

## Digestions: Analysis of Total Nitrogen using Potassium Persulfate

Modified from Lars Hedin's protocol

Important: Make sure that all glassware and pipettes are absolutely clean and free of trace N contamination. Also the efficiency of the digestions needs to be checked periodically. This digestion protocol only works for determining N, do not use this protocol to determine phosphorus concentrations.

### Materials Needed:

- Low N grade potassium persulfate ( $K_2O_8S_2$ ): Fisher # P282-100
- 10N Sodium Hydroxide (NaOH): Fisher # SS255-1
- 13 x 100 mm borosilicate tubes with polypropylene (linerless) caps: Fisher # 14-962-26D
- Autoclaveable 1L glass bottles: Fisher # 06-414-1D
- Test tube racks-autoclaveable
- 1-5 ml pipettor
- Autoclave
- Autoclaveable plastic tray to place test tube racks in
- 500 ml Erlenmeyer flask
- 500 ml volumetric flask
- 50 ml volumetric flask
- 0.5 M  $K_2SO_4$  (87.13g  $K_2SO_4$  per 1L extractant)

### Preparing Oxidizing Solution: (This solution should be made fresh every day)

- Dissolve 25.000 g potassium persulfate into 500 ml deionized water. Persulfate will go into solution in about 30 minutes if stirred on a magnetic stir plate.
- Add 19.0 ml of 10N sodium hydroxide to the persulfate solution

Standard Stock solution (1000ppm N/L): (any amino acid, i.e. Glycine, can be used as a standard as long as you make a 1000 ppm N/L solution with it)

- 3.18 g L-alanine (bring to 500 ml with DI water)
- Stock is stable for at least one year refrigerated at 4°C

Efficiency Standards: (These are used to quantify the efficiency of the digestion as they have a known concentration)

- Add 0.5 ml of stock N solution to volumetric flask (50ml)
- bring to 50 ml using 0.5 M  $K_2SO_4$
- Run 10 samples of the standard solution
- \*Prepare a new standard solution with each autoclave run

### Preparing Samples:

- Add the following to each borosilicate tube:
  - 2.5 ml of sample extract
  - 1.0 ml of oxidizing solution
- Immediately cap the tubes tightly

-Prepare the standard sample in the same way, i.e. 2.5 ml standard and 1.0 ml of oxidizing solution

Preparing Matrix solution:

- Add 28.58 ml oxidizing solution to 100 ml volumetric flask
- Bring to volume using 0.5 M K<sub>2</sub>SO<sub>4</sub> (71.42 ml)
- Separate into 2 autoclaveable glass bottles to increase surface to volume ratio in bottles. Place caps on loosely.

Autoclave

- Place test tube racks with samples and glass bottles in an autoclaveable tray.
- Fill the tray with water, to the depth of the solution in the tubes, to minimize leaks due to rapid changes in temperature and pressure
- Autoclave samples, standards and matrix solution for 50 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 plate reader right away, do not continue with the following steps. Combine the matrix solution into one bottle and store in refrigerator. Samples can be stored as long as they are sealed tightly and are not opened until they are ready to be analyzed.

After Autoclave:

- When samples have cooled, add 2.5 ml of DI water, cap and shake
- When matrix solution has cooled, combine solutions into one glass bottle and add 71.42 ml of DI water
- 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 1ul of 0.5 M ascorbic acid to unfumigated samples and shake to mix
  - add 2ul of 0.5 M ascorbic acid to fumigated samples and shake to mix

Microplate Analysis:

- Follow nutrient analysis protocol for nitrate
- Use the autoclaved matrix as the matrix to dilute the stock nitrate solution

Reference for ascorbic acid treatment:

Williams, BL, CA Shand, M Hill, C O'Hara, S Smith, and ME Young. 1995. A procedure for the simultaneous oxidation of total soluble nitrogen and phosphorus in extracts of fresh and fumigated soils and litters. Commun. Soil Sci. Plant. Anal. 26: 91-106.