The Assessment of Biodegradability in Anaerobic Digesting Sludge 1988.

Methods for the Examination of Waters and Associated Materials

London: Her Majesty's Stationery Office

First Published 1989

ISBN 0 11 752191 4

Within the Method for the Examination of Waters and Associated Materials series are four-ring binders suitable for use in storing reports. These are available from HMSO Price £4 (ISBN 0 11 7514373)

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 These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print.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 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 a committee of the Department of the Environment set up in 1972. Currently it has 9 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 Microbiological methods
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological monitoring
8.0 Sewage works control 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 and Chairman

11 August 1988

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 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. 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 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. If an ambulance is called or a hospital 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

This booklet describes a simple and reliable method for assessing the potential of an organic chemical to undergo biodegradation in anaerobically digesting sludge.

The Assessment of Biodegradability in Anaerobically Digesting Sludge 1988

1. Introduction

The assessment of the potential hazards resulting from a chemical that is discharged to the environment after sewage treatment, requires reliable tests for predicting both its aerobic and anaerobic fate during treatment. Methods for assessing aerobic biodegradability are given elsewhere in this series (Standing Committee of Analysts 1983). However, many chemicals with a low solubility in water or which adsorb to particulate matter can bind to sewage sludge. The final stage in the treatment of sewage sludge by anaerobic digestion is the production of methane (CH_4) and carbon dioxide (CO_2). Under such conditions, a biodegradable chemical will be broken down by a syntrophic consortium of fermentative and methanogenic bacteria to yield CH_4 and CO_2 as the end products. The determination of the difference in total gas (CH_4 + CO_2) produced by anaerobic digesting sludge alone, and by sludge supplemented with a test chemical, gives a simple and sensitive measurement of the chemical's potential to undergo anaerobic biodegradation under methanogenic conditions. The procedure described below is a screening test, which can also give an indication of the toxicity of the test chemical to anaerobic digestion. It is based on the method of Shelton and Tiedje (1984) for determining anaerobic biodegradation potential.

2. Performance Characteristics of the Method

2.1	Property determined	The biodegradability of an organic chemical, expressed as total net gas produced as a percentage of the theoretical production (%ThGP).
2.2	Type of Sample	Organic chemicals.
2.3	Basis of method	Measurement of the volume of gas produced when the test chemical is incubated with diluted, primary digesting sludge under anaerobic conditions.
2.4	Precision	Test chemical at 50 mg carbon/l in diluted digesting sludge from two different sewage treatment works:

Chemical	Mean % ThGP ± standard error Sludge 1*	Sludge 2*
Ethanol	107 ± 5.5	112 ± 2.2
2-Cresol	−16[1] ± 5.2	−10[1] ± 6.8
4-Cresol	96 ± 4.3	97 ± 4.6
Sodium benzoate	93 ± 7.5	no data
Di-n-butyl phthalate	24 ± 9.6	59 ± 4.5

n = 3

[1] Chemical inhibited gas production

* Data obtained at WRc Medmenham

2.5	Reproducibility of results with different batches of sludge from the same source	The number of times complete degradation occurred/number of times tested is shown below: Sodium acetate 4/4 Sodium benzoate 2/2 4-Cresol 17/19 Ethanol 22/23 Phenol 2/2 Phthalic acid 2/2
2.6	Bias	The test allows acclimatization to the test chemical to occur over a time period which may be longer than the sludge retention time of the digester. The use of diluted sludge and a high concentration of test chemical may yield false negatives.
2.7	Interferences	Ingress of oxygen; strongly acidic or sulphate-containing substances (see Section 10).
2.8	Time required for test	Up to eight weeks. Operator time: around 12 hours per batch of five test compounds and two reference chemicals.

3. Principle

Diluted primary digesting sludge is incubated with 50 mg carbon/l of the test chemical in sealed bottles. Gas (CH_4 + CO_2) production is measured by an increase in pressure in the bottles and converted to volume of gas produced. The net amount of gas produced from the degradation of the test substance (test gas production corrected for blank) is expressed as a percentage of the theoretical gas production calculated from the formula of the test chemical.

4. Interferences

Ingress of oxygen into the test bottles will reduce gas production (Section 10.1). Strongly acidic compounds may produce an abiological flux of CO_2 into the bottle headspace and elevated pressure readings (Section 10.2). Test chemicals high in sulphate may cause the terminal stage of biodegradation to be sulphate-reduction and not methanogenesis (Section 10.4).

5. Hazards

Digesting sludge may contain pathogenic microorganisms and appropriate precautions should be taken during handling to avoid the risk of infection. Primary digesting sludges produce large amounts of CO_2 and flammable CH_4. This presents a fire and explosion risk, so care must be taken when transporting the sludge (Section 6.1). Appropriate safety procedures should be followed if a stock solution of the test chemical is made up in an organic solvent. The use of a proprietary hand protector is recommended when handling the hot medium (Section 6.2) and the gas flow through the aspirator sparger (see Figure 3) should be at a rate that does not cause excessive splashing of the hot medium. Care should be exercised when using microsyringes or the pressure meter to avoid needle-stick injuries. Contaminated syringe needles should be disposed of in a suitable container and incinerated.

6. Reagents

6.1 Primary digesting sludge

Primary anaerobic digesting sludge is collected, from a properly functioning digester, in 1 or 2 litre bottles. The bottles should have a wide neck, an air-tight seal and be constructed from high density polyethylene or a similar material which can expand. Glass bottles must not be used. The bottles are filled to within 1 cm of the top and sealed tightly. The sludge can be stored overnight at room temperature under a headspace of 90% N_2/10% CO_2 without appreciable loss of activity. However, care must be taken to release excess gas pressure in the bottle either by periodically loosening the seal or by fitting a pressure-release valve to the bottle cap. Prior to use, the sludge is passed through a 1 mm square mesh and the total solids content determined (Standing Committee of Analysts 1985).

6.2 Dilution medium

The dilution medium should be made up with analytical grade reagents and contains (per litre distilled or deionised water):

KH_2PO_4	0.27g
K_2HPO_4	0.35g
NH_4Cl	0.53g
$CaCl_2.2H_20$	0.08g
$MgCl_2.6H_20$	0.10g
Trace element solution[1]	1 ml
Resazurin	1 mg

The pH of the medium is adjusted to 7.0 with 2M NaOH.

[1] Trace element solution of Pfennig *et al* (1981):

HCl (36% w/w)	5.1 ml
$FeCl_2.4H_2O$	1.5g (dissolve in acid)
H_3BO_3	60 mg
$MnCl_2.4H_2O$	100 mg
$CoCl_2.6H_2O$	120 mg
$ZnCl_2$	70 mg
$NiCl_2.6H_2O$	25 mg
$CuCl_2.2H_2O$	15 mg
$Na_2MoO_4.2H_2O$	25 mg
Distilled water to	1 litre

Store in the dark at 4°C.

The complete medium is deoxygenated by autoclaving at 121°C for 5 minutes (the medium may be cloudy after autoclaving but this should clear on cooling). As soon as the pressure inside the autoclave is down to atmospheric, empty the hot medium into the aspirator (which has been gassed-out with 90% N_2/10% CO_2). Cool the medium to 35°C, whilst sparging with the gas mixture. When the medium has cooled, add 1.20 g $NaHCO_3$/1 medium. At this stage the medium is not reduced and appears blue-purple.

6.3 Preparation of diluted sludge inoculum

Filtered sludge is added to the medium (stirred and held at 35°C in a water bath) to give a final concentration of 2-3 g dry solids/1 medium. At this stage the medium should lose its colour or change to pink as it is reduced by the anaerobic sludge.

7. Apparatus

7.1 Precision pressure meter and needle attachment

Total gas production (CH_4 + CO_2) is measured by a pressure meter adapted to take a male Luer fitting (Figure 1). A suitable instrument is the Type 300 Series (input pressure range 0-1.999 bar) hand-held precision pressure meter manufactured by John Watson and Smith Ltd (Melville Place, Leeds LS6 2EU).

Pressure readings are related to gas volumes by means of a standard curve (Figure 2) produced by injecting known volumes of gas at 35°C into serum bottles containing 100 ml medium.

7.1.1 Calibration of pressure meter

(a) Dispense 100 ml aliquots of medium (held at 35°C) into five serum bottles.

(b) Seal and place in a 35°C water bath for one hour to equilibrate.

(c) Switch on pressure meter and allow to stabilise.

(d) Zero instrument.

(e) Insert syringe needle through seal of one bottle and open valve until pressure reads zero. Close valve.

(f) Repeat for other bottles.

(g) Inject 1 ml of air at 35°C into each bottle.

(h) Insert needle through seal of one bottle and allow pressure reading to stabilise.

(i) Record pressure and open valve until pressure reads zero. Close valve.

(j) Repeat for other bottles.

(k) Repeat using 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18 and 20 ml air.

(l) Plot a calibration curve of pressure (bar) against gas volume (ml). A typical calibration curve is shown in Figure 2. It can be seen that the response of the instrument is linear over the range 0 to 0.3 bar (0-20 ml gas production). Pressures > 0.3 bar in a single reading are generally not encountered in this test.

7.2 Serum bottles

The sludge is incubated in 125 ml capacity Wheaton serum bottles (actual capacity 160 ml) sealed with No 25 (12.5 mm od) Suba-Seal septa. The re-sealing ability of a septum, after puncturing, can be extended by a blob of silicone rubber sealing compound (eg. 'Silcoset 151') on its top. This can be replaced during the course of the experiment.

7.3 Anaerobic transfer apparatus

For a valid prediction of the biodegradability of a chemical under anaerobic conditions to be made, it is essential that exposure of the sludge to the atmosphere is kept to a minimum. A suitable apparatus for the anaerobic transfer of sludge into serum bottles is shown diagrammatically in Figure 3. It is convenient to use the N_2/CO_2 mixture at an outlet pressure of 0.4 bar. The gate-clamps V3 and V4 are adjusted so that when V1 (Medi-Clamp) and V2 (Mohr clip) are fully open, gas still flows through the aspirator's sintered sparger and the gassing needles.

7.4 Incubator

An incubator which can maintain a temperature of 35°C ± 0.1°C is required.

8. Test Procedure

Step	Procedure	Notes
8.1	The aspirator should contain an appropriate volume of diluted digesting sludge at 35°C (see 6.2 and 6.3). The sludge should be stirred at a speed which keeps it dispersed but does not create a vortex. Gas should be flowing through the gassing needles and the sintered sparger. V1 and V2 should be closed.	
Test chemical addition before sludge:		
8.2	Water soluble test substances are added to triplicate bottles from an aqueous stock solution. Solids which are insoluble in water but are soluble in an organic solvent are added from stock solutions in that solvent. The solvent is evaporated before the sludge is added (note a). Insoluble solids are individually weighed out and added to triplicate bottles. (Liquids are injected into inoculated bottles by microsyringe, see 8.21).	(a) The evaporation of the solvent can be speeded up by placing the bottles under the gassing needles.
8.3	Place three serum bottles under the gassing needles and leave for two minutes.	

Step	Procedure	Notes
	Filling the pipette with sludge:	
8.4	Open V1 and release V2 to flush out the pipette with N_2/CO_2 (note b).	(b) Check that gas still flows through the gassing needles and sintered sparger.
8.5	Switch on the vacuum pump, ensuring that the three-way tap allows the pump only to draw in air from the atmosphere (position 1).	
8.6	Close V2 and place the pipette in the sludge.	
8.7	Close V1 and turn the three-way valve so that the vacuum pump exerts suction through the gas line to the pipette.	
8.8	Gradually open V2 to allow sludge to be sucked into the pipette.	
8.9	When the sludge is approximately two thirds up the pipette bulb, close V2 and turn the three-way tap to position 1.	
8.10	Using the residual vacuum in the system, gradually release V2 and suck up the sludge to the 100 ml mark. Close V2 (note c).	(c) This procedure enables the sludge to be sucked up with more control.
	Dispensing the sludge into serum bottles	
8.11	Place the tip of the pipette into the top of the serum bottle.	
8.12	Slowly open V1 to re-pressurise the gas line to the pipette (note d).	(d) Although the gas line to the sparger contains a non-return valve, rapid re-pressurisation will suck sludge back into the sparger gas line.
8.13	With V1 fully open, release V2 to dispense the sludge into the serum bottle.	
8.14	Repeat procedures 8.4 to 8.13 for the remaining two bottles.	
8.15	Whilst removing the first bottle from the gassing needle, stopper with a Suba-Seal (note e). Fold the collar of the Suba-Seal over the neck of the bottle and place in a 35°C incubator.	(e) The Suba-Seal should be lubricated with a few drops of distilled/deionised water.
8.16	Place a new bottle beneath the gassing port.	
8.17	Repeat procedure 8.15 for the remaining two bottles.	
8.18	Dispense remainder of sludge.	

Step	Procedure	Notes
	Experimental design:	
8.19	**Blanks:** Triplicate bottles containing unamended sludge.	
8.20	**Reference chemicals (50 mgC/l):** Inject triplicate bottles containing sludge with 12 μl ethanol (readily degradable) or 6.2 μl 4-cresol (more stringent test of sludge activity).	
8.21	**Liquid test chemicals:** Inject triplicate bottles containing sludge with the test chemical by microsyringe.	
8.22	**Sterile controls** (abiotic gas production): Inoculated bottles, autoclaved at 121°C for 30 minutes and amended with sterile chemical.	
8.23	Allow all bottles to equilibrate to 35°C in an incubator for two hours.	
8.24	Equalise the pressure in the bottles to atmospheric (note f).	(f) See Section 7.1.1 (e). The bottles should be shaken prior to equalisation.
8.25	Incubate the bottles at 35°C in the dark.	
8.26	Measure the pressure in the bottles (note g) after one day's incubation and then weekly. If the pressure reading is $\leqslant$ 0 bar, do not open valve.	(g) See Section 7.1.1 (h) and (i). Moisture sometimes accumulates in the syringe needle. This is often indicated by a small negative pressure reading when removing the needle from the Suba-Seal. If this happens, dry the Luer connector and fit a new needle.
8.27	Continue the test for 8 weeks or until degradation has finished (cumulative net gas production reaches a plateau, note h).	(h) Net gas production at time t = (mean gas production in test bottles at time t) − (mean gas production in blank bottles at time t). The course of degradation can be followed by plotting cumulative net gas production against time.

9. Calculation of Degradation and Interpretation of Results

The theoretical gas production (ThGP) from the anaerobic biodegradation of 50 mg carbon/l of any test chemical is:

$$\text{ThGP} = \frac{\text{mg carbon test substance in bottle} \times \text{volume of one mole gas at 35°C}}{\text{atomic weight of carbon}}$$

$$= \frac{5 \times 25.25}{12.01} = 10.5 \text{ ml}$$

It has been demonstrated that this gas will be divided between CH_4 and CO_2 based on the stoiochiometry (Buswell and Mueller 1952):

$$C_cH_hO_o + \left\{ c - \frac{h}{4} - \frac{o}{2} \right\} H_2O \rightarrow \left\{ \frac{c}{2} - \frac{h}{8} + \frac{o}{4} \right\} CO_2 + \left\{ \frac{c}{2} + \frac{h}{8} - \frac{o}{4} \right\} CH_4 \quad (1)$$

Correction must be made for the solubilities of the two gases (especially CO_2). Correction factors (*) specific for the volumes and temperatures specified above are given below. The theoretical gas production (ThGP) from a chemical is therefore:

$$\left\{ \frac{\text{moles } CO_2}{(\text{moles } CO_2 + \text{moles } CH_4)} \right\} \times 10.5 \times 0.35^* \text{ ml } CO_2$$

plus

$$\left\{ \frac{\text{moles } CO_2}{(\text{moles } CO_2 + \text{moles } CH_4)} \right\} \times 10.5 \times 0.95^* \text{ ml } CH_4$$

If the test chemical contained carboxyl groups that were neutralised before addition to the sludge, then one mole CO_2/neutralized $-COOH$ group should be subtracted from the number of moles given by equation (1).

The total volume of net gas produced (NGP) from the degradation of the test chemical at the end of the experiment is calculated as follows:

$$NGP = \frac{(V1 + V2 + V3)}{3} - \frac{(B1 + B2 + B3)}{3}$$

where, V is the total volume of gas produced by each test bottle (corrected for any gas production by sterile controls) and B is the corresponding volume for the blanks.

An assessment of the potential for anaerobic degradation of the test chemical is made as follows:

Complete degradation	$\geqslant$	80% ThGP
Not degraded	$<$	30% ThGP
Inhibitory		Negative cumulative net gas production

The level for the complete degradation of a test chemical is set at $\geqslant$ 80% ThGP as test chemical carbon may be incorporated into new biomass and CO_2 produced during the breakdown of the chemical can be trapped as bicarbonate complexes with Ca^{2+} and Mg^{2+} (Healey and Young, 1979). The disappearance of test chemical with concomitant gas production in this test has been verified by specific analysis (Battersby and Wilson, 1988).

The use of diluted sludge and a relatively high concentration of test chemical means that the test is "fail safe negative". A chemical which fails to degrade or inhibits gas production in the test may not necessarily persist in an anaerobic sludge digester. The test may be repeated with a concentration of test chemical which is $<$ 50 mg carbon/l if toxicity is indicated, although the precision with which %ThGP can be estimated decreases with decreasing test chemical concentration.

10. Sources of error

10.1 Oxygen contamination

Anaerobic methods are always subject to error from oxygen contamination. This should be minimised by the use of strict anaerobic handling techniques (see Section 8) and the redox indicator resazurin, which turns pink at a redox potential > -110 mV. The presence of oxygen in the bottles can inhibit the obligately anaerobic methane-producing bacteria. In addition, aerobic respiration by faculatively anaerobic bacteria in the sludge, will result in test chemical carbon being utilised for O_2 consumption and not $CH_4 + CO_2$ production. This will reduce the amount of gas produced in the test.

10.2 Acidic chemicals

The addition of strongly acidic test chemicals to the medium may result in a flux of CO_2 into the headspace due to the reaction:

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons \overline{CO_2}\uparrow + H_2O$$

This abiotic gas production is corrected for by the use of sterile controls (see section 8.22).

10.3 Volatile chemicals

Volatile chemicals may be released into the headspace. This may produce loss of test chemical from the system during venting of excess gas after pressure measurements.

10.4 Sulphate-containing chemicals

Sulphate-reducing bacteria (SRB) are widely distributed in sewage and in sewage sludge. Ecological studies have shown that in the presence of non-limiting concentrations of sulphate, SRB can outcompete methanogenic bacteria for acetate and H_2. Under these conditions the terminal stage in the degradation of the test chemical will be dissimilatory sulphate-reduction (end-product H_2S and CO_2) and not methanogenesis (end products CH_4 and CO_2). This invalidates equation (1) and means that the %ThGP cannot be calculated. In addition, the sulphide produced may corrode the pressure transducer.

SRB activity in the test can be detected by a blackening of the sludge (due to the formation of FeS) and a smell of H_2S (''rotten eggs'') when venting the bottle. Under these conditions the experiment should be terminated.

Unfortunately, the amount of sulphate that will allow the growth of SRB in the test will vary from sludge to sludge. The use of inhibitors of sulphate-reduction such as 20 mM molybdate (MoO_4^{2-}), is not recommended as they can also have an inhibitory effect on methanogenesis.

11. Validity of Test

Pressure readings should only be used from bottles which show no pink colouration after 24 hours incubation. The test is valid if gas production in bottles containing ethanol is $\geqslant$ 80% ThGP after 7 days incubation; and if gas production in bottles containing 4-cresol is $\geqslant$ 80% ThGP at the end of the test. Failure to achieve 80% ThGP within the specified time could be caused by ingress of air into the bottles or a sludge of low activity.

Address for Correspondence

However thoroughly a method may be tested there is always the possibility of a user encountering a hitherto unreported problem.

Correspondence about these methods should be addressed to:—

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

References

Battersby, N S and Wilson, V. Evaluation of a serum bottle technique for assessing the anaerobic biodegradability of organic chemicals under methanogenic conditions. *Chemosphere* **17,** 2441-2460. (1988).

Buswell A M and Mueller, H F. Mechanism of methane fermentation. *Industrial and Engineering Chemistry* **44,** 550-552. (1952)

Healey J B and Young L Y. Anaerobic biodegradation of eleven aromatic compounds to methane. *Applied and Environmental Microbiology* **38,** 84-89. (1979)

Pfennig N, Widdel F and Trüper H G. The dissimilatory sulfate-reducing bacteria. In: *The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria vol 1* (ed M P Starr, H Stolp, H G Trüper, A Balows and H G Schlegel) pp 926-940. Springer-Verlag, New York. (1981)

Shelton D R and Tiedje J M. General method for determining anaerobic biodegradation potential. *Applied and Environmental Microbiology* **47,** 850-857. (1984)

Standing Committee of Analysts. *Assessment of biodegradability 1981.* Methods for the Examination of Waters and Associated Materials. HMSO, London. (1983)

Standing Committee of Analysts. *The conditionability, filterability, settleability and solids content of sludges 1984 (a compendium of methods and tests).* Methods for the Examination of Waters and Associated Materials. HMSO, London. (1985)

Figure 1 Pressure meter and needle attachment

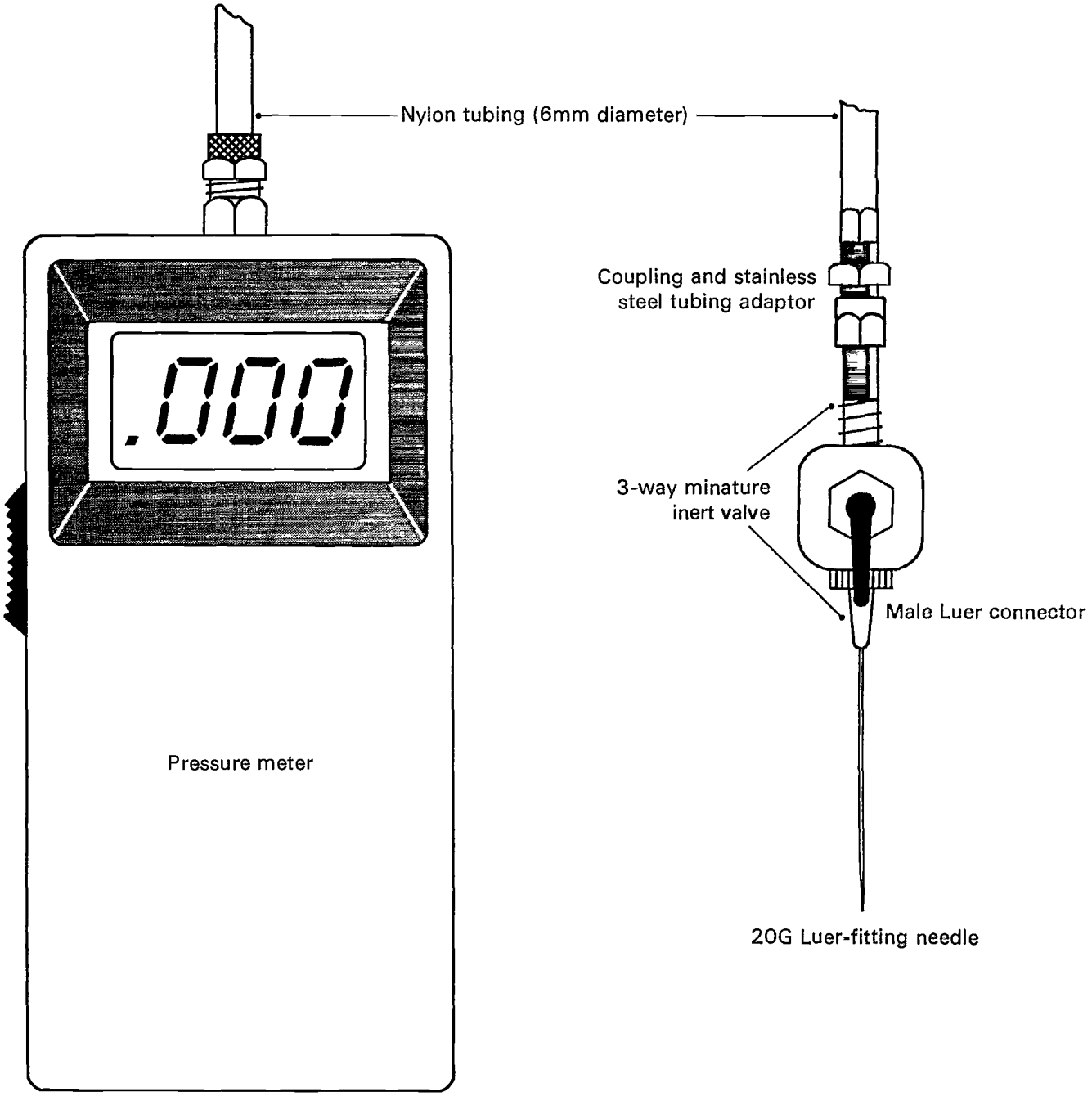


Figure 2 Calibration curve for pressure meter

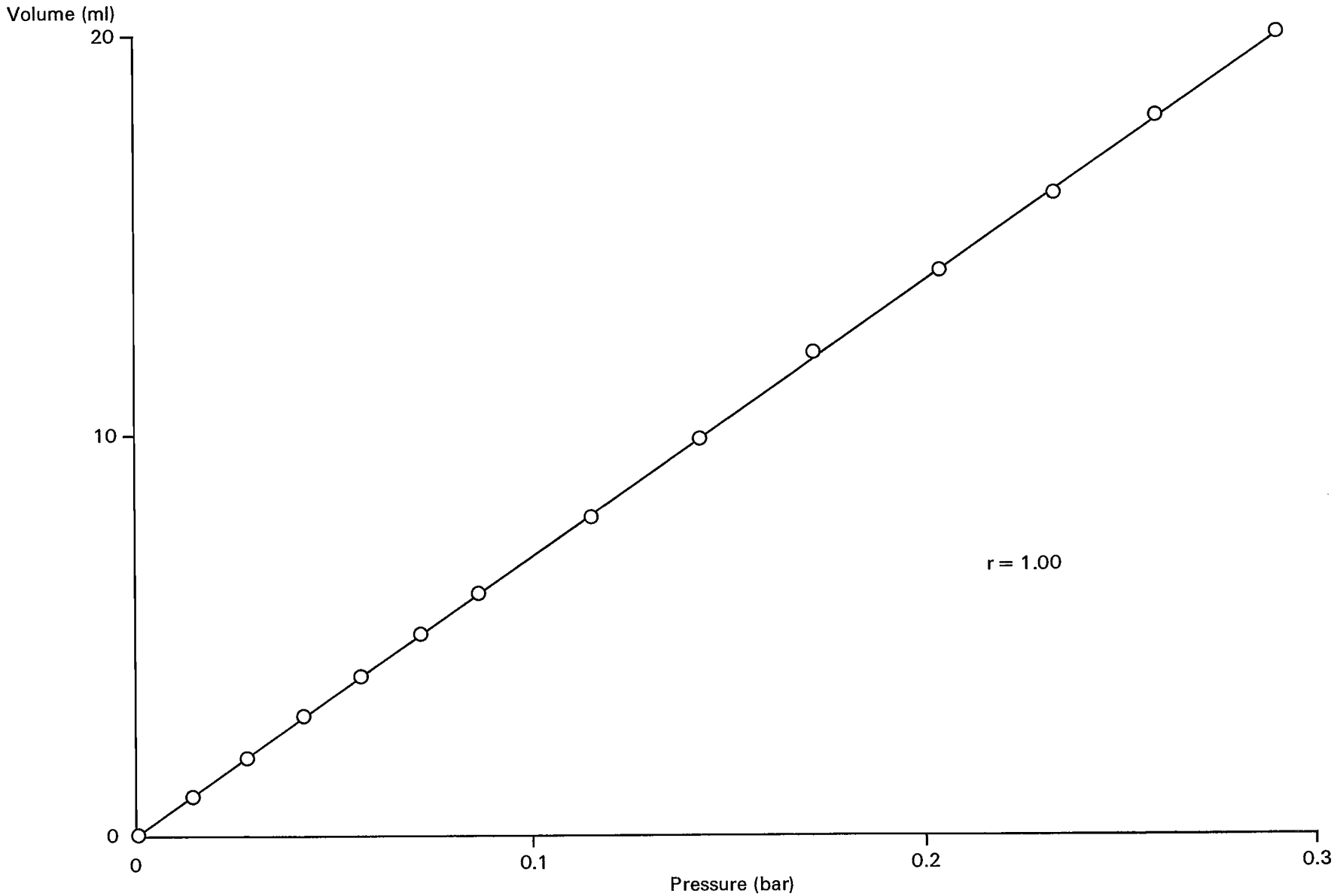
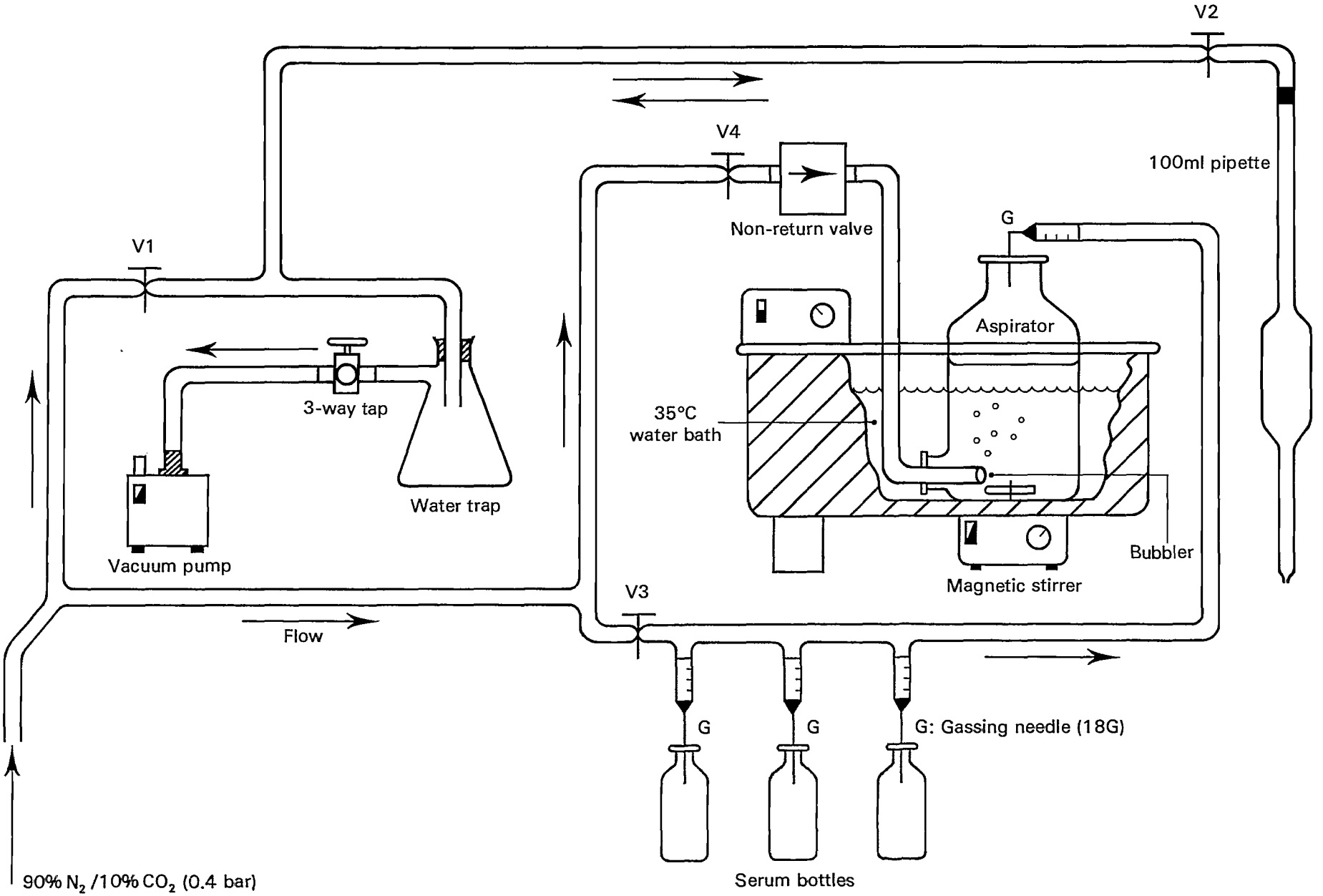


Figure 3 Apparatus for the anaerobic transfer of sludge



All tubing (except between vacuum and 3-way tap) is 4.5mm o.d. neoprene

Department of the Environment

Standing Committee of Analysts

Membership responsible for this method

A Adam 1
H T Banhoorn 1
F B Basketter 1
Dr G A Best 1
R R Birch 2
Dr N Battersby 2
P A Chave 1
E I Clark 2
Dr B T Croll 1
Dr J V Dadswell 1
E Donaldson 1
E Dryburgh 1
Dr M Evans 2
M C Finniear 1
M Gardner 1
J R Garlick 1
G I Goodfellow 1
M R Hurcombe 1
Dr J G Jones 1
R Law 1
J S Leahy 1
Dr H A Painter 1, 2
L R Pittwell 1
L D Purdie 1
L F Reynolds 2
L A Richards 1
M L Richardson 2
Prof J P Riley 1
R C Robinson 2
Dr D Taylor 1
S Turner 2
Dr A M Ure 1
R J Vincent 1
P J Walker 1
Dr R Watkinson 2

Main Committee 1
Working Group 2

Printed in the United Kingdom for Her Majesty's Stationery Office.
Dd 290847, 2/89, C10, 3385/2, 16268.

HMSO publications are available from:

HMSO Publications Centre
(Mail and telephone orders only)
PO Box 276, London SW8 5DT
Telephone orders 01-873 9090
General enquiries 01-873 0011
(queuing system in operation for both numbers)

HMSO Bookshops
49 High Holborn, London, WC1V 6HB 01-873 0011 (Counter service only)
258 Broad Street, Birmingham, B1 2HE 021-643 3740
Southey House, 33 Wine Street, Bristol, BS1 2BQ (0272) 264306
9-21 Princess Street, Manchester, M60 8AS 061-834 7201
80 Chichester Street, Belfast, BT1 4JY (0232) 238451
71 Lothian Road, Edinburgh, EH3 9AZ 031-228 4181

HMSO's Accredited Agents
(see Yellow Pages)

And through good booksellers

£2.90 net

ISBN 0 11 752191 4