TOTAL KJELDAHL NITROGEN (TKN)

TOTAL KJELDAHL NITROGEN
SEMI-MICRO KJELDAHL METHOD
ION SELECTIVE ELECTRODE METHOD

Background

Nitrogen is often the limiting factor in biological systems, especially systems that depend on bacteria for the breakdown of substances. Knowing how much nitrogen is present in a system can be very useful because a deficient supply of nitrogen can result in the inability of bacteria to grow. Obviously this situation is to be avoided.

Total Kjeldahl Nitrogen includes organic nitrogen compounds as well as ammonia nitrogen. This procedure allows for the measurement of nitrogen in amino acids, proteins, peptides and ammonia.

The ion selective electrode method described here is the most common method used at Vermont wastewater facilities for the determination of Total Kjeldahl Nitrogen. Digestion and distillation are required in all TKN methods for NPDES testing. After digestion and distillation, the following methods of TKN determination are also acceptable. Titration, Nesslerization, automated phenate, semi-automated block digester or potentiometric.

Interferences

a. Metals: The interference from metals can be eliminated with the addition of the NaI EDTA solution.

b. *High Nitrate Concentrations: Nitrate in excess of 10 mg/L can cause a negative interference. This interference can be eliminated by running the sample through an anion exchange resin (chloride form) to remove the nitrate prior to TKN analysis.

   * Unfortunately this technique is far less successful when analyzing wastewater samples when the suspended solids concentration is high.

c. Organic Matter: Large amounts of organic matter can cause a positive interference. To negate this interference add 10 ml concentrated H_2SO_4 to the digestion flask per gram of organic matter. (The organic matter can be estimated from COD results by assuming that 3 grams COD equals 1 gram of organic matter.)

d. Inorganic salts and solids cause boiling temperature to rise causing pyrolytic loss of nitrogen - addition of more H_2SO_4 gives reasonable results (1 ml additional H_2SO_4 per gram of salt).

   *NOTE: If acid addition is necessary for sample analysis the same volume of acid should be added to the blank.

Equipment

pH Meter - capable of accepting ion selective electrodes and measuring in millivolt units.
Ammonia probe - such as the Orion 95-10
Magnetic Stirring Device - with TFE coated stirring bar
Digestion apparatus - A Kjeldahl digestion apparatus with appropriately sized digestion flasks and suction to remove SO_3 fumes and water.
Block digester (Technicon BD-40) or individual heating elements or hot plates
Various graduated and volumetric pipets

Reagents

*NOTE: If samples are to be distilled see additional reagents list in Appendix A (as required for NPDES testing).

AMMONIA FREE DISTILLED WATER

Traces of ammonia in distilled water can be removed by adding 0.1 milliliter concentrated H_2SO_4 per liter to distilled water and redistilling. It's a good idea to throw out the first 100 milliliters of the distillate.

MERCURIC SULFATE SOLUTION

Dissolve 8 grams mercuric oxide (HgO) into 100 milliliters *6N H_2SO_4. This solution can be kept refrigerated at 4°C for up to one year.

* 6N H_2SO_4 is prepared by pouring 167 milliliters concentrated (36N) H_2SO_4 into a one liter volumetric flask containing about 500 milliliters distilled water. Then bring the total volume to one liter with distilled water.

DIGESTION REAGENT

a) To 650 milliliters distilled water add 200 milliliters concentrated H_2SO_4 **CAREFULLY!**

b) Dissolve 134 grams K_2SO_4 into the dilute H_2SO_4 solution

c) Then slowly add, while stirring, 25 milliliters of the mercuric sulfate solution (from #2 above). Dilute this solution to one-liter with distilled water. This solution should be stored at room temperature to prevent crystallization. It can be kept for three months.

10N NaOH

Two liters of 10 N NaOH can be prepared by partially filling a two-liter wide mouth flask with distilled water. Adding 800 grams NaOH and diluting to two liters with distilled water. This solution can be stored at room temperature for up to six months.

10N NAOH, NaI, EDTA SOLUTION

NOTE: This solution is needed only if interference from metals is suspected in the sample.

To approximately 500 milliliters distilled water add 800 grams NaOH, 600 grams NaI, and 4 grams EDTA. Dilute to 2 liters with distilled water. This solution can be stored for up to six months at room temperature.

STOCK $(NH_4)_2$ SO_4 STANDARD SOLUTION 100 mg/liter TKN-N

Dry $(NH_4)_2SO_4$ for at least one hour at 105°C. Cool for one hour in a desiccator.

Weigh out 0.4706 grams $(NH_4)_2SO_4$ add this to approximately 500 milliliters distilled water and dilute to one-liter with distilled water. This solution can be stored for 30 days refrigerated at 4°C.

WORKING $(NH_4)SO_4$ STANDARD SOLUTION 10 mg/liter TKN-N

Dilute 10 milliliters of the stock $(NH_4)_2$ SO_4 standard solution to 100 milliliters with distilled water. This solution must be used the day it is prepared.

Calibration Standards (select 5 which bracket analytical range.)

	Value of Std. in mg/l	**mg/50 ml**	**100 mg/l Std. per 50 ml**	**10 mg/l Std. per 50 ml**
	30 mg/l	1.5 mg	15 ml	
	20 mg/l	1.0 mg	10 ml	
**********	10 mg/l	0.5 mg	5 ml	
	8 mg/l	0.4 mg	4 ml	
	7 mg/l	0.35 mg	3.5 ml	
Normal range for wastewater	4 mg/l	0.2 mg	2 ml	
	2 mg/l	0.1 mg		10 ml
	1 mg/l	0.05 mg		5 ml
**********	0.6 mg/l	0.03 mg		3 ml
	0.5 mg/l	0.025 mg		2.5 ml
	0.4 mg/l	0.02 mg		2 ml
	0.2 mg/l	0.01 mg		1 ml

Procedure

1) Digestion

   a. On the day the analysis will be performed, rinse boiling flasks with dilute acid (10% H_2SO_4 or 20% HCL). Rinse three times with distilled water.

   b. Using the table provided, prepare 5 calibration standards suitable to the expected range of samples to be analyzed.

   c. Shake samples vigorously before dispensing via a graduated cylinder. 50 ml is total volume. If dilution is needed, use less and record the dilution factor on the data sheet.

   d. To each flask, add boiling stones and 10 mls of digestion reagent. (Wide-mouth serological pipettes give the quickest delivery.)

   e. Turn on vacuum for all digestion racks that are to be used - plug the unused manifold intakes with empty digestion flasks. Adjust the boiling rate and allow to boil until the solution clears or is pale straw color.

   f. Heat the solution for 30 minutes after the dense white SO_3 fumes appear.

   g. Allow flasks to cool.

   h. If you are using the type of flask that the ammonia probe can fit into: simply dilute to 50 milliliters with distilled water (in the flask); cover and refrigerate.

      If the probe will not fit into the flask: Measure out 44 milliliters distilled water into a graduated cylinder. Use this volume of distilled water to wash the contents of the digestion flask into a clean dry container capable of accommodating the ammonia probe. Refrigerate. This is a good stopping point if the procedure cannot be finished in one day. If distillation is required, go to Appendix A; if not, proceed to Section 2.

2) TKN Ammonia Probe: METER CALIBRATION (probe should be stored in filling solution when not in use).

   a. Let the meter warm up for about 20 minutes before reading. Change the filling solution before each probe use (2.5 ml).

   b. Place 100 mls distilled water into a 200 ml Berzelius beaker. Add a magnetic stirring bar.

c. Using a graduated pipette add 1 ml 10 N NaOH while stirring.

d. Place washed/dried probe into solution. Then set the meter to REL MV scale. Check for air bubbles on the probe membrane. If there are bubbles present remove them by gently tapping the probe against the side of the beaker or by removing and then reinserting the probe. Most ammonia probes are designed with a method for removing air bubbles from the probe body. Usually, this can be accomplished by gently pulling up on the probe lead wire and releasing.

e. Using a volumetric pipette add 1.0 ml of 100 mg/L $(NH_4)_2SO_4$ stock solution into the beaker.

f. Wait for the meter to stabilize then adjust the meter to read 000.0 by turning the calibration knob or pushing the zero button.

g. Using a 10.0 ml volumetric pipette transfer 10 mls of 100 mg/l $(NH_4)_2SO_4$ stock solution into the beaker. Once the meter stabilizes record the volume on a data sheet. The calibration is okay if the reading is -57.0 mv +/- 3 mv. If the response is slow the membrane should be changed according to instructions in the Ammonia Probe instruction pamphlet. If -57.0 ± 3 mv cannot be achieved, do an inner body check as outlined in the pamphlet. If that fails several times, a new probe will have to be purchased.

3) TKN Ammonia Probe: Sample Analysis (Treat standards exactly the same as samples.)

<u>IMPORTANT NOTE:</u> If samples are distilled use only 1 ml of NaOH. If samples are not distilled use a total of 10 mls NaOH as described below.

a. Add 6 ml 10 N NaOH to each Nalgene bottle containing digested sample.

b. Cool digested samples and standards in ice bath. Standards and samples should be at the same temperature.

c. Place a stir bar into the sample, place the rinsed and dried probe below the sample surface. Check for bubbles on the probe membrane - remove any bubbles by tapping the probe against the bottle (keeping the stir bar at the lowest possible RPM wile providing adequate mixing will help cut down on bubble formation).

d. Allow the meter to recover to a value at least as positive as the value corresponding to the lowest concentration standard.

e. If sample is suspected of containing interfering metals:

Inject 4 mls 10 N NaOH, NaI, EDTA into sample using a graduated pipette. Check for air bubbles.

If sample does not contain interfering metals:

Inject 4 mls 10 N NaOH solution into sample using a graduated pipette. Check for air bubbles.

If you are unsure whether or not the sample contains metals use the NaOH, NaI, EDTA solution.

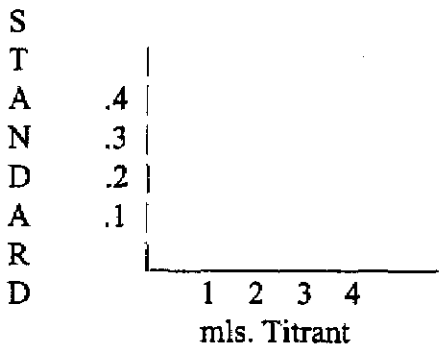
f. Record the lowest (most negative) MV reading. Be sure to watch the meter carefully so as not to miss the "bottom".

g. After removing the probe and stir bar from the sample, rinse and dry.

Calculations

Plot a curve for your standards, using semilogarithmic graph paper.

Example:



Then from this curve obtain your sample results.

Then calculate your final result by applying this formula.

$$\text{mg } NH_3\text{ - N/Liter} = A \times B \times \left[\frac{101 + C}{101} \right]$$

where:

A = dilution factor

B = concentration of NH_3-N/Liter from calibration curve

C = volume of 10 N NaOH added in excess of:

1 ml if the sample was distilled

or 10 mls if the sample was not distilled

TOTAL KJELDAHL NITROGEN
(Ion Selective Electrode Method)
TROUBLESHOOTING GUIDE

NOTE: This troubleshooting guide has been taken in its entirety from the Orion Model 95-12 Ammonia Electrode Instruction Manual.

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Off-scale or over-range reading	Defective Meter	Perform meter checkout procedure (see meter instruction manual)
	Defective inner body	Refer to Troubleshooting Guide (check inner body operation)
	Electrodes not plugged in properly	Unplug electrodes and reseat
	Internal filling solution not added	Fill outer body of electrode with proper amount of internal filling solution
	Air bubble on membrane	Remove bubble by redipping electrode
	Electrodes not in solution	Put electrodes in solution
Noisy or unstable readings (erratic - rapidly changing)	Insufficient internal filling solution	Fill outer body of electrode with proper amount of interal filling solution
	Defective Meter	Perform meter check out procedure (see meter instruction manual)
	Bottom cap loose	Ensure that bottom cap is screwed on tight enough to close gap between bottom cap and body
	Defective inner body	Check inner body operation
	ISA not used	Use recommended ISA, Orion Cat. No. 951211
	Meter or stirrer improperly grounded	Check meter and stirrer for grounding

TOTAL KJELDAHL NITROGEN
(Ion Selective Electrode Method)
TROUBLESHOOTING GUIDE (continued..)

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Drift (Reading slowly changing in one direction)	Internal filling solution leakage	Ensure that membrane is installed properly
	Incorrect internal filling solution	Refill outer body of electrode using filling solution shipped with electrode
	Total level of dissolved species above 1M	Dilute solution
	Electrode in sample too long; NH_3 loss	Reduce surface-area-to-volume ratio, slow rate of stirring, avoid high temperatures
	Membrane failure (wet, perforation, discoloration)	Replace membrane
	Solutions not at constant temperature	Allow solutions to come to room temperature before use
	Heat generated by Magnetic stirrer	Place insulating material between stirrer and beaker
	Defective inner body	Check inner body operation
	Electrode exposed to air for extended period	Hold electrode by outer body and pull up on electrode cable. Interal filling solution will flow under membrane and restore electrode response.
	Samples and standards at different temperatures	Allow solutions to come to room temperature before measurement

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Low slope or No slope	Standards contaminated or incorrectly made	Prepare fresh standards
	ISA not used	Used recommended ISA, Orion Cat. No 951211
	Standard used as ISA	Use ISA!
	Electrode exposed to air for extended period	Hold electrode by outer body and pull up on electrode cable. Interal filling solution will flow under membrane and restore electrode response.
	Membrane failure (wet, perforation, discoloration)	Replace membrane
	Defective inner body	Check inner body operation
"Wrong Answer" (But calibration curve is OK)	Incorrect scaling of semilog paper	Plot millivolts on the linear axis. On the log axis, be sure concentration numbers within each decade are increasing with increasing concentration
	Incorrect sign	Be sure to note sign of millivolt value correctly
	Incorrect standards	Prepare fresh standards
	Wrong units used	Apply correct conversion factor: 10^{-3}M = 17 ppm as NH_3 = 14 ppm as N
	Complexing agents in sample	Use known addition or titration techniques, or a decomplexing procedure
	ISA added to standards and not samples	Add same proportion of ISA to standards and samples

TOTAL KJELDAHL NITROGEN
(Ion Selective Electrode Method)
TROUBLESHOOTING GUIDE (continued...)

PROBLEM	MOST LIKELY CAUSE	SOLUTION
Frequent build-up of deposits on ammonia probe membrane	Precipitation of hydroxides from sample	Add EDTA (Ethylene dramine tetraacetic acid) to the NaOH-NaI solution

Quality Control for
TOTAL KJELDAHL NITROGEN

Document

* Supply Water Quality Conductivity
Ammonia Free

* **ALL** reagents must be made with ammonia free distilled water.

Sampling

Sample Type
Sample Time
Duration of Composite
Type of Composite
- Time/Flow - include discrete volumes
- Flow - include sample volume/discharge volume
- Straight - document <10% flow rate change during sampling event

Sample Location
Preservation - H_2SO_4 <pH2 (2mls/Liter)
- Volume of H_2SO_4 used
- Refrigerate at 4°C
- Hold Time - The maximum allowable hold time if properly preserved and refrigerated is 28 days. However, the sample should be analyzed as soon as possible due to conversion of organic nitrogen to ammonia.

Glassware

Acid washed - Distilled water rinses - Class A

Equipment

Distillation Apparatus: Properly "steamed out" before each use.
- Properly cleaned between samples

Analytical Results

Blank - Treated just as sample result
Standards - Treated just as sample. Number and concentrations used.
Preparation Method results
Standard Curve

Duplicate - Replicate Schedule

REPORTING TKN DATA

TKN Bench Sheet
Sample Type:
Sampling Time & Date:
Sample Volume:
Sample Preservation:
Analyst:
Analysis Time and Date:
Method:
Blank Result:
Standards:
Concentrations Used:
Standard Curve:
Results of Individual Standards:
Sample Results:
Raw Data (millivolt readings):
Plotted Results on Curve:
Calculations:

Date: 06-14-95
Analyst: A D F
Probe Slope Check: -57.2 MV

SAMPLE

TKN Report Sheet

	Lab ID	ml Sample	MV	Conc (mg/l)	COMMENTS
1	Blank	50 DI Water	-9.1	0.054	
2	Standard 0.2	1 ml 0.01 mg/l	-40.1	.186	Back Calculations
3	Standard 0.4	2 ml 0.01 mg/l	-62.9	.463	corr = .994
4	Standard 1.0	5 ml 0.01 mg/l	-80.5	.935	
5	Standard 4.0	20 ml 0.01 mg/l	-116.7	3.968	
6	QC TV 4.8	25	-105.0	4.92	% Bias= $\frac{4.92-4.80}{4.80}$ = +2.5%
7	12345	50	-43.1	0.16 <DL	
8	12341	2	-83.7	26.5	%RPD= $\frac{28.4-26.5}{27.5}$ = $\frac{1.9}{27.5}$ =6.9%
9	12341 Dup	2	-85.4	28.4	
10	12342	25	-103.2	4.57	%Rec= $\frac{(0.54)(53)-<DL}{3(10)}$=95.4%
11	12343	50	-64.2	0.43	
12	12344	25	-111.3	6.34	
13	12346	50	-105.1	2.44	
14	Spike 12345	50 + 3	-70.5	0.54	3 ml 10 mg/l spike
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References

The selective ion electrode method for the determination of Total Kjeldahl Nitrogen can be found on pages 4-81 through 4-94 in the 18th Edition of Standard Methods for the Examination of Water and Wastewater - Methods 4500 - NH_3F Pages 4-81 and 4-82, also 4500 - N-org (A,B,C) Pages 4-94 through 4-97

and in EPA's Methods for Chemical Analysis of Water and Wastes. Method 351.4 Storet No .00625

The troubleshooting section can be found in the Orion Model 95-12 Ammonia Electrode Instruction Manual

APPENDIX I

TOTAL KJELDAHL NITROGEN DISTILLATION

Reagents

NaOH - THIOSULFATE SOLUTION 500 g NaOH, 25 g $Na_2S_2O_3.5H_2O$ in 1 liter D.I. water.

SULFURIC ACID 0.1 ml conc. H_2SO_4 to 10 ml D.I. water in each 50 ml volumetric used to collect distillate.

Glassware Preparation

Steam out distillation apparatus before use.
Label 50 ml volumetric receiving flasks for all blanks standard and samples.
Add sulfuric acid described in reagent section to each flask.
Condenser temp. must be below 29°C.

Procedure

1) Quantitatively transfer digested sample, standard, or blank into distilling unit. Use D.I. water to make the total volume about 30 ml.

2) Add 10 ml NaOH - thiosulfate reagent.

3) Close both stopcocks and turn rheostat up to 110 to do distillation. Be sure deliver tube is below surface of sulfuric acid in receiving flask.

4) At end of distillation raise delivery tube out of liquid, then turn rheostat down to 30.

5) Open both stopcocks and drain chamber.

6) Rinse several times with D.I. water.

7) With both stopcocks open and stream off (at 30) add sample and reagent.

8) Lower delivery tube below liquid, close stopcocks and turn up to 110 for next distillation.

9) Do 2 distillation blanks at beginning and one after each high sample.

10) Bring distillates up to 50 ml volume with D.I. water.

11) Cover with parafilm and continue with analysis by phenate method (John) or by ammonia probe (see procedure section).

Reference

Standard Methods for the Examination of Water and Wastewater, 18th Edition, Method 4500-NH_3B