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Persulfate Digest for Water Total N and Total P

Introduction

This digest is applied to water samples to oxidize the nitrogen from all N compartments into nitrate and the phosphorus from all P compartments into orthophosphate. The resulting digests are analyzed by automated colorimetry (Alpkem or Technicon) for nitrate-N and orthophosphate-P. It is both safer and more effective than traditional Kjeldahl techniques. It is based on the method of Koroleff 1983 (3 below) as modified by Qualls 1989 (5).

Equipment

1) Chemical autoclave

2) 13x100 mm glass screw-cap culture tubes with teflon-lined caps. (Tubes are acid-washed in 20% HCl and muffled at 500 degrees C for two hours. Caps are acid-washed in 50% HCl)

3) autoclave-safe test tube racks

4) 100 ml, 200 ml or 500 ml acid-washed volumetric flask for oxidizing reagent (depending on how much reagent is needed)

5) 500 ml acid-washed volumetric flask for 3.75M NaOH stock

6) 1000 ul and 5000 ul automatic pipetters

7) weigh boats and clean chemical spatula

Reagents

1) fresh deionized H2O

2) low-N potassium peroxydisulfate (e.g. Fisher P282-100)

3) boric acid (e.g. Baker 0084-01)

4) low-N NaOH if stock is needed

5) EPA-certified Nutrient 2 quality control digest standard

NaOH stock:

Place ~350 ml diH2O in 500 ml volumetric on stir plate. Add 75.0 g NaOH. Stir to dissolve; remove stir bar and bring to volume. Cap with parafilm and invert to mix. Allow to stand ~30 minutes and recheck volume.

Oxidizing reagent:

Place clean volumetric of appropriate size on stir plate; into it rinse in the appropriate amounts of reagent from the table below with diH2O. Bring to about 80% of flask volume with diH2O and stir to dissolve; takes ~15 minutes on stir plate (gentle warming may help.) When dissolved, remove stir bar and

bring to final volume with diH2O. Cap with parafilm and invert to mix. Allow to stand ~30 minutes and recheck volume.

Reagent vol (mL)	100	200	250	500
persulfate (g)	5.2	10.4	13	26
boric acid (g)	3.12	6.24	7.8	15.6
NaOH stock (mL)	10	20	25	50

(This reagent may be stored 7 days at room temperature. Crystalizes when refrigerated.)

EPA Nutrient-2:

10 ml concentrate from ampoule in 1000 ml diH2O (or 5 ml concentrate in 500 ml.) yields 5.00 mg/liter total nitrogen and 1.50 mg/liter total phosphorus.

Procedure

1) bring samples to room temperature if chilled or frozen

2) make up fresh digest reagent, and NaOH stock if needed

3) obtain acid-washed, muffled digest tubes; label them.

4) for unknowns and EPA2 samples:

on first pass through the sample set, pipette 5 ml sample into each labeled digest tube. Cap loosely to exclude dust.
on second pass, pipette 1 ml oxidizing reagent into each digest tube. Cap tightly and mix well (invert several times.)

5) For reagent blanks, pipette only 1 ml oxidizing reagent into tube and cap tightly. (N.B., take care! Qualls (5) p.136: "For low level samples the variability in the reagent blanks determines the limit of detection, not the error associated with the NO3 and PO4 analyses themselves.")

6) Place capped tubes in autoclave, 30 minutes on liquid cycle. (= 30 minutes on "sterilize" in addition to all other cycle segments. If using pressure-cooker field method, time 30 minutes after coming to canning temperature in addition to warmup and cooldown times.)

7) After tubes are cool, add 5 ml diH2O to all *reagent blank tubes* so that the total volume of liquid in these tubes is the same as in the others. (N.B. Qualls (5) p.136: "Since distilled or deionized water contains significant N, the dilution water [*for the blanks*] is added after the digestion.")

8) Analyze digest-tube contents on Alpkem using the nitrate-nitrite and orthophosphate manifolds.

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Post-analysis calculations

1) Take the mean of the reagent blank determined values. Throw out any that are >2 std. deviations above the mean (for nitrogen in particular this indicates that the tube cap has cracked during the autoclave step and admitted atmospheric N to the tube.)

2) To compensate for color absorption by the digest reagents, subtract the mean reagent blank N and P values from the Alpkem determined values for each unknown or EPA2 sample.

3) The effect of diluting the samples by the addition of digest reagents must be reversed:

$$df = \frac{\text{sample volume} + \text{reagent volume}}{\text{initial sample volume}}$$

In the case of the above procedure, where initial sample volume is 5 ml and reagent volume is 1 ml,

$$df = \frac{5 \text{ ml sample} + 1 \text{ ml reagent}}{5 \text{ ml sample}} = 1.2$$

Find the actual value of the undiluted sample by multiplying the determined value (after reagent blank subtraction) by the df.

True analyte concentration = (Alpkem raw determined value - rblank value)*(df)

Comments

1) Successful digests have pH in the range 5 to 8; incomplete digests are ~2. This can be checked with wide-range pH paper. We have observed no reliable correlation between final digest pH and the yellow color developed in some digests, so the color cannot be used to spot incomplete digests.

2) Instead of using reagent blanks, it is possible to digest the calibration standards (including water blanks, i.e. calibration standards of content zero), the D3 (recalibrant) and ref3 (reference check) and the W (baseline drift correction) cups. Thus with digest reagent in both samples and calibants, the reagent's contribution to total absorbance will be compensated for automatically. (This strategy is of course useable only if all samples in the run are using the same reagent/diluent ratio.)

It may be necessary to have different dilutions of the EPA2 QC standards for nitrate and phosphate to get both into the optimum manifold range (e.g. if the PO4 manifold range is 0.2-1 ppm while the expected TP content of EPA2 is 1.5 ppm PO4, the QC digests will be offscale for phosphate unless diluted.) Perform these dilutions before digestion and then use the same reagent/diluent ratio for everything.

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References

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