Research outputs:

**Abiotic Nitrous Oxide (N$_2$O) Production Is Strongly pH Dependent, but Contributes Little to Overall N$_2$O Emissions in Biological Nitrogen Removal Systems**

Hydroxylamine (NH$_2$OH) and nitrite (NO$_2$-), intermediates during the nitritation process, can engage in chemical (abiotic) reactions that lead to nitrous oxide (N$_2$O) generation. Here, we quantify the kinetics and stoichiometry of the relevant abiotic reactions in a series of batch tests under different and relevant conditions, including pH, absence/presence of oxygen, and reactant concentrations. The highest N$_2$O production rates were measured from NH$_2$OH reaction with HNO$_2$, followed by HNO$_2$ reduction by Fe$^{2+}$, NH$_2$OH oxidation by Fe$^{3+}$, and finally NH$_2$OH disproportionation plus oxidation by O$_2$. Compared to other examined factors, pH had the strongest effect on N$_2$O formation rates. Acidic pH enhanced N$_2$O production from the reaction of NH$_2$OH with HNO$_2$ indicating that HNO$_2$ instead of NO$_2$- was the reactant. In departure from previous studies, we estimate that abiotic N$_2$O production contributes little (<3% of total N$_2$O production) to total N$_2$O emissions in typical nitritation reactor systems between pH 6.5 and 8. Abiotic contributions would only become important at acidic pH (≤ 5). In consideration of pH effects on both abiotic and biotic N$_2$O production pathways, circumneutral pH set-points are suggested to minimize overall N$_2$O emissions from nitritation systems.

**The effect of pH on N$_2$O production in intermittently-fed nitritation reactors**

The effect of pH on nitrous oxide (N$_2$O) production rates was quantified in an intermittently-fed lab-scale sequencing batch reactor performing high-rate nitritation. N$_2$O and other nitrogen (N) species (e.g. ammonium (NH$_4$+), nitrite, hydroxylamine and nitric oxide) were monitored to identify in-cycle dynamics and determine N conversion rates at controlled pH set-points (6.5, 7, 7.5, 8 and 8.5). Operational conditions and microbial compositions remained similar during long-term reactor-scale pH campaigns. The specific ammonium removal rates and nitrate accumulation rates varied little with varying reactor-scale pH. The specific ammonium removal rates and nitrate accumulation rates varied little with varying reactor-scale pH (p>0.05). The specific net N$_2$O production rates and net N$_2$O yield of NH$_4$+ removed (ΔN$_2$O/ΔNH$_4$+) increased up to...
seven-fold from pH 6.5 to 8, and decreased slightly with further pH increase to 8.5 (p<0.05). Best-fit model simulations predicted nitrifier denitrification as the dominant N2O production pathway (≥87% of total net N2O production) at all examined pH. Our study highlights the effect of pH on biologically mediated N2O emissions in nitrogen removal systems and its importance in the design of N2O mitigation strategies.

Adaption and recovery of Nitrosomonas europaea to chronic TiO2 nanoparticle exposure
Although the adverse impacts of emerging nanoparticles (NPs) on the biological nitrogen removal (BNR) process have been broadly reported, the adaptive responses of NP-impaired nitrifiers and the related mechanisms have seldom been addressed to date. Here, we systematically explored the adaption and recovery capacities of the ammonia oxidizer Nitrosomonas europaea under chronic TiO2 NP exposure and different dissolved oxygen (DO) conditions at the physiological and transcriptional levels in a chemostat reactor. N. europaea cells adapted to 50mg/L TiO2 NP exposure after 40-d incubation and the inhibited cell growth, membrane integrity, nitritation rate, and ammonia monooxygenase activity all recovered regardless of the DO concentrations. Transmission electron microscope imaging indicated the remission of the membrane distortion after the cells' 40-d adaption to the NP exposure. The microarray results further suggested that the metabolic processes associated with the membrane repair were pivotal for cellular adaption/recovery, such as the membrane efflux for toxicant exclusion, the structural preservation or stabilization, and the osmotic equilibrium adjustment. In addition, diverse metabolic and stress-defense pathways, including aminoacyl-tRNA biosynthesis, respiratory chain, ATP production, toxin-antitoxin ‘stress-fighting’, and DNA repair were activated for the cellular adaption coupled with the metabolic activity recovery, probably via recovering the energy production/conversion efficiency and mediating the non-photooxidative stress. Finally, low DO (0.5mg/L) incubated cells were more susceptible to TiO2 NP exposure and required more time to adapt to and recover from the stress, which was probably due to the stimulation limitation of the oxygen-dependent energy metabolism with a lower oxygen supply. The findings of this study provide new insights into NP contamination control and management adjustments during the BNR process.
Dynamics, pathways and mitigation of $N_2O$ production in intermittently-fed high-rate nitritation reactor

Massive quantities of inorganic nitrogen (mainly in the form of ammonium ($NH_4^+$)) in residual waters derived from human activities continue to be released in aquatic ecosystems. Among various physicochemical and biological methods for treating $NH_4^+$-rich residual waters, biological nitrogen removal (BNR) via nitrification and heterotrophic denitrification process is most widely applied. In recent years, novel processes including nitritation, anammox or a combination of partial nitritation plus anammox (PNA) have been implemented as energy and resource-efficient alternatives of conventional BNR processes. However, emissions of nitrous oxide ($N_2O$) during the operation of these novel processes may offset the claimed environmental benefits of nitritation or PNA technologies. $N_2O$ is a strong greenhouse with ca. 300 times higher global warming potential than carbon dioxide (CO2) and contributes to the destruction of stratospheric ozone. Nitrifier nitrification (NN) and nitrifier denitrification (ND) by ammonia oxidizing bacteria (AOB), heterotrophic denitrification (HD) by denitrifying bacteria and several abiotic reactions are identified as pathways of $N_2O$ production. However, the contribution of different pathways of $N_2O$ production and their environmental controls in BNR systems remain to be identified and quantified. Further, a better and quantitative understanding of the mechanisms of $N_2O$ production is warranted, in order to develop operational strategies or system designs that might mitigate $N_2O$ emissions. This PhD project investigated the kinetics and stoichiometry of the relevant abiotic reactions were quantified in a series of batch tests across a range of relevant pHs, absence/presence of nitritation process, can engage in chemical reactions that lead to $N_2O$ formation. The kinetics and stoichiometry of the relevant abiotic reactions were quantified in a series of batch tests across a range of relevant pHs, absence/presence of oxygen, and at different reactant concentrations. The highest $N_2O$ production rates were measured for $NH_4^+$ oxidation by $HNO_2$, followed by $HNO_2$ reduction by ferrous iron ($Fe^{2+}$), $NH_2OH$ oxidation by ferric iron ($Fe^{3+}$), and finally $NH_2OH$ disproportionation plus oxidation by $O_2$. Compared to other examined factors, pH had the strongest effect on $N_2O$ formation rates. Acidic pH stimulated $N_2O$ production from the oxidation of $NH_2OH$ by $HNO_2$ and we could conclude that $HNO_2$ rather than $NO_2$ is the reactant. In departure from previous studies, we estimate that abiotic $N_2O$ production is a minor source (< 3% of total $N_2O$ production) in typical nitritation reactor systems with pH between 6.5 and 8. Only at extremely acidic pH (< 5) would the abiotic pathway become significant. In consideration of the effects of pH on both abiotic and biotic $N_2O$ production pathways, circumneutral pH set-points are suggested to minimize overall $N_2O$ emissions from nitritation systems. Overall, experimental efforts were implemented to investigate dynamics, pathways and mitigation options for $N_2O$ production in nitritation reactors. This study has identified operational strategies via intermittent feeding and pH control as means to mitigate $N_2O$ emission from nitritation systems.
The pH dependency of N-converting enzymatic processes, pathways and microbes: effect on net N₂O production

Nitrous oxide (N₂O) is emitted during microbiological nitrogen (N) conversion processes, when N₂O production exceeds N₂O consumption. The magnitude of N₂O production vs. consumption varies with pH and controlling net N₂O production might be feasible by choice of system pH. This article reviews how pH affects enzymes, pathways and microorganisms that are involved in N-conversions in water engineering applications. At a molecular level, pH affects activity of cofactors and structural elements of relevant enzymes by protonation or deprotonation of amino acid residues or solvent ligands, thus causing steric changes in catalytic sites or proton/electron transfer routes that alter the enzymes' overall activity. Augmenting molecular information with, e.g., nitritation or denitrification rates yields explanations of changes in net N₂O production with pH. Ammonia oxidizing bacteria are of highest relevance for N₂O production, while heterotrophic denitrifiers are relevant for N₂O consumption at pH > 7.5. Net N₂O production in N-cycling water engineering systems is predicted to display a 'bell-shaped' curve in the range of pH 6.0-9.0 with a maximum at pH 7.0-7.5. Net N₂O production at acidic pH is dominated by N₂O production, whereas N₂O consumption can outweigh production at alkaline pH. Thus, pH 8.0 may be a favourable pH set-point for water treatment applications regarding net N₂O production.

Low nitrous oxide production in intermittent-feed high performance nitritating reactors

Nitrous oxide (N₂O) production from autotrophic nitrogen removal processes, especially nitritating systems, is of growing concern. N₂O dynamics were characterized and N₂O production factors were quantified in two lab-scale intermittent-feed nitritating SBRs. 93 ± 14% of the oxidized ammonium was converted to nitrite, with the average total net N₂O production of 2.1 ± 0.7% of the ammonium oxidized. Operation with intermittent feeding appears an effective optimization approach to mitigate N₂O emissions from nitritating systems. Net N₂O production rates transiently increased with a rise in pH after each feeding, indicating a potential role of pH in N₂O production.
Low nitrous oxide production through nitrifier-denitrification in intermittent-feed high-rate nitritation reactors

Nitrous oxide (N2O) production from autotrophic nitrogen conversion processes, especially nitritation systems, can be significant, requires understanding and calls for mitigation. In this study, the rates and pathways of N2O production were quantified in two lab-scale sequencing batch reactors operated with intermittent feeding and demonstrating long-term and high-rate nitritation. The resulting reactor biomass was highly enriched in ammonia-oxidizing bacteria, and converted ~93 ± 14% of the oxidized ammonium to nitrite. The low DO set-point combined with intermittent feeding was sufficient to maintain high nitritation efficiency and high nitritation rates at 20-26 °C over a period of ~300 days. Even at the high nitritation efficiencies, net N2O production was low (~2% of the oxidized ammonium). Net N2O production rates transiently increased with a rise in pH after each feeding, suggesting a potential effect of pH on N2O production. In situ application of 15N labeled substrates revealed nitrifier denitrification as the dominant pathway of N2O production. Our study highlights operational conditions that minimize N2O emission from two-stage autotrophic nitrogen removal systems.
Department of Environmental Engineering

Water Technologies

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