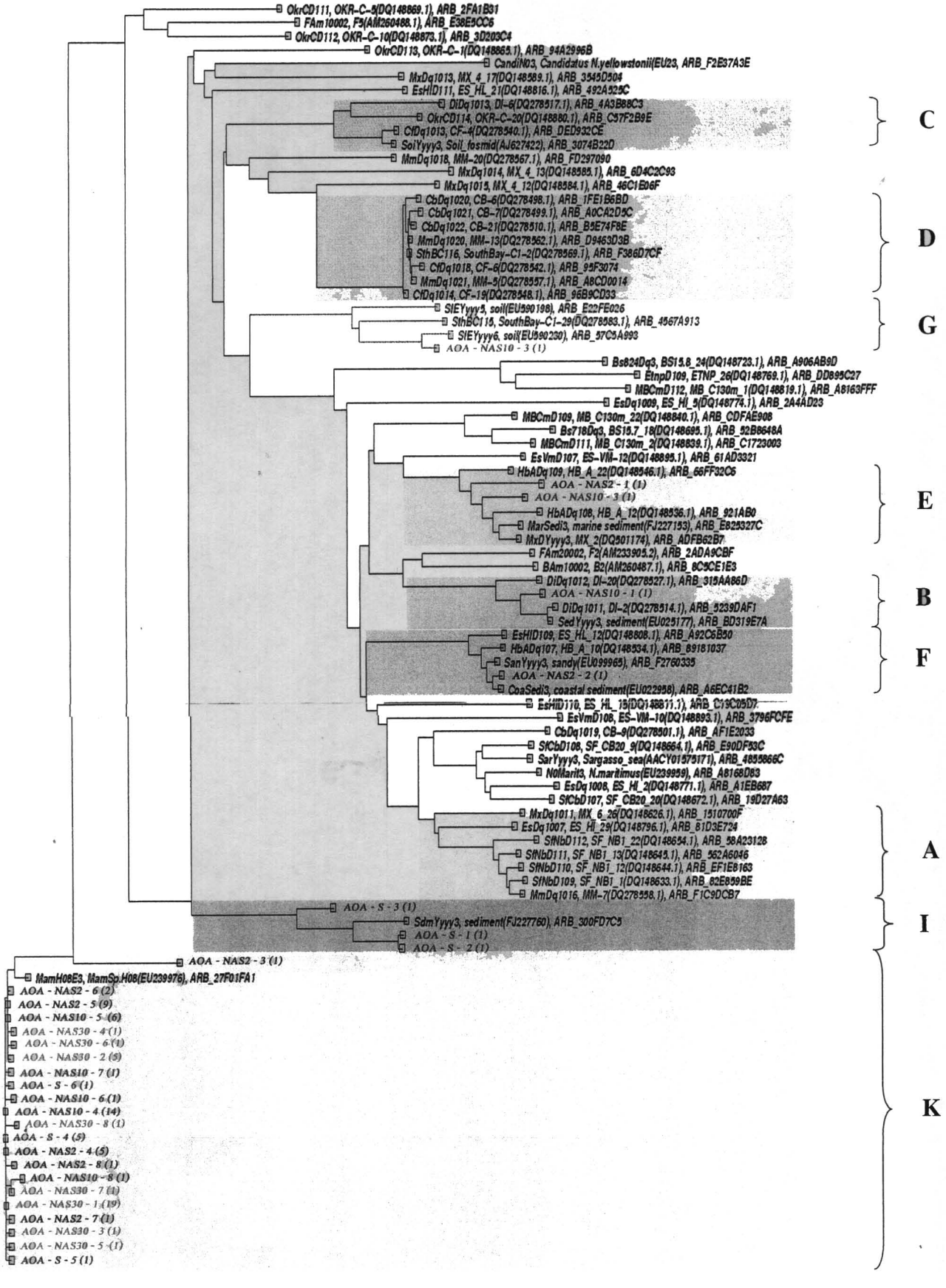


Figure 4.8 Neighbor joining tree of AOA *amo*A sequences from seed sludge and enriched NAS (Details information is provided in this tree and this tree will be used for discussion)

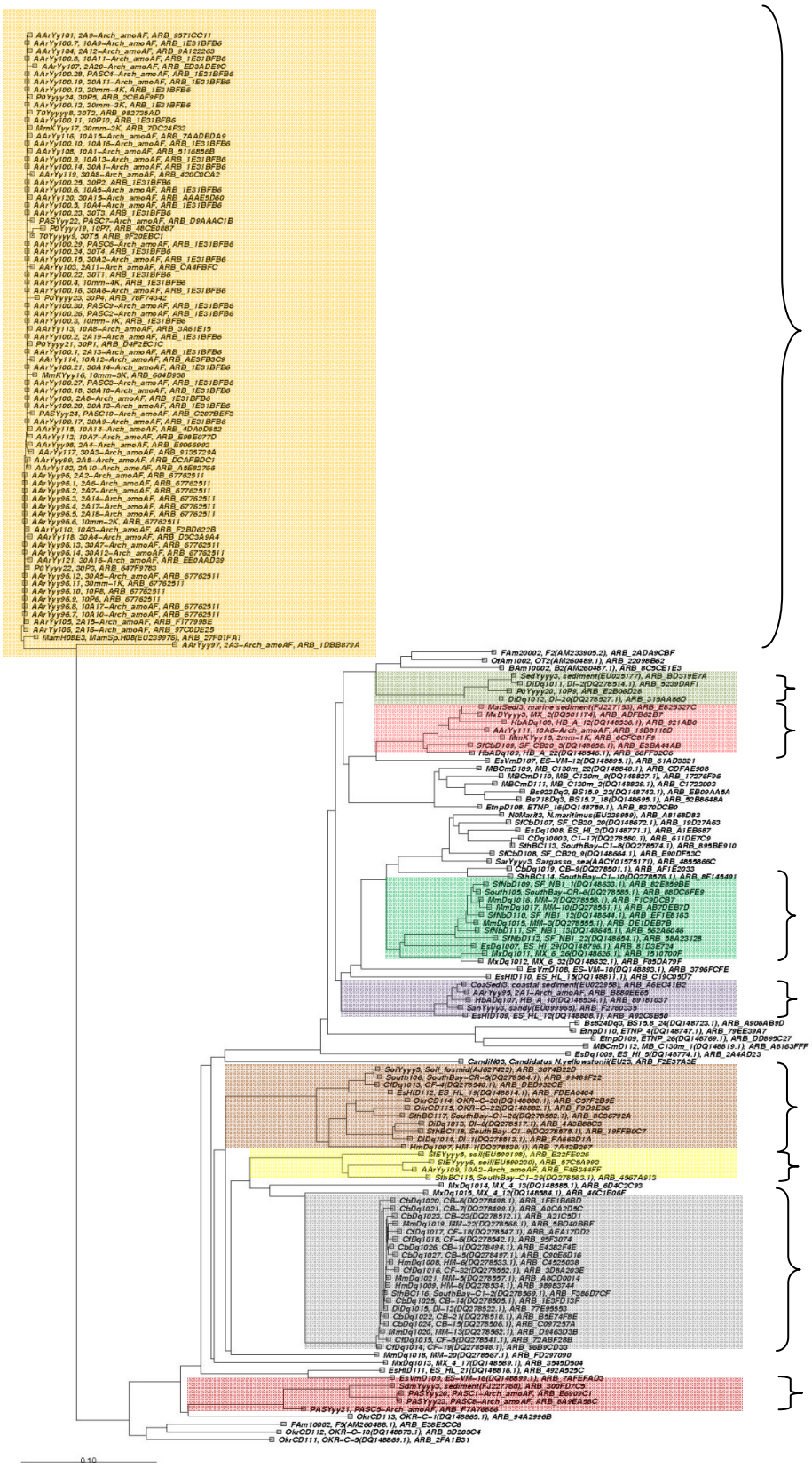


Figure 4.9 Distance matrix tree of AOA *amo*A sequences from seed sludge and enriched NAS. (It must be noted that details information of species is not provided in this tree); Clusters A, B, C, and D were indicated by Park *et al*., 2006, while other than those were found in this study.

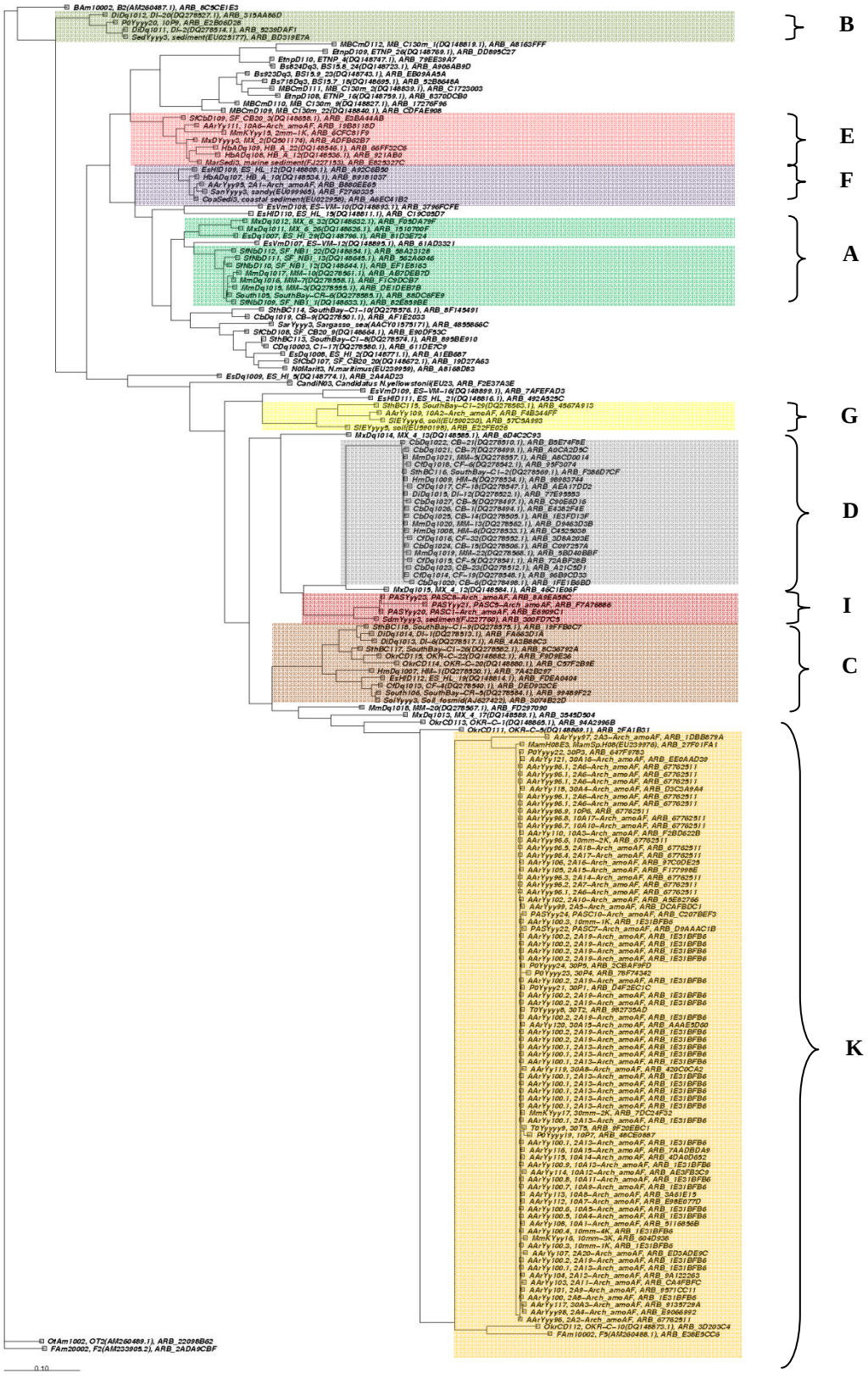


Figure 4.10 Maximum parsimony tree of AOA *amo*A sequences from seed sludge and enriched NAS. (It must be noted that details information of species is not provided in this tree); Clusters A, B, C, and D were indicated by Park *et al*., 2006, while other than those were found in this study.

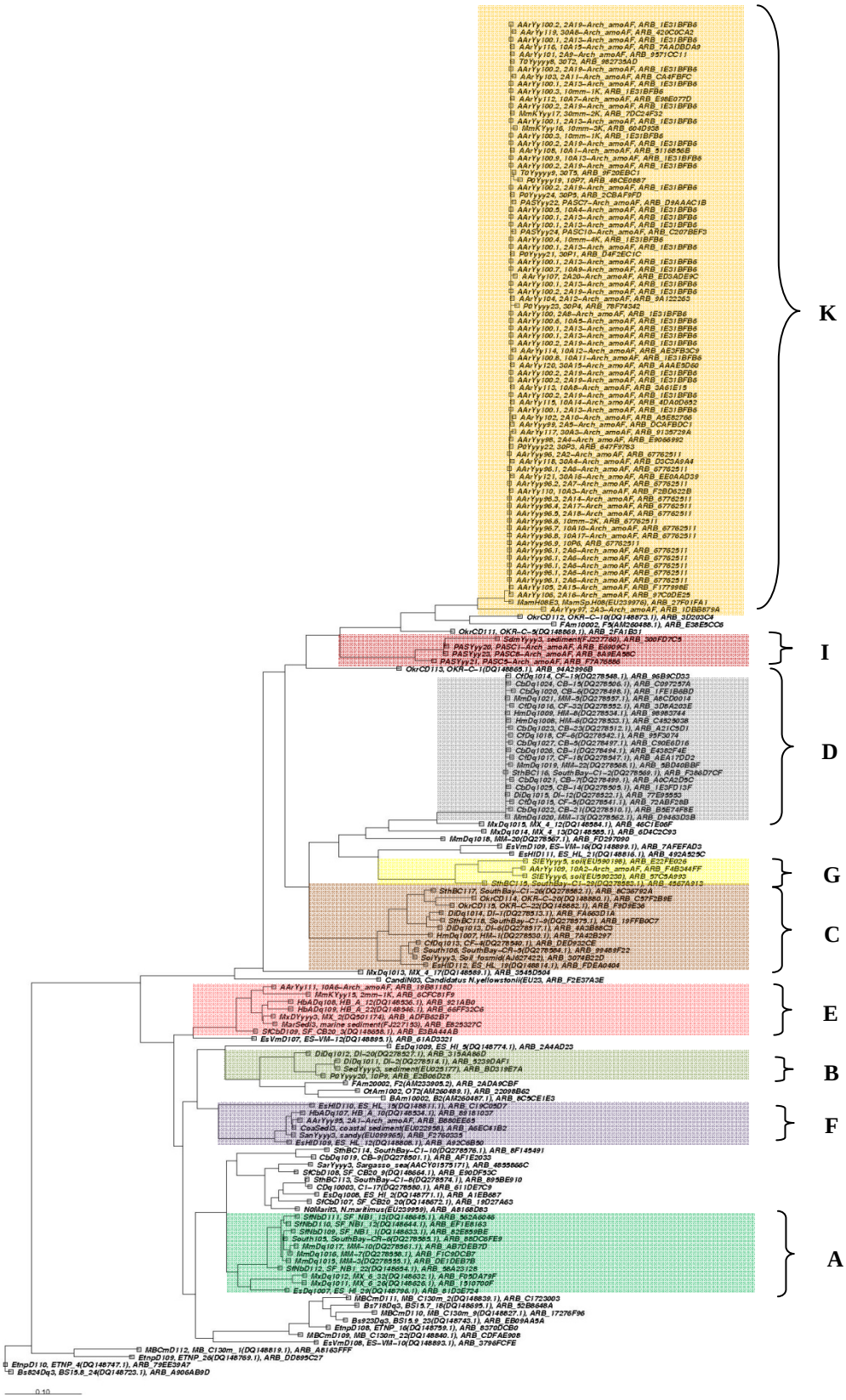


Figure 4.11 Maximum likelihood tree of AOA *amo*A sequences from seed sludge and enriched NAS. (It must be noted that details information of species is not provided in this tree); Clusters A, B, C, and D were indicated by Park *et al*., 2006, while other than those were found in this study.

For seed sludge sample, ten clones randomly selected fell in 2 clusters: 7 clones in cluster K and 3 clones in cluster I. For NAS 2, twenty-one clones were randomly selected for sequencing. All 21 cloned analyzed could be divided into 3 AOA clusters: 19 clones in cluster K, 1 clone in cluster E, and 1 clone in cluster F. In the case of NAS 10, twenty-six randomly selected clones were divided into 4 AOA clusters which were 23 clones for cluster K, 1 clone for cluster B, 1 clone for cluster E, and 1 clone for cluster G. All randomly selected clones of NAS 30 were members of cluster K only.

It seemed that AOA communities in NAS 2 and NAS 10 were more diverse than NAS 30. Unlike AOB, AOA communities seemed to be more stable by being less influence by ammonium loads. Most of the clones from seed sludge (7 out of 10), NAS 2 (19 out of 21), NAS 10 (23 out of 26), and NAS 30 (30 out of 30) fell only in the same AOA cluster K. In the case of seed sludge as mentioned above, seven out of ten clones related closely to those obtained from Mammoth hot spring sediment (cluster K) (Torre *et al*., 2008) while another 3 clones related to AOA found in sediment (cluster I) (unpublished). Interestingly, no clone from the seed sludge related closely to those in the only one study of AOA in WWTPs (Park *et al*., 2006) in spite the seed sludge in our study was taken from a municipal WWTPs (activated sludge). In the study of Park, AOA *amo*A sequences of the cluster D (Figure 4.8) were the common AOA being widespread in 5 activated sludge bioreactors. Surprisingly, the major clones in our seed sludge showed similarity to those recovered from Mammoth hot spring sediment which is in moderately thermal (42 - 50°C) environment (Torre *et al*., 2008). The temperature in the hot spring is elevated than in a municipal wastewater treatment plant in tropical (25 - 30°C) region where our seed sludge was taken from. Moreover, this group of AOA (cluster K) could survive and probably dominate in all ammonium load conditions (NAS 2, NAS 10, and NAS 30) in this study. These results suggested that they were able to adapt themselves to survive in a broad range of ammonium concentrations.

Other that cluster K, clones from NAS 2 related to the clones retrieved from coastal marine sediment (cluster E) (unpublished) and coastal sediment (cluster F) (Santoro *et al*., 2008). In addition, three clones from NAS 10 were closely related to those obtained from estuarine sediment (cluster E) (Beman *et al.,* 2006), sediment

(cluster B) (Dang *et al*., 2008), and soil (cluster G) (unpublished). Those closely related to the clones from NAS 2 and NAS 10 were from saline environments, while all enriched NASs in our study were operated without salt. These results implied that some AOA clusters were flexible to exist in both salt and non salt environments. Unlike AOA, distinct AOB species are very restrict to salt tolerance. In non salt environment *N.europaea*, *N. nitrosa*, and *N. ureae* are found, while in the salt environments *N. marina*, *N. aestuarii*, and *Nc. oceani* are found. It was questionable that whether salt is the factor influencing communities of AOA.

This experiment revealed that AOB communities obviously shifted from seed sludge to each enriched NAS, communities of AOB in each enriched NAS varied particularly. Ammonium load was confirmed to be the major factor selecting communities of AOB. AOB with high affinity to ammonia presented in NAS 2, AOB with low affinity to ammonia presented in NAS 30, and both strains can survive in NAS 10. These results corresponded to physiological properties reported in previous study on isolated AOB cultures. In the case of AOA, only isolated AOA culture (*N. maritimus*) has been obtained. Therefore, information on physiological properties of AOA is very limited. Thus this is the first study in the world that indirectly study physiological properties of AOA by using molecular tools. In contrast to AOB, AOA communities were more stable under ammonium load variation. Almost all AOA *amo*A sequences from all enriched NASs fell in the same cluster (cluster K). It was emerged the question that ammonia is the sole energy source for AOA whether it is important enough to affect their communities. However, it must be noted that the enriched NAS in this study is an ordinary reactors aimed to enriched mainly AOB. The inorganic medium used designed for AOB; consequently, no additional vitamin and trace elements being essential for AOA was supplied (Konneke *et al*., 2005). Therefore AOA found in this study might be the common one survived under these conditions only.

CHAPTER V

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORKS

5.1 Conclusions

5.1.1 Communities of ammonia-oxidizing bacteria and archaea in full-scale wastewater treatment plants

AOB communities in four industrial wastewater treatment plants (WWTPs) (I1-I4) were more diverse than those in six municipal WWTPs (LM1-LM4 and SM1, SM4). AOB found in industrial WWTPs fell into 4 clusters that are unknown *Nitrosomonas* cluster, *N. europaea-Nc. mobilis* cluster, *N. communis* cluster, and *N. oligotropha* cluster. While AOB found in municipal WWTPs were restricted to only *N. communis* cluster and *N. oligotropha* cluster. The difference of influent wastewater characteristics (ammonia concentration) might be the key factor causing distinct distribution patterns of AOB communities in both types of WWTPs. Other than wastewater characteristic, system configuration and operation can be the other factors, influencing the AOB communities in WWTPs. In this case only municipal WWTPs were considered as it is possible to avoid the effect of influent characteristics. Almost all municipal WWTPs in this study were activated sludge processes with the only exception for the plant SM4 that was aerated lagoon system. In general, the SRT of aerated lagoon system are relatively longer than the activated sludge process. SRT was firstly considered as one of a major factor for system configuration and operation. However the results suggested that the AOB communities in all municipal WWTPs were similar (comprising of only *N. communis* cluster and *N. oligotropha* cluster). Therefore, system configuration and operation were less significant than influent wastewater characteristics.

Unlike AOB, communities of AOA in the industrial WWTPs were less diverse than those in the municipal WWTPs. Only 2 industrial WWTPs (plants I3 and I4), which represented industrial WWTPs with moderate ammonium loads (40 -70 mg-N/l), contained AOA *amo*A gene fragments. Negative PCR amplification occurred

with samples I1 and I2 that represented industrial WWTPs with low (13 mg-N/l) and high (422 mg-N/l) ammonium loads, respectively. The reason that AOA were absent from samples I2 might be because of high ammonium loads for this plant. However, the reason for the case of plant I1 was unclear. In the case of the municipal WWTPs, the influent ammonium concentrations were in a narrow range of 5 – 13 mg-N/l. AOA *amo*A gene fragments were detected in all samples and they distributed in various clusters (cluster A, B, C, E, F, G, I, J, K, L, M, and N). Other than influent characteristics, system configuration and operation might be considered as the factors affecting AOA communities. Five out of six municipal WWTPs (LM1, LM2, LM3, LM4, and SM1) were activated sludge processes, whereas another (SM4) was an aerated lagoon system. For each sample from activated sludge plants, only 1 – 3 clusters of AOA were observed, while in the sample from an aerated lagoon system much more AOA clusters of 6 were found. It was implied that longer SRT might be a result of more AOA diversity in this plant. Therefore, system configuration and operation can also be important factors, influencing AOA communities in WWTPs.

5.1.2 Effect of ammonium concentrations on communities of ammonia-oxidizing bacteria and archaea in enriched nitrifying activated sludge

AOB communities obviously shifted from seed sludge to enriched NASs and in each enriched NAS, communities of AOB varied particularly. Sludge taken from a municipal WWTP used as a seed for all reactors contained members of *N. communis* cluster and *N. oligotropha* cluster. After it was enriched under various ammonium loads, members of *N. communis* cluster disappeared from all enriched NASs. In NAS 2, only AOB closely related to *N. oligotropha* cluster were found. Sequence types of unknown *Nitrosomonas* cluster and *N. europaea* cluster were recovered from NAS 10. Only AOB related to *N. europaea* cluster were observed in NAS 30. Ammonium load was confirmed to be the major factor selecting AOB communities. AOB with high affinity to ammonia presented in NAS 2, AOB with low affinity to ammonia presented in NAS 30, and both types of AOB could survive in NAS 10. These results corresponded to the physiological properties reported in the previous studies on the isolated AOB cultures.

In the case of AOA, only isolated AOA culture (*Nitrosopumilus maritimus*) has been obtained so far. Therefore, the information on physiological properties of AOA is very limited. Thus, this is the first study in the world indirectly studying the physiological properties of AOA by using molecular tools. In contrast to AOB, AOA communities were more stable under ammonium load variation. Almost all AOA *amo*A sequences from all enriched NASs fell in the same cluster (cluster K). It was emerged the question that whether ammonium, as the sole energy source for AOA, is important enough to affect the AOA communities. However, it must be noted that the enriched NASs in this study was done in an ordinary reactors that aimed to enrich mainly AOB. The inorganic medium used designed for AOB enrichment mainly; consequently, no additional vitamins and trace elements being essential for AOA was supplied. Therefore, AOA found in the enriched NASs might be the common ones survived under this limiting condition only.

5.2 Suggestions for future works

Quantitative and biochemical studies were required to reveal the role of AOA in ammonia oxidation in full-scale WWTPs. The abundance of AOA comparatively to AOB in full-scale WWTPs must be carried out first by using real-time PCR technique. Later, *in situ* activity of AOA as well as AOB can be investigated using microautoradiography combined with fluorescence *in situ* hybridization (MAR-FISH). Further clarification of environmental factors affecting AOA communities also needed to provide stability of AOA activities in the WWTPs.

REFERENCES

American Public Health Association. Standard methods for the examination of water and wastewater, 20th edition, Washington DC, USA, 1998.

Aakra, A., Utaaker, J.B., and Nes, I.F. Comparative phylogeny of the ammonia monooxygenase subunit A and 16S rRNA genes of ammonia-oxidizing bacteria FEMS Microbiology Letters 205 (2001a): 237-242.

Alexander, M. Biodegradation of organic chemicals. Environmental Science and Technology. 18, 2 (1985): 106–111.

Alzerreca, J. J., Norton, J. M., and Klotz, M. G. The amo operon in marine, ammonia-oxidizing gamma-proteobacteria. FEMS Microbiology Letters 180 (1999): 21-29.

Arciero, D.T., Vannelli, M.L., and Hooper, A.B. Degradation of trichloroethylene by the ammonia-oxidizing bacterium *Nitrosomonas europaea.* Archives of Biochemistry and Biophysics 159 (1989): 640-643.

Ballinger, S.J., Head, I.M., Curtis, T.P. and Godley, A.R. Molecular microbial ecology of nitrification in an activated sludge process treating refinery wastewater. Water Science and Technology 37 (1998): 105–108.

Beman, J.M., and Francis, C.A. Diversity of ammoniaoxidizing archaea and bacteria in the sediments of a hypernutrified subtropical estuary: Bahía del Tóbari, Mexico. Applied and Environmental Microbiology 72 (2006): 7767–7777.

Bollmann, A., and Laanbroek H.J. Continuous culture enrichments of ammonia-oxidizing bacteria at low ammonium concentrations. FEMS Microbiology Ecology 37 (2001): 211-221.

Bollmann, A. and Laanbroek, H.J. Influence of oxygen partial pressure and salinity on the community composition of ammonia-oxidizing bacteria in the schelde estuary. Aquatic Microbial Ecology 28 (2002): 239–247.

Burton, S.A., and Prosser J.I. Autotrophic ammonia oxidation at low pH through urea hydrolysis. Applied and Environmental Microbiology 67 (2001): 2952-2957.

Carballa, M., Omil, F., Lema, J.M., Llompart, M., Garcı´a-Jares, C., Rodrı´guez, I., Go´mez, M., Ternes, T. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. Water Research 38, 12 (2004): 2918–2926.

Casciotti, K.L., and Ward B.B. Dissimilatory nitrite reductase genes from autotrophic ammonia-oxidizing bacteria. Applied and Environmental Microbiology 67 (2001): 2213-2221.

Ceccherini, M.T., Castialdini, M., Piovanelli, C., Hastings, R.C., McCarthy, A.J., Bazzicalupoet, M., and Miclaus, N. Effects of swine manure on autotrophic ammonia-oxidizing bacteria in soil microcosms. Applied Soil Ecology 7 (1998): 149-157.

Coolen, M.J.L., Abbas, B., Van Bleijswijk, J., Hopmans, E.C., Kuypers, M.M.M., Wakeham, S.G. Putative ammonia-oxidizing Crenarchaeota in suboxic waters of the Black Sea: a basin-wide ecological study using 16S ribosomal and functional genes and membrane lipids. Environmental Microbiology 9 (2007): 1001–1016.

Clara, M., Kreuzingera, N., Strenna, B., Gansb, O., Kroiss, H. The solids retention time a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. Water Research 39, 1 (2005): 97–106.

Damsté, J.S., Rijpstra, W.I.C., Hopmans, E.C., Prahl, F.G., Wakeham, S.G., and Schouten, S. Distribution of membrane lipids of planktonic Crenarchaeota in the Arabian sea. Applied and Environment Microbiology 68 (2002b): 2997–3002.

Dang, H., Zhang, X., Sun, J., Li, T., Zhang, Z., and Yang, G. Diversity and spatial distribution of sediment ammonia-oxidizing crenarchaeota in response to estuarine and environmental gradient in the Changjiang Estuary and East China Sea. Microbiology 154 (2008): 2084-2095.

De Boer, W., and Laanbroek, H.J. Ureolytic nitrification at low pH by Nitrosospira species. Archives of Microbiology 152 (1989): 178-181.

De Boer, W., Klein-Gunnewiek, P.J.A., Veenhuis, M., Bock, E., and Laanbroek, H.J. Nitrification at low pH by aggregated autotrophic bacteria. Applied and Environmental Microbiology 57 (1991): 3600-3604.

DeLong, E.F. Archaea in coastal marine environments. Proceeding of the National Academy of Sciences USA 89 (1992): 5685–5689.

Dionisi, H.M., Layton, A.C., Harms, G., Gregory, IR., Robinson, KG., and Sayler, GS. 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 (2002): 245–253.

Ely, R.L., Hyman, M.R., Arp, D.J., Guenther, R.B., Williamson, K.J. A cometabolic kinetics model incorporation enzyme inhibition, inactivation, and recovery: II. Trichloroethylene degradation experiments. Biotechnology and Bioengineering 46 (1995): 232-245.

Foesel, B.U., Gieseke, A., Schwermer, C., Stief, P., Koch, L., Cytryn, E., de la Torre, J.T., van Rijn, J., Minz, D., Drake,H.L. and Schramm, A. *Nitrosomonas* Nm143-like ammonia oxidizers and *Nitrospira* marina-like nitrite oxidizers dominate the nitrifier community in a marine aquaculture biofilm. FEMS Microbiology Ecology 63, 2 (2008): 192-204.

Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., Oakley, B.B. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proceeding of the National Academy of Sciences USA 102 (2005): 14683–14688.

Gieseke, A. Community structure and activity dynamics of nitrifying bacteria in a phosphate-removing biofilm. Applied and Environment Microbiology 67 (2001): 1351–1362.

Golovacheva, R.S. Thermophilic nitrifying bacteria from hot springs. Journal of Microbiology 45 (1976): 329-331.

Goreau, T.W., Kaplan, W.A., Wofsy, S.C., McElroy, M.B., Valois, F.W., and Watson, S.W. Production of NO_2^- and N_2O by nitrifying bacteria at reduced concentrations of oxygen. Applied and Environment Microbiology 40 (1980.): 526-532.

Hall, G.H. Nitrification in lakes. Prosser, J.I. (Ed.), Nitrification, 127–156. Oxford UK: IRL Press, 1986.

Harms, G., Layton, A.C., Dionisi, H.M., Gregory, I.R., Garrett, V. M., Hawkins, S. A., Robinson, K.G., and Sayler, G.S. Real-time PCR quantification of nitrifying bacteria in a municipal wastewater treatment plant. Environmental Science and Technology 37 (2003): 343-351.

Head, I.M., Hiorns, W.D., Embley, T.M., McCarthy, A.J., and Saunders, J.R. The phylogeny of autotrophic ammonia-oxidizing bacteria as determined by analysis of 16S ribosomal RNA gene sequences. Journal of General Microbiology 139 (1993): 1147-1153.

Hermansson, A., and Lindgren, P. E. Quantification of ammonia-oxidizing bacteria in Arable soil by real-time PCR. Applied and Environment Microbiology 67, 2 (2001): 972 -976.

Hommes, N.G., Sayavedra-Soto, L.A., and Arp, D.J. Mutagenesis and expression of *amo*, which codes for ammoniamonooxygenase in *Nitrosomonas europaea*. Journal of Bacteriology 180 (1998): 3353-3359.

Horz, H.P., Rotthauwe, J.H., Lukow, T., and Liesack, W. Identification of major subgroups of ammonia-oxidizing bacteria in environmental samples by T-RFLP of *amo*A PCR products. Journal of Microbiological Methods 39 (2000): 197-204.

Hyman, M.R., Russell, S.A., Ely, R.L., Willamson, K.J., Arp, D.J. Inhibition, inactivation, and recovery of ammonia-oxidizing activity in cometabolism of trichloroethylene by *Nitrosomonas europaea*. Applied and Environment Microbiology 61 (1995) 1480-1487.

International Union of Pure and Applied Chemistry (IUPAC). Endocrine disruptors in the environment (IUPAC Technical Report). Pure and Applied Chemistry 75, 5 (2003): 631-381.

Jiang, Q.Q., and Bakken, L.R. Comparison of *Nitrosospira* strains isolated from terrestrial environments. FEMS Microbioloy Ecology 30 (1999): 171-186.

Jones, R.D., Morita, R.Y., Koops, H.P., and Watson, S.W. A new marine ammonium-oxidizing bacterium, *Nitrosomonas cryotolerans* sp. nov. Can. Journal of Microbiology 34 (1988): 1122-1128.

Juretschko, S., Timmermann, G., Schmid, M., Schleifer, K.H., Pommerening-Roser, A., Koops, H.P., and Wagne, M. Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: *Nitrosococcus mobilis* and *Nitrospira*-like bacteria as dominant populations. Applied and Environment Microbiology 64 (1998): 3042–3051.

Klotz, M.G., and Norton, J.M. Sequence of an ammonia monooxygenase subunit A-encoding gene from *Nitrosospira* sp. NpAV. Gene 163 (1995): 159-160.

Konneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B., Stahl D.A. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437 (2005): 543–546.

Koops, H. P., and Harms, H. Deoxyribonucleic acid homologies among 96 strains of ammonia-oxidizing bacteria. Archives of Microbiology 141 (1985): 214-218.

Koops, H.P., Böttcher, B., Möller, U.C., Pommerening-Röser, A., and Stehr, G. Description of a new species of *Nitrosococcus*. Archives of Microbiology 154 (1990): 244-248.

Koops, H.P., Böttcher, B., Möller, U. C., Pommerening-Röser, A., and Stehr, G. Classification of eight new species of ammonia-oxidizing bacteria: *Nitrosomonas communis* sp. nov., *Nitrosomonas ureae* sp. nov., *Nitrosomonas aestuarii* sp. nov., *Nitrosomonas marina* sp. nov., *Nitrosomonas nitrosa* sp. nov., *Nitrosomonas eutropha* sp. nov., *Nitrosomonas oligotropha* sp. nov., and *Nitrosomonas halophila* sp. nov. Journal of General Microbiology 137 (1991): 1689-1699.

Koops, H.P., and Pommerening-Roser A. Distribution and ecophysiology of the nitrifying bacteria emphasizing cultured species. FEMS Microbiology Ecology 37 (2001): 1–9.

Koops, H. P., Purkhold, U., Pommeren, R. A., Timmermann, G., and Wagner, M. The Lithoautotrophic Ammonia-Oxidizing Bacteria. M. Dworkin et al (eds.), The Prokaryotes: An Evolving Electronic Resource For the Microbiological Community, 1-22. New York, 2003.

Kowalchuk, G.A. Analysis of ammonia-oxidizing bacteria of the βsubdivision of the class Proteobacteria in costal sand dunes by denaturing gradient gel electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments. Applied and Environment Microbiology 63, 4 (1997): 1489–1497.

Kowalchuk, G.A., Naoumenko, Z.S., Derikx, P.J.L., Felske, A., Stephen, J.R., and Laanbroek, H.J. Community analysis of ammonia-oxidising bacteria, in relation to oxygen availability in soils and root-oxygenated sediments, using PCR, DGGE and oligonucleotide probe hybridization. FEMS Microbiology Ecology 27 (1998): 339-350.

Kowalchuk, G.A., and Stephen, J.R. Ammonia-oxidizing bacteria: a model for molecular microbial ecology. Annual Review of Microbiology 55 (2001): 485-529.

Kreuzinger, N., Clara, M., Strenn, B., Kroiss, B. Relevance of the sludge retention time (SRT) as design criteria for wastewater treatment plants for the removal of endocrine disruptors and pharmaceuticals from wastewater. Water Science and Technology 50, 5 (2004): 149–156.

Kurisu, F., Satoh, H., Mino, T., and Matsuo, T. Microbial community analysis of thermophilic contact oxidation process by using ribosomal RNA approaches and the quinone profile method. Water Research 36 (2002): 429–438.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442 (2006): 806-809.

Limpiyakorn T., Shinihara, Y., Kurisu, F., and Yagi, O. Communities of ammonia oxidizing bacteria in activated sludge of various sewage treatment plants in Tokyo. FEMS Microbiology Ecology 54 (2005): 205–117.

Limpiyakorn, T., Kurisu, F., and Yagi, O. Development and application of real-time PCR quantification for particular ammonia-oxidizing bacteria in full-scale sewage activated sludge systems and continuous enrichment cultures. Applied and Environmental Microbiology 72 (2006a): 1004-1013.

Limpiyakorn, T., Kurisu, F., and Yagi, O. Quantification of ammonia-oxidizing bacteria populations in full-scale sewage activated sludge systems and assessment of system variables affecting their performance. Water Science and Technology 54 (2006b): 91–99.

Limpiyakorn, T., Kurisu, F., Sakamoto, Y., and Yagi, O. Effects of ammonium and nitrite on communities and populations of ammonia-oxidizing bacteria in laboratory-scale continuous-flow reactors. FEMS Microbiology Ecology 60 (2007): 501-512.

Liu Xin-chun, Zhang Yu, Yang Min, Wang Zhen-yu, Lv Wen-zhou. Analysis of bacterial community structures in two sewage treatment plants with different sludge properties and treatment performance by nested PCR-DGGE method. Journal of Environmental Sciences 19 (2007): 60-66.

MacDonald, R. M. Nitrification in soil: a introductory history J.I. Prosser (Eds.), Nitrification, 1-16. Oxford UK: IRL Press, 1986

McTavish, H., Fuchs, J.A., and Hooper, A.B. Sequence of the gene coding for ammonia monooxygenase in *Nitrosomonas europaea*. Journal of Bacteriology 175 (1993): 2436-2444.

Mobarry, B.K., Wagner, M., Urbain, V., Rittmann, B.E., and Stahl, D.A. Phylogenetic probes for analyzing abundance and spatial organization of nitrifying bacteria. Applied and Environment Microbiology 62, 6 (1996): 2156–2162.

Nicol, G.W., and Schleper, C. Ammonia-oxidising Crenarchaeota: important players in the nitrogen cycle? Trends in Microbiology 14, 5 (2006): 207-212.

Nicolaisen, M.H. and Ramsing, N.B. Denaturing gradient gel electrophoresis (DGGE) approaches to study the diversity of ammonia-oxidizing bacteria. Journal of Microbiologigal Methods 50 (2002): 189–203.

Nold, S.C., Zhou, J., Devol, A.H., and Tiedje, J.M. Pacific Northwest marine sediments contain ammonia-oxidizing bacteria in the beta subdivision of the Proteobacteria. Applied and Environment Microbiology 66 (2000): 4532-4535.

Norton, J.M., Alzerreca, J.J., Suwa, Y., and Klotz, M.G. Diversity of ammonia monooxygenase operon in autotrophic ammonia-oxidizing bacteria. Archieves of Microbiology 177 (2002): 139-149.

Okabe, S., Satoh, H., and Watanabe, Y. In situ analysis of nitrifying biofilms as determined by in situ hybridization and the use of microelectrodes. Applied and Environment Microbiology 65 (1999): 3182-3191.

Painter, H.A. Nitrification in the Treatment of Sewage and Waste-waters. Prosser, J.I. (Ed.), Nitrification, 185–211. Oxford UK: IRL Press, 1986.

Park, H.D, Wells, G.F., Bae, H., Criddle, C.S., Francis, C.A. Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. Applied and Environment Microbiology 72 (2006): 5643–5647.

Prosser, J.I., and Embley, T.M. Cultivation-based and molecular approaches to characterisation of terrestrial and aquatic nitrifiers. Antonie Van Leeuwenhoek 81 (2002): 165–179.

Purdom, C.E., Hardiman, P.A., Bye, V.J., Eno, N.C., Tyler C.R., Sumpter, J.P. Estrogenic effects of effluents from sewage treatment works. Journal of Chemical Ecology 8 (1994): 275–285.

Purkhold, U., Pommering-Röser, A., Juretschko, S, Schmid, M. C., Koops, H.P., and Wagner, M. Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and amoA sequence analysis: implications for molecular diversity surveys. Applied and Environment Microbiology 66 (2000): 5368-5382.

Purkhold, U., Wagner, M., Timmermann, G., Pommerening-Röser, A., and Koops, H.P. 16S rRNA and amoA-based Phylogeny of 12 Novel Betaproteobacterial Ammonia Oxidizing Isolates: Extension of the Data Set and Proposal of a New Lineage within the *Nitrosomonads*. International Journal of Systematic and Evolutionary Microbiology [online] (2003) Available from: http://dx.doi.org/10.1099/ijs.0.02638-0 [10/5/2007]

Regan, J.M., Harrington, G.W., and Noguera, D.R. Ammonia and nitrite-oxidizing bacterial communities in a pilot-scale chloraminated drinking water distribution system. Applied and Environment Microbiology 68 (2002): 73-81.

Regan, J.M., Harrington, G.W., Baribeau, H., De Leon, R., and Noguera, D.R. Diversity of nitrifying bacteria in full-scale chloraminated distribution systems. Water Research 37 (2003): 197–205.

Rotthauwe, J.H., de Boer, W., and Liesack, W. Comparative analysis of gene sequences encoding ammonia monooxygenase of *Nitrosospira* sp. AHB1 and *Nitrosolobus multiformis* C-71. FEMS Microbiology Letters 133 (1995): 131-135.

Rotthauwe, J.H., Witzel, K.P., and Liesack, W. The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Applied and Environment Microbiology 63 (1997): 4704-4712.

Routledge, EJ. Identification of estrogenic chemicals in STW effluent-II. In vivo responses in trout and roach. Environmental Science and Technology 32 (1998): 1559-1565.

Santoro, A.E., Francis, C.A., de Sieyes, N.R., and Boehm, A.B. Shifts in the relative abundance of ammonia-oxidizing bacteria and archaea across physicochemical gradients in a subterranean estuary. Environmental Microbioogy 10 (2008): 1068-1079.

Schleper, C. Genomic studies of uncultivated archaea. Nature Reviews Microbiology 3 (2005): 479–488.

Schloss, P.D., and Handelsman, J. Introducing DOTUR, a computerprogram for defining operational taxonomic units and estimating speciesrichness. Applied and Environment Microbiology 71 (2005): 1501-1506.

Schmidt, I., and Bock, E. Anaerobic ammonia oxidation with nitrogen dioxide by *Nitrosomonas eutropha*. Archives of Microbiology 167 (1997): 106-111.

Schmidt, I., and Bock, E. Anaerobic ammonia oxidation with nitrogen dioxide by cell-free extracts of *Nitrosomonas eutropha*. Antonie van Leeuwenhoek 73 (1998): 271-278.

Schramm, A., De Beer, D., Wagner, M., and Amann, R. Identification and activities in situ of *Nitrosospira* and *Nitrospira* spp. as dominant population in nitrifying fluidized bed reactor. Applied and Environment Microbiology 64 (1998): 3480–3485.

Shi, J., Fujisawa, S., Nakai, S., Hosomi, M. Biodegradation of natural and synthetic estrogen by nitrifying activated sludge and ammonia-oxidizing bacterium *Nitromonas europaea*. Water Research 38,9 (2004): 2323-2330.

Siripong, S., and Rittmannb, B.E. Diversity study of nitrifying bacteria in full-scale municipal wastewater treatment plants. Water Research 41 (2007): 1110 – 1120.

Stehr, G., Böttcher, B., Dittberner, P., Rath, G., and Koops, H.P. The ammonia oxidizing nitrifying population of the River Elbe estuary. FEMS Microbiology Ecology 17 (1995a): 177-186.

Stehr, G., Zörner, S., Böttcher, B., and Koops, H.P. Exopolymers: A ecological Characteristic of a Floc-Attached, Ammonia-Oxidizing Bacterium. Microbial Ecology 30 (1995b): 115-126.

Stephen, J.R., Chang, Y.J., Macnaughton, S.J., Kowalchuk, G.A., Leung, K.T., Flemming, C.A., and White, D.C. Effect of toxic metals on indigenous soil beta-subgroup proteobacterium ammonia oxidizer community structure and protection against toxicity by inoculated metal-resistant bacteria. Applied and Environment Microbiology 65 (1999): 95-101.

Strickland, J.D., and Parson, T.R. Fishery research board of Canada. A practical handbook of seawater analysis, 310-315. Otawa, 1972.

Suwa, Y., Imamura, Y., Suzuki, T., Tashiro, T., and Urushigawa, Y. Ammonia-oxidizing bacteria with different sensitivities to $(NH_4)_2SO_4$ in activated sludge Water Research 28 (1994): 1523-1532.

Suwa, Y., Sumino, T., and Noto, K. Phylogenetic relationships of activated sludge isolates of ammonia oxidizers with different sensitivities to ammonium sulfate. Applied Microbiology 43 (1997): 373-379.

Taewoo Ti and Willief. Harper, JR. The Link between Nitrification and Biotransformation of 17α-Ethinylestradiol. Environmental Science and Technology 41 (2007): 4311-4316**.**

Torre, José R., Christopher B. Walker, Anitra E. Ingalls, Martin Könneke, and David A. Stahl. Cultivation of a thermophilic ammonia oxidizingarchaeon synthesizing crenarchaeol. Environmental Microbiology 10,1 (2008): 810–818.

Treusch, A.H., Leininger, S., Kletzin, A., Schuster, S.C., Klenk, H.P., Schleper, C. Novel genes or nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Environmental Microbiology 7 (2005): 1985–1995.

Vader, J.S. Degradation of ethinyl estradiol by nitrifying activated sludge. Chemosphere 41 (2000): 1239-1243.

Venter, J.C., Remington, K., Heidelberg, J.F., Halper, A.L., Rusc, D., Eisen, J.A. Environmental genome shotgun sequencing of the Sargasso Sea. Science 304 (2004): 66–74.

Vogel, T.M., and McCarty, P.L. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions, Applied and Environment Microbiology 49 (1985): 1080-1083.

Wagner, M., Rath, G., Amann, R., Koops, H.P., and Schleifer, K.H. In situ identification of ammonia-oxidizing bacteria. Systematic and Applied Microbiology 18 (1995): 251–264.

Wagner, M., Noguera, D.R., Juretschko, S., Rath, G, Koops, H.P., and Schleifer , H.K.H. Combining fluorescent in situ hybridization (FISH) with cultivation and mathematical modeling to study population structure and function of ammonia-oxidizing bacteria in activated sludge. Water Science and Technology 37 (1998): 441–449.

Wagner, M., Loy, A. Bacterial community composition and function in sewage treatment systems. Current Opinion Biotechnology 13 (2002): 218-227.

Ward, B.B., Martino, D.P., Diaz, M.C., and Joye, S.B. Analysis of ammonia-oxidizing bacteria from hypersaline Mono Lake, California, on the basis of 16S rRNA sequences. Applied and Environment Microbiology 66 (2000): 2873-2881.

Watson, S.W., and Mandel, M. Comparison of the morphology and deoxyribonucleid acid composition of 27 strains of nitrifying bacteria Journal of Bacteriology 107 (1971): 563-569.

William, K.K., and Daniel, J.A. Kinetic studies of ammonia monooxygenase inhibition in *Nitrosomonas europaea* by hydrocarbons and halogenated hydrocarbons in an optimized whole-cell assay. Applied and Environment Microbiology 59 (1993): 501-510.

Wilson, J.T., and Wilson, B.H. Biodegradation of trichloroethylene in soil. Applied and Environment Microbiology 49 (1985). 242-243.

Woese, C.R., Weisburg, W.G., Paster, B.J., Hahn, C.M., Tanner, R.S., Krieg, N.R., Koops, H.P., Harms, H., and Stackebrandt, E. The phylogeny of the purple bacteria: the beta subdivision system. Applied Microbiology 5 (1984): 327-336.

Woese, C.R., Weisburg, W.G., Hahn, C.M., Paster, B.J., Zablen, L.B., Lewis, B.J. , Macke, T.J., Ludwig, W., and Stackebrandt, E. The phylogeny of the purple bacteria: the gamma subdivision system. Applied Microbiology 6 (1985): 25-33.

Wuchter, C., Abbas, B., Coolen, M.J.L., Herfort, L., van Bleijswijk, J., Timmers, P. Archaeal nitrification in the ocean. Proceeding of the National Academy of Sciences USA 103 (2006)**:** 12317–12322.

You, S.J., Hsu, C.L., Chuang, S.H. and Ouyang, C.F. Nitrification efficiency and nitrifying bacteria abundance in combined AS-RBC and A_2O systems. Water Research 37 (2003): 2281–2290.

Yamagata, A., Kato, J., Hirota, R., Kuroda, A., Ikeda, T., Takiguchi, N., and Ohtake, H. Isolation and characterization of two cryptic plasmids in the ammonia-oxidizing bacterium *Nitrosomonas* sp. Strain ENI-11. Journal of Bacteriology 181 (1999): 3375-3381.

Zart, D., Schmidt, I., and Bock, E. Significance of gaseous NO for ammonia oxidation by *Nitrosomonas eutropha*. Antonie Van Leeuwenhoek 77 (2000): 49-55.

APPENDICES

APPENDIX A

Sequences of ammonia-oxidizing bacteria found in full-scale WWTPs

>AOB-I1-1
NGNNNATCCCTCAACTCAANGGGCNACGGATACAGTAGTTGGTCTGAGAGGACGACCAGCCACACTGGG
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TCCAGCAATGCCGCGTGAGTGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTTAGTTGAAAAGAAAAAACA
GCGATTAATAATCGCTGTTATGACGGTATCAACAGAAAAAGCACCGGCTAACTACGTGCCAGCAGCCGC
GGTAATACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGGGTGCGCAGGCGGTTTTGTAAG
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>AOB-I2-1
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GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCC
AGCCATGCCGCGTGAGTGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTTAGTCGGAAAGAAAGAGTTGCA
ATGAATAATTGTGATTTATGACGGTACCGACAGAAAAAGCACCGGCTAACTACGTGCCAGCAGCCGCGG
TAATACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGGGTGCGCAGGCGGTCTTGCAAGTC
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>AOB-I3-1
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ATGCCGCGTGAGTGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTCAGTCGAGAAGAAAAGGCTATGCCGA
ATAAGTATAGTTAATGACGGTATCGAAAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAAT
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GTGAAATCCCCGGGCTTAACCTGGGAATTGCGTTTGAAACTCNNAGCTAGACCGTTTGANGCTTGAAAG
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CAATGCCGCGTGAGTGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTCAGTCGAGAAGAAAAGGCTGCAGT
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>AOB-LM1-1
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CTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGA
GTGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTCGGTCGGAAAGAAATATCTATAAAAAATATTTATAGA
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AGCGTTAATCGGAATTATTGGGCGTAAAGGGTGCGCAGGCGGTGCTGTAAGTCAGATGTGAAATCCCCG
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> AOB-LM2-1
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TAACCTGGGAATTGCGTTTGAAACTCNAG
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APPENDIX B

Sequences of ammonia-oxidizing archaea found in full-scale WWTPs

>AOA-I3-1
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APPENDIX C

Sequences of ammonia-oxidizing bacteria found in seed sludge and enriched NAS

>AOB-S-1
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APPENDIX D

Sequences of ammonia-oxidizing archaea found in seed sludge and enriched NAS

>AOA-S-1
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International presentations

- Sonthiphand P. and Limpiyakorn T. Evidence of Ammonia-oxidizing Archaea in Municipal Full-scale Wastewater Treatment Plants in Bangkok. The 6th International Symposium on Southeast Asian Water Environment, Bandung, Indonesia, October 29-31, 2008.
- Sonthiphand P. and Limpiyakorn T. Effect of Ammonium Loads on Communities of Ammonia-oxidizing Bacteria and Archaea in Laboratory-scale Continuous Flow Reactors. The 6th International Symposium on Southeast Asian Water Environment, Bandung, Indonesia, October 29-31, 2008.
- Sonthiphand P. and Limpiyakorn T. Distribution of ammonia-oxidizing bacteria and archaea in full-scale wastewater treatment plants in Thailand. 11th International Conference on Environmental Science and Technology, September 3-5, 2009

Journal publications

- Sonthiphand P. and Limpiyakorn T. Effect of Ammonium Loads on Communities of Ammonia-oxidizing Bacteria and Archaea in enriched nitrifying activated sludge. (in preparation 60% completed submitted to **Applied and environmental microbiology** in **June 2009**)
- Sonthiphand P. and Limpiyakorn T. Evidence of ammonia-oxidizing bacteria and archaea in full – scale municipal wastewater treatment plants in Bangkok. (in preparation submitted **to FEMS Microbiology Ecology** in **September 2009**)
- Sonthiphand P. and Limpiyakorn T. Communities of ammonia-oxidizing bacteria and archaea in full – scale industrial wastewater treatment plants. (in preparation submitted to **Applied and environmental microbiology** in **November 2009**)