UNISENSE

ENABLING MICROSCALE RESEARCH

Understanding the denitrification dynamics of nitrifying microorganisms

The real-time detection of NO and N_2O production and consumption profiles of nitrifying microorganisms in the MicroRespiration System

The application note is written by:
Dr. Chris Sedlacek
University of Vienna
Centre for Microbiology and Environmental Systems Science
Division of Microbial Ecology

Introduction

Nitrifying microorganisms are ubiquitous in the environment and perform the essential step of nitrification in the biogeochemical global nitrogen cycle. Depending on the environment, the process of nitrification can be viewed as a positive and desirable process (during wastewater treatment) or as a negative and undesired process (leading to agricultural fertilizer loss).

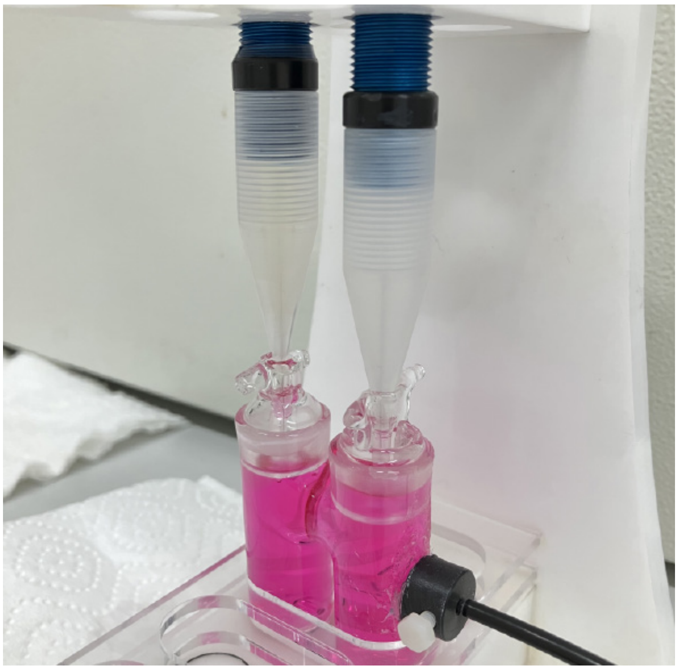

Figure 1: The MicroRespiration setup of a 10 mL double MicroRespiration chamber with NO and N_2O sensors inserted through sensors ports. The injection lids allow for the addition of substrates during experimental runs.

Interestingly, under anoxic conditions, some nitrifying microorganisms are also able to perform the process of denitrification which produces nitric oxide (NO) and nitrous oxide (N_2O).
Therefore, regulating where nitrification is occurring, and where nitrifying microorganisms are active in the environment is an important part of our global nitrogen management strategy.
To accomplish this, the first step is to characterize the nitrifying microorganisms themselves.

With our research we aim to determine how nitrifying microorganisms are able to produce nitrous oxide through denitrification. We use an approach that combines comparative genomics and culture-based physiological assays. Together, these techniques allow us to identify genes or proteins that are essential for the process, along with being able to describe different physiological phenotypes. The Unisense MicroRespiration System enables us to look at the physiological process of microbially driven denitrification under highly controlled conditions.

"The Unisense suite of sensors provides our research team the flexibility we need to investigate the profiles of several dissolved gases simultaneously. Great products, helpful people!"

Dr. Chris Sedlacek, University of Vienna

Laboratory setup

A MicroRespiration System equipped with a 10 mL double MR chamber and two injections lids was used to monitor the production and consumption profile of NO and N_2O during anoxic denitrification. An oxygen optode was attached to the side of the chamber and NO / N_2O microsensors were used in order to measure O_2, NO, and N_2O simultaneously (Figure 1). Here, the MR chamber was filled headspace-free with a pure nitrifying microorganism culture. After an initial substrate injection, microbial respiration depleted the dissolved oxygen in the MR chamber. Once anoxic, nitrite was injected and the production and consumption of NO and N_2O was observed.

Results and conclusion

One example of an anoxic NO and N_2O production and consumption profile of a nitrifying microorganism is provided (Figure 2). The introduction of nitrite into the anoxic MR chamber is immediately followed by a burst of NO and N_2O production. Here, over the course of ~9 hours, the NO intermediate is completely depleted, but there is a stable amount of N_2O that remains. As shown, multiple nitrite injections can be performed subsequently in a single chamber. Each time, NO is produced an intermediate which is fully consumed after several hours, while a stable amount of N_2O remains.

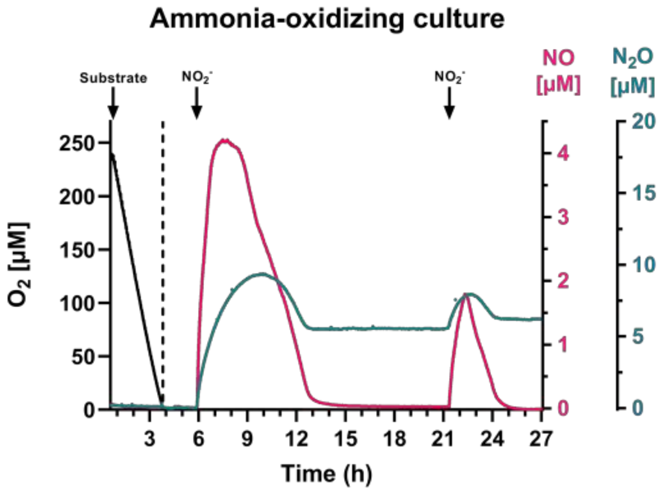

Figure 2: O_2, NO, and N_2O dynamics of an ammonia-oxidizing culture. The arrows indicate when substrate or nitrite was injected into the chamber.

Although only a single example is shown here, the NO and N_2O production and consumption profiles of different species of nitrifying microorganisms can be compared using this method. These profiles, together with comparative genomics, can be used to identify and characterize the essential genes needed for nitrifier denitrification. With this information, the ability of individual nitrifier species or nitrifier communities to produce N_2O can more accurately be predicted solely with insilico or molecular techniques.

Suggested products

- NO-MR, N2O-MR, OX-MR, opto-MR
- MicroRespiration System
- SensorTrace Rate
- MR Double Chamber

Related publications

Jung, MY et al. Ammonia-oxidizing archaea possess a wide range of cellular ammonia affinities. ISME journal. 16:1, 272-283, 2022.

Kits, KD et al. Low yield and abiotic origin of N_2O formed by the complete nitrifier Nitrospira inopinata. Nature Communications 10:1, 1836, 2019.

Kitzinger, K et al. Characterization of the first “Candidatus Nitrotoga” isolate reveals metabolic versatility and separate evolution of widespread nitrite-oxidizing bacteria. MBio. 9:4, e01186-18, 2018.

Kits, KD et al. Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle. Nature. 549:7671, 269-272, 2017.

Version: June 2024