# Determination of the Inhibitory Effects of Chemicals and Waste Waters on the Anaerobic Digestion of Sewage Sludge (1986)

**Methods for the Examination of Waters and Associated Materials**

Her Majesty's Stationery Office

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**Methods for the Examination of Waters and Associated Materials**

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## Contents

# About this Series

This booklet is part of a series intended to provide both recommended methods for the determination of water quality, and in addition, 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 These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print.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 series of booklets on single or related topics; 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 and notes 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 technical staff to decide which of these methods to use for the determination in hand. Whilst the attention of the users 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 a committee of the Department of the Environment set up in 1972. Currently it has seven 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
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological methods
9.0 Radiochemical methods

The actual methods and reviews 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.

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 and minor additions to published booklets not warranting a new booklet in this series will be issued periodically as the need arises. 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 that booklet.

**L R PITTWELL**
*Secretary*

*1 July 1986*

# 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 for one's self, one's colleagues, those outside the laboratory or work place, or subsequently for maintenance or waste disposal workers. 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. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specifications. 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.

Lone working, whether in the laboratory or field, should be discouraged.

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 waste, 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. Hazardous reagents and solutions should always be stored in plain sight and below face level. Attention should also be given to potential vapour and fire risks. 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.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: "Guide to Safe Practices in 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; and "The Prevention of Laboratory Acquired Infection" Public Health Laboratory Service Monograph 6, HMSO, London.

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 required 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. If an ambulance is called or a hosptial notified of an incoming patient give information on the type of injury, especially if poisoning is suspected, as the patient may be taken directly to a specialized hospital.

# Summary

The final stages of anaerobic digestion in the important process for the treatment of sewage and other sludges depend on the activity of methane-producing bacteria, which are particularly susceptible to inhibition by a range of toxic substances. A 5-day batch test, intended to determine whether chemicals or waste-waters are inhibitory to gas production is described for use as a screening procedure in place of the much more costly and time-consuming continuous test simulating large-scale digesters. Mixtures of raw and digesting sludges, containing a range of concentrations of the chemical or waste-water, are incubated in closed vessels fitted with devices to allow the manual measurement of the volume of gas production. The degrees of inhibition of the rate of gas production, compared to the control rate, during 5 d are calculated for each mixture and the concentration (EC50) giving 50% inhibition is calculated graphically.

However, it must be borne in mind that, as with other short term batch tests, the toxicity may be overestimated since in continuous digesters with retention times of 20–30 d acclimatisation to the chemical could take place. Also, little or no effect may be observed in batch tests with growth-inhibiting chemicals which can have serious effects in continuous digesters. In the former case, if the expected concentration of the chemical approaches the batch EC50, a longer, continuous digester test would resolve the problem but the course of action in the latter case is more problematical.

## 1 Introduction

Anaerobic digestion processes for the treatment of sewage sludge depend on the activity of methane-producing bacteria which are particularly susceptible to inhibition by toxic substances present in the sludge. This batch test is a screening procedure (based on the 'Amenability of Sewage Sludge to Anaerobic Digestion' (HMSO 1977) and on the work of Swanwick and Foulkes (1971)), intended to ascertain whether substances are inhibitory to methane production, in place of the more costly continuous tests simulating large-scale digesters.

However, it must be remembered that short-term batch tests, in common with other screening tests for toxicity may over-estimate toxic effects which, for certain chemicals, could be largely overcome in full-scale digesters with a retention time of 20 days, allowing a longer period of acclimatisation.

This short-term test is generally useful for fairly rapid prediction of 'worst-case' effects of shock loads of chemicals on the digestion process.

Conversely, the batch test may show little or no effect for a substance which over a longer period proves highly toxic in the full-scale digester.

Since the solids content, the proportion of undigested solids, the level of bacterial activity and the presence of other toxic compounds may be important variables influencing inhibitory effects, it is important that sludge from a constant source be used and that the reaction mixture be kept as constant as possible in respect of solids content. If the total solids concentration of, say 2% or less is used, the EC50 of the chemical may be misleadingly low.

## 2 Performance Characteristics of the Method

### 2.1 Parameter determined

Toxicity of the chemicals to anaerobic digestion of sewage sludge.

### 2.2 Types of sample

Chemicals, formulations, waste waters.

### 2.3 Nature of test

EC50 is determined.

### 2.4 Basis of method

Comparison of the volume of gas produced by incubation of mixtures of raw sludge and a digesting seed sludge with those produced by incubation of similar mixtures containing a range of concentrations of the test substance.

### 2.5 Relative standard deviation of gas volume

$$\frac{s\,d}{mean} \times 100)\%$$

| | Lab 1* | Lab 2* |
|---|---|---|
| Control | 2.2 | 1.3–3.4 |
| + 30mg/l pentachlorophenol | 2.2 | – |
| n= | 8 | 4 |

* Lab 1 = Water Research Centre, Stevenage
* Lab 2 = Unilever Research Laboratory, Port Sunlight.

### 2.6 Interferences

Chemically unstable or biodegradable toxic substances may give misleading results (Section 4)

### 2.7 Time taken

Total time of up to 6 days. Operator time of 4 hours per sample

## 3 Principle

The effect is determined of a range of concentrations of the test chemical on the rate of gas production of mixtures of raw and digesting sewage sludge. All mixtures have the same content of total solids and this should be in the range 3–6% (w/w), although lower concentrations may be used. The degree of inhibition of gas production during 5 days is calculated for each concentration of test substance used and the concentration giving 50% inhibition (EC50) is calculated graphically.

## 4 Interferences

No specific tests of interfering substances have been made. Toxic substances which are chemically unstable or biodegradable may give misleading results because they could indicate toxicity in the batch tests but may not be toxic in a continuous digester. Examples of such possible interfering substances are free chlorine, chromates and high concentrations of propionic acid.

Care must be taken to carry out the tests in a part of the laboratory where chlorinated hydrocarbon solvents are not in use, since minute traces of chlorinated hydrocarbon vapours (eg chloroform) can seriously inhibit gas production. The raw sludge may contain interfering substances (Section 6.1).

## 5 Hazards

Samples of sludge should be transported in wide-necked non-rigid polyethylene containers with loose-fitting caps or stoppers. Particular care should be taken when transporting sludges which are in a state of active digestion, due to fire and explosion hazards from generated gases. The explosive range of concentrations of methane given by HSE is 5–15%. During the test, ensure that all lines carrying gas are free from obstruction to avoid pressurising glass vessels. The tests should be carried out in a well-ventilated laboratory to avoid the possibility of forming explosive mixtures of methane and air. Sludges may contain potentially pathogenic micro-organisms and should be handled with care. Test chemicals may be hazardous and these too should be handled with care.

## 6 Reagents

### 6.1 Raw sludge

A reliable supply of raw sludge, containing no substances toxic to methane producing bacteria, of total solids content greater than 3% (w/w) is normally required. (Sludges of lower solids concentration have been successfully used.) The raw sludge should be obtained freshly each week from a local treatment works and stored at a temperature between 0 and 4°C. Before use or storage it helps in subsequent handling if the sludge is sieved through a 4 mm sieve.

### 6.2 Digesting seed sludge

A digesting seed sludge of uniform activity is required. This may be obtained from a continuous breeder digester operated in the laboratory at 33 ± 2°C, or from a properly functioning digester at a local sewage-treatment works. The sludge temperature should be maintained during transportation and before use. If the temperature of the digesting sludge is not maintained, an unacceptably long lag phase before gas evolution at a constant rate may result. It has been found suitable to run the laboratory digester by feeding daily with a volume of raw sludge so as to give a hydraulic retention time in the digester of about 25 days. An equal volume of digester contents is discarded prior to feeding. Alternatively, it has been found satisfactory to feed the digester daily on Monday to Friday, the dose on Friday being twice that on other days. It follows that the capacity of the digester needs to be at least 25 times the volume of digesting seed sludge required per day.

It is recommended that two laboratory continuous breeder digesters are operated in parallel in case one of them malfunctions or is accidentally damaged.

### 6.3 Stock solution of test substance

A solution of the test chemical of between 3 and 5 g/l in distilled water is usually suitable. Weighed amounts of insoluble compounds may be added directly to the

reaction mixture or after first being dissolved in solvent, eg ethanol. If an organic solvent is being used, separate tests must be carried out to determine any effect it might have.

## 7 Apparatus

### 7.1 Batch digestion (incubation) apparatus

This apparatus is constructed in the laboratory. A suitable apparatus of the type used at the Water Research Centre (Stevenage Laboratory) is shown diagrammatically in Figure 1.

The following appropriate dimensions are to be recommended:

Incubation bottles 500-ml capacity

Gas collecting tubes 110 cm length, 5 cm internal diameter
1500 ml useable capacity

The gas-collecting tubes should be calibrated in volume (ml) or height (mm) with the zero mark at the top of the tube. Self-adhesive graduations are suitable. The gas is collected over water acidified with hydrochloric acid to a pH value of 4.0 and containing a suitable pH-indicator. The presence of acid, though not essential to prevent absorption of carbon dioxide, prevents microbial growth in the collecting tubes, and the indicator facilitates reading of the volumes.

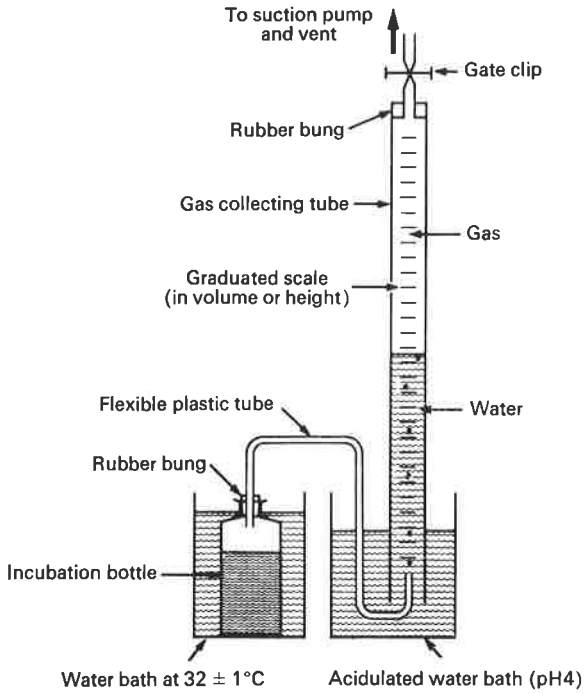


Figure 1 Batch digestion apparatus (side view—not to scale)

### 7.2 Water bath

A water bath with adequate circulation to maintain a temperature of 33 ± 2°C in all parts of the bath is required.

### 7.3 Vacuum pump

A water ejector vacuum pump is used to evacuate methane from the apparatus at the end of the test.

### 7.4 Glassware

Measuring cylinders are preferred to pipettes for measuring out volumes of sludge. With the thicker sludges care should be taken to ensure that the measured volume is completely dispensed.

## 8 Test Procedure

| Step | Procedure | Notes |
|---|---|---|
| | **Standardisation of sludges** | |
| 8.1 | Determine the total solids content and volatile solids content of the raw sludge and digesting sludges by suitable methods (see appropriate methods in this series (Note a). | (a) Sludges of total solids contents of 3–6% w/w are recommended, though lower concentrations have been used sucessfully. |
| | **Incubation of sludge mixtures** | |
| 8.2 | Warm the bath to 33 ± 2°C. | |
| 8.3 | Label six incubation bottles (1–6) and transfer to them the appropriate raw sludge as, for example, shown in Table 1 (Note b). Place the incubation bottles in the warm bath. Add the volumes of solution of test substance or waste water and distilled water (Table 1) to the appropriate bottles (Note c) to give concentrations centrations of test substance of, for example 0, 1, 5, 20, 200 mg/l (note d). Shake. | (b) To dispense representative volumes of sub-samples of sludges, shake the bulk sample vigorously in a closed bottle and then rapidly measure the required volume into a measuring cylinder.<br><br>(c) The pH value of the mixtures should be between 7 and 8, as determined in identical mixtures made up prior to incubation. If outside this range add a suitable amount of HCl or NaOH. However, especially in the case of waste waters, the report of the test should contain a reference to the original pH of the mixture and to the fact that the pH of the mixture was adjusted. It may be appropriate to carry out a test without pH adjustment.<br><br>(d) It may be necessary to carry out a sighting experiment to obtain the range of concentration giving 0–80% inhibition. |
| 8.4 | Allow the bottles 15–20 min to warm up to 33 ± 2°C and then transfer the required volume of digesting seed sludge (Note e), eg as shown in Table 1, to the bottles (Note b).<br><br>Alternatively, carry out Step 8.3 without the raw sludge. Warm the latter to the required temperature, mix with digesting seed sludge in the required proportion and dispense to the bottles as required. | (e) If the solids contents of the sludges are lower than the recommended range, the proportions of raw and digesting sludges may be altered to take this into account. |
| 8.5 | Connect the incubation bottles to the gas collecting tubes, gently shake or swirl the bottles and wait about 15 min for the temperatures to equilibrate (Note f). Adjust the water level in the gas collecting tubes to the zero mark by suction. Close the clip and note the time; this is the start of the incubation period (Note f). | (f) Some gas will be displaced into the collecting tube indicating a gas-tight seal. |
| 8.6 | After the commencement of incubation and for up to 5 d thereafter (Note g), gently shake or swirl the incubation bottles twice daily (at the beginning and end of the working day), wait for about 2 min and read the volume or height of gas produced from each incubation bottle. | (g) The total time for a full test may be up to 6.5 days made up of approximately 1.5 days determining the total and volatile solids content of the sample sludge and preparing the apparatus followed by up to 5 days incubation. |

Table 1 An example: for soluble substances

(assumes stock solution of test substance to be 3.7 g/l)

| Incubator bottle number | Volume of raw sludge | Volume of solution of test substance | Volume of distilled water | Volume of digesting seed sludge | Total volume |
|---|---|---|---|---|---|
| | | (ml) | (ml) | (ml) | (ml) |
| 1 | 80 | 0 | 50 | 240 | 370 |
| 2 | 80 | 0.1 | 49.9 | 240 | 370 |
| 3 | 80 | 0.5 | 49.5 | 240 | 370 |
| 4 | 80 | 2 | 48 | 240 | 370 |
| 5 | 80 | 10 | 40 | 240 | 370 |
| 6 | 80 | 50 | 0 | 240 | 370 |

Note: The volumes of distilled water and solution of test substance may be varied (keeping the same total volume) to obtain the required range of concentrations of test substance.

## 9 Calculation of Results

| Step | Procedure | Notes |
|---|---|---|
| 9.1 | Plot a graph of the cumulative gas volumes against incubation time for each incubation mixture. | |
| 9.2 | Select a time when the rate of gas production is constant (Note h) for each of the mixtures (usually between 24 and 72 hours) and from the graph read the volumes of gas produced $V_1$, $V_2$, $V_3$, $V_4$, $V_5$, and $V_6$ in the 6 bottles at the selected time. If the rate is not constant, extrapolate from the initial (constant) rate. | (h) This is the time when the concentration of substrate is not rate-limiting. |
| 9.3 | Calculate the percentage inhibition I, caused by each concentration of substance: in bottles 2–6: $I_2 = \frac{V_1 - V_2}{V_1} \times 100$ $I_3 = \frac{V_1 - V_3}{V_1} \times 100$, etc | |
| 9.4 | Calculate the concentration of test substance as mg/g dry solids (ie mg/l test substance divided by the concentration (g/l) either of the total solids of the mixture or of solids of the raw sludge) and plot percentage inhibition against log of concentration of test substance. | |
| 9.5 | From the graph, read off EC50, the concentration giving 50% inhibition. Similarly, other values may be read off, eg EC20, EC80. | |

## 10 General Comments

If the range of inhibition was not 80.0% or if insufficient data were collected to be able to read off an EC50 value, repeat with appropriate concentrations of test substance, up to a maximum of 2.5% (wt of substance/wt of sludge solids). The concentration of total solids of the sludge mixtures should be reported, together with the method of calculation of concentration of test substance.

If required, the test may be extended up to a maximum of 20 days to see whether the toxic effect of the test substance may be overcome.

It may be advisable from time to time to use a standard inhibitory substance (eg pentachlorophenol) to check that the raw and digesting sludges are behaving normally (ie not adapted).

The usual range of amounts of gas produced from the incubated bottles containing no inhibitor will be found by experience and any appreciable deviation from this which cannot be explained by the total solids content may indicate a failing breeder digester. A regular check of the pH, total alkalinity and volatile solids content of the breeder digester should be made.

## References

Amenability of sewage sludge to anaerobic digestion. (1977) ISBN 0 11 751250 8. Her Majesty's Stationery Office, in this series.

Methods for assessing the treatability of chemicals and industrial waste waters and their toxicity to sewage treatment processes. (1982) ISBN 0 11 75 19596, Her Majesty's Stationery Office, in this series.

Swanwick J D and Foulkes M Inhibition of anaerobic digestion of sewage sludge by chlorinated hydrocarbons. *Wat Pollut Control* **70**, 58 (1971).

## Address for Correspondence

However thoroughly a method may be tested, there is always the possibility of users discovering a hitherto unknown problem. Users with queries or information should write to:

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The Standing Committee of Analysts
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