学　位　論　文　内　容　の　要　旨
DISSERTATION ABSTRACT

博士の専攻分野の名称　　博士（工学）　　氏名　RATHNAYAKE MUDIYANSELAGE LASHITHA DINESH RATHNAYAKE

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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)

Since nitrogen (N) is an essential element to maintain life, some of its fixed forms are used as key ingredients of fertilizer to achieve high crop yields. However, N is one of the major pollutants causing many problems in the aquatic environment. For example, excess N input leads to eutrophication, in which dissolved oxygen is depleted, septic conditions are caused and odor problem arises in water bodies. Moreover, ammonia is toxic to aquatic life and nitrite and nitrate cause several health effects, such as methemoglobinemia and vitamin A shortage. Therefore, stringent water quality standards for N are established to protect aquatic environment. Since municipal, industrial, and agricultural wastewaters are the major N source, N removal from the wastewaters is critical. N is generally removed from wastewaters by biological processes and a conventional biological N removal technology is a combination of nitrification and denitrification processes. However, an alternative and innovative approach, a partial nitrification (PN) process followed by an anaerobic ammonium oxidation (anammox) process (a PN-anammox process), has recently drawn attention. It has several advantages, such as no need for external carbon addition, less energy and oxygen requirement, and less sludge production. Especially, granular sludge reactors for PN and anammox processes are promising because of because of good sludge settleability, long-term retention of slow-growing bacteria in a reactor and high specific reaction rate. 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. Besides its environmental adverse effect, a NO molecule has some ecological influences on microbial consortia by regulating the specific microbial activities. To gain the insight into N_2O and NO production and consumption rates, their pathways, and the factors influencing them is essential to control N_2O and NO emissions from a PN-anammox process. However, very limited studies have been conducted to characterize N_2O and NO production in granular PN-anammox process. Therefore, this thesis started with estimation of N_2O emission rates and identification of a key N_2O production pathway in a granular PN reactor. Then the effects of dissolved oxygen (DO) and pH on the N_2O production rates and pathways were investigated. Furthermore, we experimentally identified NO emission pathways in anammox granules and investigated physicochemical parameters affecting the NO emissions. To achieve the objectives, we conducted batch tests, microsensor measurements, isotopomer analysis, fluorescence in situ hybridization (FISH) and microbial community structure analysis.

We investigated the emission of N_2O from a lab-scale granular sequencing batch reactor (SBR) for PN treating synthetic wastewater without organic carbon.The average N_2O emission rate from the SBR was 0.32 ± 0.17 mg $-$ NL^{-1}h^{-1}, corresponding to the average emission of N_2O of $0.8 \pm 0.4\%$ of the incoming N load ($1.5 \pm 0.8\%$ of the converted ammonia). Analysis of dynamic concentration profiles during one cycle of the SBR operation demonstrated that N_2O concentration in off-gas was the highest just after starting aeration whereas dissolved N_2O concentration in effluent gradually in-

creased in the initial 40 min of the aeration period and decreased thereafter. Isotopomer analysis was conducted to identify the main N_2O production pathway in the reactor during one cycle. The hydroxylamine (NH_2OH) oxidation pathway accounted for 65% of the total N_2O emission in the initial phase during a 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 FISH. PN 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. Based on these results, although N_2O was produced mainly via NH_2OH oxidation pathway in the autotrophic partial nitrification reactor, N_2O production mechanism is complex and could involve multiple N_2O production pathways.

Moreover, the effects of DO and pH on N_2O production rates and pathways in autotrophic PN granules were investigated at the granular level. We conducted batch experiments to investigate the effects of DO and pH on N_2O emission rates. Allylthiourea (ATU) was used to distinguish the amount of N_2O produced by nitrification (NH_2OH oxidation) and denitrification (nitrifier denitrification and heterotrophic denitrification). N_2O emission and ammonia oxidation rates increased with increasing bulk DO concentration from 0.6 to 2.3 mg L^{-1}. The inhibition tests with ATU revealed that N_2O was mainly produced via NH_2OH oxidation and DO dominantly affected this pathway. The linear correlation between N_2O emission and ammonia oxidation rates emphasized that an increase in DO concentration promoted NH_2OH oxidation and then stimulated N_2O production via NH_2OH oxidation. To investigate the effect of pH on the N_2O emission rates from the PN granules, batch tests were conducted at different pH values from 6.5 to 8.5. In contrast to the effects of DO, the change in pH affected the both N_2O production via NH_2OH oxidation and denitrification. Although the ammonia oxidation rate 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 from microsensor measurements, FISH analysis and microbial community analysis revealed that DO and pH mainly influenced N_2O production by *Nitrosomonas europaea* and *Nitrosomonas europaea*, in the oxic surface layer ($< 200\,\mu$m) of the autotrophic PN granules. However, the mechanisms underlying pH effect on N_2O production via NH_2OH oxidation are currently unclear. This study suggests that in situ analysis of PN granules is essential to gaining insight into N_2O emission mechanisms in a PN granule in order to establish a strategy to mitigate N_2O emissions in PN processes.

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 accounted for 88% of the total bacteria in the granules.Stable isotope-labeling studies indicated that most of N_2 was emitted by anammox bacteria, NO was produced only by nitrite reduction and the inhibitors for anammox bacteria reduced N_2 and NO emissions. Both anammox and denitrifying bacteria were responsible for NO emission from the anammox granules. The NO emitted from the anammox granules accounted for < 1.1% of the total gaseous N. In situ analysis showed that the density and activity of the anammox bacteria and NO production rate were higher in the outer layer of the anammox granules. NO emissions were strongly influenced by ammonia, nitrite and pH levels. The results presented in this study are useful for strategies to control NO emissions from anammox processes.

In summary, NH_2OH oxidation contributed mainly to N_2O emission from a granular PN-SBR treating autotrophic wastewater. Nitrification occurred in the surface layer of the granules. DO and pH influenced the N_2O production rates of NH_2OH oxidation. Increase in DO concentrations increase N_2O emission from PN granules and N_2O production rate was highest at pH 7.5. Anammox and denitrifying bacteria were responsible for NO emission from the anammox granules. This study concludes that the combined use of multiple analytical techniques is indispensable to our knowledge of N_2O and NO production mechanisms in PN and anammox granules.