Hofmockel Lab Protocol
ANALYSIS OF TOTAL NITROGEN USING POTASSIUM PERSULFATE DIGESTION

Ammonium Determination

Modified from: Suding & Isenhart Lab protocols

MATERIALS

- Low N grad potassium persulfate ($K_2O_8S_2$) (VWR#:)
- 10N sodium hydroxide (NaOH) (Fisher # SS255-1)
- Potassium nitrate (Fisher # P38100)
- 13 x 100 mm borosilicate tubes (small) (Fisher#)
- Caps for tubes (not acid washed)
- Test tube racks
- Autoclave
- Autoclave tray
- Matrix

Note: With very autoclave run of samples, run a standard curve of urea and a standard curve of nitrate. These curves can be used to directly quantify nitrite concentrations of samples; curves should match. See below for dilution details.

SOLUTIONS

1) 10N NaOH, 100 ml ***highly corrosive: where gloves, lab coat, and goggles when making****
Cautiously add 40 g NaOH to ultrapure DI in 100 ml volumetric. Dissolve with stir bar on hot plate. 10N NaOH will corrode glassware, so as soon as the solution has been made, *transfer to a 125 ml nalgene bottle*.

2) Oxidizing solution – ***(prepare fresh daily)***
Dissolve 25.000 g potassium persulfate into 500 ml ultrapure DI water. Persulfate will go into solution in about 3 hours if stirred on a magnetic stir plate.

Add 19.0 ml 10N NaOH to the persulfate

3) Urea standard stock solution, 1000 ppm urea-N/L – *(store at 4˚C for several months)*
1 ppm N = 1 mg N/L; 1000 ppm N = 1 g N/L
Urea: 60.06 g/mol with 28.0 g N/mol,

For 1000 ppm, add 1.0725 g urea and bring to 500 ml in a volumetric with ultrapure = 0.5 g N in 500 ml
* Weigh volumetric + water for final weight to find ppm*

Dilute 1000 ppm urea to 100 ppm in ultrapure water: 10 mL 1000 ppm into a 100 mL volumetric.
Dilute 100 ppm standard directly into test tubes as follows:

final ppm	mL of 100ppm to add to test tube	mL of matrix to add to test tube
15	0.375	2.125
10	0.25	2.25
5	0.125	2.375
2	0.05	2.45
1	0.025	2.475
0.5	0.0125	2.4875
0.1	0.0025	2.4975
0	0	2.5

4) Nitrate standard stock solution, 100 ppm nitrate-N/L – *(store at 4˚C for several months)*
Add 0.3609 g potassium nitrate and bring to 500 ml in a volumetric with ultrapure

Dilute 100 ppm standard directly into test tubes as above.

Sample Preparation
Check for chips on the borosilicate tubes and do not use tubes that are chipped.
Label tubes *and **make a layout** of the samples in the test tube rack, as labels on tubes may disappear in autoclave!*
Label corner of test tube racks to identify orientation.

To each borosilicate tube:
2.5 ml sample/standard extract
1.0 ml oxidizing solution (use repeat pipettor)
Immediate cap the tubes *tightly*

Protocol

1. **Autoclave**

- Place test tube racks with samples and glass bottles with matrix in an autoclave tray
- Fill the tray with water, to the depth of the solution in the tubes. This will minimize leaks due to rapid changes in temperature and pressure
- Autoclave samples, standards, and matrix solution for **60 minutes on liquid cycle**
- *After digestion, check water height of samples. If they are inconsistent, there were likely leaks during the digestion, and the digestion should be redone.*

* If samples cannot be analyzed on the plate reader right away, do not continue with the post-autoclave dilution for the samples. Samples can be stored as long as they are sealed tightly and are not opened until they are ready to be analyzed. Combine the matrix solution into one bottle and store in refrigerator. *

2. **Post-autoclave dilution**

When samples have cooled, **add 2.5 ml ultrapure DI water (use repeat pipettor)**, cap, and shake

- If samples turn pink, a small addition of 0.5M ascorbic acid (176.13 g ascorbic acid per liter of DI water) should eliminate the pink color:
    - Add 1 ul of 0.5M ascorbic acid to unfumigated samples shake to mix
    - Add 2 ul of 0.5M ascorbic acid to fumigated samples and shake to mix

3. **Nitrate analysis of samples**

Nutrient Analysis of Inorganic N. Use the oxidized standard curves as standards for nutrient analysis.

Reference for ascorbic acid treatment:
Williams et al. 1995. A procedure for the simultaneous oxidation of total soluble N and P in extracts of fresh and fumigated soils and litters. Commun. Soil Sci. Plant Anal. 26:91-106.

Calculations
1. Output in ppm = ug N/ml extract Note: assume 1 ml extract = 1 g; therefore 1 nl = 1 ug
2. ug N / ml extract x [volume extract + (water weight of sample = fresh weight – dry weight)] = ug N
3. ug N_{sample} – ug N_{blank}
4. ug N / g dry weight soil = ug N / g dry weight soil