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 nitrite accumulation rates varied little with varying pH levels.
The specific net N2O production rates and net N2O yield of NH4+ removed (ΔN2O/ΔNH4+) 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.
Nitrous oxide production in intermittently aerated Partial Nitritation-Anammox reactor: oxic N₂O production dominates and relates with ammonia removal rate

Emissions of the greenhouse gas nitrous oxide from the Partial Nitritation-Anammox process are of concern and can determine the carbon footprint of the process. In order to reduce nitrous oxide emissions intermittent aeration regimes have been shown to be a promising mode of operation, possibly due to an effective control of accumulation of nitrogen intermediates. However, due to frequent changes of redox conditions under intermittent aeration regimes, nitrous oxide production and emissions are dynamic. In this study the production and emission dynamics of nitrous oxide in an intermittently aerated sequencing batch reactor were monitored in high temporal resolution, the contribution of different redox conditions to overall nitrous oxide production was quantified and the most relevant factors for nitrous oxide production were identified. The average fraction of nitrous oxide produced (per unit ammonium removed) was 1.1 ± 0.5%. Cycle-averaged approx. 80% of nitrous oxide was produced during aerated phases, the remaining 20% were produced during non-aerated phases. Yet, the intra-cycle dynamics of nitrous oxide were substantial. The net-production rate of nitrous oxide during aerated phases correlated with the ammonia removal rate, whereas the concentration of nitrite determined the production during non-aerated phases. While aerated phases contributed predominantly at the beginning of reactor cycles, non-aerated phases became the dominant source of nitrous oxide at the end. Particularly low net-production rates were observed at ammonia removal rates below 5 mg NH₃-N*gVSS−1*L−1, when the fraction of nitrous oxide produced was 0.011 ± 0.004% (per ammonia removed). Based on the nitrous oxide dynamics and correlations, reactor operation at relatively low nitrogen loadings (below 100 mg NH₄+-N*L−1), ammonia removal rates of approx. 5 mg NH₃-N*gVSS−1*L−1 and nitrite concentrations below 1 mg NO₂−1-N*L−1 appears as beneficial for low emission of nitrous oxide.
Reactor staging influences microbial community composition and diversity of denitrifying MBBRs- Implications on pharmaceutical removal

The subdivision of biofilm reactor in two or more stages (i.e., reactor staging) represents an option for process optimisation of biological treatment. In our previous work, we showed that the gradient of influent organic substrate availability (induced by the staging) can influence the microbial activity (i.e., denitrification and pharmaceutical biotransformation kinetics) of a denitrifying three-stage Moving Bed Biofilm Reactor (MBBR) system. However, it is unclear whether staging and thus the long-term exposure to varying organic carbon type and loading influences the microbial community structure and diversity. In this study, we investigated biofilm structure and diversity in the three-stage MBBR system (S) compared to a single-stage configuration (U) and their relationship with microbial functions. Results from 16S rRNA amplicon libraries revealed a significantly higher microbial richness in the staged MBBR (at 99% sequence similarity) compared to single-stage MBBR. A more even and diverse microbial community was selected in the last stage of S (S3), likely due to exposure to carbon limitation during continuous-flow operation. A core of OTUs was shared in both systems, consisting of Burkholderiales, Xanthomonadales, Flavobacteriales and Sphingobacteriales, while MBBR staging selected for specific taxa (i.e., Candidate division WS6 and Deinococcales). Results from quantitative PCR (qPCR) showed that S3 exhibited the lowest abundance of 16S rRNA but the highest abundance of atypical nosZ, suggesting a selection of microbes with more diverse N-metabolism (i.e., not-complete denitrifiers) in the stage exposed to the lowest carbon availability. A positive correlation (p<0.05) between removal rate constants of several pharmaceuticals with abundance of relevant denitrifying genes was observed, but not with biodiversity. Despite the previously suggested positive relationship between microbial diversity and functionality in macrobial and microbial ecosystems, this was not observed in the current study, suggesting a need to further investigate structure-function relationships for denitrifying systems.

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The pH dependency of N-converting enzymatic processes, pathways and microbes: effect on net N₂O production

Nitrous oxide (N₂ O) is emitted during microbiological nitrogen (N) conversion processes, when N₂ O production exceeds N₂ O consumption. The magnitude of N₂ O production vs. consumption varies with pH and controlling net N₂ O 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 N₂ O production with pH. Ammonia oxidizing bacteria are of highest relevance for N₂ O production, while
heterotrophic denitrifiers are relevant for N2 O consumption at pH > 7.5. Net N2 O 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 N2 O production at acidic pH is dominated by N2 O production, whereas N2 O consumption can outweigh production at alkaline pH. Thus, pH 8.0 may be a favourable pH set-point for water treatment applications regarding net N2 O production.

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**Where does N2O from Partial Nitritation-Anammox processes come from? – A high temporal resolution study of a lab-scale system gives answers**

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**Aquatvalens Project - Report on the evaluation of data in comparison to "classical" data: Deliverable D10.5**

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Dynamics of N2O production pathways analyzed by 15N18O isotope labeling

Nitrous oxide production associated with biological nitrogen transformations can contribute substantially to the CO2 footprint of both man-made and natural systems, but the pathways and regulation of N2O production are poorly understood. We developed a 15N/18O dual isotope labelling technique to distinguish and quantify these pathways in mixed communities. The use of 18O-O2 permits differentiation of hydroxylamine oxidation and nitrifier-denitrification driven N2O production by ammonium oxidizing bacteria. We analysed N2O production pathways during biological nitrogen removal at Lynetten wastewater treatment plant. Under anoxia, N2O accumulated due to denitrification, but N2O accumulation was ~3 and 1.7 times higher at 30 and 100 µM O2, respectively. Oxic N2O production was dominated by nitrifier-denitrification, reaching 73% of the total with the remainder due to hydroxylamine oxidation. Our results demonstrate three active pathways of N2O production, each with different environmental controls. The dual 15N/18O isotope labelling approach can contribute to the development of strategies to minimise N2O emissions from man-made and natural systems.

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Heterotrophs are key contributors to nitrous oxide production in mixed liquor under low C-to-N ratios during nitrification - batch experiments and modelling

Nitrous oxide (N2O), a by-product of biological nitrogen removal during wastewater treatment, is produced by ammonia-oxidizing bacteria (AOB) and heterotrophic denitrifying bacteria (HB). Mathematical models are used to predict N2O emissions, often including AOB as the main N2O producer. Several model structures have been proposed without consensus calibration procedures. Here, we present a new experimental design that was used to calibrate AOB-driven N2O dynamics of a mixed culture. Even though AOB activity was favoured with respect to HB, oxygen uptake rates indicated HB activity. Hence, rigorous experimental design for calibration of autotrophic N2O production from mixed cultures is essential. The proposed N2O production pathways were examined using five alternative process models confronted with experimental data inferred. Individually, the autotrophic and heterotrophic denitrification pathway could describe the observed data. In the best-fit model, which combined two denitrification pathways, the heterotrophic was stronger than the autotrophic contribution to N2O production. Importantly, the individual contribution of autotrophic and heterotrophic to the total N2O pool could not be unambiguously elucidated solely based on bulk N2O measurements. Data on NO would increase the practical identifiability of N2O production pathways.

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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.

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Pathways and Controls of $\text{N}_2\text{O}$ Production in Nitritation-Anammox Biomass

Nitrous oxide (N$_2$O) is an unwanted byproduct during biological nitrogen removal processes in wastewater. To establish strategies for N$_2$O mitigation, a better understanding of production mechanisms and their controls is required. A novel stable isotope labeling approach using 15N and 18O was applied to investigate pathways and controls of N$_2$O production by biomass taken from a full-scale nitritation-anammox reactor. The experiments showed that heterotrophic denitrification was a negligible source of N$_2$O under oxic conditions ($>0.2$ mg O$_2$ L$^{-1}$). Both hydroxylamine oxidation and nitrifier denitrification contributed substantially to N$_2$O accumulation across a wide range of conditions with varying concentrations of O$_2$, NH$_4^+$, and NO$_2^-$. The O$_2$ concentration exerted the strongest control on net N$_2$O production with both production pathways stimulated by low O$_2$, independent of NO$_2^-$ concentrations. The stimulation of N$_2$O production from hydroxylamine oxidation at low O$_2$ was unexpected and suggests that more than one enzymatic pathway may be involved in this process. N$_2$O production by hydroxylamine oxidation was further stimulated by NH$_4^+$, whereas nitrifier denitrification at low O$_2$ levels was stimulated by NO$_2^-$ at levels as low as 0.2 mM. Our study shows that 15N and 18O isotope labeling is a useful approach for direct quantification of N$_2$O production pathways applicable to diverse environments.

General information
Simple control rules for mitigating $\text{N}_2\text{O}$ emissions in phase isolated fullscale WWTPs

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**ABSTRACT BOOK**

**ABSTRACT**

Simple control rules for mitigating $\text{N}_2\text{O}$ emissions in phase isolated fullscale WWTPs

Nitrous oxide ($\text{N}_2\text{O}$) is a strong greenhouse gas (GHG) and ozone depleter, with a warming potential 300 times higher than carbon dioxide (CO2). 1.2% of the total anthropogenic $\text{N}_2\text{O}$ emissions are believed to originate from the wastewater treatment (WWT) sector. Conventional biological nutrient removal processes relying on nitrification and denitrification are known to produce $\text{N}_2\text{O}$. A one year long-term study of $\text{N}_2\text{O}$ production and emissions was performed at Lynetten, Denmark’s largest WWTP. Nitrification and denitrification takes place by alternating process conditions as well as influent and effluent flows in 20 pairs of interconnected and surface aerated reactors. The long-term data revealed that the $\text{N}_2\text{O}$ emissions contribute to as much as 30% of the total CO2 footprint from the WWTP. High ammonium concentrations and long aeration phases lead to high $\text{N}_2\text{O}$ production and emissions rates. Nitrification phases were identified to produce and emit most of the $\text{N}_2\text{O}$. High production and emissions were also associated with the afternoon loading peaks at the WWTP. During denitrification phases $\text{N}_2\text{O}$ was produced initially but consumed consequently. An effective control strategy was implemented, whereby $\text{N}_2\text{O}$ emissions were reduced from 0.8% to 0.3% of the nitrogen load during the mitigation period.

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**Research output**
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**ABSTRACT BOOK**

**ABSTRACT**

Simple control strategy for mitigating $\text{N}_2\text{O}$ emissions in phase isolated full-scale WWTPs

Nitrous oxide ($\text{N}_2\text{O}$) is a strong greenhouse gas (GHG) and ozone depleter, with a warming potential 300 times higher than carbon dioxide (CO2). 1.2% of the total anthropogenic $\text{N}_2\text{O}$ emissions are believed to originate from the wastewater treatment (WWT) sector. Conventional biological nutrient removal processes relying on nitrification and denitrification are known to produce $\text{N}_2\text{O}$. A one year long-term study of $\text{N}_2\text{O}$ production and emissions was performed at Lynetten, Denmark’s largest WWTP. Nitrification and denitrification takes place by alternating process conditions as well as influent and effluent flows in 20 pairs of interconnected and surface aerated reactors. The long-term data revealed that the $\text{N}_2\text{O}$ emissions contribute to as much as 30% of the total CO2 footprint from the WWTP. High ammonium concentrations and long aeration phases lead to high $\text{N}_2\text{O}$ production and emissions rates. Nitrification phases were identified to produce and emit most of the $\text{N}_2\text{O}$. High production and emissions were also associated with the afternoon loading peaks at the WWTP. During denitrification phases $\text{N}_2\text{O}$ was produced initially but consumed consequently. An effective control strategy was implemented, whereby $\text{N}_2\text{O}$ emissions were reduced from 0.8% to 0.3% of the nitrogen load during the mitigation period.

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Does reactor staging influence microbial structure and functions in biofilm systems? The case of pre-denitrifying MBBRs

To date, a number of treatment technologies and configurations have been tested to improve the elimination of conventional and trace (e.g., pharmaceutical residues) pollutants via biological wastewater treatment. Bioreactor staging and the moving bed biofilm reactor (MBBR) technology have emerged as promising bioengineered solutions (Plósz et al., 2010) for this purpose. In this study, we combined the two solutions and investigated microbial functions (heterotrophic denitrification, pharmaceutical removal) and structure of the microbial community in staged MBBRs for pre-denitrification. A three-stage MBBR system (S1+S2+S3), fed with pre-clarified wastewater, was operated at laboratory-scale with (i) controlled biomass exposure to organic substrate (COD); and (ii) enhanced the physical retention of biomass, thus inducing adaptation to different substrate exposure conditions. During long-term operation (~500 days) of the three-stage MBBR under continuous-flow conditions, biofilm samples were collected to assess the temporal evolution of the microbial structure in terms of functional gene abundance and biodiversity. A set of batch experiments (day 471) was performed to assess denitrification and pharmaceutical removal in each MBBR, following prolonged biofilm exposure to specific COD availability.

Results from batch experiments showed declining denitrification potential and pharmaceutical biotransformation rate constants \( k_{bio} (L \cdot gTSS^{-1} \cdot d^{-1}) \) from MBBR S1 (exposed to highest COD availability) to S3 (exposed to lowest availability). These findings indicate that the exposure to tiered substrate availability influenced the capacity of utilizing a different range of carbon sources in each MBBR, thus impacting denitrification and pharmaceutical biotransformation. Preliminary analysis on the microbial community based on qPCR (quantitative polymerase chain reaction) showed differences in the abundance of genes (nirS, nirK, nosZ) encoding for denitrifying enzymes in the three staged MBBRs. Further microbial characterization through 16S rRNA sequencing (Illumina) is currently under investigation to determine whether differences in microbial functions should be associated to differences in the microbial diversity in the three MBBRs.

Dynamics of \( \text{N}_2\text{O} \) production pathways in a full-scale activated sludge system analysed by \( ^{15}\text{N}^{18}\text{O} \) dual isotope labelling

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Organisations: Department of Environmental Engineering, Water Technologies, University of Southern Denmark, Max Planck Institute
The influence of reactor staging on microbial structure and functions in pre-denitrifying MBBRs

Aerobic Microbial Respiration in Oceanic Oxygen Minimum Zones

Aerobic Microbial Respiration in Oceanic Oxygen Minimum Zones

Oxygen minimum zones are major sites of fixed nitrogen loss in the ocean. Recent studies have highlighted the importance of anaerobic ammonium oxidation, anammox, in pelagic nitrogen removal. Sources of ammonium for the anammox reaction, however, remain controversial, as heterotrophic denitrification and alternative anaerobic pathways of organic matter remineralization cannot account for the ammonium requirements of reported anammox rates. Here, we explore the significance of microaerobic respiration as a source of ammonium during organic matter degradation in the oxygen-deficient waters off Namibia and Peru. Experiments with additions of double-labelled oxygen revealed high aerobic activity in the upper OMZs, likely controlled by surface organic matter export. Consistently observed oxygen consumption in samples retrieved throughout the lower OMZs hints at efficient exploitation of vertically and laterally advected, oxygenated waters in this zone by aerobic microorganisms. In accordance, metagenomic and metatranscriptomic analyses identified genes encoding for aerobic terminal oxidases and demonstrated their expression by diverse microbial communities, even in virtually anoxic waters. Our results suggest that microaerobic respiration is a major mode of organic matter remineralization and source of ammonium (~45-100%) in the upper oxygen minimum zones, and reconcile hitherto observed mismatches between ammonium producing and consuming processes therein.
Challenges encountered calibrating N2O dynamics from mixed cultures

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Vivianite formation and its role in phosphorus retention in Lake Ørn, Denmark

Vivianite [(Fe₃(PO₄)₂·8H₂O)] may precipitate in anoxic lake sediments affecting the porewater orthophosphate concentration, and thereby the trophic status of lakes. We have investigated changes in lake diagenesis of Fe and P (1969-2009), with particular attention focused on vivianite formation with sediment depth (0-20cm) in an iron-silica-carbon rich lake sediment (Ørnsø, Denmark). Porewaters were supersaturated for vivianite by two to five orders of magnitude (upper 10cm) with porewater phosphate concentrations ranging between 0.69 and 10μmol/l, in winter, and summer concentrations ranging between 9.8 and 40μmol/l. Significant formation of vivianite was confirmed by X-ray diffraction while scanning electron microscopy and electron dispersive X-ray spectroscopy indicated an increase in vivianite crystal size with depth (~20 to ~70μm across). Variations in elemental composition of vivianite crystals in relation to at.% P and Fe were especially seen going from 9.5cm to 24.5cm. The total sediment Fe pool was very large ~3000μmol/g and total P increased from 200μmol/g to 400μmol/g descending down the sediment profile. Differential extraction experiments of P release at pH3 estimated that vivianite amounts to between 3 and 5% of the total Fe pool. The total P burial fluxes estimate that ~38μmolcm⁻²yr⁻¹ or ~26% of sedimentary P in the lower sediments is sequestered as vivianite. There are seasonal variations in the porewater composition with lower Fe, orthophosphate and higher sulfate concentrations during winter (5°C), than during summer (15°C). This suggests that temperature modulates the rate of organic matter degradation which in turns affects the rate of Fe(III) phase reduction, release of phosphate, and thereby the porewater Fe²⁺ and orthophosphate concentrations and hence vivianite formation. This work highlights the role vivianite can play for P retention in a Si-Fe-C rich lake sediment.

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Organisations: Department of Environmental Engineering, Urban Water Engineering, Center for Electron Nanoscopy, Geological Survey of Denmark and Greenland, University of Copenhagen, University of Southern Denmark
Aeration Strategies To Mitigate Nitrous Oxide Emissions from Single-Stage Nitritation/Anammox Reactors

Autotrophic nitrogen removal is regarded as a resource efficient process to manage nitrogen-rich residual streams. However, nitrous oxide emissions of these processes are poorly documented and strategies to mitigate emissions unknown. In this study, two sequencing batch reactors performing single-stage nitritation/anammox were operated under different aeration strategies, gradually adjusted over six months. At constant but limiting oxygen loading, synthetic reject water was fed (0.75g-N/L.d) and high nitrogen removal efficiencies (83 +/- 5 and 88 +/- 2%) obtained. Dynamics of liquid phase nitrous (N2O) and nitric oxide (NO) concentrations were monitored and N2O emissions calculated. Significant decreases in N2O emissions were obtained when the frequency of aeration was increased while maintaining a constant air flow rate (from >6 to 1.7% Delta N2O/Delta TN). However, no significant effect on the emissions was noted when the duration of aeration was increased while decreasing air flow rate (10.9 +/- 3.2% Delta N2O/Delta TN). The extant ammonium oxidation activity (mgNH(4)(+)-N/gVSS.min) positively correlated with the specific N2O production rate (mgN(2)O-N/gVSS.min) of the systems. Operating under conditions where anaerobic exceeds aerobic ammonium oxidation activity is proposed to minimize N2O emissions from single-stage nitritation/anammox reactors; increasing the frequency of aeration cycling is an efficient way of obtaining those conditions.
Modelling N2O dynamics in the engineered N cycle: Evaluation of alternate model structures

Research on nitrous oxide (N2O) formation in engineered wastewater systems has experienced an exponential development in the recent years due to the important environmental impact of this greenhouse gas. These efforts have crystalized in a large number of publications that aim to identify the importance of the main microbial processes responsible for its production and consumption. The conceptualization of these pathways in mathematical models has the potential to become a key tool to increase our understanding on the complex interrelationships within these ecosystems and develop strategies to minimize the carbon footprint of wastewater treatment plants. The present contribution aims to summarize the recent developments in this field and makes use of standard indentifiability measures to show how the choice of experimental protocols and model structures can potentially impact their calibration.

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The microbial nitrogen cycle

General information
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Organisations: Department of Environmental Engineering, Urban Water Engineering, Princeton University
Contributors: Ward, B. B., Jensen, M. M.
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Source-ID: 102205401
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Bakterier gi'r den gas

**General information**

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Organisations: Department of Environmental Engineering, Urban Water Engineering, BIOFOS Renseanlæg Lynetten, University of Southern Denmark
Contributors: Thamdrup, B., Jensen, M. M., Thornberg, D.
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**Publication information**

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**Diversity of total and functional microbiome of anammox reactors fed with complex and synthetic nitrogen-rich wastewaters**

There are few comparative studies of microbial structure, composition and phylogenetic diversity of the anammox reactors as a function of substrate complexity exist, representing a large gap in the scientific literature.

In this study, we applied 16S rRNA gene (rDNA) tag-based 454 pyrosequencing as a deep sequencing approach to 59 biomass samples from 24 different anammox bioreactors together with proper biological replication in order to compare their total and functional (wrt anaerobic ammonium oxidation) microbial diversity. Among 24 sampled bioreactors, 10 of them were full scale implementations treating complex nitrogen-rich wastewaters and 14 were lab-scale implementations treating synthetic wastewaters. We found that nitritation/anammox bioreactors treating complex nitrogen-rich wastewaters were more diverse in terms of total microbial diversity but less diverse at anammox functional diversity than the bioreactors treating synthetic wastewaters inferred from observed OTUs, Chao1, Shannon index and Phylogenetic distance calculations. Differences in total microbial diversity agreed with the ecological theory concerning the positive correlation between substrate complexity and biodiversity (Parrott 2010), but, not (Harris et al. 2012) in the anammox functional guild diversity (functional diversity term was used based on phylogenetic groups known to harbor the anammox metabolic pathway). Classifying the microbial structure of bioreactors according to substrate complexity using weighted UniFrac algorithm explained 29% of the variance where the bioreactor samples of complex nitrogen-rich wastewater feeding was clearly separated from the bioreactor samples of synthetic feeding.

Here we examined and compared for the first time microbial diversity of nitritation-anammox reactors that are designed and built individually for treating complex nitrogen-rich and synthetic wastewaters across the world using 16 rRNA gene pyrosequencing. With the aid of replicated genetic snapshots, we revealed the relationship between the microbial diversity of nitritation-anammox reactors operated by different substrate complexity in terms of microbial composition, structure, richness and phylogenetic diversity from two points of view: total and functional diversity.

**General information**

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Poster presentation
Source: dtu
Modelling N\textsubscript{2}O dynamics in the engineered N cycle: Observations, assumptions, knowns, and unknowns

Research on nitrous oxide formation in engineered wastewater systems has experienced an exponential development in the recent years due to the important environmental impact of this greenhouse gas. These efforts have crystallized in a large number of publications that aim to identify the importance of the main microbial processes responsible for its production and consumption. The conceptualization of these pathways in mathematical models has the potential to become a key tool to increase our understanding on the complex interrelationships within these ecosystems and develop strategies to minimize the carbon footprint of wastewater treatment plants. Unfortunately, existing model structures are limited to describe the emissions of individual microbial pathways in an attempt to decrease their complexity and facilitate their calibration. The present contribution summarizes the recent developments in this field and makes use of sensitivity analyses, and an in-depth study of model uncertainties to establish experimental protocols that facilitate the calibration and predictive ability of a new generation of more realistic models describing N\textsubscript{2}O production during wastewater treatment.

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Publication status: Published
Organisations: Department of Environmental Engineering, Department of Chemical and Biochemical Engineering, Computer Aided Process Engineering Center
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Research output: Contribution to conference › Conference abstract for conference – Annual report year: 2013 › Research › peer-review

N\textsubscript{2}O production dynamics in nitrifying/denitrifying activated sludge under defined environmental conditions

Nitrous oxide (N\textsubscript{2}O) is a gaseous pollutant emitted as an unwanted product in wastewater treatment plants during the nitrification-denitrification process. Even though the emission capacity of the process with respect to this compound is still under debate, N\textsubscript{2}O has been identified as an important contributor to global warming and the destruction of the ozone layer. The present study makes use of unique datasets collected during controlled batch tests with activated sludge biomass to test and calibrate a pseudo-mechanistic model that predicts N\textsubscript{2}O production by nitrifying and heterotrophic bacteria. The proposed model described successfully the observed N\textsubscript{2}O production dynamics and confirmed that the availability of ammonia, low dissolved oxygen and nitrite accumulation were the main factors triggering N\textsubscript{2}O production. Nitrifier-denitrification was proposed as the main pathway catalyzing the conversion of fixed nitrogen to N\textsubscript{2}O. Heterotrophic denitrification rates were one order of magnitude lower than nitrification rates and contributed marginally to the overall N\textsubscript{2}O production. Further data analysis allowed derivation of the overall mass transfer coefficients describing gaseous stripping and revealed that a minor portion of the N\textsubscript{2}O produced was actually released to the gas phase. This work represents a step further in the use and calibration of process models to control and understand better N\textsubscript{2}O production and emissions during conventional wastewater treatment.

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Organisations: Department of Environmental Engineering
Contributors: Pellicer i Nàcher, C., Jensen, M. M., Petersen, M. S., Smets, B. F.
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Architecture evolution of biomass aggregates in single stage nitritation/anammox reactors

General information
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Organisations: Department of Environmental Engineering, Environmental Chemistry, Department of Chemical and Biochemical Engineering, Computer Aided Process Engineering Center
Publication date: 2012
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Research output: Contribution to conference › Poster – Annual report year: 2012 › Research › peer-review

Intensive and extensive nitrogen loss from intertidal permeable sediments of the Wadden Sea

Nitrogen (N) loss rates were determined in permeable sediments of the Wadden Sea using a combination of stable N isotope incubation experiments and model simulation approaches during three seasons. Three different incubation methods that employed the isotope pairing technique were used: intact core incubations simulating either (1) diffusive or (2) advective transport conditions and (3) slurry incubations. N loss rates from core incubations under simulated advective transport conditions exceeded those rates measured under diffusive transport conditions by 1–2 orders of magnitude, but were comparable to those observed in slurry incubations. N loss rates generally showed little seasonal and spatial variation (207 ± 30 mmol m⁻² h⁻¹) in autumn 2006 and spring and summer 2007. Utilizing an extensive time series of nutrient concentrations and current velocities obtained from a continuous monitoring station, nitrate and nitrite (i.e., NOₓ⁻) flux into the sediment was modeled over a full annual cycle. Fluxes were sufficient to support the experimentally derived N loss rates. Combining the measured rates with the modeled results, an annual N removal rate of 745 ± 109 mmol N m⁻² yr⁻¹ was estimated for permeable sediments of the Wadden Sea. This rate agrees well with previous N loss estimates for the Wadden Sea based on N budget calculations. Permeable sediments, accounting for 58–70% of the continental shelf area, are an important N sink and their contribution to the global N loss budget should be reevaluated.

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Publication status: Published
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Scopus rating (2012): CiteScore 3.81 SJR 2.474 SNIP 1.499
Web of Science (2012): Impact factor 3.405
ISI indexed (2012): ISI indexed yes
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Research output: Contribution to journal › Journal article – Annual report year: 2012 › Research › peer-review
Nitrite oxidation in the Namibian oxygen

Nitrite oxidation is the second step of nitrification. It is the primary source of oceanic nitrate, the predominant form of bioavailable nitrogen in the ocean. Despite its obvious importance, nitrite oxidation has rarely been investigated in marine settings. We determined nitrite oxidation rates directly in (15)N-incubation experiments and compared the rates with those of nitrate reduction to nitrite, ammonia oxidation, anammox, denitrification, as well as dissimilatory nitrate/nitrite reduction to ammonium in the Namibian oxygen minimum zone (OMZ). Nitrite oxidation (≤372 nM NO(2)(-) d(-1)) was detected throughout the OMZ even when in situ oxygen concentrations were low to non-detectable. Nitrite oxidation rates often exceeded ammonia oxidation rates, whereas nitrate reduction served as an alternative and significant source of nitrite. Nitrite oxidation and anammox co-occurred in these oxygen-deficient waters, suggesting that nitrite-oxidizing bacteria (NOB) likely compete with anammox bacteria for nitrite when substrate availability became low. Among all of the known NOB genera targeted via catalyzed reporter deposition fluorescence in situ hybridization, only Nitrospina and Nitrococcus were detectable in the Namibian OMZ samples investigated. These NOB were abundant throughout the OMZ and contributed up to ~9% of total microbial community. Our combined results reveal that a considerable fraction of the recently recycled nitrogen or reduced NO(3)(-) was re-oxidized back to NO(3)(-) via nitrite oxidation, instead of being lost from the system through the anammox or denitrification pathways.

15N-labeling experiments to dissect the contributions of heterotrophic denitrification and anammox to nitrogen removal in the OMZ waters of the ocean.

In recent years, (15)N-labeling experiments have become a powerful tool investigating rates and regulations of microbially mediated nitrogen loss processes in the ocean. This chapter introduces the theoretical and practical aspects of (15)N-labeling experiments to dissect the contribution of denitrification and anammox to nitrogen removal in oxygen minimum zones (OMZs). We provide a detailed description of the preparation and realization of the experiments on board. Subsequent measurements of N(2) isotopes using gas chromatography mass spectrometry as well as processing of data and calculation of anammox and denitrification rates are explained. Important supplementary measurements are specified, such as the measurement of nanomolar concentrations of ammonium, nitrite, and nitrate. Nutrient profiles and (15)N-experiments from the Peruvian OMZ are presented and discussed as an example.
High nitrite oxidation rates in the Namibian oxygen minimum zone

General information
Publication status: Published
Organisations: Max Planck Institute for Marine Microbiology
Contributors: Füssel, J., Lam, P., Lavik, G., Jensen, M. M., Kuypers, M. M. M.
Publication date: 2011
Peer-reviewed: No
Event: Abstract from ASLO Aquatic Sciences Meeting 2011, San Juan, Puerto Rico.
Source: orbit
Source-ID: 285532
Research output: Contribution to journal › Journal article – Annual report year: 2011 › Research › peer-review

Intensive nitrogen loss over the Omani Shelf due

A combination of stable isotopes (15N) and molecular ecological approaches was used to investigate the vertical distribution and mechanisms of biological N2 production along a transect from the Omani coast to the central–northeastern (NE) Arabian Sea. The Arabian Sea harbors the thickest oxygen minimum zone (OMZ) in the world’s oceans, and is considered to be a major site of oceanic nitrogen (N) loss. Short (

Keyword: Oxygen minimum zone,Denitrification,Marine nitrogen loss,Anammox,Central–northeastern Arabian Sea,Functional gene expression

General information
Publication status: Published
Organisations: Max Planck Institute, Aarhus University, GKSS-Research Centre, University of Hamburg, Radboud University Nijmegen
Pages: 1660-1670
Publication date: 2011
Peer-reviewed: Yes
**Micro2-Managed Microbial Communities: Next Generation Environmental Bio/Technologies**

Microbes are amazingly diverse in terms of the reactions that they catalyze. This diversity can be exploited to create competitive biotechnological solutions for many environmental challenges, where the right combination of existing microbial reactions can convert unwanted pollutants into a useful or harmless end-product. There are, however, significant scientific and technical challenges in order to combine potentially useful microbial reactions into a workable biotechnology, especially for processes that require the cooperation between microbial groups with very different properties and preferences. Simple empirical approaches often fail, and our intent is therefore to rationally manipulate the composition, that is, the microbial diversity of such systems, towards a target performance. Our hypothesis is that controlled biofilm or bioaggregate-based systems, wherein microbes grow in spatially structured assemblies, are suitable to harness these microbial potentials. We specifically aim to develop, implement and validate the feasibility of generic approaches for the rapid and efficient selection and management of the microbial composition and the micro-scale structure (micro2-management) of biofilms and bioaggregates for a target performance goal. These approaches are being implemented for the rapid start-up and high-rate operation of membrane-supported biofilm reactors and granular biomass reactors to attain a community consisting of aerobic and anaerobic ammonium oxidizing bacterial guilds (AeAOB and AnAOB) for autotrophic nitrogen (N) removal from wastewaters.

**General information**

Publication status: Published  
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Electronic versions: prod21324202499748.MRM.pdf  
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Source-ID: 314829  
Research output: Contribution to conference » Conference abstract for conference – Annual report year: 2011 » Research peer-review

**Origin and fate of the secondary nitrite maximum in the Arabian Sea**

The Arabian Sea harbours one of the three major oxygen minimum zones (OMZs) in the world’s oceans, and it alone is estimated to account for ~10–20 % of global oceanic nitrogen (N) loss. While actual rate measurements have been few, the consistently high accumulation of nitrite (NO2−) coinciding with suboxic conditions in the central-northeastern part of the Arabian Sea has led to the general belief that this is the region where active N-loss takes place. Most subsequent field studies on N-loss have thus been drawn almost exclusively to the central-NE. However, a recent study measured only low to undetectable N-loss activities in this region, compared to orders of magnitude higher rates measured towards the Omani Shelf where little NO2− accumulated (Jensen et al., 2011). In this paper, we further explore this discrepancy by comparing the NO2−-producing and consuming processes, and examining the relationship between the overall NO2−-balance and active N-loss in the Arabian Sea. Based on a combination of 15N-incubation experiments, functional gene expression analyses, nutrient profiling and flux modeling, our results showed that NO2− accumulated in the central-NE Arabian Sea due to a net production via primarily active nitrate (NO3−) reduction and to a certain extent ammonia oxidation. Meanwhile, NO2− consumption via anammox, denitrification and dissipatory nitrate/nitrite reduction to ammonium (NH4+) were hardly detectable in this region, though some loss to NO2− oxidation was predicted from modeled NO3− changes. No significant correlation was found between NO2− and N-loss rates (p>0.05). This discrepancy between NO2− accumulation and lack of active N-loss in the central-NE Arabian Sea is best explained by the deficiency of labile organic matter that is directly needed for further NO2− reduction to N2O, N2 and NH4+, and indirectly for the remineralized NH4+ required by anammox. Altogether, our data do not support the long-held view that NO2− accumulation is a direct activity indicator of N-loss in the Arabian Sea or other OMZs. Instead, NO2− accumulation more likely corresponds to long-term integrated N-loss that has passed the prime of high and/or consistent in situ activities.

**General information**

Publication status: Published  
Organisations: Max Planck Institute, Leibniz Institute of Marine Sciences, University of Oldenburg, Princeton University  
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Peer-reviewed: Yes

**Publication information**

Journal: Biogeosciences
Oxygen dependency of aerobic and anaerobic N-transformations in Oxygen Minimum Zones

Nutrient measurements indicate that 30–50% of the total nitrogen (N) loss in the ocean occurs in oxygen minimum zones (OMZs). This pelagic N-removal takes place within only 0.1% of the ocean volume, hence moderate variations in the extent of OMZs due to global warming may have a large impact on the global N-cycle. We examined the effect of oxygen (O2) on anammox, NH3 oxidation and NO3 2 reduction in 15N-labeling experiments with varying O2 concentrations (0–25 mmol L⁻¹) in the Namibian and Peruvian OMZs. Our results show that O2 is a major controlling factor for anammox activity in OMZ waters. Based on our O2 assays we estimate the upper limit for anammox to be 20 mmol L⁻¹. In contrast, NH3 oxidation to NO2 2 and NO3 2 reduction to NO2 2 as the main NH4 + and NO2 2 sources for anammox were only moderately affected by changing O2 concentrations. Intriguingly, aerobic NH3 oxidation was active at non-detectable concentrations of O2, while anaerobic NO3 2 reduction was fully active up to at least 25 mmol L⁻¹ O2. Hence, aerobic and anaerobic N-cycle pathways in OMZs can co-occur over a larger range of O2 concentrations than previously assumed. The zone where N-loss can occur is primarily controlled by the O2-sensitivity of anammox itself, and not by any effects of O2 on the tightly coupled pathways of aerobic NH3 oxidation and NO3 2 reduction. With anammox bacteria in the marine environment being active at O2 levels 20 times higher than those known to inhibit their cultured counterparts, the oceanic volume potentially acting as a N-sink increases tenfold. The predicted expansion of OMZs may enlarge this volume even further. Our study provides the first robust estimates of O2 sensitivities for processes directly and indirectly connected with N-loss. These are essential to assess the effects of ocean de-oxygenation on oceanic N-cycling.

Oxygen sensitivity of anammox and coupled N-cycle processes in oxygen minimum zones

Nutrient measurements indicate that 30–50% of the total nitrogen (N) loss in the ocean occurs in oxygen minimum zones (OMZs). This pelagic N-removal takes place within only 0.1% of the ocean volume, hence moderate variations in the extent of OMZs due to global warming may have a large impact on the global N-cycle. We examined the effect of oxygen (O2) on anammox, NH3 oxidation and NO3 2 reduction in 15N-labeling experiments with varying O2 concentrations (0–25 mmol L⁻¹) in the Namibian and Peruvian OMZs. Our results show that O2 is a major controlling factor for anammox activity in OMZ waters. Based on our O2 assays we estimate the upper limit for anammox to be 20 mmol L⁻¹. In contrast, NH3 oxidation to NO2 2 and NO3 2 reduction to NO2 2 as the main NH4 + and NO2 2 sources for anammox were only moderately affected by changing O2 concentrations. Intriguingly, aerobic NH3 oxidation was active at non-detectable concentrations of O2, while anaerobic NO3 2 reduction was fully active up to at least 25 mmol L⁻¹ O2. Hence, aerobic and anaerobic N-cycle pathways in OMZs can co-occur over a larger range of O2 concentrations than previously assumed. The zone where N-loss can occur is primarily controlled by the O2-sensitivity of anammox itself, and not by any effects of O2 on the tightly coupled pathways of aerobic NH3 oxidation and NO3 2 reduction. With anammox bacteria in the marine environment being active at O2 levels 20 times higher than those known to inhibit their cultured counterparts, the oceanic volume potentially acting as a N-sink increases tenfold. The predicted expansion of OMZs may enlarge this volume even further. Our study provides the first robust estimates of O2 sensitivities for processes directly and indirectly connected with N-loss. These are essential to assess the effects of ocean de-oxygenation on oceanic N-cycling.
Aerobic denitrification in permeable Wadden Sea sediments.
Permeable or sandy sediments cover the majority of the seafloor on continental shelves worldwide, but little is known about their role in the coastal nitrogen cycle. We investigated the rates and controls of nitrogen loss at a sand flat (Janssand) in the central German Wadden Sea using multiple experimental approaches, including the nitrogen isotope pairing technique in intact core incubations, slurry incubations, a flow-through stirred retention reactor and microsensor measurement. Results indicate that permeable Janssand sediments are characterized by some of the highest potential denitrification rates (> or =0.19 mmol N m(-2) h(-1)) in the marine environment. Moreover, several lines of evidence showed that denitrification occurred under oxic conditions. In intact cores, microsensor measurements showed that the zones of nitrate/nitrite and O(2) consumption overlapped. In slurry incubations conducted with (15)NO(3)(-) enrichment in gas-impermeable bags, denitrification assays revealed that N(2) production occurred at initial O(2) concentrations of up to approximately 90 microM. Initial denitrification rates were not substantially affected by O(2) in surficial (0-4 cm) sediments, whereas rates increased by twofold with O(2) depletion in the at 4-6 cm depth interval. In a well mixed, flow-through stirred retention reactor (FTSRR), (29)N(2) and (30)N(2) were produced and O(2) was consumed simultaneously, as measured online using membrane inlet mass spectrometry. We hypothesize that the observed high denitrification rates in the presence of O(2) may result from the adaptation of denitrifying bacteria to recurrent tidally induced redox oscillations in permeable sediments at Janssand.
Organisations: Department of Environmental Engineering, Urban Water Engineering, University of Copenhagen, University of Southern Denmark, Geological Survey of Denmark and Greenland
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Web of Science (2010): Indexed yes
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URLs:
http://goldschmidt.info/2010/abstracts/view.html
Source: PublicationPreSubmission
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nitrogen loss and microbial nitrogen cycling in three different oceanic oxygen minimum zones

General information
Publication status: Published
Organisations: Max Planck Institute, Unknown
Contributors: Lam, P., Jensen, M. M., Lavik, G., Kuypers, M. M. M.
Publication date: 2010
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Event: Abstract from 2010 ASLO Ocean Sciences Meeting, Portland, OR, United States.
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Source-ID: 315561
Research output: Contribution to conference › Conference abstract for conference – Annual report year: 2010 › Research

Active expression of nitrogen cycling functional genes in the Arabian Sea oxygen minimum zone

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Organisations: Max Planck Institute
Contributors: Lam, P., Jensen, M. M., Kuypers, M. M. M.
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Event: Poster session presented at ASLO Aquatic Science Meeting 2009, Nice, France.
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Biogeochemistry of electron transfer at the iron oxide-solution interface in sediments

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Publication status: Published
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Publication information
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New perspectives on nitrogen loss in oceanic oxygen minimum zones

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Organisations: Max Planck Institute, Unknown
Contributors: Lam, P., Jensen, M. M., Lavik, G., Kuypers, M. M. M.
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Peer-reviewed: No
Event: Abstract from Society of General Microbiology Meeting 2009, Edinburgh, United Kingdom, .
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Research output: Contribution to conference » Conference abstract for conference – Annual report year: 2009 » Research

New perspectives on water-column nitrogen cycling in oceanic oxygen minimum zones

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Contributors: Lam, P., Jensen, M. M., Lavik, G., Kuypers, M. M. M.
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Event: Abstract from Gordon Research Conference – Chemical Oceanography, Tilton, NH, USA, .
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Nitrogen transformations in the Arabian Sea oxygen minimum zone as revealed by combined 15N-incubation experiments and functional gene expression analyses

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Organisations: Max Planck Institute, Unknown
Contributors: Lam, P., Jensen, M. M., Lavik, G., Kuypers, M. M. M.
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Source: orbit
Source-ID: 315567
Research output: Contribution to conference » Poster – Annual report year: 2009 » Research

Pathways, rates, and regulation of N2 production in the chemocline of an anoxic basin, Mariager Fjord, Denmark
Pathways of N2 production and the influence of oxygen, nitrate/nitrite, and sulfide on the activity of denitrifying bacteria were studied in the chemocline of the shallow, brackish Mariager Fjord, by means of incubation experiments with 15N-labeled compounds. Denitrification was an important process, with potential rates up to 18.6±0.12 µM N2 d⁻¹. The potential for denitrification increased with water depth across the chemocline towards higher hydrogen sulfide concentrations, which indicates the utilization of sulfide as an electron donor for denitrification. The anammox process was not detected, and therefore denitrification is the dominant sink for bioavailable nitrogen in Mariager Fjord. The absence of anammox may be explained by an inhibitory effect of low sulfide levels and/or an unstable environment caused by chemocline perturbations that generate unfavorable conditions for slow-growing anammox bacteria. Nitrate reduction to nitrite and denitrification (nitrite reduction to N2O and, mainly, N2) fueled by sulfide oxidation was evident from the direct dependence of rates on endogenous sulfide concentrations, with first-order rate constants of 1.90±0.31 and of 0.93±0.11 d⁻¹ for the two processes, respectively, and from the correlation of N2 production with sulfide consumption in anoxic
incubations. Elemental sulfur (S0) was the immediate product of sulfide oxidation with both nitrate and nitrite. The nitrate and nitrite dependence of the processes followed Michaelis–Menten kinetics with higher apparent Km and Vmax for nitrate reduction to nitrite than for denitrification of nitrite, leading to an accumulation of nitrite at nitrate concentrations ≥5 µM. The apparent Km of nitrate consumption during denitrification was 2.9±0.1 µM, which is close to or even higher than the range of nitrate concentrations encountered by denitrifying bacteria in the chemocline, and nitrate is therefore continuously a limiting factor for chemolithotrophic denitrification in Mariager Fjord. In addition to substrate availability, oxygen is an important controlling factor for denitrification and inhibited the process at concentrations of 8–15 µM. The experimentally-derived kinetics were validated for the conditions in situ in a 1-D reaction transport model, which reproduced the nitrate distribution at the chemocline well, suggesting that our incubation results provide a good quantitative description of the regulation of denitrification by nitrate and sulfide in situ. Our study demonstrates that the chemocline is an important site for removal of bioavailable nitrogen by chemolithotrophic denitrification in Mariager Fjord.

Keyword: Nitrogen loss, Euxinic basins, Chemolithotrophy, Kinetics, Denitrification, Anammox

Revising the nitrogen cycle in the Peruvian oxygen minimum zone.

The oxygen minimum zone (OMZ) of the Eastern Tropical South Pacific (ETSP) is 1 of the 3 major regions in the world where oceanic nitrogen is lost in the pelagic realm. The recent identification of anammox, instead of denitrification, as the likely prevalent pathway for nitrogen loss in this OMZ raises strong questions about our understanding of nitrogen cycling and organic matter remineralization in these waters. Without detectable denitrification, it is unclear how NH(4)(+) is remineralized from organic matter and sustains anammox or how secondary NO(2)(-) maxima arise within the OMZ. Here we show that in the ETSP-OMZ, anammox obtains 67% or more of NO(2)(-) from nitrate reduction, and 33% or less from aerobic ammonia oxidation, based on stable-isotope pairing experiments corroborated by functional gene expression analyses. Dissimilatory nitrate reduction to ammonium was detected in an open-ocean setting. It occurred throughout the OMZ and could satisfy a substantial part of the NH(4)(4)(+) requirement for anammox. The remaining NH(4)(4)(+) came from remineralization via nitrate reduction and probably from microaerobic respiration. Altogether, deep-sea NO(3)(3)(-) accounted for only approximately 50% of the nitrogen loss in the ETSP, rather than 100% as commonly assumed. Because oceanic OMZs seem to be expanding because of global climate change, it is increasingly imperative to incorporate the correct nitrogen-loss pathways in global biogeochemical models to predict more accurately how the nitrogen cycle in our future ocean may respond.

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Publication information
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Rates and regulation of anaerobic ammonium oxidation and denitrification in the Black Sea

We performed incubation experiments with N-15-labeled nitrogen compounds to investigate the vertical distribution of pathways of N-2 production through the suboxic zone of the central Black Sea and the impact of oxygen and sulfide on the anammox process. Anammox rates increased with depth through the upper suboxic zone and reached a maximum of similar to 11 nmol N-2 L-1 d(-1) at the sharp interface between nitrate and ammonium, below which rates decreased toward the depth of sulfide accumulation. Heterotrophic denitrification was not detected, and therefore anammox was the prevailing sink for fixed nitrogen in the central Black Sea. In incubations with low oxygen concentrations, anammox activity was only partially inhibited, with a decrease in anammox rates to similar to 70% and 50% of the anoxic level at similar to 3.5 and similar to 8 mu mol L-1 O-2, respectively, and complete inhibition at similar to 13.5 mu mol L-1 O-2. Thus, the anammox process is not constrained to anoxic marine waters. This increases the volume of the major open-ocean oxygen-deficient zones, where anammox is potentially active, which has important implications for the contribution of anammox to the marine nitrogen cycle. We observed an inhibitory effect of micromolar sulfide concentrations on anammox activity, indicating that the vertical and likely horizontal distribution of active anammox bacteria is constrained to nonsulfidic water layers, which may explain the absence of the process in sulfidic basins with no suboxic zone.

Effects of Specific Inhibitors on Anammox and Denitrification in Marine Sediments

The effects of three metabolic inhibitors (acetylene, methanol, and allylthiourea [ATU]) on the pathways of N-2 production were investigated by using short anoxic incubations of marine sediment with a N-15 isotope technique. Acetylene inhibited ammonium oxidation through the anammox pathway as the oxidation rate decreased exponentially with increasing acetylene concentration; the rate decay constant was 0.10 +/- 0.02 mu M-1, and there was 95% inhibition at similar to 30 mu M. Nitrous oxide reduction, the final step of denitrification, was not sensitive to acetylene concentrations below 10 mu M. However, nitrous oxide reduction was inhibited by higher concentrations, and the sensitivity was approximately one-half the sensitivity of anammox (decay constant, 0.049 +/- 0.004 mu M-1; 95% inhibition at -70 mu M). Methanol specifically inhibited anammox with a decay constant of 0.79 +/- 0.12 mM(-1), and thus 3 to 4 mM methanol was required for nearly complete inhibition. This level of methanol stimulated denitrification by similar to 50%. ATU did not have marked effects on the rates of anammox and denitrification. The profile of inhibitor effects on anammox agreed with the results of studies of the process in wastewater bioreactors, which confirmed the similarity between the anammox bacteria in bioreactors and natural environments. Acetylene and methanol can be used to separate anammox and denitrification, but the effects of these compounds on nitrification limits their use in studies of these processes in systems where nitrification is an important source of nitrate. The observed differential effects of acetylene and methanol on anammox and denitrification support our current understanding of the two main pathways of N-2 production in marine sediments and the use of N-15 isotope
methods for their quantification.

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Organisations: University of Southern Denmark, Danish Centre for Environment and Energy
Contributors: Jensen, M. M., Thamdrup, B., Dalsgaard, T.
Pages: 3151-3158
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Peer-reviewed: Yes

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**Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea.**
Active expression of putative ammonia monooxygenase gene subunit A (amoA) of marine group I Crenarchaeota has been detected in the Black Sea water column. It reached its maximum, as quantified by reverse-transcription quantitative PCR, exactly at the nitrate maximum or the nitrification zone modeled in the lower oxic zone. Crenarchaeal amoA expression could explain 74.5% of the nitrite variations in the lower oxic zone. In comparison, amoA expression by gamma-proteobacterial ammonia-oxidizing bacteria (AOB) showed two distinct maxima, one in the modeled nitrification zone and one in the suboxic zone. Neither the amoA expression by crenarchaea nor that by beta-proteobacterial AOB was significantly elevated in this latter zone. Nitrification in the suboxic zone, most likely microaerobic in nature, was verified by (15)NO(2)(-) and (15)N(15)N production in (15)NH(4)(+) incubations with no measurable oxygen. It provided a direct local source of nitrite for anammox in the suboxic zone. Both ammonia-oxidizing crenarchaeae and gamma-proteobacterial AOB were important nitrifiers in the Black Sea and were likely coupled to anammox in indirect and direct manners respectively. Each process supplied about half of the nitrite required by anammox, based on (15)N-incubation experiments and modeled calculations. Because anammox is a major nitrogen loss in marine suboxic waters, such nitrification-anammox coupling potentially occurring also in oceanic oxygen minimum zones would act as a short circuit connecting regenerated ammonium to direct nitrogen loss, thus reducing the presumed direct contribution from deep-sea nitrate.

Keyword: Crenarchaeota, AmoA gene expression, Marine group, Marine nitrogen loss, Ammonia-oxidizing bacteria

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10.1073/pnas.0611081104
Source: orbit
Anaerobic ammonium oxidation in the oxygen-deficient waters off northern Chile

We investigated the pathways of N2 production in the oxygen-deficient water column of the eastern tropical South Pacific off Iquique, Chile, at 20°S, through short anoxic incubations with 15N-labelled nitrogen compounds. The location was characterized by steep chemical gradients, with oxygen decreasing to below detection at 50-m depth, while nitrite reached 6 mmol L−1 and ammonium was less than 50 nmol L−1. Ammonium was oxidized to N2 with no lag phase during the incubations, and when only NH4+ was 15N-labeled, 15N appeared in the form of 14N15N, whereas 15N15N was not detected. Likewise, nitrite was reduced to N2 at rates similar to the rates of ammonium oxidation, and when only NO2− was 15N-labeled, 15N appeared mainly as 14N15N, whereas 15N15N appeared in only one incubation. These observations indicate that ammonium was oxidized and nitrite was reduced through the anammox reaction, whereas denitrification was generally not detected and, therefore, was a minor sink for nitrite. Anammox rates were highest, up to 0.7 nmol N2 L−1 h−1, just below the oxycline, whereas rates were undetectable, <0.2 nmol N2 L−1 h−1, deeper in the oxygen-deficient zone. Instead of complete denitrification to N2, oxidation of organic matter during the incubations may have been coupled to reduction of nitrate to nitrite. This process was evident from strong increases in nitrite concentrations toward the end of the incubations. The results point to anammox as an active process in the major open-ocean oxygen-deficient zones, which are generally recognized as important sites of denitrification. Still, denitrification remains the simplest explanation for most of the nitrogen deficiency in these zones.

General information
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Organisations: University of Southern Denmark, Danish Centre for Environment and Energy, Universidad de Concepcion
Contributors: Thamdrup, B., Dalsgaard, T., Jensen, M. M., Ulloa, O., Farias, L., Escribano, R.
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Journal: Limnology and Oceanography
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Scopus rating (2006): SJR 2.375 SNIP 1.64
Web of Science (2006): Indexed yes
Original language: English
Source: orbit
Source-ID: 285526

Composition and diagenesis of neutral carbohydrates in sediments of the Baltic-North Sea transition

The distribution and composition of neutral carbohydrates in the solid phase and porewater, and their role in carbon cycling were investigated in contrasting marine sediments of the Baltic-North Sea region. Depth-invariant profiles of particulate carbohydrates (PCHO) and low PCHO yields (PCHO/organic carbon) indicated that a small inert carbohydrate fraction deposits on the sediment at the deeper stations in the northern Kattegat and Skagerrak compared to the shallower stations further South. This was supported by long-term sediment incubations, in which the PCHO concentrations remained unchanged during 480 days, revealing that neutral carbohydrates play a minor role in carbon mineralization at the deeper sites. In contrast, the reactivity of PCHO was high (first-order rate constant of 3.2 yr−1) at one shallow site in the Belt Sea. Monosaccharide spectra were uniform with sediment depth and between sites with the exception of the shallowest site in the middle of Kattegat, where glucose dominated the polymers at the surface. This was likely due to benthic diatoms. Addition of fresh algae to surface sediment from the deeper sites resulted in a preferential mineralization of particulate glucose polymers. The addition of algae also resulted in an initial pulse of glucose in the porewater pools of total hydrolyzable carbohydrates (THCHO), indicating a faster hydrolysis of glucose polymers in the particulate phase than the subsequent hydrolysis and bacterial consumption of oligo- and polymers of glucose in the porewater. This study shows that some carbohydrates such as glucose polymers are selectively utilized by heterotrophic bacteria during the settling of organic particles through the water column, and a relatively inert fraction arrives to the sediments where much of it escapes mineralization and becomes permanently buried. In shallow coastal environments, where the degradation in the water column is less extensive and where benthic algae may represent a local carbohydrate source, neutral carbohydrates appear to be more important in organic matter mineralization.

General information
Publication status: Published
Organisations: University of Southern Denmark
Rates and regulation of microbial iron reduction in sediments of the Baltic-North Sea transition

The rates and pathways of anaerobic carbon mineralization processes were investigated at seven stations, ranging from 10 to 56 m water depth, in the Kattegat and Belt Sea, Denmark. Organic carbon mineralization coupled to microbial Mn and Fe reduction was quantified using anaerobic sediment incubation at two stations that were widely separated geographically within the study area. Fe reduction accounted for 75% of the anaerobic carbon oxidation at the station in the northern Kattegat, which is the highest percentage so far reported from subtidal marine sediment. By contrast, sulfate reduction was the dominant anaerobic respiration pathway (95%) at the station in the Great Belt. Dominance of Fe reduction was related to a relatively high sediment Fe content in combination with active reworking of the sediment by infauna. The relative contribution of Fe reduction to anaerobic carbon oxidation at both stations correlated with the concentration of poorly crystalline Fe(III), confirming that the concentration of poorly crystalline Fe(III) exerts a strong control on rates of Fe reduction in marine sediments. The dependence of microbial Fe reduction on concentrations of poorly crystalline Fe(III) was used to quantify the importance of Fe reduction at sites where anaerobic incubations were not applied. This study showed that Fe reduction is an important process in anaerobic carbon oxidation in a wider area of the seafloor in the northern and eastern Kattegat (contribution 60 – 75%). By contrast, Fe reduction is of little significance (6 – 25%) in the more coarse-grained sediments of the shallower western and southern Kattegat, where a low Fe content was an important limiting factor, and in fine-grained sediments of the Belt Sea (4 – 28%), where seasonal oxygen depletion limits the intensity of bioturbation and thereby the availability of Fe(III). A large fraction of the total deposition of organic matter in the Kattegat and Belt Sea occurs in the northern Kattegat, and we estimate 33% of benthic carbon oxidation in the whole area is conveyed by Fe reduction.
Organisations: Danish Centre for Environment and Energy, University of Southern Denmark
Contributors: Rysgaard, S., Fossing, H., Jensen, M. M.
Pages: 77-91
Publication date: 2001
Peer-reviewed: Yes

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Web of Science (2001): Indexed yes
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Source: orbit
Source-ID: 285524
Research output: Contribution to journal › Journal article – Annual report year: 2001 › Research › peer-review

Projects:

Mechanisms and regulation of nitrous oxide (N2O) production during biological wastewater treatment
Su, Q., PhD Student, Department of Environmental Engineering
Smets, B. F., Main Supervisor
Jensen, M. M., Supervisor
Thamdrup, B., Supervisor
Andersen, H. R., Examiner
Canestro, J. P., Examiner
Persson, F., Examiner
Stipendie fra udlandet
01/10/2015 → 15/01/2019
Award relations: Mechanisms and regulation of nitrous oxide (N2O) production during biological wastewater treatment
Project: PhD

Engineering microbial communities in granular autotrophic nitrogen removal systems by steering metabolic networks
Blum, J., PhD Student, Department of Environmental Engineering
Smets, B. F., Main Supervisor
Jensen, M. M., Supervisor
Thamdrup, B., Supervisor
Andersen, H. R., Examiner
Vlaeminck, S. E., Examiner
Wilén, B., Examiner
01/03/2015 → 15/08/2018
Project: PhD

Controlling factors for nitrification in biological rapid sand filters for drinking water treatment
Wagner, F. B., PhD Student, Department of Environmental Engineering
Albrechtsen, H., Main Supervisor
Boe-Hansen, R., Supervisor
Jensen, M. M., Examiner
Roslev, P., Examiner
van der Wielen, P. W. J. J., Examiner
Samfinansieret - Andet
15/11/2013 → 25/10/2017
Award relations: Controlling factors for nitrification in biological rapid sand filters for drinking water treatment
Project: PhD

Biogas enhancement and upgrading
Bassani, I., PhD Student, Department of Environmental Engineering
Angelidaki, I., Main Supervisor
Kougias, P., Supervisor
Jensen, M. M., Examiner
Activities:

**Dynamics of N2O production pathways analyzed by 15N18O isotope labeling**
Period: 12 Aug 2018 → 17 Aug 2018
Marlene Mark Jensen (Other)
Water Technologies
Department of Environmental Engineering

**Related event**
17th International Symposium on Microbial Ecology (ISME)
12/08/2018 → 17/08/2018
Leipzig, Germany
Activity: Talks and presentations › Conference presentations

**Diagnostics, monitoring and mitigation of N2O emissions from wastewater treatment operation - outcome of the LaGas project**
Period: 30 Jan 2018
Marlene Mark Jensen (Lecturer)
Water Technologies
Department of Environmental Engineering

**Related event**
12th Annual Water Research Meeting of Danish Water Forum
30/01/2018 → 30/01/2018
Lyngby, Denmark
Activity: Talks and presentations › Conference presentations

**DTU Sustain 2017**
Period: 6 Dec 2017
Marlene Mark Jensen (Organizer)
Department of Environmental Engineering
Water Technologies
Degree of recognition: National

**Related event**
DTU Sustain 2017
06/12/2017 → 06/12/2017
Kgs. Lyngby, Denmark
Activity: Attending an event › Participating in or organising workshops, courses, seminars etc.

**DTU Sustain 2017**
Period: 6 Dec 2017
Carlos Domingo-Felez (Participant)
Marlene Mark Jensen (Participant)
Water Technologies
Related event

DTU Sustain 2017
06/12/2017 → 06/12/2017
Kgs. Lyngby, Denmark
Keywords: n2o, wastewater, mitigation
Activity: Attending an event › Participating in or organising a conference

Controls of N2O production pathways in nitritation-anammox biomass
Period: 13 Nov 2017
Marlene Mark Jensen (Invited speaker)
Department of Environmental Engineering
Water Technologies
Degree of recognition: National

Related event

Danish Microbiological Society 2017 Congress
13/11/2017 → 13/11/2017
Copenhague, Denmark
Activity: Talks and presentations › Conference presentations

International workshop on marine geomicrobiology - A matter of energy
Period: 28 Aug 2017 → 1 Sep 2017
Marlene Mark Jensen (Participant)
Department of Environmental Engineering
Water Technologies
Degree of recognition: International

Related event

International workshop on marine geomicrobiology - A matter of energy
28/08/2017 → 01/09/2017
Sønderborg, Denmark
Activity: Attending an event › Participating in or organising workshops, courses, seminars etc.

Kortlægning af den bakterielle lattergasproduktion i aktivt slam ved hjælp af stabile isotoper
Period: 9 Nov 2016
Marlene Mark Jensen (Speaker)
Department of Environmental Engineering
Water Technologies

Related event

Dansk Vand Konference
08/11/2016 → 09/11/2016
Århus, Denmark
Activity: Talks and presentations › Conference presentations

When is it produced, why is it produced and how to prevent nitrous oxide emissions? Main findings from a large nitrous oxide project in Denmark
Period: 27 Sep 2016
Marlene Mark Jensen (Invited speaker)
Department of Environmental Engineering
Water Technologies

Related event

**Swedish Water & Waterwater Fair 2016: Leading Edge Wastewater Treatment**
27/09/2016 → 27/10/2016
Jönköping, Sweden
Activity: Talks and presentations › Conference presentations

**Application of microbial communities in environmental biotechnology: from wastewater treatment to resource recovery**
Period: 1 Dec 2015
Marlene Mark Jensen (Invited speaker)
Department of Environmental Engineering

Related event

**Application of microbial communities in environmental biotechnology: from wastewater treatment to resource recovery**
01/12/2015 → 01/12/2015
Copenhagen, Denmark
Activity: Talks and presentations › Guest lectures, external teaching and course activities at other universities

**Frem mod det energineutrale vandselskab**
Period: 30 Nov 2015
Marlene Mark Jensen (Invited speaker)
Department of Environmental Engineering

Description
Frem mod det energineutrale vandselskab. Workshop arrangeret af Dansk Miljøteknologi, DANVA og Dansk Industri

Related event

**Frem mod det energineutrale vandselskab**
30/11/2015 → 30/11/2015
Skanderborg, Denmark
Activity: Talks and presentations › Talks and presentations in private or public companies and organisations

**EcoDesign MBR Centre**
Period: 10 Nov 2011 → 11 Nov 2011
Marlene Mark Jensen (Speaker)
Department of Environmental Engineering

Related external organisation

**EcoDesign MBR Centre**
Aalborg, Denmark
Activity: Talks and presentations › Talks and presentations in private or public companies and organisations

**Nitrogen loss in the intertidal permeable sediment: ph.d. defence**
Period: 1 Jun 2011
Marlene Mark Jensen (External examiner)
Environmental Chemistry
Department of Environmental Engineering
Activity: Examinations and supervision › External examination

Press clippings:
De tre millimeter mellem os og Solen
Marlene Mark Jensen
15/05/2014

Subject
Gasser, Ozonlaget, Lattergas
Department of Environmental Engineering, Urban Water Engineering

Media contribution (1)

De tre millimeter mellem os og Solen
15/05/2014
Weekendavisen, Print
Christoffer Muusmann
Marlene Mark Jensen
Department of Environmental Engineering, Urban Water Engineering
Press/Media: Press / Media