UNIVERSITY OF KWAZULU-NATAL	*Standard Operating Procedure* PRG pollution research group	**Effective Date:** 20 June 2013	**Version:** ***003***
		Reviewed:	
SOP_Chem_010 Chemical Analysis_ Total Kjeldahl Nitrogen			Page #: ***1 of 5***

Standard Operation Procedure – Total Kjeldahl Nitrogen

1. Scope and Application

- This Kjeldahl method determines nitrogen in the tri-negative state. This fails to account for nitrogen in the form of ozide, azine, azo, hydrazone, nitrate, nitrite, nitro.
- Kjeldahl nitrogen is the sum of organic nitrogen and ammonia nitrogen. Organic nitrogen: includes proteins, peptides, nucleic acids and urea.
- Typical organic nitrogen concentrations vary from a few hundred mg/L in some lakes to more than 20mg/L in raw sewage.
- This macro-Kjeldahl method is applicable for samples containing either low or high concentrations of organic nitrogen but requires but requires a relatively large sample volume for low concentrations.

2. Summary

- In the presence of sulphuric acid, potassium sulfate, and cupric sulphate catalyst, amino nitrogen of many organic materials is converted to ammonium.
- Free ammonia is also converted to ammonium.
- After addition of base, the ammonia is distilled from an alkaline medium and absorbed in boric or sulphuric acid.
- The ammonia may be determined colorimetrically, by ammonia selective electrode or by titration with a standard mineral acid.
- The titrimetric and selective electrode methods of measuring ammonia in the distillate are suitable for determining a wide range of organic nitrogen concentrations.

3. Apparatus and Glassware

- Velp Digestion apparatus: DK 20 slim. F30100181, S/N 214130 .
- SMS Scrubber F307C0199
- JP recirculating water pump F30620198

- Kjeldahl flasks with a total capacity of 800ml yield the best results. Digest over a heating device adjusted so that 250ml water at an initial temperature of 25ºC can be heated to a rolling boil in about 5 min. The temperature range should be 375 to 385ºC for effective digestion.
- Distillation apparatus: UDK 127 Distilling Unit F30200183 s/N 126145
- 300ml TKN flasks.

4. Interferences

- The most reliable results are obtained on fresh samples.
- If an immediate analysis is not possible, preserve samples for Kjeldahl digestion by acidifying to pH 1.5 to 2.0 with concentrated sulphuric acid and storing at 4°C.
- Do not use HgCl2 because it will interfere with ammonia removal.
- Nitrate: During Kjeldahl digestion, nitrate in excess of 10mg/L can oxidize a portion of the ammonia released from the digested organic nitrogen, producing N2O, resulting in a negative interference.
- Inorganic salts and solids: The acid and salt content of the Kjeldahl digestion reagent is intended to produce a digestion temperature of about 380°C.
- If the sample contains a very large quantity of salts or inorganic solids, the temperature may rise to 400°C during digestion at which point pyrolytic loss of nitrogen occurs. To prevent this increase in temperature add more sulphuric acid to maintain an acid-salt balance.

5. Collection, Sampling and Storage

- Collect samples in 1L plastic buckets.
- Preferably, analyse samples immediately after sampling.
- Store samples at 4 °C or freeze dry samples.
- Preserve wastewater samples by acidifying with concentrated sulphuric acid to pH 2 and faecal samples by freeze drying or freezing.
- Determine TKN on well- homogenised samples.

6. Safety Precautions

- Handle concentrated sulphuric acid with care.
- Always use safety goggles, gloves and laboratory coat while working in laboratory.
- Wear face shield and protect hands from heat produced when contents of the vessels are mixed.
- After the analysis, clean bottles and beakers with water then dry.
- Dispose used gloves after completion of analysis.
- Clean hands using antiseptic soap.
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.
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7. Sampling Preparation – Faecal Sludge

- Weigh out between 1.8g and 2g of well-mixed faecal sludge sample.
- Place the weighed out sample into a blender with 250ml of distilled water.
- Blend for 30 seconds.
- Transfer the blended mixture into a volumetric flask and top up to 1L with distilled water.
- Transfer the 1L solution to a plastic bottle and store in the cold room.

8. Reagents

Digestion Reagent

- Kjeldahl tablets or powder: Free of Hg, Se.
- 3.5g K_2SO_4 and 0.5g $CuSO_4$.

Boric Acid – 4%

- Dissolve 40g of Boric acid into 1L of distilled water.

Concentrated Sulphuric acid

Sulphuric acid – 0.1N

- Dissolve 2.8ml of concentrated sulphuric acid into 1L of distilled water.

Sodium Hydroxide – 35%

- Dissolve 350g of NaOH into 1L of distilled water.

Mixed Indicator

- Mix methyl red (20mg) and bromocresol green indicator (100mg) top up to 100ml ethanol. Make up every month.

9. Calibration

- Prepare a standard $K_2Cr_2O_7$ solution daily to correct any variation in the concentration of the Ferrous Ammonium Sulphate.
- Prepare a blank with each set of samples consisting of 5ml distilled water in place of sample together with all the reagents and digest together with samples.

Standard

- A solution of 30mg/N is prepared by weighing 0.1607g glycine dissolving in distilled water and diluting to 1L in a volumetric flask.

Waste water / Sludge	20-100ml (70ml)	15-20 TKN

Final effluent	140ml	
Outfalls	100ml	4-5 TKN

10. Procedure

- **Sample Preparation**
- Weigh out between 1.8g and 2g of well-mixed faecal sludge sample.
- Place the weighed out sample into a blender with 250ml of distilled water.
- Blend for 30 seconds.
- Transfer the blended mixture into a volumetric flask and top up to 1L with distilled water.
- Transfer the 1L solution to a plastic bottle and store in the cold room.

Heating Block

- Place 50ml of mixed diluted sample into 300ml Kjeldahl flask for raw or primary sewage, wastewater or 140ml for ponds, rivers or final effluents.
- Add a glass rod to the tube, and 5 boiling stones.
- Add slowly 10ml of concentrated sulphuric acid, 1kjeltab (or 2 spatulas powder), swirl to dissolve- wait approx. 15 min or overnight if sample as a high organic/fat content and then place onto digestion unit.
- Add 1000ml of 32% NaOH into reagent bottle 2, screw in bottle (clockwise) and push the bubbling tube to the bottom.
- Temperature range is set, follow program 1: 380°C for 60 min,
- Place suction cap onto tubes and open tap until a steady flow of water is reached (2L/min).
- Set pump to Mode A, air flow No: 4 until temperature of heating block reaches 200°C.
- Then set pump to Mode B: air flow No: 4 until end of digestion.
- Reactivate Mode B -100% of the maximum air flow – if SO3 gas emission is too much.
- Boil briskly at 380°C until dense fumes of SO3 are evolved and a pale green colour is obtained.
- The required temperature i.e. 380°C is usually reached after an hour.
- Keep pump running for 30 min after samples are fully digested and heating block is switched off.
- If sample is too little before fully digested, add 10ml concentrated sulphuric acid and remember to increase the vol. of sodium hydroxide used during the distillation.
- Digestion takes about 3 hours – colour changes from blue to dark green to black to colourless/pale green.
- Switch off the heating block, pump and the water supply.
- Replace water in the water bath and replace the NaOH in reagent bottle 2.
- Wait for samples to cool then distil samples.

Distillation

- Prepare absorption solution by placing 25ml of 4% boric acid in a 250ml conical flask and insert under the condenser outlet with the tip below the surface of boric acid.
- Lower collected distillate free from contact with the condenser tip and continue distillation for 1 or 2 minutes to cleanse the condenser.

- Enter distillation programme as follows:
- Vol of water Add 50ml to tube manually
- Phenolphthalein indicator 10 drops to tube manually
- Vol of NaOH 50ml (if 10ml sulphuric acid used in digestion)
- 200ml (if 30ml sulphuric acid used in digestion**)**
- **Distillation time** 3min
- Sample in tube turns purple with addition of NaOH - above pH 11 before distillation
- Distillate in flask should reach around pH 8 before titrating.
- **Titration**
- Titrate distillate against 0.1N sulphuric acid with mixed methyl red (0.02g) bromocresol green indicator (0.1g) top up to 100ml ethanol.
- Colour change: from blue to pale pink

11. Waste Disposal

- Collect waste in a 2.5L bottle for Waste Tech collection.

12. Calculation and Data Analysis

$$Nitrogen\ (mg/L) = \frac{(Titration - Blank)(0.1)(14)(1000)}{Sample\ Volume\ (ml)}$$

0.1 - Concentration of sulphuric acid used in titration
14 - Atomic weight of Nitrogen
1000 - Conversion of g to mg

$$Nitrogen\ in\ Wet\ Sample\ (mg/g) = \frac{(Titration - Blank)(0.1)(14)(1000)}{Sample\ Volume\ (ml)} \times \frac{V}{M}$$

$$Nitrogen\ in\ Wet\ Sample\ (g/g) = \frac{Nitrogen\ in\ Wet\ Sample\ (mg/g)}{1000}$$

$$Nitrogen\ in\ Dry\ Sample\ (g/g) = \frac{Nitrogen\ in\ Wet\ Sample\ (g/g)}{Total\ Solids\ (g/g)}$$

Where:

M = mass of sludge used in sample preparation (g)
V = Volume of dilution (L)

13. Data Quality

14. References

- Standards Methods for the Examination of Water and Wastewater, 18th Edition, p. 4-132, Methods 4500 N_{org} B Macro-Kjeldahl (1992).

APPROVAL OF STANDARD OPERATING PROCEDURE

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