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.