

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 *amoA* 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 *amoA* 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 *amoA* 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.