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DISSERTATION SUMMERY

Nitrous oxide and nitric oxide production in partial nitrification and anaerobic ammonium oxidation granular sludge
(部分硝化および嫌気性アンモニア酸化グラニュール汚泥中における一酸化二窒素および一酸化窒素の生成)

A partial nitrification (PN) process followed by an anaerobic ammonium oxidation (anammox) process (a PN-anammox process) is an alternative and innovative approach for microbial nitrogen (N) removal from wastewaters. It is generally accepted that N removal process in a wastewater treatment system are an anthropogenic source of nitrous oxide (N_2O) and nitric oxide (NO). N_2O is an important greenhouse gas with a global warming potential of about 300 times higher than CO_2 and the major stratospheric ozone-depleting substance. NO is a highly reactive free radical and is toxic to a wide range of organisms. The main aim of this research was to investigate the N_2O and NO production from the N removal processes via PN-anammox process using multiple analytical techniques.

The emission of N_2O from a lab-scale sequencing batch partial nitrification reactor treating synthetic wastewater without organic carbon was therefore investigated in this study. The average N_2O emission rate from the SBR was 0.32 ± 0.17 mg-N L^{-1} h^{-1}, corresponding to the average emission of N_2O of 0.8 ± 0.4% of the incoming N load (1.5 ± 0.8% of the converted ammonia). Analysis of dynamic concentration profiles during one cycle of the sequencing batch reactor operation demonstrated that both N_2O concentrations in off-gas and effluent were high in the initial phase in one cycle. Isotopomer analysis revealed that the hydroxylamine (NH_2OH) oxidation pathway accounted for 65% of the total N_2O production in the initial phase in one cycle, whereas contribution of the NO_2^- reduction pathway to N_2O production was comparable with that of the NH_2OH oxidation pathway in the latter phase. In addition, spatial distributions of bacteria and their activities in single microbial granules taken from the reactor were determined with microsensors and by in situ hybridization. Partial nitrification occurred mainly in the oxic surface layer of the granules and ammonia-oxidizing bacteria were abundant in this layer. N_2O production was also found mainly in the oxic surface layer.

The effects of dissolved oxygen (DO) and pH on N_2O production rates and pathways in autotrophic PN granules were investigated at the granular level. Polyphasic analysis revealed that N_2O was primarily produced by the betaproteobacterial ammonia-oxidizing bacteria, mainly *Nitrosomonas* *europaea*, in the oxic surface layer (< 200 μm) of the autotrophic PN granules. Therefore, N_2O production increased significantly with increasing bulk DO concentration owing to activation of the ammonia (*i.e.*, hydroxylamine) oxidation rate (AOR) in this oxic layer. Although the AOR was unchanged in the range of pH 6.5 to 8.5, the highest N_2O emission was observed at pH 7.5. The results of our present study suggest us that DO and pH are two of most important parameters to control N_2O emission from PN reactors and engineers should develop operational strategies based on our findings.

NO emission from wastewater treatment processes has great impacts on human health, microorganisms and the environment. Although, NO emissions from anammox processes have been reported, the knowledge about responsible microorganisms and pathways the factors affecting for the NO emissions from anammox granules is limited. Therefore, we investigated the microorganisms and pathways responsible for and the factors affecting the NO emissions from the microbial granules taken from an anammox reactor. Anammox bacteria were identified as the members of "*Candidatus* Brocadia sinica" and they were enriched in the granules. According to the results of inhibition batch tests and trancriptome analysis, it was likely that both anammox and heterotrophic denitrifiers contributed in NO production in the anammox granules. In situ analyses showed that the density and activity of the anammox bacteria and NO production rate were higher in the outer layer of the anammox granule. Ammonium, nitrite and pH levels significantly affected the NO production rates in the anammox granules. The results like presented in this study are useful for strategies to control NO emissions from anammox processes. Since the detected NO concentration in the anammox granules was higher as 2.7 μM, the role of NO molecule in the anammox granules should be deeply investigated in the future researches.

Applying of multiple analytical techniques very indispensable to our knowledge of complicated N_2O and NO production mechanisms in PN and anammox granules.