Technical Assistance
HYDROLOGY PROJECT

World Bank & Government of The Netherlands funded

Training module # WQ - 38

How to measure Ammonia and Organic Nitrogen: Kjeldahl Method

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DHV Consultants BV & DELFT HYDRAULICS

with
HALCROW, TAHAL, CES, ORG & JPS

Table of contents

1. Module context

This module deals with the significance of nitrogen to water quality and Kjeldahl method for measuring ammonia and organic nitrogen. Modules in which prior training is required to complete this module successfully and other available, related modules in this category are listed in the table below.

While designing a training course, the relationship between this module and the others would be maintained by keeping them close together in the syllabus and placing them in a logical sequence. The actual selection of the topics and the depth of training would, of course, depend on the training needs of the participants, i.e. their knowledge level and skills performance upon the start of the course.

No.	Module title	Code	Objectives
1.	Basic water quality concepts	**WQ - 01**	• Discuss the common water quality parameters • List important water quality issues
2.	Basic chemistry concepts	**WQ - 02**	• Convert units from one to another • Discuss the basic concepts of quantitative chemistry • Report analytical results with the correct number of significant digits.
3.	Basic ecology concepts	**WQ - 26**	• Explain how energy flows through an aquatic ecosystem • Explain how nutrients are cycled in the environment • Explain the causes and problems of eutrophication
4.	Surface water quality planning concepts	**WQ - 27**	• Understand principles of WQ monitoring and assessment • Know of simple data analysis methods
5.	Use of ion selective probes	**WQ - 33**	• Precautions required in use of ion selective electrodes
6.	Absorption spectroscopy	**WQ - 34**	• Understand theory and applications of absorption spectroscopy
7.	How to measure Oxidised Nitrogen: Cd reduction method and UV Spectrophotometric methods	**WQ - 37**	• Measure oxidised nitrogen by Cd-reduction and UV spectrophotometric methods • Appreciate limitations of the UV method

2. Module profile

Title	:	How to measure Ammonia and Organic Nitrogen: Kjeldahl Method
Target group	:	HIS function(s): Q2, Q3, Q5, Q6
Duration	:	1 Theoretical session of 30 min., plus 1 Practical Laboratory session of 120 min, plus 1 Report writing session of 30 min.
Objectives	:	After the training the participants will be able to*:* • Understand the relevance of nitrogen to water quality • Know how to make analysis of ammonia and organic nitrogen by Kjeldahl Method
Key concepts	:	• Kjeldahl titrimetric method
Training methods	:	Lecture, Laboratory Analytical Exercise, Report preparation
Training tools required	:	Board, flipchart, OHS, Complete Laboratory Facilities for Ammonia and Organic Nitrogen Analysis
Handouts	:	As provided in this module, Including SAP for Analysis of Nitrogen, Ammonia and Organic
Further reading and references	:	• Chemistry for environmental engineers - C. N. Sawyer, P. L. McCarty & G. F. Parkin, McGraw - Hill, Inc., 1994 • Standard methods for the examination of water and wastewaters, AWWA, 19th edition, 1995

3. Session plan

No	Activities	Time	Tools
1	***Preparations*** • Reagents and glassware for the test • Collect samples A, B and C as described in the text		
2	***Introduction***: • Introduce the session and the subject of ammonia and organic nitrogen • Discuss the forms of ammonia found in water • Discuss the problems of ammonia and organic nitrogen in water and why it is a useful parameter	10 min	OHS
3	***Ammonia & Organic Nitrogen Methods*** • Describe the basis of the Kjeldahl method in terms of its chemistry. • Refer to SAP and allow time to read it.	20 min	OHS, SAP
4	***Practical Session:*** • Divide the class in working group of 3-4 persons. • Allow participants to conduct analysis according to SAP.	120 min	Laboratory
5	***Report Writing and wrap up*** • Allow participants to complete their reports • Discuss results and discrepancies	30 min	Board

4. Overhead/flipchart master

OHS format guidelines

Type of text	Style	Setting
Headings:	OHS-Title	Arial 30-36, with bottom border line (not: underline)
Text:	OHS-lev1 OHS-lev2	Arial 24-26, maximum two levels
Case:		Sentence case. Avoid full text in UPPERCASE.
Italics:		Use occasionally and in a consistent way
Listings:	OHS-lev1 OHS-lev1-Numbered	Big bullets. Numbers for definite series of steps. Avoid roman numbers and letters.
Colours:		None, as these get lost in photocopying and some colours do not reproduce at all.
Formulas/Equations	OHS-Equation	Use of a table will ease horizontal alignment over more lines (columns) Use equation editor for advanced formatting only

Ammonia and Organic Nitrogen

- Nitrogen cycle
  - *atmospheric fixation precesses, chemical and biochemical*
  - *conversion of ammonia nitrogen into organic nitrogen*
  - *wastes: organic and ammonia nitrogen*
  - *nitrification and denitrification*
- Ammonia and organic nitrogen in water bodies is an important parameter of their quality.

Nitrogen cycle

- Fixation of atmospheric nitrogen, N_2
  - *electrical discharge, chemical production, biological fixation*
- NO_3 ⟶ plants ⟶ animals ⟶ death & wastes
- Waste org. N ⟶ decomposers ⟶ NH_3
- NH_3 ⟶ nitrifiers ⟶ NO_2 & NO_3
- NO_2 & NO_3 ⟶ denitrifiers ⟶ N_2

Nitrogen and Water quality (1)

- Water which has been polluted by organic wastes contains:
  - *ammonia nitrogen (free gas NH_3 or ionic specie NH_4^+)*
  - *organic nitrogen (proteins, urea)*
- Both forms influence water quality

Nitrogen and water quality (2)

- Ammonia in water can be problematic in two ways:
  - *The free (un-ionised) form (NH_3) is toxic to fish*
  - *Nitrification of ammonia removes dissolved oxygen from the water:*

$$NH_3 + 2O_2 \longrightarrow HNO_3 + H_2O$$

Nitrogen and Water Quality (3)

- Organic nitrogen compounds are:
  - *Excreted by animals or released when animals and plants die*
  - *Present in sewage effluents*
- Bacterial decomposition of organic wastes produces ammonia

Kjeldahl Method (1)

- Ammonia by distillation followed by titration of distillate with sulphuric acid
- Organic nitrogen, on the residue from the above, by conversion to ammonia followed by determination as above

Kjeldahl Method (2)

- Ammonia
  - *conversion to gaseous from*

    $NH_4^+ + OH^- \longrightarrow NH_3 + H_2O,\ pH = 9.5$

  - *distillation and absorption*

    $NH_3 + H_3BO_3 \longrightarrow NH_4^+ + H_2BO_3^-$

  - *titration*

    $H_2BO_3^- + H^+ \longrightarrow H_3BO_3$

Kjeldahl Method (3)

- Organic nitrogen
  - *Acid digestion*

$$\text{Organic nitrogen} + H_2SO_4 \longrightarrow (NH_4)_2SO_4 + CO_2 + H_2O$$

  - *K_2SO_4 & $CuSO_4$ added to raise digestion temperature to 370^oC*
  - *Digestion is completed when digesting liquor clarifies with release of fumes.*

Experiment

- Aim
  - *To determine the concentration of ammonia and organic nitrogen using Kjeldahl method*

Sample	**Source**	**Expected concentration, mg/L**	
		NH3 – N	Organic - N
A	Surface water	0 – 5	1 – 10
B	Polluted drain	10 – 20	10 – 50
C	Distilled water	0	0

Report

- the aim of the investigation
- the results you have produced
- the results compared to original predictions and the reasons for any differences
- possible reasons for presence of nitrogen in blank, if any
- what the ammonia and organic nitrogen results mean in terms of water quality

5. Evaluation sheets

6. Handout

Ammonia and Organic Nitrogen

- Nitrogen cycle
  - *atmospheric fixation precesses, chemical and biochemical*
  - *conversion of ammonia nitrogen into organic nitrogen*
  - *wastes: organic and ammonia nitrogen*
  - *nitrification and denitrification*
- Ammonia and organic nitrogen in water bodies is an important parameter of their quality.

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Report

- the aim of the investigation
- the results you have produced
- the results compared to original predictions and the reasons for any differences
- possible reasons for presence of nitrogen in blank, if any
- what the ammonia and organic nitrogen results mean in terms of water quality

Add copy of Main text in chapter 8, for all participants.

7. Additional handout

8. Main text

Contents

How to measure Ammonia and Organic Nitrogen: Kjeldahl Method

1. Introduction

An understanding of the chemistry and biochemistry of nitrogen is a very important factor in the management of water quality. As an aid to this understanding, Figure 1 shows a simplified representation of the nitrogen cycle in nature. Many of the interactions shown in the diagram also apply in the aquatic environment as discussed below.

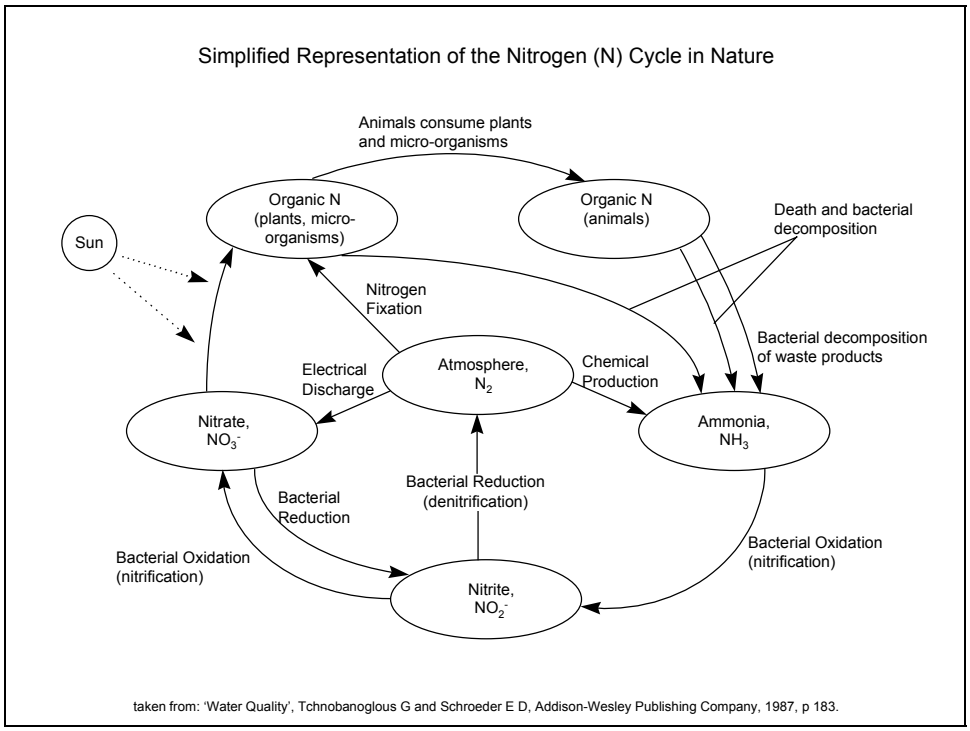

Figure 1: Simplified Representation of the Nitrogen Cycle in Nature

Water which has been subject to pollution from waste materials, including sewage, often contains nitrogenous organic compounds and ammonia. Ammonia is also produced when compounds, such as, proteins, urea, etc., are decomposed through microbial action.

Ammonia in water can be problematic in two ways. Firstly, the free (un-ionised) form of the gas is toxic to fish in reasonably low concentrations (approximately 0.2mg/L can cause death in some species). Secondly, as can be seen from Figure 1, ammonia in the aquatic environment is normally oxidised by bacteria to nitrite (NO_2^-) and then nitrate (NO_3^-) in a process known as 'nitrification'. This process consumes dissolved oxygen in the water which can lead to distress or death for aquatic life if sufficient oxygen is lost. In addition, nitrate is an important factor in the eutrophication of surface waters which can lead to further water quality problems such as explosive algal growth, low dissolved oxygen and fish deaths. Further, when present in drinking water, nitrate has been associated with methaemoglobinaemia (blue baby disease) in human infants.

This experiment deals with measurement of nitrogen existing as free or ionised ammonia and that occurring in combination with organic compounds. Waters that contain mostly organic and ammonia nitrogen are considered to have been recently polluted and therefore potentially dangerous from public health view point.

2. Kjeldahl Method of analysis

This method, when used to determine ammonia and organic nitrogen, consists of two steps as follows:

- distillation of the ammonia from the sample into a solution of boric acid and titration of the ammonia against a standard sulphuric acid solution
- after the ammonia has been distilled from the sample, the residue is used to determine organic nitrogen. Nitrogen contained in many organic species is converted to ammonium sulphate when heated in the presence of sulphuric acid. Potassium sulphate and copper sulphate catalysts are added to raise the boiling point of sulphuric acid to 370^{o}C. The organic matter is oxidised to carbon dioxide. Once the ammonia from organic nitrogen has been released, it is distilled and determined as above.

In both cases, before distillation the pH of the sample or digested liquor is raised to pH of 9.5 to encourage formation of ammonia which can be distilled with steam:

$$NH_4^+ + OH^- \longrightarrow NH_3 + H_2O$$

The distilled ammonia is absorbed in boric acid:

$$NH_3 + H_3BO_3 \longrightarrow NH_4^+ + H_2BO_3^-$$

The ammonia which reacts with Boric Acid can be determined by back titrating with a strong standard acid

$$H_2BO_3^- + H^+ \longrightarrow H_3BO_3$$

3. Experiment

Aim

a. To determine the concentration of ammonia and organic nitrogen in water samples by Kjeldahl method.

Method

a. Read the SAP for determination of ammonia nitrogen by distillation titrimetric method and organic nitrogen by Kjeldahl method.
b. Collect samples for analysis. The source of samples and expected analyte concentrations are given below.

Sample	Source	Expected concentration, mg/L	
		NH3 – N	Organic - N
A	Surface water	0 – 5	1 – 10
B	Polluted drain	10 – 20	10 – 50
C	Distilled water	0	0

c. Choose appropriate sample aliquots and start with the determination of ammonia nitrogen.
d. After ammonia distillation is complete and the residue in the boiling (Kjeldahl) flask has cooled down start digestion for the determination of organic nitrogen.
e. While the digestion is proceeding, titrate the distillate obtained in ‘d’ above.
f. Complete digestion and start distillation for organic nitrogen and titrate for organic nitrogen.

Observations & calculations

a) Calculate the concentration of the two forms of nitrogen and fill in the following table. Use the values obtained for the blank to correct the values for the samples A and B

Sample	Nitrogen, mg/L	
	NH3 – N	Organic - N
A		
B		
C		

Report

When writing your report the following aspects should be addressed:

- the aim of the investigation
- the results that you have produced
- whether the results were as originally predicted and, if they were not, the reasons why they deviated
- possible reasons for the presence of nitrogen in the blank, if any
- the ammonia and organic nitrogen concentration of the samples and what this could mean in terms of water quality