The Assessment of the Nitrifying Ability of Activated Sludge 1980 (Tentative Methods)

Methods for the Examination of Waters and Associated Materials

London Her Majesty's Stationery Office £2.40 net

The Assessment of the Nitrifying Ability of Activated Sludge 1980 (Tentative Methods)

Methods for the Examination of Waters and Associated Materials

Contents

London Her Majesty's Stationery Office

Warning to users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary. Local safety regulations must be observed. Laboratory procedures should be carried out only in properly equipped laboratories. Field operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried. Care should be taken against creating hazards. Lone working, whether in the laboratory or field, should be discouraged. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specification. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Code of Practice for Chemical Laboratories' and 'Hazards in the Chemical Laboratory' issued by the Royal Society of Chemistry, London; 'Safety in Biological Laboratories (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological Hazards; Public Health Laboratory Service Monograph Series No 6 'The Prevention of Laboratory Acquired Infection', HMSO, London.

Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. It cannot be too strongly emphasised that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radio chemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes and the accessibility of the correct and properly maintained first-aid, fire-fighting, and rescue equipment. If in doubt; it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

First published 1983

ISBN 0 11 751655 4

About this series

This booklet is part of a series intended to provide recommended methods for the determination of water quality. In addition, the series contains short reviews of the more important analytical techniques of interest to the water and sewage industries. In the past, the Department of the Environment and its predecessors in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as individual methods, thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods being issued when necessary. The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users – the senior analytical chemist, biologist, bacteriologist etc to decide which of these methods to use for the determination in hand. Whilst the attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysts is one of the joint technical committees of the Department of the Environment and the National Water Council. It has nine Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
*2.0 Instrumentation and on-line analysis
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General non-metallic substances
6.0 Organic impurities
7.0 Biological methods
*8.0 Sludge and other solids analysis
9.0 Radiochemical methods

The actual methods etc are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee. The names of those associated with this method are listed inside the back cover.

Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No 5, and the current status of publication and revision will be given in the biennial reports of the Standing Committee of Analysts.

Whilst an effort is made to prevent errors from occurring in the published text, a few errors have been found in booklets in this series. Correction notes for booklets in this series are given in the Reports of The Standing Committee of Analysts, published by the Department of the Environment but sold by the National Water Council, 1 Queen Anne's Gate, London SW1H 9BT. Should an error be found affecting the operation of a method the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in the booklet.

* These two Working Groups are in process of being wound up. Their tasks are being redistributed among the other Working Groups.

T A DICK
Chairman

L R PITTWELL
Secretary

25 September 1981

The Assessment of the Nitrifying Ability of Activated Sludge 1980

Introduction

The microbial oxidation of ammonia to nitrate, via nitrite, (nitrification) is an important reaction in waste-water treatment. When it occurs it accounts for a significant proportion of the oxygen requirements of a treatment process. Nitrification is essential in situations where nitrate is preferable in the receiving river to the equivalent amount of ammonia. The process is a pre-requisite of microbial denitrification in situations where nitrogen compounds have to be eliminated or severely reduced in discharges to avoid eutrophication in the receiving water. Nitrification also occurs (inevitably) in situations where exceptionally high quality effluents are produced, eg where the receiving river water is to be used for abstraction to produce potable water.

The bacteria responsible for the oxidation of ammonia to nitrite and of nitrite to nitrate grow at much lower specific rates than the heterotrophic bacteria* which oxidize the bulk of the organic pollutants in waste waters (eg $0.2d^{-1}$, generation time of 3–4d compared with $0.1h^{-1}$, 6h). Using these data, the operating conditions necessary to achieve nitrification in the activated sludge system have been set out (Downing, Painter and Knowles, 1964). The autotrophic nitrifiers are much more sensitive to a wide range of inhibitory substances, organic and inorganic, than are the heterotrophs. The nitrifiers also require higher concentrations of dissolved oxygen for optimal growth and grow over a narrower range of pH value than heterotrophs. Because of their low growth rate and these more fastidious requirements, nitrification is often difficult to achieve in any treatment plants which otherwise produce acceptable effluents and is transient in others receiving fluctuating organic loads and varying concentrations of toxic agents. Also because of their low specific growth rates, nitrifying bacteria are particularly sensitive to changes in temperature and this must be borne in mind when carrying out experiments.

The methods described here are designed to help the operator decide why nitrification does not occur in his plant, whether it can be achieved, and if so, how. The first method determines the nitrifying activity of a sludge under 'ideal' conditions as well as in the presence of the sewage it has to treat. The second method assesses the degree of inhibition to nitrification brought about by a trade waste water or by single compounds and is useful in defining conditions for acceptance of trade wastes into the sewer and also for tracing troublesome compounds back to their sources.

The methods available fall into two categories:–

a. Those (described elsewhere in this series) depending on short-term respiration rate measurements using oxygen electrodes in the presence and absence of allylthiourea, one of the most powerful and specific inhibitors of *Nitrosomonas* (see another method in this series SCA 1981 (b)).

and

b. Those in which the change in the concentration of oxidized nitrogen (or of ammoniacal nitrogen) is determined after incubation.

The 'loss' of inorganic nitrogen in activated sludge processes is a commonly observed phenomenon and is usually ascribed to the production of gaseous nitrogen by denitrification occurring in parts of the process which are anaerobic either deliberately or fortuitously (settlement tank, within large flocs) (Painter, 1970; Wood *et al*, 1981.) However, even when no anaerobic or anoxic zones are present, as in the test procedures described here, there is evidence (Wood *et al*, 1981) of 'loss' of inorganic nitrogen, that is, ammonia-N plus oxidized-N initially is greater than the total after incubation. The loss increases as the degree of nitrification increases, and hence is not due to formation of organic (cellular) nitrogen but may be due to the formation of gaseous oxides of nitrogen.

It is therefore advisable that periodic balances of inorganic nitrogen be made on the sludge in question by determining the removal of ammonia nitrogen as well as the production of oxidized nitrogen.

---

* Those species (the vast majority) which derive their energy and carbon from organic compounds.

† Nitrification in sewage treatment has been shown to be carried out by autotrophs, ie bacteria which use inorganic carbon as their source of carbon and the oxidation of ammonia and nitrite as their source of energy.

Interpretation

Good agreement was found between the two types of method (Painter and Jones, 1963) when used for determining rates of nitrification of sludge – domestic sewage mixtures when the formation of nitrite was low. Differences between the methods would result if significant concentrations of nitrite were formed, unless appropriate corrections were made.

In the case of inhibition, differences between the two types of methods could arise if the nitrifying bacteria recover from the presence of the toxic substance during the incubation test (2–4h) or if the compound is metabolized by heterotrophs during that periods.

Some toxic compounds, and presumably also some trade wastes, which are not metabolized in the incubation test, may be removed in treatment plants after periods of acclimatization (days, or even weeks) or the nitrifying population may become adapted, or partially so, to the toxic agent (Tomlinson, Boon and Trotman, 1966). In these cases it would be necessary, if economic considerations justified it, to carry out longer-term tests using model activated-sludge plants (SCA 1981(a) or rotating tubes (SCA 1982).

Determination of the Actual and Potential Nitrifying Ability of Activated Sludge

1. Performance Characteristics of the Method

1.1	Property determined	Specific nitrification rate
1.2	Type of sample	Activated sludge (mixed liquor or returned sludges).
1.3	Basis of method	Determination of oxidized nitrogen formed during aeration at constant temperature for 2–4h of a sample containing known suspended solids concentration plus sewage or standard medium.
1.4	Range of application	Up to 10 mg oxidized nitrogen per g activated sludge suspended solids per hour.
1.5	Interferences	In the determination of potential nitrifying ability of sludge, substances not removed by washing which are inhibitory to the oxidation of ammonia.
1.6	Time required for determination	For 8 determinations, excluding chemical analysis, total time is about 3–5h; operator time is about 1h.

2. Principle

The *actual* nitrifying ability is determined by incubating, with adequate aeration a mixture of the sludge and appropriate sewage for 2-4h. The specific rate of nitrification is calculated from the concentration of suspended solids and the increase in concentration of oxidized nitrogen.

The *potential* nitrifying ability is assessed by centrifuging, washing and recentrifuging the sludge sample to remove any oxidized nitrogen and inhibitory compounds. The re-suspended sludge is mixed with a standard medium containing an excess of ammonium salts at pH 7.6 and the mixture is incubated with adequate aeration for 2–4h. The specific rate of nitrification is calculated as above.

3. Interferences

Any substances present in the sludge or sewage which inhibits the oxidation of ammonia will give low values. Such substances are usually removed on washing the sludge; strongly absorbed compounds may not be removed by this treatment.

Inhibitory substances may be formed when sewage is allowed to stand anaerobically; samples of sewage should be used in the same state (dissolved oxygen, etc) as would be found in the full-scale plant. Interferences in the methods for the determination of nitrite and nitrate should be borne in mind.

4. Hazards

Since some of the micro-organisms in activated sludge and sewage may be potential pathogens, appropriate caution should be used in handling such samples. Allyl-thiourea can cause skin disorders; thiourea is reported to be carcinogenic and should not be used as an alternative.

5. Reagents

5.1 **Distilled or de-ionized water**

5.2 **Settled sewage**

Sewage should be collected from the overflow of the appropriate settlement tank, used as soon as possible and kept aerated before use.

5.3 **Activated Sludge**

Samples of sludge, collected from the aeration tank or the return sludge line, should be kept aerobic from the time of sampling, since prolonged anoxic conditions may reduce the nitrifying activity of the sludge.

5.4 **Medium**

Dissolve 252 mg sodium bicarbonate ($NaHCO_3$) and 264 mg ammonium sulphate ($(NH_4)_2SO_4$) in 1 litre distilled water (5.1); this medium contains 56 mg N/l and will maintain a pH value of about 7.6 during the production of 10 mg oxidized N per litre.

5.5 **Allylthiourea**

Dissolve 1.16 g allylthiourea in 1 litre distilled water (5.1). (See Section 4).

5.6 **Suggested Buffer Solutions**

A buffer solution is only required if use of distilled water in section 8.2 reduces the activity of the sample, see that section. One of the following solutions should then be used:

5.6.1. (i) Dissolve 0.125 ± 0.005 g of ferric chloride hexahydrate in water and dilute with water to 1 litre in a measuring cylinder.
(ii) Dissolve 27.5 ± 0.5 g of anhydrous calcium chloride in water and dilute with water to 1 litre in a measuring cylinder.
(iii) Dissolve 25.0 ± 1.0 g of magnesium sulphate heptahydrate in water and dilute with water to 1 litre in a measuring cylinder
(iv) Dissolve 42.5 ± 0.5 g of potassium dihydrogen phosphate in 700 ± 10 ml of water in a beaker, add 8.8 ± 0.1 g of sodium hydroxide, dissolve, add 2.0 ± 0.1 g of ammonium sulphate, dissolve and make g to 1 litre in a measuring cylinder with water.
(v) To each 1 litre ± 10 ml of water used in section 8.2 add 1 ml of each of the above solutions (i) – (iv) in that order and mix.

5.6.2. Dissolve 2.25 ± 0.01 g sodium chloride, 0.105 ± 0.001 g potassium chloride, 0.120 ± 0.005 g calcium chloride, 0.050 ± 0.002 g sodium bicarbonate in water and make up to 1 litre in a measuring cylinder with water.

5.6.3. Analyse the discarded liquor for major cations and ions and prepare a similar solution, but replace any nitrate on nitrite by chloride or sulphate on an equivalent basis.

6. Apparatus

(see also 9.8)

6.1 **250 ml conical flasks**

6.2 **Shaking machine:** a suitable machine (Kantorowicz 1951) had an amplitude of 12 cm and a periodicity of 68/min.

7. Sampling

Representative samples of activated sludge and sewage, if required, should be taken in accordance with reference SCA 1977.

8. Pre-treatment of Samples

Samples of sludge should be kept aerated before use; further pre-treatment depends on the object of the determination.

8.1 **Actual nitrifying ability**
No further treatment is given.

8.2 **Potential nitrifying ability**
The sample is centrifuged (1100G (gravity) for 5 min has been found suitable) and the supernatant liquor is discarded. The residue is washed with distilled water (5.1), re-centrifuged and again the supernatant liquor is discarded. Finally, the sludge is re-suspended in an equal volume of distilled water to give a slurry and aerated until required. The sludge should be used within a few hours of sampling.

In some cases, such as might occur with sewages containing high levels of dissolved solids, distilled water may not be satisfactory because of osmotic effects. In such cases an isotonic buffer of appropriate strength and composition, in respect of the nature of the dissolved salts, should be used. If there is doubt when testing unfamiliar or anomalous samples, it may be advisable to try both water and buffer solution. As there is no one ideal buffer solution, Section 5.6 gives preparation details for the two most commonly used solutions (BOD dilution water, and quarter strength Ringer's Solution) and suggests a third possible alternative.

9. Analytical Procedure

Step	Procedure	Notes
	Actual nitrifying ability	
	9.1 Add a volume of aerated sludge to a measured volume of the appropriate sewage such that the required concentration of suspended solids (note a) is reached and place 100 ml in a 250 ml conical flask.	(a) The concentration would normally be that at which the treatment plant is operated.
	9.2 Set up a control containing the same mix of sludge and sewage, but also containing 1 ml allylthiourea solution (5.5) (note b). This mixture is 10^{-4}M with respect to allylthiourea, which is equivalent to 11.6 mg/l.	(b) 10^{-4}M allylthiourea has been shown to inhibit nitrification in many sludges without affecting the oxidation of organic compounds (Painter 1970).
	Potential nitrifying ability	
9.3	Add 4 or 5 ml washed sludge (note c) to a 250 ml conical flask and add a volume of standard medium (5.4) to give a final volume of 100 ± 1 ml.	(c) The concentration of suspended solids in the final mixture should be such as to give readily detectable amounts of oxidized nitrogen after 2–4h incubation. A concentration of 1500 mg/l is normally adequate.
9.4	Set up a control containing the same volume of sludge diluted to 100 ± 1 ml with standard medium. Add a nitrifying inhibitor (1 ml allylthiourea solution, 5.5).	
	Incubation (see also 9.8)	
9.5	Secure the flasks on the shaker (note d) and incubate at a constant temperature, the value depending on the application. Remove the flasks after 2–4h depending on the activity of the sludge.	(d) The shaking should ensure that the concentration of dissolved oxygen at no time falls below 2 mg/l. The shaker described (6.2) gave an aeration rate of 12 mg oxygen/l.h for each 1 mg/l dissolved oxygen deficit.
	Then either:	
9.6	Filter the contents of the test and control flasks through glass fibre discs (Grade C) for determination of suspended solids (note e) and collect the filtrate for determination of oxidized nitrogen by a suitable method (note f). See also note (g).	(e) See SCA 1979. (f) See SCA 1981 (a) (g) The removal of ammonia-nitrogen should be determined from time to time to ensure that little or no oxidation of ammonia to products other than nitrite and nitrate is occurring. See SCA 1980. Also, it must be ascertained that at least 5 mg NH^{+}_{4} N/l remains at the end of the incubation. Nitrite determination should be performed from time to time in order to ensure that no significant quantities accumulate during any experiment. See SCA 1981 (a).

Step	Procedure	Notes
	or	
9.7	Alternatively (to 9.6), the contents of the flasks may be centrifuged. The supernatant liquor is used for the determination of oxidized nitrogen (note f) while the pellet is transferred quantitatively to a weighed vessel for determination of suspended solids (note e). See also note (g).	
	Alternative Apparatus	
9.8	The 250 ml flask/shaker combination may be replaced with aeration stones and 250 ml beakers or conical flasks, provided that solids are maintained in suspension without excessive agitation and that the dissolved oxygen concentration remains above 2 mg/l. A suitable supply of clean compressed air would also be required.	

10. Calculation

The specific nitrifying ability (potential or actual, as appropriate)

$$= \frac{C_t - C_c}{SS \times t} \text{ mg nitrogen per gram of dry solids per hour (mg N/g h)}$$

where C_t = concentration of oxidized nitrogen present in test flask at end of incubation (mg/1),

C_c = concentration of oxidized nitrogen present in inhibited control flask at end of incubation (mg/1),

SS = concentration of activated sludge solids present in the test flask (g/1),

and t = incubation period (h).

The specific nitrifying ability also

$$= \frac{C'_c - C'_t}{SS \times t} \text{ mg nitrogen per gram of dry solids per hour (mg N/g h)}$$

where C'_c = concentration of ammonium nitrogen (in mg/1), present in the inhibited control flask at end of incubation,

and C'_t = concentration of ammonium nitrogen (in mg/1) present in the test flask at end of incubation.

Assessment of Inhibition to Nitrification

1. Performance Characteristics of Method

1.1	Property determined	Inhibitory effect of the sample on the bacterial oxidation of ammonia.
1.2	Type of sample	Specific chemicals, formulations, trade waste waters, sewage.
1.3	Basis of method	Comparison of the oxidized nitrogen formed by a nitrifying sludge in the absence and presence of the test material.
1.4	Range of application	0–100% inhibition.
1.5	Interferences	Reaction between inhibitor and component of sludge. Possible acclimatization of sludge to the inhibitor if already present in sewage.
1.6	Time required for determination	For one compound or industrial waste water at six concentrations, excluding chemical analysis, total time is about 3–5h; operator time is about 1h.

2. Principle

The degree of inhibition of nitrification by a compound, formulation or waste water is calculated by assessing the difference in concentration of nitrate formed after parallel aeration of a nitrifying sludge in the presence and absence of the test material.

It must be stressed that sludges respond differently to the same concentration of an inhibitor and this is probably due, at least in part, to reaction between the inhibitor and components of the sludge resulting in a partial nullifying of the toxic effect. Examples are reactions between copper or mercury and thiol groups. If there is some suspicion that the sludge used may be acclimatized to the potential inhibitor, then the opportunity should be taken to repeat the test with a sludge from another source.

3. Interferences

Some components of activated sludge can react with potential inhibitors to give lower degrees of inhibition. Also, if the plant from which the nitrifying sludge is taken has received sewage containing the test material, low values for its inhibitory ability will result if acclimatization has taken place.

4. Hazards

Since some of the micro-organisms in activated sludge may be potential pathogens, appropriate caution should be used in handling such samples.

Precautions should be taken in handling substances and formulations, which might have harmful properties.

Allylthiourea has been reported to give rise to skin irritations. Thiourea has been reported to be carcinogenic and should not be used as an alternative.

5 Reagents

5.1 **Distilled or deionized water**

5.2 **Nitrifying activated sludge**
This may be obtainable from a nearby treatment works or it may be necessary to operate a pilot or laboratory-scale plant treating domestic sewage or a 'synthetic sewage'. The sludge must be maintained in an aerobic condition.

Before use, the sludge is centrifuged (1100G for 5 min) and the supernatant liquid is discarded. The residue is washed with distilled or deionized water (5.1), re-centrifuged and again the supernatant liquid is discarded. Finally, the sludge is re-suspended in an equal volume of distilled water to give a slurry, which is aerated until required.

5.3 **Medium**
Dissolve 504 mg sodium bicarbonate ($NaHCO_3$) and 528 mg ammonium sulphate ($(NH_4)_2 SO_4$) in 1 litre of distilled water (5.1). This medium when diluted 1:1 contains 56 mg N/1 and will maintain a pH value of about 7.6 during the production of 10 mg/1 oxidized Nitrogen.

5.4 **Allylthiourea**
Dissolve 1.16 g allylthiourea in 1 litre of distilled water (5.1) (See Section 4).

6 Apparatus

6.1 **250 ml conical flasks**

6.2 **100 ml measuring cylinders**

6.3 **Shaking machine:** a suitable machine (Kantorowicz 1951) had an amplitude of 12 cm and a periodicity of 68/min.

7. Analytical Procedure

Step	Procedure	Notes
7.1	Add equal volumes (note a) of washed nitrifying sludge to a series of 250 ml conical flasks such that the final concentration of suspended solids is approximately 1500 mg/1 in the mixture described in 7.2 or 7.3.	(a) It is important to have the same concentration of suspended solids in each flask since inhibition may depend on the ratio of concentration of solids to concentration of inhibitor. Under the conditions described 1500 ± 200 mg/1 was found suitable.
	Specific chemicals or formulations	
7.2	To a series of 100 ml cylinders add graded volumes (0–50 ml maximum) of a solution of the test substance (note b) to give a suitable range of final concentrations (note c). Add 50 ml medium (5.3) and distilled water to the 100 ml marks. Mix and add the mixtures to the series of flasks (note d).	(b) If the pH value of the solution is outside the range 7–8, it may be necessary to adjust its value by addition of appropriate acid or alkali to ensure that the pH values of the subsequent mixtures are within this range.
	Industrial Waters	
7.3	Prepare 100 ml portions of various mixtures of industrial waste water (note b) and medium (or appropriate sewage), containing 0–5% (maximum) industrial waste water (note c) and add to the series of flasks (note d).	(c) The concentration range will have to be determined by trial and error, or by the use of the respirometer technique (SCA 1982). (d) On no account should the stock solution of test substance be added to the sludge directly since irreversible effects on the nitrifying organisms might result.
7.4	Repeat 7.2 using allylthiourea in place of the test substance if a comparison is required with a 'standard' inhibitor (note e).	(e) A suitable range of concentrations is 0.12–11.6 mg/1.

Step	Procedure	Notes
7.5	Incubate control and test flasks at constant temperature (depending on the application) on the shaker (note f) for 2–4 h (note g). (See also 7.8).	(f) The shaking should ensure that the concentration of dissolved oxygen at no time falls below 2 mg/l. The shaker described here gave 12 mg O_2/1.h for each 1 mg/l deficit.
	Then either:	
7.6	Filter the contents of the flasks through glass-fibre discs (Grade C) for determination of suspended solids (note h) and collect the filtrate for the determination of oxidized N and, if required, ammonia N by a suitable method (notes i and j).	(g) It is important that at the end of incubation the pH value remains at 7.6 ± 0.2 and that at least 5 mg NH^+_4–N/l remains. See SCA 1980. If not, repeat the test using double-strength standard medium or a lower concentration of activated sludge.
	or	
7.7	Alternatively (to 7.6), the contents of the flasks may be centrifuged. The supernatant liquid is used for the determination of oxidized N (notes i and j), while the pellet is transferred quantitatively to a weighed vessel for the determination of suspended solids (note h).	(h) See SCA 1979. (i) The removal of ammonium-N should be determined from time to time to ensure that little or no oxidation of ammonia to products other than nitrite and nitrate is occurring. See SCA 1980.
	Alternative apparatus	
7.8	The 250 ml flask/shaker combination may be replaced with aeration stones and 250 ml beakers or conical flasks, provided that solids are maintained in suspension without excessive agitation and that the dissolved oxygen concentration remains above 2 mg/l. A suitable supply of compressed air would also be required.	(j) For methods for the determination of nitrate and nitrite see SCA 1981.

8. Calculation

$$\text{The degree of inhibition} = \frac{(C_c - C_i)}{C_c} \times 100\%$$

Where C_c = concentration of oxidized-nitrogen formed or of ammonia-N control (no inhibitor present),

and C_i = concentration of oxidized-nitrogen formed or of ammonia-N removed in the presence of the inhibitor.

A graph is then drawn, plotting degree of inhibition against the concentration of inhibitor and each substance can then be characterized by the concentration which inhibited nitrification by 75% (Tomlinson et al 1966) or more usually by 50%.

From the results obtained with an industrial waste water and knowledge of the volume of waste discharged to the sewer, an assessment may be made as to whether or not the waste will be inhibitory to nitrification in the treatment plant, assuming no degradation of the compounds present in the waste or acclimatization of the organisms.

References

1. Downing, A L, Painter, H A and Knowles, C (1964) *J Inst Sew Purif.* **63,** 130.
2. Kantorowicz, O (1951) *J gen Microbiol,* **5,** 276.
3. Painter, H A and Jones, K (1963) *J appl Bact,* **26,** 471–483.
4. Painter, H A (1970) *Wat Res,* **4,** 393–450.
5. Tomlinson, T G, Boon, A G and Trotman, G N A (1966) *J appl Bact,* **29,** 266–291.
6. Wood, L B, Hurley B J E and Matthews, P J (1981), *Wat Res,* **15,** 543–551.

SCA Methods for the Examination of Waters and Associated Materials. The following booklets in this series are referred to:

The Sampling and Initial Preparation of Sewage and Waterworks' Sludge, Soils Sediments and Plant Materials 1977.
(There will be extra information on sludge and related sampling in Additions, Corrections and Index 1983).

Suspended Matter 1979, Total Dissolved and Settleable Solids 1980.

Ammonia in Waters 1980.

Oxidized Nitrogen in Waters 1981. (a)
Biochemical Oxygen Demand 1981. (b)

Methods for Assessing the Treatability of Chemicals and of Industrial Waste Waters, and their Toxicity to Sewage Treatment Processes 1982.

Address for Correspondence:

However thoroughly a method may be tested, there is always the possibility of a user discovering a hitherto unknown problem. Users with information on this method are requested to write to:

The Secretary
The Standing Committee of Analysts
The Department of the Environment
Romney House
43 Marsham Street
LONDON SW1P 3PY
England

Printed in England for Her Majesty's Stationery Office
by Linneys of Mansfield
Dd 718267 C12 4/83

Standing Committee of Analysts

Members of the Committee Responsible for this Method

Mr D A Bailey[1]
Mr P J Ballinger[2]
Dr G I Barrow[3]
Dr M L Berrow[2]
Mr R R Birch[1]
Mr J Borland[3] until February 1978
Dr J M Carter[3]
Dr H Crabtree[1]
Dr G W Clayfield[3]
Mr E I Clark[1]
Mr B E P Clement[3] from February 1978
Dr V Collins[3] until January 1977
Dr R L Cooper[3]
Dr B Cocksey[1]
Dr B T Croll[3]
Mr T A Dick[3]
Mr J W R Dutton[3]
Dr W H Evans[2]
Dr C Foster[1]
Mr G I Goodfellow[3] from January 1980
Mr K Goodhead[3] from July 1978
Mr T R Graham[3] from April 1977
Mr I Harper[3] from July 1978
Mr E Hodges[3]
Mr J G Holland[3]
Dr D T E Hunt[3] from May 1980
Mr W M Lewis[3] until January 1980
Mr P J Long[3]
Dr P J Matthews[2]
Mr J C McCullins[3]
Mr P Morries[3]
Mr A H Nield[3]
Mr P E Norris[1]
Dr H A Painter[1, 2, 3]
Dr S J Patterson[3] until July 1979
Mr L R Pittwell[3]
Dr J E Portman[2, 3]
Mr L D Purdie[3]
Mr B D Raverscroft[3]
Mr B Rhodes[3] from July 1978
Prof J P Riley[3]
Mr R Sinar[3] died April 1979
Mr J Street[1]
Mr J A Tetlow[3] from July 1980
Mr A M Ure[3] from August 1979
Mr J L Vosser[1]
Mr B T Whitham[3]
Mr A L Wilson[3] until May 1980
Mr A A Wood[2]
Dr R Wood[3] until July 1978
Mr P Worthington[2]

Panel member[1]
Working Group member[2]
Main Committee member[3]

HER MAJESTY'S STATIONERY OFFICE

Government Bookshops

49 High Holborn, London WC1V 6HB
13a Castle Street, Edinburgh EH2 3AR
41 The Hayes, Cardiff CF1 1JW
Brazennose Street, Manchester M60 8AS
Southey House, Wine Street, Bristol BS1 2BQ
258 Broad Street, Birmingham B1 2HE
80 Chichester Street, Belfast BT1 4JY

Government publications are also available through booksellers

ISBN 0 11 751655 4