High-throughput analysis of $\delta^{15}N$ and $\delta^{18}O$ of nitrates by the bacterial denitrifier method

Christopher Yarnes, Katharine Pecsok Ewert, Emily Ngo Schick

University of California, Davis

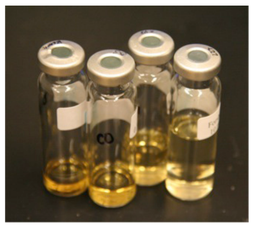
INTRODUCTION

The "bacterial denitrifier" method for the analysis of $\delta^{15}N$ and $\delta^{18}O$ of nitrates has been widely adopted for its ability to easily analyze low-concentration samples (~1µM), simplified preparation, and suitability for saline samples (Sigman et al. 2001, Casciotti et al. 2002). The method, as originally published and typically implemented, relies on a double-needle (or concentric needle) purge and trap system, whereby the bacteria culture is bubbled with a stream of helium provided by one needle, while headspace N_2O is quantitatively sampled with the other.

The viscosity of the bacterial suspension can create problems for traditional purge and trap methods. The excess water vapor, hydrocarbons, and tendency for the unintended sampling of the suspension itself are potential hurdles to uninterrupted analysis over long periods. This has required the liberal use of antifoaming agents, extra nafion dryers, and the addition of so-called "snot tubes" to trap marauding bacterial suspension before it reaches expensive instrumentation. Recently, McIlvin and Casciotti (2011) provide a dedicated solution for overcoming many of these analytical difficulties. However, their method requires extensive modification of commercially available instrumentation and does not yet represent a turn-key solution for many laboratories. Further, many laboratories with interest in the bacterial denitrifier method may not have the resources to dedicate an instrument to the analysis and/or may require flexibility of instrumentation to provide additional analytical capabilities.

Here we tested a static-headspace alternative to purge and trap sampling of nitrous oxide produced by bacterial denitrifier suspensions. Our primary focus was fractionation and recovery with variable headspace volume. This method has been applied using two commercially-available preconcentration-GC-IRMS systems from Thermo Scientific (Bremen, Germany) and Sercon Limited (Crewe, UK). No modification was made to either pre-concentration interface to accommodate this method. The data presented here is from a Thermo Scientific PreCon-GasBench II interface with Delta V Plus isotope-ratio mass spectrometer.

METHODS



Preparation

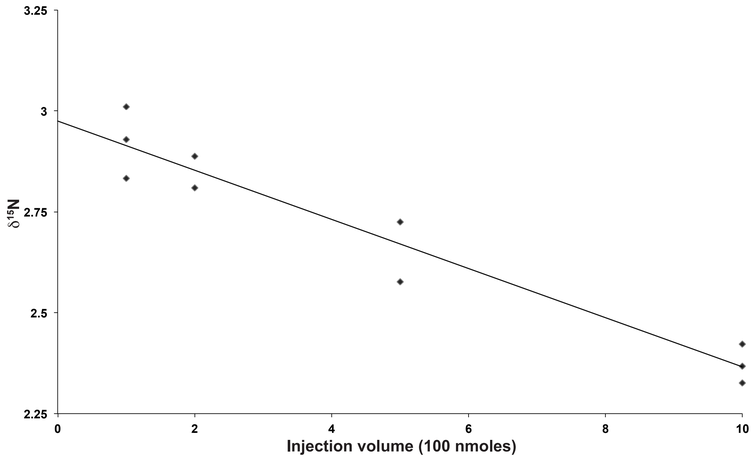
Bacterial cultures are prepared similar to McIlvin and Casciotti (2011) and others (Sigman et al. 2001, Casciotti et al. 2002). We have found that the use of UHP nitrogen is unnecessary for vial purging. We have also greatly reduced the use of antifoaming agent (one drop per batch in nitrate-free TSB re-suspension medium), and are testing the elimination of antifoam altogether. Without a purge stream, excessive antifoam prevents the release of N_2O into the vial headspace. For this study, batches were prepared consisting of 100nmoles of NIST 8568 and 8569 at 1, 5, and 10mL injection volumes.

Measurement

Sample headspace is sampled for 630s at 30mL min^{-1}; longer sampling times did not result in measurable changes in N_2O recovery or isotopic measurement. Our maximum injection volume was 10mL to allow for sufficient headspace for both Thermo and Sercon needle types.

Standardization

Each batch contains a minimum four separate nitrates that have been calibrated to international standards. Standards were chosen based on $\delta^{15}N$, $\delta^{18}O$, and homogeneity. Standards include NIST 8568, NIST 8569, as well as two nitrates from Fisher Scientific ("FA", $\delta^{15}N$:-4.61, $\delta^{18}O$:+26.01; "P383", $\delta^{15}N$:+59.94, $\delta^{18}O$:+41.67) and one from Acros Organics ("ACR", $\delta^{15}N$:+14.57, $\delta^{18}O$:+30.01).



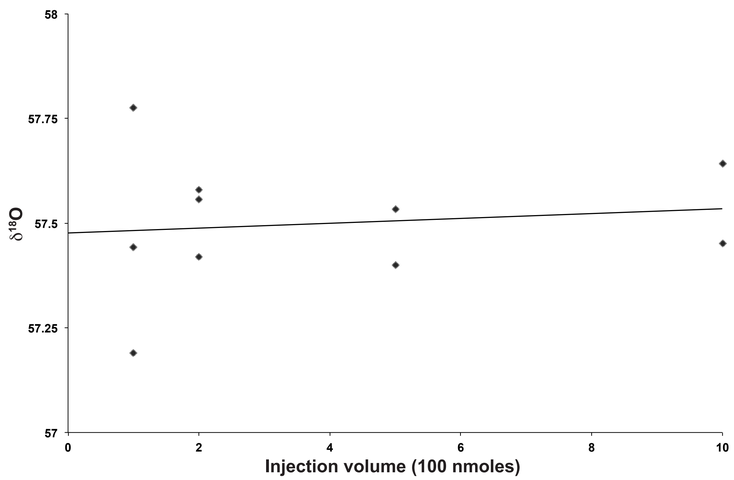
Example of $\delta^{15}N$ variability with injection volume

Inj. Vol.	Area	$\delta^{15}N$ mean	$\delta^{15}N$ MSD	$\delta^{15}N$ range SD	$\delta^{18}O$ mean	$\delta^{18}O$ MSD	$\delta^{18}O$ range SD
1 mL	12.3	2.73	0.23	0.16-0.25	57.38	0.61	0.43-0.89
5 mL	12.8	2.72	0.12	0.05-0.15	57.50	0.43	0.21-0.64
10 mL	13.0	2.69	0.29	0.16-0.37	57.39	0.53	0.06-0.61

NIST 8569, 100 nmoles, *P. aureofaciens*, mean and range across four batches, vol. uncorrected

METHODS, CONT.

Overall, a batch includes: two blanks; four linearity standards, FA, 10-300 nmoles; four drift standards, ACR; two each of two scaling standards, NIST 8568 and P383; and two each of three headspace standards, ACR, at 1mL (from ACR drift), 5mL, and 10mL. Enriched standards are also included for the analysis of nitrates enriched in $\delta^{15}N$ and/or $\delta^{18}O$. This format allows for appropriate standardization for scale compression, headspace volume, instrument drift, and linearity. While nitrate data is often known, we have found that nitrate concentration data can occasionally be unreliable. We include concentration-variable standards to account for linearity.



Example of $\delta^{18}O$ variability with injection volume

RESULTS

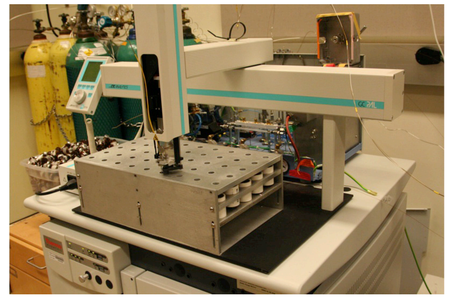
Sample analysis time was 16 minutes, with throughput at ~90 samples/24 hours. During a standard five-day workweek, two trays of 54 samples could be loaded per day under rolling start times. Long-term standard deviation for $\delta^{15}N$ and $\delta^{18}O$ measured from a static headspace are 0.2‰ and 0.5‰, respectively. Detection limit is 10 nmoles.

Mean standard deviation of samples uncorrected for volume was ~0.2‰ for $\delta^{15}N$ and ~0.5‰ for $\delta^{18}O$ (see Table). The strength and function of the relationship between isotopic value and headspace volume was variable between batches and isotopes, but was typically linear (see Figures). Recovery from the 1mL injection volume was statistically lower than the 5 and 10 mL injections (ANOVA, $F_{3,85}$= 6.37, P<0.005; Student's t), perhaps due to higher viscosity. The highest N_2O recovery was observed from headspace volumes that correspond with those used when analyzing low [NO_3^-] samples.

KEY POINTS

1. Provide for appropriate standardization for samples of variable injection volume and nitrate concentration, instrument drift, and scale calibration.

2. Severely limit or eliminate the use of antifoaming agent (e.g. Antifoam B).



CONCLUSION

A range of solutions has been developed to prevent bacterial suspension and excess water from entering the pre-concentration system. The incomplete nature of these solutions poses a potential barrier to uninterrupted, high-throughput analysis of nitrate by the bacterial denitrifier method. The intention of this study was to determine if a simplified method where N_2O is quantitatively sampled from the headspace above the bacteria culture, coupled with appropriate standardization could improve sample throughput without risk to data quality. At injection volumes of 1, 5, and 10mL (100 nmoles each), the mean standard deviation (σ) was ±0.23‰, ±0.12‰, and ±0.29‰ respectively for $\delta^{15}N$, and ±0.61‰, ±0.43‰, and ±0.53‰ respectively for $\delta^{18}O$ across four separate batches of *Pseudomonas aureofaciens*. Headspace-associated effect on accuracy was variable between batches, but generally <0.5‰ for $\delta^{15}N$, and <1‰ for $\delta^{18}O$, uncorrected for headspace volume. Precision and accuracy are maintained by the inclusion of volume-variable standards. Standardization for a range of analytical scenarios (e.g. headspace volume, samples artificially-enriched in $\delta^{15}N$ and/or $\delta^{18}O$, samples of variable NO_3^- concentration) is prudent for service-oriented facilities.

ACKNOWLEDGEMENTS

We thank Linda Huynh and Irene Yen for assistance with bacteria preparation and Joy Matthews and David Harris for comments and thoughtful discussions.