

Novel denitrifier method for measuring ^{15}N and ^{18}O of nitrate

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The 'Denitrifier Method' using *Pseudomonas aureofaciens* to convert NO_2^- and NO_3^- quantitatively to N_2O , first devised by Sigman & Casciotti *et al.* [1, 2] has become a state-of-the-art method for nitrate preparation prior to IRMS analysis of ^{15}N & ^{18}O . This method is, however, relatively laborious and requires numerous successive steps of culturing, purging and concentration, all of which increase the risk of contamination by non-sample NO_3^- or N_2O . Moreover, the viability of the used *P. aureofaciens* cultures typically remains unreported, making it difficult to assess fractionation biases through incomplete NO_2^- and NO_3^- conversion. Here we present a novel denitrifier method that uses *Paracoccus denitrificans* (a complete denitrifier) to remove any NO_3^- and N_2O background from the growth medium before culturing *P. aureofaciens*. We found that *P. aureofaciens* cultures growing oxically in sterilized *P. denitrificans* treated medium can be switched successfully to anoxic respiration in the presence of 20 mM NH_4^+ with the sample NO_2^- and NO_3^- as the sole electron acceptor. This significantly shortens preparation times and reduces the risk of contamination (Tab. 1). We successfully tested the method for water samples with pH as low as 4 and for soil extracts containing 0.5 M KCl. First results applying the novel method to pore water samples from an N-saturated subtropical forest in southwest China will be presented.

	Precision (nmol)	^{15}N accuracy (‰)	^{18}O accuracy (‰)	Duration (days)
Original	20	0.4	20	10
New	≤ 25	1.9	7.9	3

Table 1: Comparison of the original with the newly developed denitrifier method

[1] Sigman *et al.* (2001) *Analytical Chemistry* **73**, 4145–4153.

[2] Casciotti *et al.* (2002) *Analytical Chemistry* **74**, 4905–4912.

Large sulfur isotope fractionation does not require disproportionation

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Microbial sulfate reduction (MSR) controls the partitioning of sulfur isotopes among various sulfur reservoirs, leaving a sedimentary sulfur isotope record that is used to track the oceanic budgets of oxidants, the progressive oxygenation of Earth's surface, and the evolution of microbial metabolisms through geologic history. Although previous environmental studies and models suspected that MSR alone could produce sulfur isotope offset between sedimentary sulfides and sulfates as large as ~ 75‰, all culture studies to date reported enrichment factors for MSR ($^{34}\epsilon$) smaller than 47‰. ^{34}S fractionation larger than 47‰ and its relationship to ^{33}S fractionation ($^{33}\lambda$) were thus thought to indicate active microbial disproportionation and oxidative recycling of sulfur.

A pure, actively growing culture of the recently isolated marine sulfate reducing bacterium (DMSS-1) produces sulfide depleted in ^{34}S by 6 to 66‰. The largest isotope effects occur during the very slow growth of cultures grown on glucose, a recalcitrant organic substrate. The large isotope effects and the associated $^{33}\lambda$ values produced by DMSS-1 during sulfate reduction approach the equilibrium value between sulfate and sulfide at low temperatures (<40 °C). These findings bridge the long-standing discrepancy between the upper limit for $^{34}\epsilon$ in laboratory cultures and the corresponding observations in nature and indicate that near-equilibrium $^{34}\epsilon$ and $^{33}\lambda$ do not unambiguously record the stepwise oxygenation of Earth's surface environment. Instead, the strong dependence of $^{34}\epsilon$ on the availability and quality of natural organic matter suggests that temporal or regional changes in the sulfur isotope systematics may reflect the changing nature of organic material that fueled sulfate reduction during the Proterozoic and the Phanerozoic.