

# LIMNOLOGY and OCEANOGRAPHY: METHODS

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## Effect of chloride on the chemical conversion of nitrate to nitrous oxide for $\delta^{15}\text{N}$ analysis

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### Abstract

We investigate the influence of chloride concentration on the performance of the chemical reduction method for measurement of the nitrogen isotopic ratio ( $\delta^{15}\text{N}$ ) in  $\text{NO}_3^-$  in natural waters (McIlvin and Altabet, 2005). In this method,  $\text{NO}_3^-$  is first reduced to  $\text{NO}_2^-$  using activated cadmium metal, with further reduction to  $\text{N}_2\text{O}$  using sodium azide in an acetic acid buffer.  $\text{N}_2\text{O}$  is introduced into an isotope ratio mass spectrometer (IRMS) for isotopic measurement. Previously, it was recognized that the presence of halides was necessary for the speed and efficiency of the second step but not thought to be important for the first step. Whereas quantitative Cd reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  had been noted for seawater samples, here we report, for freshwater and low-salinity ( $S < 30$ ) samples, a variable conversion efficiency (both under- and overreduction were observed) and significant variation in  $\delta^{15}\text{N}$  determination. Addition of 5 M NaCl to all samples resulted in rapid (<4 h) and quantitative (>99%) reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  as well as stable  $\delta^{15}\text{N}$  values that closely matched expected values for standards (within 0.3‰ of standard value). The positive effect of NaCl is likely due to a decrease in free  $\text{Cd}^{2+}$  produced over the course of the reaction due to formation of  $\text{CdCl}_2$ .

### Introduction

The nitrogen and oxygen isotope ratios of nitrate ( $\delta^{15}\text{N}$ – $\text{NO}_3^-$ ,  $\delta^{18}\text{O}$ – $\text{NO}_3^-$ ) provide powerful tools to investigate nitrate sources (Aravena et al. 1993, Böttcher et al. 1990, Casciotti et al. 2002, Schmidt et al. 2004) as well as mechanisms in the nitrogen cycle (Brandes et al. 2007). A number of important biogeochemical processes result in isotopic fractionation and alteration of  $\delta^{15}\text{N}$  values that can be measured using isotope ratio mass spectrometry (IRMS). This allows for studies of  $\text{NO}_3^-$  cycling (e.g., assimilation, remineralization, and nitrification) as well as identification of sinks and sources of nitrogen in the ocean (e.g., nitrogen fixation and denitrification).

Within the past two decades, several approaches have been developed to analyze nitrogen and oxygen isotope ratios in

dissolved nitrogen compounds. Usually, these methods contain a step transforming  $\text{NO}_3^-$  via chemical reduction into a gaseous compound suitable for IRMS analysis. Various studies have been published that use the conversion of  $\text{NO}_3^-$  into  $\text{N}_2$  (Silva et al. 2000, Voss et al. 1997),  $\text{NH}_4^+$  (Sigman et al. 2000, Slawyk and Raimbault 1995, Thunell et al. 2004), or  $\text{N}_2\text{O}$  (Casciotti et al. 2002; Kaiser et al. 2007; Sigman et al. 2001, 2005). As part of a “chemical only” approach, Cd metal reduction to  $\text{NO}_2^-$  has been adapted from the methodology for colorimetric  $\text{NO}_3^-$  concentration determination (Burakham et al. 2004, Gal et al. 2004, Hales et al. 2004, Nydahl 1976, Thabano et al. 2004). All of these methods have advantages and disadvantages. Pyrolytic methods (Silva et al. 2000) have been useful only in the analysis of freshwater samples, and their application is unsuitable for measuring oceanic nitrate isotope compositions. Chemical reduction methods have the potential for over- or underreduction and corresponding difficulty in maintaining stable 100% reduction yield (Gal et al. 2004). In the ammonia diffusion method (Slawyk and Raimbault 1995), both nitrate and nitrite are converted to ammonia, and then the N isotopic composition is measured. Similarly, the denitrifier method (Casciotti et al. 2002, Sigman et al. 2001) does not distinguish between the respective signals imparted by nitrite and nitrate. In our laboratory, we have applied the method

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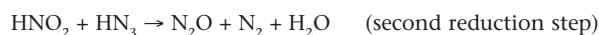
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developed by McIlvin and Altabet (2005), which uses azide for quantitative nitrite conversion to  $\text{N}_2\text{O}$  for the isotopic analysis of seawater and freshwater. The method allows separate analysis of nitrite without interference from the isotopic signature of nitrate and has a standard deviation of less than 0.2‰ for  $\delta^{15}\text{N}$  in nitrate samples ranging in concentration from 40 to 0.5  $\mu\text{M}$ . We refer to the McIlvin and Altabet method as MA (2005).

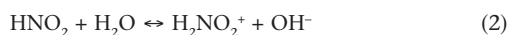
For  $\text{NO}_3^-$  concentration measurements (e.g., by an autoanalyzer), a quantitative (100%) reduction is not necessary, because  $\text{NO}_3^-$  standards are run under exactly the same conditions as the samples, so that sample concentrations are corrected for any over- or underreduction. For  $^{15}\text{N}$  analysis, on the other hand, a quantitative (100%) reduction of  $\text{NO}_3^-$  is essential to avoid potentially large and variable isotopic fractionation.

Application of the MA (2005) method at IFM-GEOMAR includes analysis of samples collected from the Atlantic and Pacific Oceans and the Baltic Sea covering a wide range in salinity (equivalent to 0–0.5 M NaCl). A salinity effect on  $\text{NO}_3^-$  reduction yields has been discussed in several articles, which examined the effect of Cd column methods for  $\text{NO}_3^-$  concentration measurements (Gal et al. 2004, Nydahl 1976). For example, Nydahl (1976) argued: “Considering the reaction equation for the reduction, the reducing power of cadmium should increase when the concentration of cadmium ions decreases, in this case by complex formation with the chloride ions, and the reduction should be accelerated instead of retarded.”

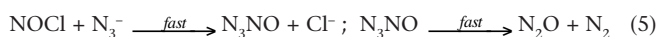
The MA (2005) method has two independent reduction steps. The first reduction step consists of a  $\text{NO}_3^-$  to  $\text{NO}_2^-$  reduction using cadmium metal. In the second step, the  $\text{NO}_2^-$  is reduced further to  $\text{N}_2\text{O}$  using a reaction with sodium azide in an acetic acid buffer.



The mechanism of the azide reaction was described by Stedman (1959a, 1959b). First, the nitrous acidium ion is formed, followed by slow nucleophilic attack by the azide ion on the nitrous acidium ion to form nitrosyl azide. This decomposes in a fast step to nitrogen and nitrous oxide.



Addition of chloride, bromide and thiocyanate ions catalyzes the reaction, via the formation of the corresponding nitrosyl compounds, thereby improving competition with exchange of  $\text{H}_2\text{NO}_2^+$  with water:



Until now, no systematic treatment of the effect of salt concentration on the reduction yield, especially for first reduction step, appears to have been conducted. This article presents results of experiments designed to investigate the efficiency of the first reduction step and the influence of salinity on the chemical reduction method for  $\delta^{15}\text{NO}_3^-$  analysis.

## Materials and methods

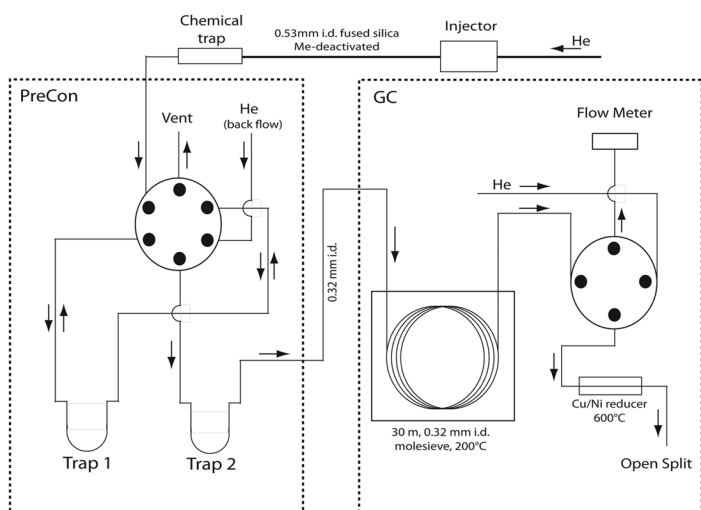
*Chemical conversion of nitrate to nitrous oxide*—Off the shelf cadmium powder (Alfa Aesar, –325 mesh, 99.5%) was found to be as effective as lab-generated spongy Cd. It was prepared by washing with 10% HCl to activate particle surfaces followed by multiple rinsing with deionized water (DIW) until the pH became neutral. After use, the cadmium could be collected for reuse, by repeating the acid wash and rinse steps. Samples containing 70 mL of 0.5–20  $\mu\text{M}$   $\text{NO}_3^-$  were reduced to  $\text{NO}_2^-$  by adding 3 g (dry weight) activated cadmium powder. Effectiveness of Cd powder is likely sensitive to the activation process and pH. On the one hand, if Cd is not properly activated, the reduction process will take longer. On the other, if Cd is not brought to pH neutral, there is the risk of  $\text{NO}_2^-$  loss under acidified conditions. NaCl used for manipulating sample  $\text{Cl}^-$  concentration was precombusted for 20 h to remove contaminants.

After the addition of Cd and NaCl (where indicated), the sample bottles were capped tightly with Teflon-faced rubber septa (Macherey-Nagel, rubberstoppers N20 gray, 702931) and aluminum crimp seals. The pH was adjusted to 9 by addition of 1 mL of 1 M imidazole solution, via a medical syringe. Samples were shaken on a horizontal shaker (GFL 3018) for between 2 and 20 h at a rate of ~220 cycles/min. Sample volumes of 70 mL were decanted into fresh 120-mL bottles with Teflon-lined septa.

For the next stage (second reduction step), the samples were reduced to nitrous oxide using sodium azide in an acetic acid buffer, exactly as described in the MA (2005) method. For a separate analysis of  $^{15}\text{NO}_2^-$ , the first reduction step can be bypassed.

*IRMS*—In contrast to the MA (2005) method, we did not quantitatively purge the samples by bubbling He through the liquid, but rather performed a  $\text{N}_2\text{O}$  headspace analysis. Inoue and Mook (1994) reported isotopic fractionation between dissolved and gaseous  $\text{N}_2\text{O}$  that may accordingly influence our observations. Under equilibrium conditions at 25°C, they found a small but significant fractionation of –0.75‰ for nitrogen and –1.06‰ for oxygen isotopes that did not vary from 0 to 44.5°C. The heavier isotopes ( $^{15}\text{N}$  and  $^{18}\text{O}$ ) were concentrated in the aqueous solution.

For  $^{15}\text{N}$  measurements (IRMS), 2 mL of the headspace, containing  $\text{N}_2\text{O}$  released in the reaction, were injected with a syringe into a PreCon system (Nuß 2007) and transferred onto a gas chromatography column (Fig. 1). Unlike the MA (2005) method,  $\text{N}_2\text{O}$  was further reduced to  $\text{N}_2$  at the end of the chromatographic column, using a Cu/Ni metal reducer at 640°C with Pt added as catalyst before reaching the continuous-flow



**Fig. 1.** Scheme of PreCon, GC, and the Cu/Ni metal reduction furnace.

isotope ratio mass spectrometer (CF-IRMS) via an open split. Quantitative conversion of  $\text{N}_2\text{O}$  to  $\text{N}_2$  was tested and verified. We run  $\text{N}_2$  and  $\text{N}_2\text{O}$  standards every day for a minimum of 5 times for validation of the signal and  $^{15}\text{N}$  data. It is also verified through monitoring mass 44. When  $\text{N}_2\text{O}$  standard is injected, there is no detectable mass 44 peak, only a stable background signal. Subsequently, the conversion efficiency was tracked by monitoring the  $\delta^{15}\text{N}$  value and peak height of the  $\text{N}_2\text{O}$  standards on a daily basis. If the response was changed, then the efficiency of the reducing furnace was checked. This approach allowed  $\text{N}_2$  from a gas cylinder, calibrated against atmospheric  $\text{N}_2$ , to be used as a direct reference standard for nitrogen isotopic analysis. Disadvantages of this approach are that oxygen isotope information is lost and care must be taken to ensure 100% conversion of  $\text{N}_2\text{O}$  into  $\text{N}_2$  to avoid further fractionation.

**$\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{NH}_4^+$  concentration analysis (DIN)**—To examine the reduction kinetics of the first reaction step, we measured the concentration of the  $\text{NO}_2^-$  product and the remaining  $\text{NO}_3^-$  as well as  $\text{NH}_4^+$  on an autoanalyzer equipped with a Cd-copperized column (Grasshoff et al. 1999).  $\text{NO}_3^-$  in the sample is reduced to  $\text{NO}_2^-$  in a column packed with copperized cadmium granules. Nitrite and ammonium concentrations were detected and measured spectrophotometrically. The  $\text{NO}_3^-$  concentration was calculated as the difference between the  $\text{NO}_2^- + \text{NO}_3^-$  and the  $\text{NO}_3^-$  absorbance data.

**Nitrous oxide analysis**—A gas-chromatography method was used to determine  $\text{N}_2\text{O}$ . An aliquot (10 mL) of the sample headspace was used to flush a 2-mL sample loop after passing through a moisture trap (filled with Sicapent; Merck). The gas chromatographic separation was performed at 190°C on a packed molecular sieve column (6 feet by 1/800 inch, 5 A, mesh 80/100; Alltech GmbH), and  $\text{N}_2\text{O}$  was detected with an electron capture detector (GC-ECD). A mixture of argon and methane (95:5 by volume) was used as carrier gas with a flow

of 21 mL/min. For the two-point calibration procedure, we used standard gas mixtures with  $311.8 \pm 0.2$  ppb and  $346.5 \pm 0.2$  ppb  $\text{N}_2\text{O}$  in synthetic air (Deuste Steiningering GmbH). The standard mixtures have been calibrated against the US National Oceanic and Atmospheric Administration (NOAA) standard scale in the laboratories of the Air Chemistry Division of the Max Planck Institute for Chemistry, Mainz, Germany (Walter et al. 2006).

## Results

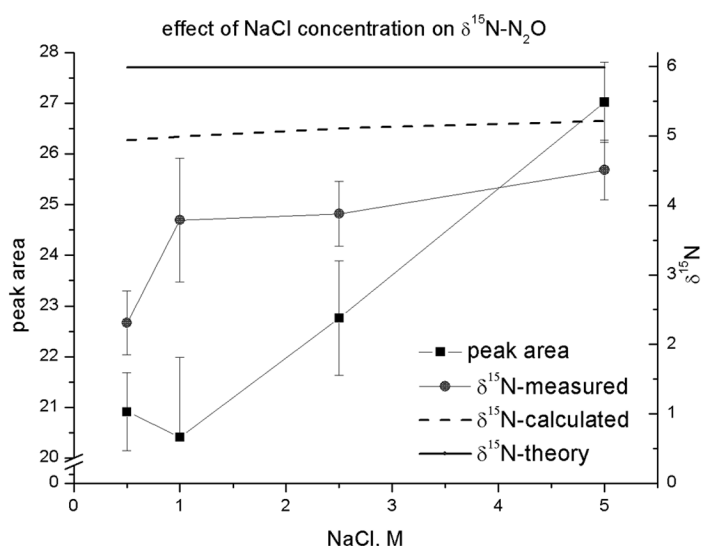
**Influence of salt concentration on  $\delta^{15}\text{NO}_3^-$  determination**—To check the effect of salt concentration on the first reduction step, test samples with five replicates were prepared. Samples containing  $20 \mu\text{M}$   $^{15}\text{N-NO}_3^-$  standard of initial concentration and  $\delta^{15}\text{N} = 14.67 \pm 0.03\text{‰}$ , 1 mL imidazole buffer, and 3 g Cd were diluted to 70-mL volume with distilled water and placed in a 120-mL bottle. NaCl was added to the samples to produce solutions with concentrations ranging from 0.5 to 5 M. The NaCl was precombusted for 20 h before sample preparation. The samples were shaken for 20 h. Sample volumes of 60 mL were then decanted into fresh 120-mL bottles. The  $\text{NO}_2^-$  produced was converted into  $\text{N}_2\text{O}$  using the azide ( $\delta^{15}\text{N} = -2.69 \pm 0.08\text{‰}$ ) reaction step and injected into the IRMS-PreCon system.

Along with an increase of peak area, there is a trend in the measured  $\delta^{15}\text{N-N}_2$  with salt concentration. Assuming that the conversion to  $\text{NO}_2^-$  is quantitative and that there is 1:1 combination of nitrite-N and azide-N without fractionation, a theoretical value of  $\delta^{15}\text{N}$  in the  $\text{N}_2\text{O}$  produced from the  $\text{NO}_3^-$  standard would be  $+5.99\text{‰}$  (McIlvin and Altabet 2005), assuming no isotopic fractionation with respect to the N contributed by the azide reagent. The  $\text{N}_2\text{O}$  produced is partitioned between liquid and gas phases (1:1 volume ratio in our study). Under equilibrium conditions at 25°C, the fractionation of  $-0.75\text{‰}$  between dissolved and gaseous  $\text{N}_2\text{O}$  (Inoue and Mook 1994) implies a theoretical  $\delta^{15}\text{N}_2\text{O}$  value of  $+5.24\text{‰}$  in the headspace. With increasing NaCl concentration in the sample solution, progressively more  $\text{N}_2\text{O}$  was detected in the headspace and there was an increase in its measured  $\delta^{15}\text{N}$  value, which became closer to the theoretical  $\delta^{15}\text{N}_2\text{O}$  value expected for the headspace. With 5 M salt concentration, the difference between the measured and calculated  $\delta^{15}\text{N}_2\text{O}$  headspace values was about 1.4‰. This offset, which we attribute to isotopic fractionation of the N originating from azide, is still significant but much smaller than the offset in MA (2005) (ca. 4.4‰) (see Table 1 and Fig. 2).

With an increasing NaCl concentration, there is a ca. 30% increase in the peak area associated with  $\text{N}_2\text{O}$  in the headspace. This could be explained by either incomplete conversion of initial  $\text{NO}_3^-$  to  $\text{NO}_2^-$  at low salt concentrations or the “salting out” effect: i.e., the decrease of  $\text{N}_2\text{O}$  solubility due to an increase of the salt concentration. Blank samples showed that there was no  $\text{NO}_3^-$  or  $\text{NO}_2^-$  contamination from the salt (data not shown; see also “Efficiency and recovery of the first reduction step” under “Results”).

**Table 1.** Effect of salt concentration in the sample on  $\delta^{15}\text{N}_2\text{O}$  product.

NaCl concentration, M	$\text{N}_2\text{O}$ peak area	Standard deviation	n	$\delta^{15}\text{N}_2\text{O}$ (‰)	Standard deviation	n
0.5	20.9	0.8	5	2.3	0.5	5
1.0	20.4	1.6	5	3.8	0.9	5
2.5	22.8	1.1	5	3.9	0.5	5
5.0	27.0	0.8	5	4.5	0.4	5



**Fig. 2.** Effect of salt concentration on the peak area and  $\delta^{15}\text{N}_2\text{O}$ .  $\delta^{15}\text{N}$ -theory shows the calculated value, assuming 1:1 combination of nitrite-N and azide-N and no isotopic fractionation.  $\delta^{15}\text{N}$ -calculated takes account of fractionation between dissolved and gaseous  $\text{N}_2\text{O}$  and changes in headspace-liquid partitioning as a function of salt concentration ( $\delta^{15}\text{N}_{\text{calculated}} = \delta^{15}\text{N}_{\text{theory}} - 0.75\alpha$ ). Error bars represent standard deviations.

The effect of the salt concentration on the solubility of gases was first described by Sechenov (1889) and was modified for mixed electrolyte solutions (Schumpe 1993):

$$\log(c/c_0) = \sum(h_i + h_g)c_i,$$

where  $h_i$  is an ion-specific parameter,  $h_g$  a gas-specific parameter, and  $c_i$  the ion concentration. Table 2 shows the predicted effect of  $\text{N}_2\text{O}$  solubility change on the expected headspace concentration in relation to the salt concentration.  $\delta^{15}\text{N}_{\text{total}}$  presented in the table is calculated under different solubility conditions as

$$\delta_{\text{total}} = (1 - \alpha)\delta_{\text{gas}} + \alpha\delta_{\text{liq}} = (1 - \alpha)\delta_{\text{gas}} + \alpha(\delta_{\text{gas}} + 0.75),$$

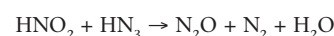
$$\delta_{\text{total}} = \delta_{\text{gas}} + 0.75\alpha,$$

where  $\alpha$  is  $\text{N}_2\text{O}$  solubility under different salt concentrations, calculated from the Sechenov equation.

The calculated increase of  $\text{N}_2\text{O}$  in the headspace of about 30% correlates well with our results. However this does not explain the shift in  $\delta^{15}\text{N}$  values. The  $\delta^{15}\text{N}$  could be altered via changes in fractionation for the N contributed from  $\text{HN}_3$  during the second step or by incomplete conversion and isotopic fractionation of  $\text{NO}_3^-$  during the first reduction step (e.g., at

low salt concentrations). To further study the source(s) of isotopic fractionation for the resulting  $\text{N}_2\text{O}$ , we designed a set of experiments that would outline kinetics and pathways for both reduction steps.

*Influence of the salt concentration on second reduction step—*



To check the effect of salt concentration on the fractionation effect and the  $\text{N}_2\text{O}$  peak area for the second reduction step, test samples were prepared (60 mL) containing an initial concentration of  $20 \mu\text{M}$   $\text{NO}_2^-$  ( $\delta^{15}\text{N} = -16.67 \pm 0.07\text{‰}$ ), which was converted into  $\text{N}_2\text{O}$  and injected into the IRMS-PreCon system.

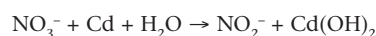
These experiments showed that with an increase of the NaCl concentration in the sample solution there is a ca. 30% increase in the peak area associated with the  $\text{N}_2\text{O}$  concentration in the sample headspace, as was seen with the overall method and which is consistent with the salting-out effect. Comparison of the salting out effect with the result from the overall method and the second step are also shown in Fig. 3.

The slopes of both curves are close to the predicted trend.  $\delta^{15}\text{N}_{\text{total}}$  in Table 3 represents the isotope ratio for the  $\text{N}_2\text{O}$  in both the gas and liquid phases and has been calculated for the different solubility conditions as described earlier. Increasing NaCl does not change  $\delta^{15}\text{N}_{\text{total}}$  when only the second reduction step is carried out.

The similar offset of 1.6‰ for the overall method with 5 M NaCl addition ( $\text{NO}_3^-$  standard) and the second reduction step ( $\text{NO}_2^-$  standard) suggests that this offset is a result of azide fractionation, which was also observed by McIlvin and Altabet (2005).

This result suggests that the source of the increase of  $\delta^{15}\text{N}$  with an increase of salt concentration in the overall method (Table 1) is due to fractionation during the first reduction step, especially at salt concentrations below or close to 0.5 M which correspond, very approximately, to salinities <30–32. (We note that the exact equivalent salinity cannot be calculated without knowledge as to whether it is  $[\text{Cl}^-]$  or the ionic strength of the solution that controls the reduction efficiency).

*Efficiency and recovery of the first reduction step—*



To measure the reduction efficiency of the first reduction step, we measured the concentration of the  $\text{NO}_2^-$  product and the remaining  $\text{NO}_3^-$  on an autoanalyzer. The samples, which we made up in distilled water, initially contained 60 mL of  $20 \mu\text{M}$   $\text{NO}_3^-$ , 1 mL imidazole buffer, and 3 g Cd in a 120-mL bot-

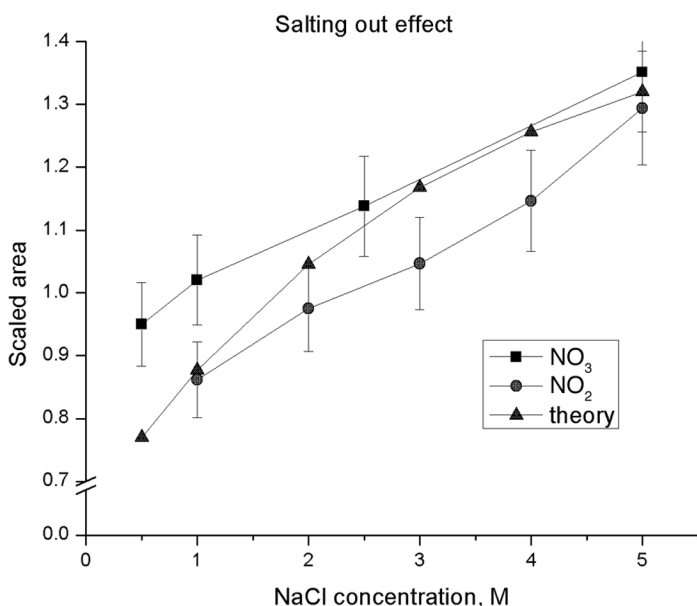


**Table 2.** Calculation of  $\text{N}_2\text{O}$  partitioning into the gas phase and associated  $\delta^{15}\text{N}$  fractionation.

	NaCl concentration, M	$\text{N}_2\text{O}$ in the headspace, %	$\delta^{15}\text{N}_{\text{gas}}$ (measured)	$\delta^{15}\text{N}_{\text{liq}}$	$\delta^{15}\text{N}_{\text{total}}$	$\delta^{15}\text{N}_{\text{offset}}$
Overall method				6.0*		
	0.5	51.76	2.3	3.1	2.7	3.3
	1	58.95	3.8	4.6	4.1	1.9
	2.5	74.71	3.9	4.7	4.1	1.9
	5	88.72	4.5	5.3	4.6	1.4

$h_i(\text{Na}^+) = 0.1171 \text{ M}^{-1}$ ,  $h_i(\text{Cl}^-) = 0.1171 \text{ M}^{-1}$ , and  $h_c(\text{N}_2\text{O}) = 0.011 \text{ M}^{-1}$  (Schumpe 1993) for a headspace volume that is 58% of the total volume.

\*Calculated from 1:1 combination of nitrite-N and azide-N.



**Fig. 3.** Salting out effect on the second reduction step ( $\text{NO}_2^-$ ) and overall reduction ( $\text{NO}_3^-$ ). Scaled peak area is calculated from peak area divided by the initial concentration ( $[\text{NO}_3^-]$  or  $[\text{NO}_2^-]$ ). Dashed line shows the theoretical increase of  $\text{N}_2\text{O}$  in the headspace with an increase of the salt concentration.

tle. NaCl was added to samples at concentrations varying from 0 to 5 M, and the time on the shaker was varied from 1 to 18 h to investigate reduction kinetics under different salt concentrations. The reference samples contained  $20 \mu\text{M}$   $\text{NO}_3^-$  standard solution that did not undergo reduction with Cd (Table 4).

From these results, it is obvious that *without* salt addition, the recovery of DIN decreases with time and the loss of DIN to an unknown form is very large after 18 h (ca. 80%) (Fig. 4). Moreover, there is a measurable increase ( $0.8 \mu\text{M}$ ) of dissolved  $\text{NH}_3$ , which may suggest an overreduction of  $\text{NO}_3^-$ . From these measurements, however, it is not clear which nitrogen species are being produced. With intermediate NaCl concentrations (0.2 and 0.5 M; see Figs. 5–6, respectively), all the added nitrogen can be accounted for; however, reduction to  $\text{NO}_2^-$  is incomplete even after 18 h (ca. 50%).

Quantitative yields for this step are routinely obtained overnight in the laboratory at SMAST, and we suspect that this step is very sensitive to details of the Cd preparation. For example, previous work done at IFM-GEOMAR (Nuß 2007) showed that complete  $\text{NO}_3^-$  conversion with 0.5 M NaCl was achieved only after 35 h.

These results demonstrate overreduction of  $\text{NO}_3^-$  (i.e., beyond  $\text{NO}_2^-$ ) in freshwater, with  $\text{N}_2\text{O}$  or  $\text{N}_2$  as the most likely products. A set of experiments was therefore designed and conducted in our laboratory to measure  $\text{N}_2\text{O}$  accumulated during

**Table 3.**  $\delta^{15}\text{N}$  correlation between the overall method and the second reduction steps.

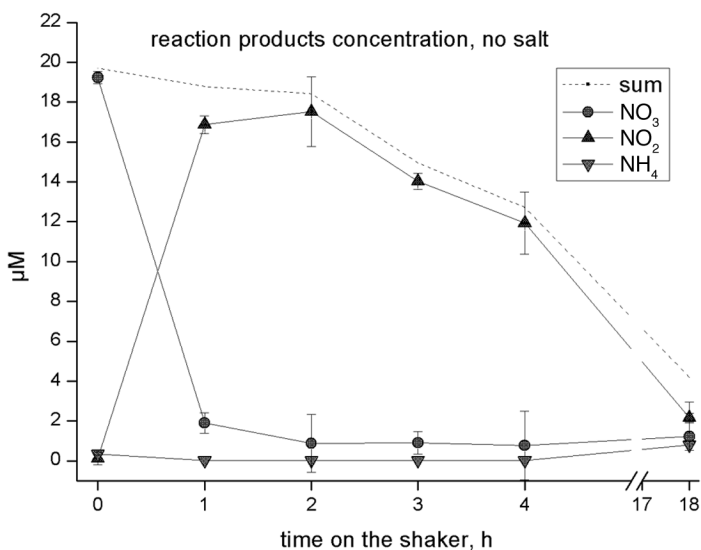
	NaCl concentration, M	$\text{N}_2\text{O}$ in the headspace, %	$\delta^{15}\text{N}_{\text{gas}}$ (measured)	$\delta^{15}\text{N}_{\text{liq}}$	$\delta^{15}\text{N}_{\text{total}}$	$\delta^{15}\text{N}_{\text{offset}}$
Second step				-9.7*		
	1	58.95	-11.7	-11.0	-11.4	1.7
	2	70.28	-11.0	-10.3	-10.8	1.1
	3	78.48	-11.7	-11.0	-11.5	1.9
	4	84.42	-11.3	-10.6	-11.2	1.5
	5	88.72	-11.4	-10.7	-11.3	1.6
Overall method				6.0*		
	0.5	51.76	2.3	3.1	2.7	3.3
	1	58.95	3.8	4.6	4.1	1.9
	2.5	74.71	3.9	4.7	4.1	1.9
	5	88.72	4.5	5.3	4.6	1.4

Standard deviation of 0.3‰ was calculated for  $n = 3$ .

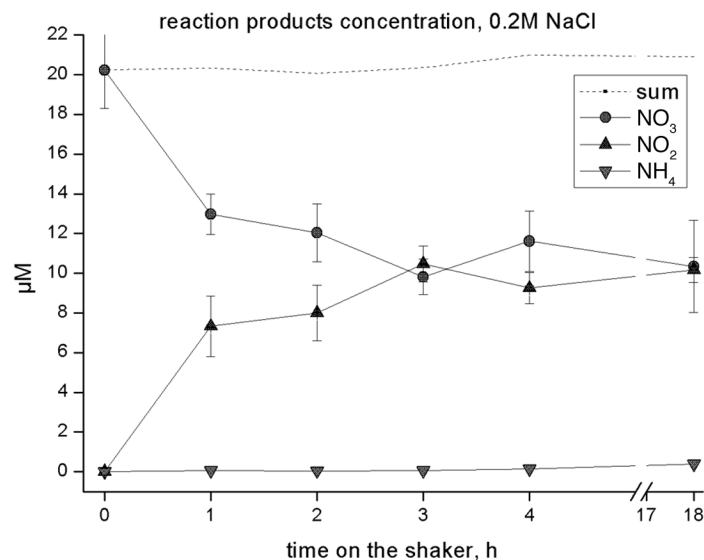
\*Calculated for 1:1 combination of nitrite-N and azide-N.

**Table 4.** Kinetics of first reduction step.

NaCl concentration, M	Time, h	$\text{NO}_3^-$ , $\mu\text{M}$	$\text{NO}_2^-$ , $\mu\text{M}$	$\text{NH}_4^+$ , $\mu\text{M}$	Sum, $\mu\text{M}$
No salt addition	Reference	19.23	0.13	0.33	19.69
	1	1.90	16.87	0.01	18.78
	2	0.88	17.53	0.01	18.42
	3	0.91	14.03	0.01	14.95
	4	0.77	11.93	0.02	12.72
	18	1.22	2.17	0.80	4.19
0.2 M	Reference	20.23	0.01	0.00	20.24
	1	12.96	7.33	0.05	20.34
	2	12.03	8.00	0.04	20.07
	3	9.82	10.47	0.06	20.35
	4	11.61	9.25	0.13	20.99
	18	10.34	10.17	0.39	20.90
0.5 M	Reference	20.06	0.43	0.01	20.50
	1	15.78	4.33	0.03	20.14
	2	13.93	7.00	0.04	20.97
	3	14.15	6.37	0.04	20.56
	4	13.40	7.25	0.04	20.69
	18	8.68	11.95	0.05	20.68
5 M	Reference	19.58	0.13	1.30	21.01
	1	4.73	15.84	0.21	19.78
	2	1.55	18.27	1.32	21.14
	3	0.25	19.53	1.27	21.05
	4	0.01	20.20	0.10	20.31
	18	0.14	20.53	0.20	20.87

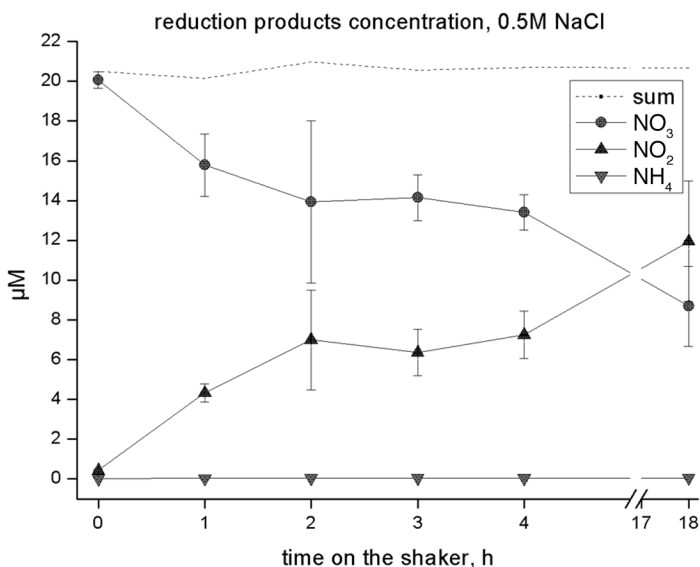
**Fig. 4.** DIN speciation of first reduction step without salt addition. Standard deviation of 1  $\mu\text{M}$  was calculated for  $n = 3$ .

the first reduction step. The samples contained 60 mL  $\text{NO}_3^-$  solution of 20  $\mu\text{M}$  initial concentration, 1 mL imidazole buffer, 5 M NaCl, and 3 g Cd in a 120-mL bottle and were left on the shaker for 2 and 18 h. The reference samples contained no

**Fig. 5.** DIN speciation of first reduction step with 0.2 M salt addition. Standard deviation of 1  $\mu\text{M}$  was calculated for  $n = 3$ .

$\text{NO}_3^-$  standard solution but had 3 g Cd and imidazole buffer in the solution. All bottles were flushed with an  $\text{N}_2$  flow for 5 min before closing the bottles and placing them on the shaker.

The results from the GC-ECD measurements show some



**Fig. 6.** DIN speciation of first reduction step with 0.5 M salt addition. Standard deviation of 3  $\mu\text{M}$  was calculated for  $n = 3$ .

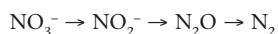
production of  $\text{N}_2\text{O}$  without salt addition, but it was very small, representing only about 13 nmol  $\text{N}_2\text{O}$  or 0.07% of the initial  $\text{NO}_3^-$  concentration. This cannot explain the loss of more than 90% of the DIN after 18 h (Fig. 3).  $\text{N}_2\text{O}$  is probably an intermediate product in the reduction mechanism to  $\text{N}_2$ .

From these data, it is also clear that already (after only 4 h on the shaker) there is a complete conversion of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  performed with salty samples (5 M NaCl, Fig. 7), whereas without salt addition we risk either overreduction to  $\text{N}_2$  or incomplete reduction to  $\text{NO}_2^-$ .

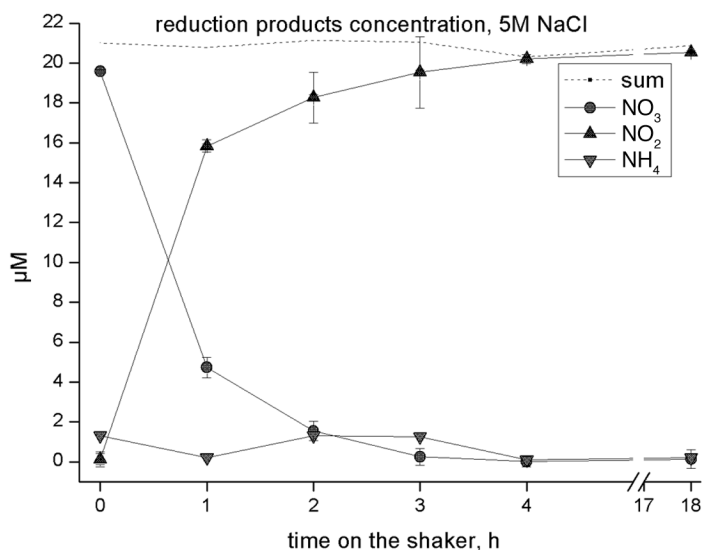
### Discussion and conclusions

Whereas it was recognized previously that  $\text{Cl}^-$  was needed for the azide reaction, in this work we show that it promotes the efficient reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  by Cd. Therefore  $[\text{Cl}^-]$  has a significant influence on the overall reduction mechanism and the resulting  $\delta^{15}\text{N}$  values. Very low salt concentrations can lead to overreduction of  $\text{NO}_3^-$  and significant fractionation of  $^{15}\text{N}$  in the products. By adding 5 M NaCl, a quantitative reduction to  $\text{NO}_2^-$  after only 4 h of treatment with Cd on the shaker is achieved and overreduction on the first reduction step is excluded.

We hypothesize that the sequence for overreduction by Cd is



Without salt addition, Cd metal likely reduces  $\text{NO}_3^-$  to  $\text{N}_2$ , whereas with 5 M NaCl the reduction of  $\text{NO}_3^-$  effectively stops with  $\text{NO}_2^-$ . With intermediate salt concentrations, there is no overreduction apparent, but the conversion to  $\text{NO}_2^-$  is not complete. At 5 M NaCl, there is quantitative conversion of  $\text{NO}_3^-$  to  $\text{NO}_2^-$ , as required by the method. We speculate that the effect of  $\text{Cl}^-$  on acceleration and completion of the reaction may be associated with formation of a complex between



**Fig. 7.** DIN speciation of first reduction step with 5 M salt addition. Standard deviation of 0.5  $\mu\text{M}$  was calculated for  $n = 3$ .

the  $\text{Cd}^{2+}$  and  $\text{Cl}^-$  during the reduction.

Addition of 5 M NaCl in our experiments sped up the conversion to  $\text{NO}_2^-$  and resulted in avoidance of fractionation effects along with improved yield of  $\text{N}_2\text{O}$  from the headspace (due to the salting out effect). The salting out effect resulted in a sensitivity of the headspace method comparable to that of the purge/trap method of MA (2005).

Thus we suggest a modification of the MA (2005) method for  $^{15}\text{N}$  analysis by adding 5 M NaCl into the initial samples to achieve quantitative and rapid reduction. The modified method is suitable for water analysis over a wide range of salinities.

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