

$\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotopic analysis by “*bacterial denitrifier*” method

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Overview:

A method used to determine the N and O isotopic composition of ‘Total Oxidized Nitrogen’ (TON) in fresh or sea waters where (TON = $\text{NO}_3^- + \text{NO}_2^-$). Note: most surface and groundwaters under ‘*natural*’ conditions contain dissolved nitrite \ll dissolved nitrate, such that dissolved TON \approx dissolved nitrate and is used here interchangeably.

The ‘*bacterial denitrifier*’ method is based on bacterial reduction of TON to nitrous oxide gas (N_2O) via a bacterium that lacks nitrous oxide reductase, so further reduction to N_2 does not occur. This enables simultaneous determination of both $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the sample NO_3^- by measuring $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the produced $\text{N}_2\text{O}_{(\text{g})}$. The bacterial strain *Pseudomonas aureofaciens* (ATCC# 13985) is used to reduce TON to $\text{N}_2\text{O}_{(\text{g})}$.

Dissolved TON extraction:

Briefly, *P.aureofaciens* bacteria are grown in a special Tryptic Soy Broth (TSB) prepared in advance. Batches of TSB are inoculated using sterile techniques and allowed 7 days of growth, after which time the bacteria will have reached maximum population density and exhausted the TSB nutrients. The bacteria are then harvested and subdivided into individual 20ml, crimp sealed sample vials (pre-flushed with inert N_2 (UHP 4.8) for ~3-4 hours to lower the atmospheric blank). Standards and raw sample waters are then injected into individual vials in a preset order and left overnight at room temperature. Note: the target amount of NO_3^- depends on the sensitivity of the mass spec. but is generally in the range of 20 nmol [NO_3^- - NO_3^-]. The next day, each vial is lysed by injecting 0.15mL of 10 N NaOH and the vials mounted in an autosampler for automated analysis.

The analytical system is comprised of an autosampler + PreCon[®] + HP 6890[®] gas chromatogram + Conflo-IV[®] and Thermo DeltaVPlus[®] mass spectrometer. The entire instrumental process is automated and computer controlled using ISODAT 3.88 software. Prior to sample analysis, instrument conditions (oven temperatures, carrier gas flows, ion beam background(s), ion source stability and signal linearity) are checked and recorded by the operator as part of the “*Daily check*” routine.

The instrumental technique is as follows: the sample/broth solution in a vial is sparged, and the headspace of the vial flushed, with UHP 5.0 helium at a flow rate of 15 ml/min using a custom concentric needle. The $\text{N}_2\text{O}_{(\text{g})}$ + carrier passes through a series of traps: alcohol/slush \rightarrow Ascarite[®]/ H_2O \rightarrow Tekmar purge/trap F[®] (Supelco p/n: 20293) to remove:

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excess moisture, CO₂ and VOCs respectively before being cryo-focused in the PreCon. The N₂O_(g), once released from the PreCon, passes through a GC column (HP Plot-Q, 30m x 320µm @ 40°C) to separate N₂O from any remaining CO₂, then into the open split interface of the Conflo-IV which leads to the ion source of the mass spectrometer. The 'raw' δ¹⁵N and δ¹⁸O values are calculated by the ISODAT software.

Stable isotope ratios are expressed as delta (δ) and are measures of a 'per mill' (‰), or parts per thousand difference between the isotope ratio of a sample and that of a known (International) standard material. Values are reported relative to 'VSMOW' for Oxygen and 'Atmospheric air' for Nitrogen.

Data correction:

Ion currents of masses 44, 45 and 46 are measured simultaneously and the ^{15/14}Nitrogen and ^{18/16}Oxygen ratios of the sample are compared to that of a working-reference N₂O gas (4.8, 'Semiconductor Process Gas', Praxair Air, Canada). Raw data is corrected to the International 'air-N₂' and 'VSMOW' scales by normalization with reference materials analyzed in the same sequence as the samples (the "IT" principal described in Werner & Brand, 2001). An ¹⁷O correction is applied to the δ¹⁵N calculation by the ISODAT software.

Reference materials:

<u>Identifier</u>	<u>δ¹⁵N (‰)_{Air-N₂}</u>	<u>δ¹⁸O (‰)_{VSMOW}</u>
IAEA NO ₃	+4.7 ± 0.2	+25.6 ± 0.4
USGS 32	+180 ± 1.0	+25.7 ± 0.4
USGS 34	-1.8 ± 0.2	-27.9 ± 0.6
USGS 35	n/a*	+57.5 ± 0.6

* The non-mass dependent ¹⁸O:¹⁷O:¹⁶O ratios of USGS35, a commercially purified NaNO₃ from the natural nitrate ore deposits of the Atacama Desert, preclude its use in δ¹⁵N normalization calculations. See: Bohlke et al. (2003).

Accuracy and Precision:

Accuracy and precision of δ¹⁵N determinations based on a long-term record of in-house references is: 0.3 per mil

Accuracy and precision of δ¹⁸O determinations based on a long-term record of in-house references is: 0.7 per mil

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