

METHOD #: 351.1 Approved for NPDES, CWA (Ed. Rev. 1974, 1978)

TITLE: Nitrogen, Kjeldahl, Total (Colorimetric, Automated Phenate)

ANALYTE: CAS # N Nitrogen 7727-37-9

INSTRUMENTATION: Autoanalyzer

STORET No. 00625

1.0 Scope and Application

- 1.1 This automated method may be used to determine Kjeldahl nitrogen in surface and saline waters. The applicable range is 0.05 to 2.0 mg N/L. Approximately 20 samples per hour can be analyzed.

2.0 Summary of Method

- 2.1 The sample is automatically digested with a sulfuric acid solution containing potassium sulfate and mercuric sulfate as a catalyst to convert organic nitrogen to ammonium sulfate. The solution is then automatically neutralized with sodium hydroxide solution and treated with alkaline phenol reagent and sodium hypochlorite reagent. This treatment forms a blue color designated as indophenol. Sodium nitroprusside, which increases the intensity of the color, is added to obtain necessary sensitivity for measurement of low level nitrogen.

3.0 Definitions

- 3.1 Total Kjeldahl nitrogen is defined as the sum of free-ammonia and of organic nitrogen compounds which are converted to $(\text{NH}_4)_2\text{SO}_4$ under the conditions of digestion which are specified below.
- 3.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free- ammonia value from the total Kjeldahl nitrogen value. Also, organic Kjeldahl nitrogen may be determined directly by removal of ammonia before digestion.

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 refrigeration 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 Interferences

- 5.1 Iron and chromium ions tend to catalyze while copper ions tend to inhibit the indophenol color reaction.

6.0 Apparatus

- 6.1 Technicon AutoAnalyzer consisting of:
 - 6.1.1 Sampler II, equipped with continuous mixer.
 - 6.1.2 Two proportioning pumps.
 - 6.1.3 Manifold I.
 - 6.1.4 Manifold II.
 - 6.1.5 Continuous digester.
 - 6.1.6 Planetary pump.
 - 6.1.7 Five-gallon Carboy fume-trap.
 - 6.1.8 80°C Heating bath.
 - 6.1.9 Colorimeter equipped with 50 mm tubular flow cell and 630 nm filters.
 - 6.1.10 Recorder equipped with range expander.
 - 6.1.11 Vacuum pump.

7.0 Reagents

- 7.1 Distilled water: Special precaution must be taken to insure that distilled water is free of ammonia. Such water is prepared by passage of distilled water through an ion exchange column comprised of a mixture of both strongly acidic cation and strongly basic anion exchange resins. Furthermore, since organic contamination may interfere with this analysis, use of the resin Dowex XE-75 or equivalent which also tends to remove organic impurities is advised. The regeneration of the ion exchange column should be carried out according to the instruction of the manufacturer.
NOTE 1: All solutions must be made using ammonia-free water.
- 7.2 Sulfuric acid: As it readily absorbs ammonia, special precaution must also be taken with respect to its use. Do not store bottles reserved for this determination in areas of potential ammonia contamination.
- 7.3 EDTA (2% solution): Dissolve 20 g disodium ethylenediamine tetraacetate in 1 liter of distilled water. Adjust pH to 10.5-11 with NaOH (7.4).
- 7.4 Sodium hydroxide (30% solution): Dissolve 300 g NaOH in 1 liter of distilled water.
NOTE 2: The 30% sodium hydroxide should be sufficient to neutralize the digestate. In rare cases it may be necessary to increase the concentration of sodium hydroxide in this solution to insure neutralization of the digested sample in the manifold at the water jacketed mixing coil.
- 7.5 Sodium nitroprusside, (0.05% solution): Dissolve 0.5 g $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}\cdot 2\text{H}_2\text{O}$ in 1 liter distilled water.
- 7.6 Alkaline phenol reagent: Pour 550 mL liquid phenol (88-90%) slowly with mixing into 1 liter of 40% (400 g per liter) NaOH. Cool and dilute to 2 liters with distilled water.
- 7.7 Sodium hypochlorite (1% solution): Dilute commercial "Clorox"-200 mL to 1 liter with distilled water.. Available chlorine level should be approximately 1%. Due to the instability of this product, storage over an extended period should be avoided.
- 7.8 Digestant mixture: Place 2 g red HgO in a 2 liter container. Slowly add, with stirring, 300 mL of acid water (100 mL H_2SO_4 + 200 mL H_2O) and stir until cool. Add 100 mL 10% (10 g per 100 ml) K_2SO_4 . Dilute to 2 liters with conc. sulfuric acid (approximately 500 mL at a time, allowing time for cooling). Allow 4

- hours for the precipitate to settle or filter through glass fiber filter.
- 7.9 Stock solution: Dissolve 4.7193 g of pre-dried (1 hour at 105°C) ammonium sulfate in distilled water and dilute to 1.0 liter in a volumetric flask. 1.0 mL = 1.0 mg N.
- 7.10 Standard solution: Dilute 10.0 mL of stock solution (7.9) to 1000 mL. 1.0 mL = 0.01 mg N.
- 7.11 Using the standard solution (7.10), prepare the following standards in 100 mL volumetric flasks:

Conc., mg N/L	mL Standard Solution/100 mL
0.00	0.0
0.05	0.5
0.10	1.0
0.20	2.0
0.40	4.0
0.60	6.0
0.80	8.0
1.00	10.0
1.50	15.0
2.00	20.0

8.0 Procedure

- 8.1 Set up manifolds as shown in Figures 1, 2, and 3.
- 8.1.1 In the operation of manifold No. 1, the control of four key factors is required to enable manifold No. 2 to receive the mandatory representative feed. First, the digestant flowing into the pulse chamber (PC-1) must be bubble free; otherwise, air will accumulate in A-7, thus altering the ratio of sample to digestant in digester. Second, in maintaining even flow from the digester helix, the peristaltic pump must be adjusted to cope with differences in density of the digestate and the wash water. Third, the sample pick-up rate from the helix must be precisely adjusted to insure that the entire sample is aspirated into the mixing chamber. And finally, the contents of the "Mixing Chamber" must be kept homogeneous by the proper adjustment of the air bubbling rate.
- 8.1.2 In the operation of manifold No. 2, it is important in the neutralization of the digested sample to adjust the concentration of the NaOH so that the waste from the C-3 debubbler is slightly acid to Hydrion B paper.
- 8.1.3 The digester temperature is 390°C for the first stage and 360°C for the second and third stages.
- 8.2 Allow both colorimeter and recorder to warm up for 30 minutes. Run a baseline with all reagents, feeding distilled water through the sample line. Adjust dark current and operative opening on colorimeter to obtain stable baseline.
- 8.3 Set sampling rate of Sampler II at 20 samples per hour, using a sample to wash ratio of 1 to 2 (1 minute sample, 2 minute wash).
- 8.4 Arrange various standards in sampler cups in order of increasing concentration. Complete loading of sampler tray with unknown samples.

8.5 Switch sample line from distilled water to sampler and begin analysis.

9.0 Calculation

9.1 Prepare standard curve by plotting peak heights of processed standards against concentration values. Compute concentration of samples by comparing sample peak heights with standard curve.

9.2 Any sample that has a computed concentration that is less than 10% of the sample run immediately prior to it must be rerun.

10.0 Precision and Accuracy

10.1 Six laboratories analyzed four natural water samples containing exact increments of organic nitrogen compounds, with the following results:

Increment as Kjeldahl-Nitrogen mg N/liter	Precision as Standard Deviation Kjeldahl-N mg N/liter	Bias, %	Accuracy as Bias, mg N/liter
1.89	0.54	-24.6	-0.46
2.18	0.61	-28.3	-0.62
5.09	1.25	-23.8	-1.21
5.81	1.85	-21.9	-1.27

Bibliography

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2. McDaniel, W. H., Hemphill, R. N., Donaldson, W. T., "Automatic Determination of Total Kjeldahl Nitrogen in Estuarine Waters". Presented at Technicon Symposium on Automation in Analytical Chemistry, New York, October 3, 1967.
3. B. O'Connor, Dobbs, Villiers, and Dean, "Laboratory Distillation of Municipal Waste Emuents". JWPCF 39, R 25, 1967.

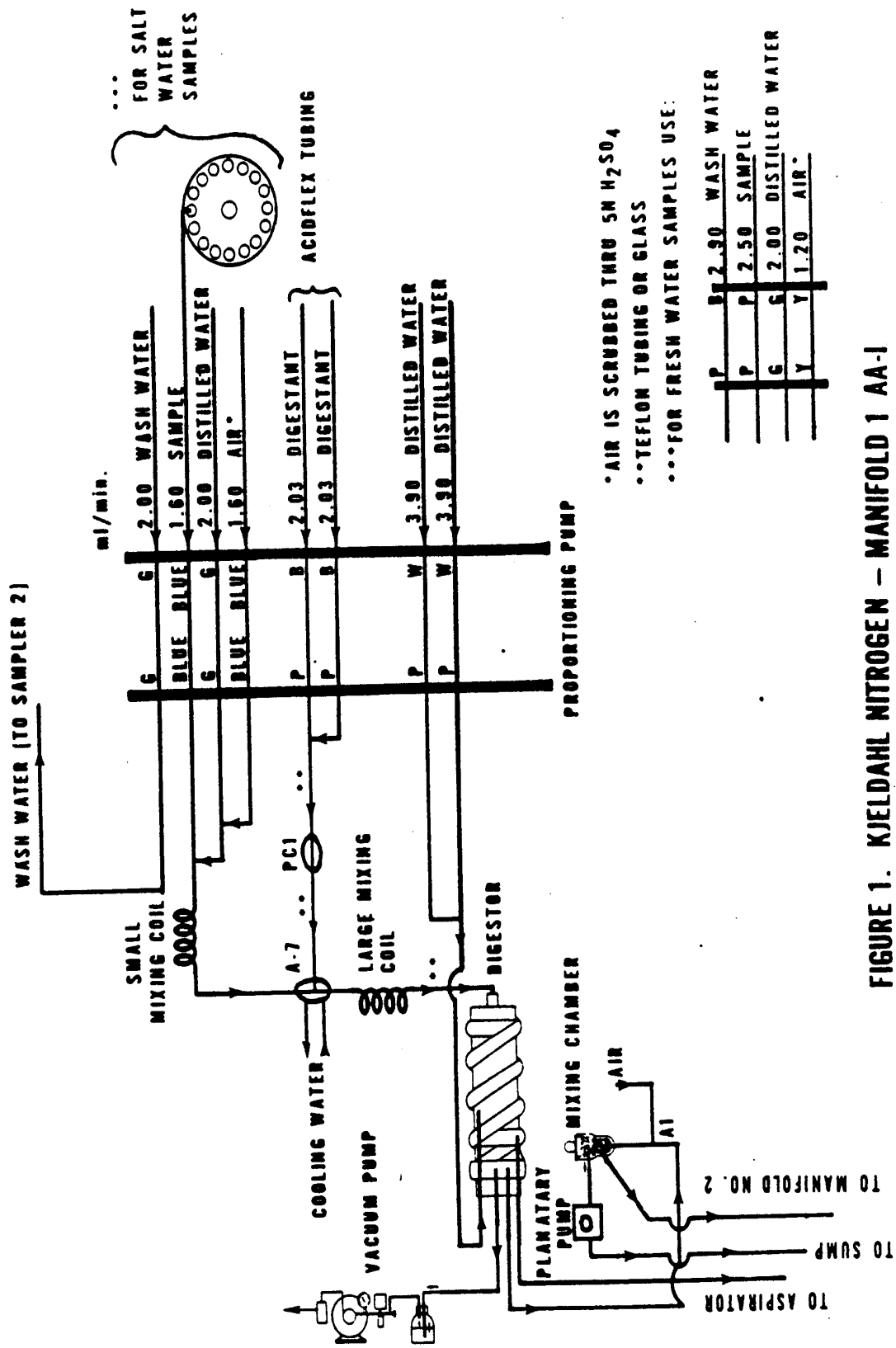


FIGURE 1. KJELDAHL NITROGEN - MANIFOLD 1 AA-I

CONTINUOUS DIGESTER & MIXING CHAMBER ASSEMBLY

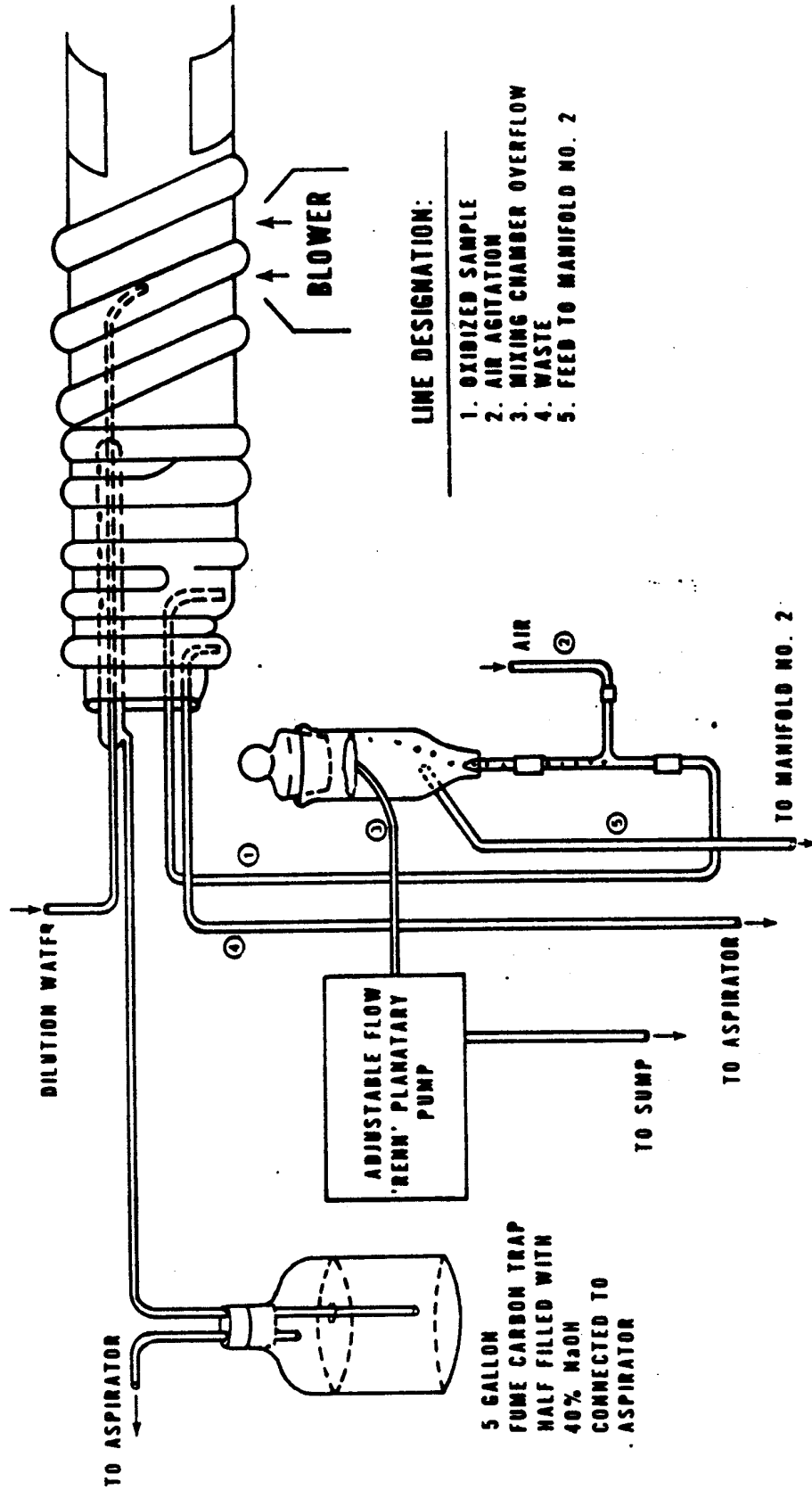


FIGURE 2. KJELDAHL NITROGEN AA-1

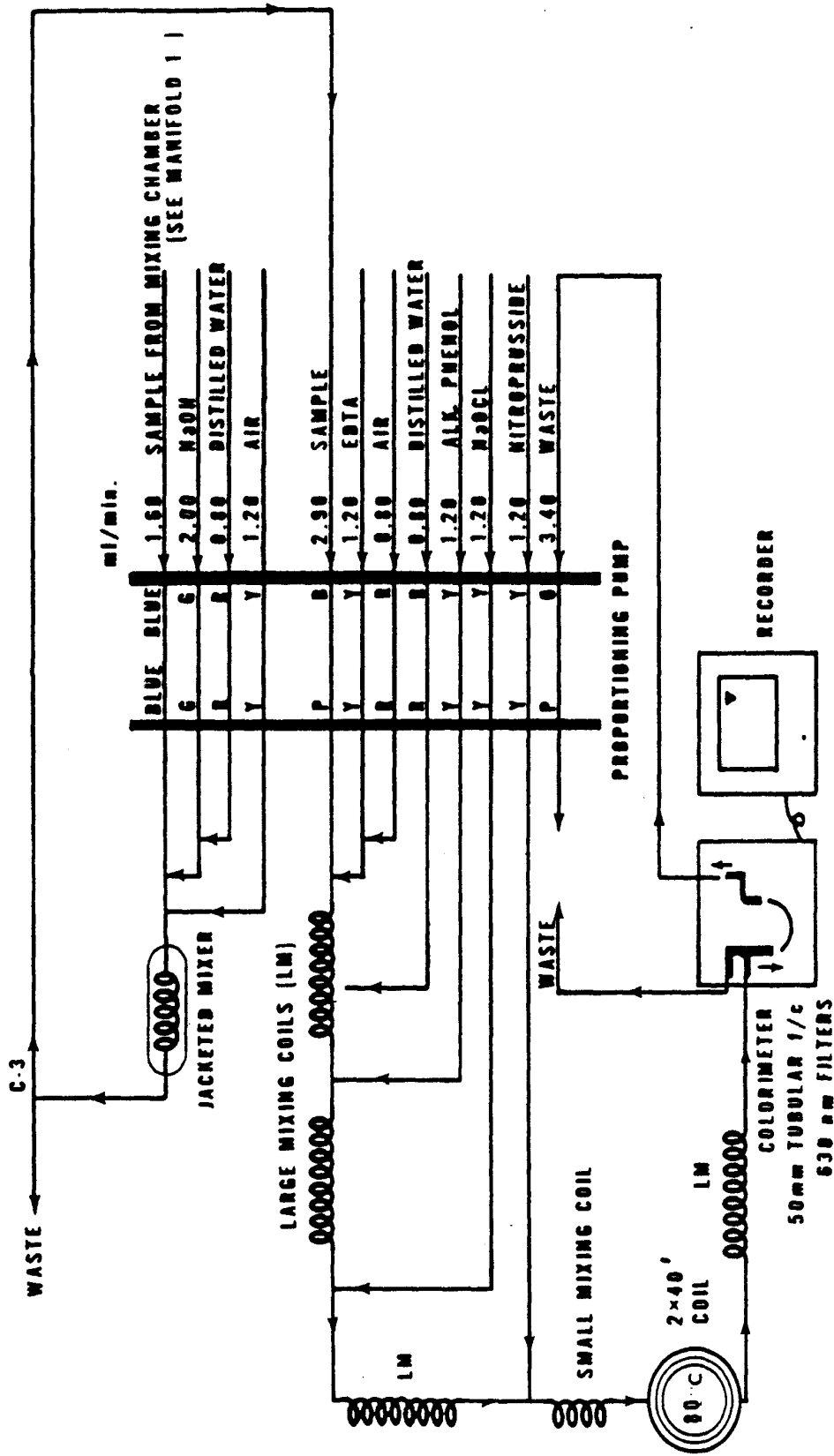


FIGURE 3. KJELDAHL NITROGEN MANIFOLD 2. AA-1