

METHOD #: 351.2 Pending Approval for NPDES (Issued 1978)

TITLE: Nitrogen, Kjeldahl, Total (Colorimetric, Semi-Automated Digester, AAI)

ANALYTE: CAS # N Nitrogen 7727-37-9

INSTRUMENTATION: Autoanalyzer

STORET No. 00625

1.0 Scope and Application

1.1 This method covers the determination of total Kjeldahl nitrogen in drinking and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines. The applicable range of this method is 0.1 to 20 mg/L TKN. The range may be extended with sample dilution.

2.0 Summary of Method

2.1 The sample is heated in the presence of sulfuric acid, K_2SO_4 and $HgSO_4$ for two and one half hours. The residue is cooled, diluted to 25 mL and placed on the AutoAnalyzer for ammonia determination. This digested sample may also be used for phosphorus determination.

3.0 Definitions

3.1 Total Kjeldahl nitrogen is defined as the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described below.

3.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free-ammonia value (Method 350.2, Nitrogen, Ammonia, this manual) from the total Kjeldahl nitrogen value.

4.0 Sample Handling and Preservation

4.1 Samples may be preserved by addition of 2 mL of conc H_2SO_4 per liter and stored at 4°C. Even when preserved in this manner, conversion of organic nitrogen to ammonia may occur. Therefore, samples should be analyzed as soon as possible.

5.0 Apparatus

5.1 Block Digester-40

5.2 Technicon Manifold for Ammonia (Figure 1)

5.3 Chemware TFE (Teflon boiling stones), Markson Science, Inc., Box 767, Delmar, CA 92014)

6.0 Reagents

- 6.1 Mercuric Sulfate: Dissolve 8 g red mercuric oxide (HgO) in 50 mL of 1:4 sulfuric acid (10 mL conc H₂SO₄: 40 mL distilled water) and dilute to 100 mL with distilled water.
- 6.2 Digestion Solution: (Sulfuric acid-mercuric sulfate potassium sulfate solution): Dissolve 133 g of K₂SO₄ in 700 mL of distilled water and 200 mL of conc H₂SO₄. Add 25 mL of mercuric sulfate solution and dilute to 1 liter.
- 6.3 Sulfuric Acid Solution (4%): Add 40 mL of conc. sulfuric acid to 800 mL of ammonia free distilled water, cool and dilute to 1 liter.
- 6.4 Stock Sodium Hydroxide (20%): Dissolve 200 g of sodium hydroxide in 900 mL of ammonia-free distilled water and dilute to 1 liter.
- 6.5 Stock Sodium Potassium Tartrate Solution (20%): Dissolve 200 g sodium potassium -tartrate in about 800 mL of ammonia-free distilled water and dilute to 1 liter.
- 6.6 Stock Buffer Solution: Dissolve 134.0 g of sodium phosphate, dibasic (Na₂HPO₄) in about 800 mL of ammonia free water. Add 20 g of sodium hydroxide and dilute to 1 liter.
- 6.7 Working Buffer Solution: Combine the reagents in the stated order; add 250 mL of stock sodium potassium tartrate solution (6.5) to 200 mL of stock buffer solution (6.6) and mix. Add xx mL sodium hydroxide solution (6.4) and dilute to 1 liter. See concentration ranges, Table I, for composition of working buffer.
- 6.8 Sodium Salicylate/Sodium Nitroprusside Solution: Dissolve 150 g of sodium salicylate and 0.3 g of sodium nitroprusside in about 600 mL of ammonia free water and dilute to 1 liter.
- 6.9 Sodium Hypochlorite Solution: Dilute 6.0 mL sodium hypochlorite solution (clorox) to 100 mL with ammonia free distilled water.
- 6.10 Ammonium chloride, stock solution: Dissolve 3.819 g NH₄Cl in distilled water and bring to volume in a 1 liter volumetric flask. 1 ml= 1.0 mg NH₃-N.

7.0 Procedure

Digestion

- 7.1 To 20 or 25 mL of sample, add 5 mL of digestion solution (6.2) and mix (use a vortex mixer).
- 7.2 Add (4-8) Teflon boiling stones (5.3). Too many boiling chips will cause the sample to boil over.
- 7.3 With Block Digester in manual mode set low and high temperature at 160°C and preheat unit to 160°C. Place tubes in digester and switch to automatic mode. Set low temperature - timer for 1 hour. Reset high temperature to 380°C and set timer for 2 1/2 hours.
- 7.4 Cool sample and dilute to 25 mL with ammonia free water.

Colorimetric Analysis

- 7.5 Check the level of all reagent containers to ensure an adequate supply.
 - 7.6 Excluding the salicylate line, place all reagent lines in their respective containers, connect the sample probe to the Sampler IV and start the proportioning pump.
 - 7.7 Flush the Sampler IV wash receptacle with about 25 mL of 4.0% sulfuric acid (6.3).
 - 7.8 When reagents have been pumping for at least five minutes, place the salicylate line in its respective container and allow the system to equilibrate. If a precipitate forms after the addition of salicylate, the pH is too low. Immediately stop the proportioning pump and flush the coils with water using a syringe. Before restarting the system, check the concentration of the sulfuric acid solutions and/or the working buffer solution.
 - 7.9 To prevent precipitation of sodium salicylate in the waste tray, which can clog the tray outlet, keep the nitrogen flowcell pump tube and the nitrogen Colorimeter "To Waste" tube separate from all other lines or keep tap water flowing in the waste tray.
 - 7.10 After a stable baseline has been obtained start the Sampler.
- 8.0 Calculations
- 8.1 Prepare standard curve by plotting peak heights of processed standards against concentration values. Compute concentrations by comparing sample peak heights with standard curve.
- 9.0 Precision and Accuracy
- 9.1 In a single laboratory (EMSL), using sewage samples of concentrations of 1.2, 2.6, and 1.7 mg N/L, the precision was +/- 0.07, +/- 0.03 and +/-0.15, respectively.
 - 9.2 In a single laboratory (EMSL), using sewage samples of concentrations of 4.7 and 8.74 mg N/L, the recoveries were 99 and 99%, respectively.

Bibliography

1. McDaniel, W.H., Hemphill, R.N. and Donaldson, W.T., "Automatic Determination of Total Kjeldahl Nitrogen in Estuarine Water", Technicon Symposia, pp. 362-367, Vol. 1, 1967.
2. Gales, M.E., and Booth, R.L., "Evaluation of Organic Nitrogen Methods", EPA Office of Research and Monitoring, June, 1972.
3. Gales, M.E. and Booth, R.L., "Simultaneous and Automated Determination of Total Phosphorus and Total Kjeldahl Nitrogen", Methods Development and Quality Assurance Research Laboratory, May, 1974.
4. Technicon "Total Kjeldahl Nitrogen and Total Phosphorus BD-40 Digestion Procedure for Water", August, 1974.
5. Gales, M.E., and Booth, R.L., "Evaluation of the Block Digestion System for the Measurement of Total Kjeldahl Nitrogen and Total Phosphorus", EPA-600/4-78-015, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

TABLE 1. CONCENTRATION RANGES (NITROGEN)

No.	Dilution loops				Approx. std. cal. setting	Range PPM N (±10%)	ml stock NaOH per liter working buffer solution
	Initial sample Sample line	Diluent line	Resample Resample line	Diluent line			
1	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	700	0-0.5	250
2	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	100	0-1.5	250
3	.16 (ORN/YEL)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	700	0-1	120
4	.16 (ORN/YEL)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	100	0-5	120
5	.16 (ORN/YEL)	.80 (RED/RED)	.16 (ORN/YEL)	.80 (RED/RED)	700	0-2	80
6	.16 (ORN/YEL)	.80 (RED/RED)	.16 (ORN/YEL)	.80 (RED/RED)	100	0-10	80

