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15 N-Diffusion Protocol for Freshwater Samples

15NO3

1. Tare a clean, acid-washed 1000mL flask on a balance to nearest 0.1g.
2. Add enough sample up to ~500g (or 100g for smaller volumes) to the flask and record the exact mass.
3. Add 10g of ashed NaCl (for ionic balance, want final concentration after boil down to be about 50 g/L) and 0.6 g ashed MgO (to raise pH) to sample (add proportionately less NaCl if boiling down smaller volumes of sample to achieve a 50 g/L concentration after boil-down, add same amount of MgO (initial 3 g/L) regardless of sample size to boil down).
4. Add stir bar and place on hot plate with stirring capability (SETTING 400). Heat until volume is reduced to roughly 100 ml to 200mL (doesn't have to be exact). Aim for a simmering boil (SETTING ~400C). Stir during the boil. It takes about 2 hours of gentle boiling to reduce 0.5 L to about 100 mL.
5. After cooling somewhat, pour the boiled-down sample into an acid-washed 250 mL HDPE bottle (suggest rigid rectangular bottles). Top off volume of the sample to 200 mL with ultrapure deionized water. These can be stored in refrigerator until you have an entire series ready for next step (reduction of nitrate to NH_3 and diffusion of NH_3 into headspace).
6. Add another 0.6 g of ashed MgO and then 0.15 g of Devardas alloy to boiled-down sample in 250 mL bottle.
7. Immediately after adding Devardas alloy, place filter pack in bottle (see subsection on steps for constructing filter packs below) and cap very tightly.
8. Place bottles in oven at 60^{o}C for 48 hours (be careful not to pack too closely in a tray since the bottles will swell slightly).
9. Remove bottles from oven and place on shaker and shake gently for 7 days at 40 ^{o}C.
10. Open bottles and remove filter pack. Gently blot water droplets from filter pack making sure not to burst any swollen packs and place in labeled scintillation vial and into desiccator. Also place an open vial of 2.5 M $KHSO_4$ (to absorb any ammonium in air) in desiccator.
11. Let filters dry in desiccator for 3 or 4 days or so. Remove and cap the scintillation vials containing filter packs very tightly and store until ready to encapsulate in tins.
12. Encapsulating filters: Remove filter pack from scintillation vial on clean surface (use alcohol to clean). Using cleaned forceps, open filter pack and remove small glass fiber filter. Place filter in silver capsule and fold opening of capsule down once and compress. Crimp sides of tin to form small packet (all dimensions < 2 mm). Place capsule packet into a well in the well tray recording the well location and sample ID. Place well cap strip over wells as soon as possible to minimize any further exposure of encapsulated filter to air. Store plate in a desiccator with an open vial of 2.5 M $KHSO_4$ until they can be run on the mass spec.
13. Tell the isotope lab to enter a dry mass value of 1 mg for all filter samples and to report a %N value for each sample. This will allow calculation of N recovery during processing of each sample. Poor N recovery may be a reason to eliminate data points.

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Reference: Sigman, D. M., M. A. Altabet, R. Michener, D. C. McCorkle, B. Fry, and R. M. Holmes. 1997. Natural abundance-level measurement of nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. Marine Chemistry 57:227-242.

NH4

1. Add 50 g of NaCl per liter of sample (10 g of NaCl for a 200-mL sample).
2. Add 3 g of MgO per liter of sample (0.6 g MgO for a 200-mL sample) and place filter pack (see subsection on steps for constructing filter packs below) on a strip of parafilm over top of the bottle, and cap very tightly. Incubate the samples for three weeks at 40 C on a shaker.
3. Open bottles and remove filter pack. Gently blot water droplets from filter pack making sure not to burst any swollen packs and place in labeled scintillation vial and into desiccator. Also place an open vial of 2.5 M $KHSO_4$ (to absorb any ammonium in air) in desiccator.
4. Let filters dry in desiccator for 3 or 4 days or so. Remove and cap the scintillation vials containing filter packs very tightly and store until ready to encapsulate in silver capsules.
5. Encapsulating filters: Remove filter pack from scintillation vial on clean surface (use alcohol to clean). Using cleaned forceps, open filter pack and remove small glass fiber filter. Place filter in silver capsule and fold opening of capsule down once and compress. Crimp sides of tin to form small packet (all dimensions < 2 mm). Place capsule packet into a well in the well tray recording the well location and sample ID. Place well cap strip over wells as soon as possible to minimize any further exposure of encapsulated filter to air. Store plate in a desiccator with an open vial of 2.5 M $KHSO_4$ until they can be run on the mass spec.
6. Tell the isotope lab to enter a dry mass value of 1 mg for all filter samples and to report a %N value for each sample. This will allow calculation of N recovery during processing of each sample. Poor N recovery may be a reason to eliminate data points.

Reference: Holmes, R. M., J. W. McClelland, D. M. Sigman, B. Fry, and B. J. Peterson. 1998. Measuring 15N-NH4 in marine, estuarine and fresh waters: an adaption of the ammonium diffusion method for samples with low ammonium concentrations. Marine Chemistry 60:235-243.

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Filter Pack Construction:

1. Ash 1cm-diameter GF/D filters.
2. Cut 1" Teflon tape into 1" squares with alcohol-cleaned scissors. Cut enough for 2X the number of samples.
3. Spread out aluminum foil over a layer of paper towels and clean by rubbing down with alcohol. Clean forceps with alcohol.
4. Place a teflon square down and GF/D filter centered on top. Pipet 25 µL of 2.5 M KHSO4 onto GF/D filter (it will be completely absorbed by the filter).
5. Place second teflon square centered on top of GF/D filter.
6. Seal teflon pack holding acidified GF/D filter by rolling the open end of a plastic 20-mL scintillation vial around the outside portion of the teflon filter, or using a custom press. To ensure that the filter pack remains stuck together, you can also create another ring around the filter with a smaller diameter vial. You should notice a thinning of the teflon filters around the edge where it is sealed. Hold the filter pack up to the light to verify this. If you press too hard the membrane will tear, but if you press too lightly the membranes will not be truly stuck together and may come apart during the diffusion. You will need to practice this. Press really hard and break through a test filter pack so that you know how much is too much. Check your filter-pack-making capabilities by making a batch of dummy filter packs and shaking them with NaCl, MgO, and Devarda's for a week. See if any fall apart.
7. Place fresh filter packets into a small clean air-tight bottle and cap very tightly. It is best to make up filter packets within a few days of use. If necessary, filter packets can be stored for several weeks, but it's critical that they be tightly capped to prevent any exposure to air.

Reference: Sigman, D. M., M. A. Altabet, R. Michener, D. C. McCorkle, B. Fry, and R. M. Holmes. 1997. Natural abundance-level measurement of nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. Marine Chemistry 57:227-242.

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