

## Research Project

Advanced understanding of autotrophic nitrogen removal and associated N<sub>2</sub>O emissions in mixed nitrification-anammox systems through combined stable ISOTopic and MOLEcular constraints (IsoMol)

### Third-party funded project

**Project title** Advanced understanding of autotrophic nitrogen removal and associated N<sub>2</sub>O emissions in mixed nitrification-anammox systems through combined stable ISOTopic and MOLEcular constraints (IsoMol)

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Autotrophic nitrogen (N) removal by anaerobic ammonium oxidation (anammox) is an important mechanism of fixed N elimination, both in engineered and natural systems. In wastewater treatment plants it may permit operation under energy autarky and with a better carbon footprint. However, its process control and engineering is still under development: An optimized removal process using nitrification-anammox systems must combine stable operation, high N removal efficiency and minimized greenhouse gas emissions. Yet the biogeochemical and microbiological controls on the production of N<sub>2</sub>O in wastewater treatment are poorly understood. Similarly, the links between system stability, activity, and microbial population shifts are not well constrained. Equally important, in natural aquatic environments, the anammox process probably plays a much greater role than it was assumed for decades, challenging the traditional perception of the natural N-cycle. The questions regarding the prime pathways of fixed N loss in natural environments (i.e., denitrification versus anammox), the controls on the balance between fixed N loss and N<sub>2</sub>O production, and the associated microbial population dynamics are important rationales of current research. In this applied and environmental context, an important focus of the project will be on the use of stable isotope measurements and microbiological analyses to understand and identify N metabolism in pure and mixed anammox cultures, to assess the controls on N<sub>2</sub>O production in nitrification-anammox systems, and to verify ties between microbial dynamics, N-turnover and optimal process control.

The collaborative and highly interdisciplinary project “IsoMol”, led by M. Lehmann (Unibas), seeks to understand the functioning of mixed microbial populations featuring alternative biogeochemical pathways and to characterize its dependence on environmental conditions and microbial composition. Each subproject will provide important information to improve our understanding of anammox on multiple levels (process engineering, isotope dynamics, microbiology). Subproject 1, led by A. Joss (EAWAG) has a strong applied component, which includes the set-up of enriched and mixed culture nitrification-anammox reactors at the laboratory and pilot scales. It aims at understanding both process controls and N<sub>2</sub>O emission in these systems (e.g., oxygen supply and temperature), and to evaluate the benefits of anammox-based wastewater treatment with respect to conventional approaches. Sub-projects 2 and 3, each with completely different analytical approaches, will develop and test stable isotope methods to understand and fingerprint N metabolism in pure and mixed cultures. Ammonium, nitrite and nitrate N

and oxygen (O) isotope fractionation will be studied by M. Lehmann (Unibas), with a particular focus on the isotopic effect of anaerobic nitrate formation by anammox, as well as the potential of N and O isotope measurements to diagnose chemical transformations and reaction rates for different inorganic N substrates in general. Gas phase isotopic composition studies, led by J. Mohn (Empa), will identify the main processes leading to the emission of N<sub>2</sub>O across the range of typical operating conditions – in particular, with respect to a possible new pathway associated with the anammox metabolism. Sub-project 4, led by H. Burgmann (Eawag), will make use of cutting-edge techniques in microbiology and genetics, specifically metagenomics and metatranscriptomics, to understand the structure and function of mixed-culture anammox consortia in response to key external variables, and the mechanisms that control stability. It will apply concepts of ecological stability to the anammox process, aiming for fundamental advances in our understanding of what drives resistance and resilience of the involved microbial communities and processes.

Energy-neutral municipal wastewater treatment is feasible with autotrophic N removal, but requires an optimized process design and control based on fundamental understanding of bacterial behavior and interactions. N (and O) isotope measurements in different N-species hold a strong potential to discriminate between alternative biochemical pathways and quantify reaction rates, to assess process stability and to understand the conditions that are most conducive to the emission of N<sub>2</sub>O. Similarly, the engineered nature of the community provides a unique level of control over what is nonetheless a complex real-life microbial community, and results will be immediately applicable to develop improvements for process engineering. In addition, the proposed research will provide the first in-depth transcriptomic analysis of anammox enrichments, and will significantly deepen our understanding of the physiological and regulatory differences between anammox strains. Our research may also contribute to the discovery and understanding of new modes of N<sub>2</sub>O production directly or indirectly related to anammox...

**Keywords** anammox, nitrification, wastewater, stable isotopes, isotope fingerprinting, metagenomics, wastewater, N<sub>2</sub>O, nitrate

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