

Protocol for Persulfate Oxidation to Measure Total Nitrogen

(This is the procedure Christine Hawkes gave me, and she has used it successfully for diffusions. It is mainly Robert Northup's method.)

Equipment:

- 50 mL polypropylene screw-cap "Evergreen" vials (used for Lachat calibration standards)
- Pasteur pipets
- two-place balance
- Autoclave

Reagents:

- 0.5 M K_2SO_4 (87.14 g L^{-1}) for reagent blank
- 10 mg N/L as alanine in 0.5 M K_2SO_4 for standards
- 5% Alkaline potassium persulfate reagent
 - Sodium hydroxide, NaOH (15 g L^{-1}) (low N)
 - Potassium persulfate, $K_2S_2O_8$ (50 g L^{-1}) (low N)
 - Boric Acid, H_3BO_4 (30 g L^{-1})

Note: To make the alkaline potassium persulfate, first dissolve low-N NaOH into DDI water, then add H_3BO_4 , and finally add low-N $K_2S_2O_8$ and bring to volume with DDI water. Stable for up to a week at room temperature. Keep out of light or in a dark bottle.

Digestion Procedure:

1. Thaw the K_2SO_4 extracts.
2. Label polypropylene vials and caps with the sample numbers (black or blue Sharpie will survive autoclaving). Alternatively, number the vials 1 to N, and keep a datasheet of which number vial contains which sample.
3. Invert the sample (K_2SO_4 extract) several times to mix. Using the guides on the side of the Evergreen vial, add 25 mL of the sample to the tube. To date, these markings have been sufficiently accurate. However, if greater accuracy is needed, the vial can be placed (without cap) on a two-place balance and tared, and 26.45 g (25 mL) can be measured out into the vial. A pasteur pipet can be used for accurate addition of final drops. Vials can stand on their own or can be held in tube racks for convenience.

4. For every 10 samples include 1 reagent blank and 1 standard:

Blank = 25 mL (26.45 g) of 0.5 M K_2SO_4

Standard = 25 mL (26.45 g) of 10 mg N/L as alanine in 0.5 M K_2SO_4

5. To each sample, reagent blank, or standard, add 25 mL (26.65 g) of alkaline potassium persulfate reagent either using the guidemarks on the side of the vial or a balance. *NOTE: for samples where there are less than 25 mL, simply use a volume of alkaline potassium persulfate that is equal to the sample volume.*

6. Immediately after adding the persulfate reagent, tightly cap the vial to prevent gaseous loss of ammonia. Cap will “snap” into tightly-locked position, but be sure that it is flat. Caps will occasionally snap into place in a lopsided position, allowing vapor loss.

7. With balance zeroed, weigh and record entire weight of each capped vial.

8. Place labeled, capped and weighed vials into autoclave for 40 minutes. The tubes can either be packed into a plastic autoclave pan without tube racks or placed in racks inside an autoclave pan. Ensure the autoclave gets up to temperature.

NOTE: As little as 15 minutes may be sufficient for complete digestion of organic nitrogen, but more autoclave time is needed to ensure complete degradation of the persulfate. Otherwise, residual persulfate will prevent efficient reduction and capture of ammonia for ^{15}N diffusion, and shortens life of the cadmium column for Lachat nitrate analysis.

9. After autoclaving, allow sufficient time for the digests to cool and the outside of the vials to dry, then reweigh the vials to calculate vapor mass loss. Samples can be stored in these same vials until further analysis (lachat and diffusions).

10. Corrections

(i) Mass loss correction. During autoclaving, a small volume of water is lost as vapor. With autoclave time sufficient for complete degradation of persulfate (40 min), mass loss fraction can be as high as 0.04, or 4% weight loss. Pre- and post-autoclave weights are therefore recorded to be able to calculate and correct for mass loss. The difference is divided by the pre-autoclave mass minus average weight of the capped vials (14.6g in Robert's run)

$$\frac{[(\text{pre-autoclave weight}) - (\text{post-autoclave weight})]}{[(\text{pre-autoclave weight}) - (\text{avg vial wt})]} = \text{mass loss fraction}$$

This mass loss fraction is then multiplied by the Lachat reading to correct the value.

$$(\text{Lachat reading}) - (\text{Lachat reading} \times \text{mass loss fraction}) = (\text{mass loss corrected value})$$

(ii) Reagent blank correction. Since the Lachat is calibrated with standards in DDI water (including the 0 ppm standard), all Lachat values for samples must be corrected by subtracting the average reading from reagent blanks. This average is different for each run, and so far has ranged from about 0.123 to 0.23 ppm N. This potential source of error can be reduced in future work with greater care to use nitrogen-free reagents. Ironically, the "cleanest" persulfate so far was obtained by repurifying (dissolving and recrystallizing) some very old reagent, giving lower "blank" values than the brand new (certified less than 0.001% N) persulfate purchased. At just 0.001% (10 ppm), a 1:1 reagent:sample mix of 5% persulfate would give "blanks" of 0.25 ppm N.

$$(\text{mass loss corrected value}) - (\text{reagent blank average for run}) = (\text{blank corrected value})$$

(iii) Dilution correction. Having calculated the mass-loss and blank corrected concentration in the sample plus reagent mix, the concentration of the original sample is calculated according to the composition of the mix. Since the persulfate procedure combines an equal volume of sample and reagents (1:1, sample:reagent), the concentration of the mix is multiplied by 2 to get the actual concentration of the sample.

$$(\text{Dilution corrected value}) = (\text{blank corrected value} \times 2)$$

Any other dilutions performed on samples can also be accounted for in this step

11. Calculation of actual N concentration (recovery corrected value)

The 10 ppm N as alanine standards digested in each set provide a means to calculate percent recovery.

$$\text{Actual Concentration (ppm N)} = (\text{dilution corrected value})/(\text{proportion recovery})$$

In Robert's runs, 10 standards had recoveries ranging from 97.32% to 100.28% with an average of 98.8%. *In this case*, actual concentration is calculated as:

$$\text{Actual Concentration (ppm N)} = (\text{dilution corrected value})/(0.988)$$