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Nitrous oxide and nitric oxide production in partial nitrification and anaerobic ammonium oxidation granular sludge

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$_2$O) and nitric oxide (NO). N$_2$O 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$_2$O and NO production and consumption rates, their pathways, and the factors influencing them is essential to control N$_2$O and NO emissions from a PN-anammox process. However, very limited studies have been conducted to characterize N$_2$O and NO production in granular PN-anammox process. Therefore, this thesis started with estimation of N$_2$O emission rates and identification of a key N$_2$O production pathway in a granular PN reactor. Then the effects of dissolved oxygen (DO) and pH on the N$_2$O 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$_2$O from a lab-scale granular sequencing batch reactor (SBR) for PN treating synthetic wastewater without organic carbon. The average N$_2$O emission rate from the SBR was 0.32 ± 0.17 mg - NL$^{-1}$h$^{-1}$, corresponding to the average emission of N$_2$O 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 SBR operation demonstrated that N$_2$O concentration in off-gas was the highest just after starting aeration whereas dissolved N$_2$O 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\textsubscript{2}O production pathway in the reactor during one cycle. The hydroxylamine (NH\textsubscript{2}OH) oxidation pathway accounted for 65% of the total N\textsubscript{2}O emission in the initial phase during a cycle, whereas contribution of the NO\textsubscript{\textsuperscript{-}} reduction pathway to N\textsubscript{2}O production was comparable with that of the NH\textsubscript{2}OH 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\textsubscript{2}O production was also found mainly in the oxic surface layer. Based on these results, although N\textsubscript{2}O was produced mainly via NH\textsubscript{2}OH oxidation pathway in the autotrophic partial nitrification reactor, N\textsubscript{2}O production mechanism is complex and could involve multiple N\textsubscript{2}O production pathways.

Moreover, the effects of DO and pH on N\textsubscript{2}O 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\textsubscript{2}O emission rates. Allylthiourea (ATU) was used to distinguish the amount of N\textsubscript{2}O produced by nitrification (NH\textsubscript{2}OH oxidation) and denitrification (nitrifier denitrification and heterotrophic denitrification). N\textsubscript{2}O emission and ammonia oxidation rates increased with increasing bulk DO concentration from 0.6 to 2.3 mg L\textsuperscript{-1}. The inhibition tests with ATU revealed that N\textsubscript{2}O was mainly produced via NH\textsubscript{2}OH oxidation and DO dominantly affected this pathway. The linear correlation between N\textsubscript{2}O emission and ammonia oxidation rates emphasized that an increase in DO concentration promoted NH\textsubscript{2}OH oxidation and then stimulated N\textsubscript{2}O production via NH\textsubscript{2}OH oxidation.

To investigate the effect of pH on the N\textsubscript{2}O 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\textsubscript{2}O production via NH\textsubscript{2}OH oxidation and denitrification. Although the ammonia oxidation rate was unchanged in the range of pH 6.5 to 8.5, the highest N\textsubscript{2}O 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\textsubscript{2}O production by Nitrosomonas europaea and Nitrosomonas europaea, in the oxic surface layer (< 200 μm) of the autotrophic PN granules. However, the mechanisms underlying pH effect on N\textsubscript{2}O production via NH\textsubscript{2}OH oxidation are currently unclear. This study suggests that in situ analysis of PN granules is essential to gaining insight into N\textsubscript{2}O emission mechanisms in a PN granule in order to establish a strategy to mitigate N\textsubscript{2}O 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\textsubscript{2} was emitted by anammox bacteria, NO was produced only by nitrite reduction and the inhibitors for anammox bacteria reduced N\textsubscript{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\textsubscript{2}OH oxidation contributed mainly to N\textsubscript{2}O emission from a granular PN-SBR treating autotrophic wastewater. Nitrification occurred in the surface layer of the granules. DO and pH influenced the N\textsubscript{2}O production rates of NH\textsubscript{2}OH oxidation. Increase in DO concentrations increase N\textsubscript{2}O emission from PN granules and N\textsubscript{2}O 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\textsubscript{2}O and NO production mechanisms in PN and anammox granules.