Determination of Volatile Fatty Acids in Sewage Sludge 1979

Methods for the Examination of Waters and Associated Materials

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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 a properly equipped laboratory. 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 others. 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. One such publication is 'Code of Practice for Chemical Laboratories' issued by the Royal Institute of Chemistry, London. Another such publication, which includes biological hazards, is 'Safety in Biological Laboratories' (editors E Hartree and V Booth), Biochemical Society Special Publication No 5, The Biochemical Society, 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 radiochemical 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 the 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 a hazard may exist and take reasonable precautions rather, than to assume that no hazard exists until proved otherwise.

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About this series

This booklet is one of a series intended to provide recommended methods for the determination of water quality. In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, has 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 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.

TA DICK
Chairman

LR PITTWELL
Secretary

20 July 1977

About this booklet

1 This volume contains two methods for the determination of volatile fatty acids (chiefly $C_2 - C_6$). A rapid control test is mentioned but not described in detail.

2 Chromatographic methods are very sensitive to minor physical and chemical variations in the quality of the materials and apparatus used. The method therefore mentions the actual materials used for the evaluation tests. This in no way endorses these materials as superior to other similar materials. Equivalent materials are acceptable but it must be understood that the performance characteristics may be different and may vary with batch. It is left to the senior supervising analyst to evaluate and choose from the appropriate brands available.

Determination of Volatile Fatty Acids in Sewage Sludge 1979

Introduction

1 Use of the Determinand

The lower aliphatic carboxylic acids (volatile fatty acids) are formed during the anaerobic digestion of sewage sludge. Under normal digester operating conditions these acids are converted to methane and carbon dioxide. Satisfactory operation of digesters is characterized by a relatively low and fairly constant concentration of volatile fatty acids, often less than 300 mg/l expressed as acetic acid. One of the earliest indications of digester malfunction is a sudden substantial rise in the concentration of volatile fatty acids. An increase in the concentration of volatile acids or changes in their proportions are usually apparent before the pH value is affected.

2 Gas Liquid Chromatographic Method

The recommended method for the determination of the concentration of volatile fatty acids is based on gas liquid chromatography. This method enables the 'total' volatile fatty acids concentration and a number of individual fatty acid concentrations to be determined. It should be used wherever practicable.

3 Spectrophotometric Method

For laboratories in which the gas liquid chromatography method cannot be used, an empirical method based on the spectrophotometric determination of ferric hydroxamates is recommended. This method enables the 'total' volatile fatty acids expressed as acetic acid to be determined.

4 Rapid Control Test

Volatile fatty acids may also be determined by electrometric titrimetry on the neutralized sludge obtained from the determination of alkalinity (see a publication in this series). Carbon dioxide must be removed first by boiling under reflux at pH value 3·3 and the sludge pH value adjusted to 4·0. The volatile fatty acids are taken to be equivalent to the sodium hydroxide required to raise the pH value to 7·0. This method may only be employed to provide approximate comparative results and has not been included as a full recommended method.

5 Sample Storage

It should be noted that sludge samples may change composition because of continuing biological activity and therefore they must be analyzed as soon as possible after sampling, preferably within 8 hours. They should be stored in a flame proof refrigerator.

Method A Gas-Liquid Chromatographic Determination [1]

A1 Performance Characteristics of the Method

(For further information on the determination and definition of performance characteristics see a publication in this series)

A1.1	Substances determined	The lower fatty acids ($C_2 - C_6$).
A1.2	Type of sample	Raw, digesting and digested sewage sludge.
A1.3	Basis of method	Separation of the sludge liquor followed by gas chromatographic determination using a flame ionization detector. Confirmed by using a second column.
A1.4	Range of application	Up to 2000 mg/l.
A1.5	Calibration curve	Linear over range of application.
A1.6	Standard deviation	See Table 1.
A1.7	Limit of detection	See Table 1.
A1.8	Sensitivity	See Table 1.
A1.9	Bias	No significant bias known (see Table 1).
A1.10	Interferences	None normally encountered; (see Section A3).
A1.11	Time required for analysis	25 minutes per determination 15 determinations per day (using primary column only): operator time, 5 minutes per sample.

Table 1 Statistics of Method for the Determination of Volatile Fatty Acids by Gas Chromatography Using the Primary Column

Acid	Type of sample	S_w	S_b[d]	S_t	Mean conc mg/l	95% mg/l confidence limits	Sensitivity millivolts equivalent to 500 mg/l	Limit of[c] detection mg/l	%Recovery from sludge	
									100 mg/l spike	1000 mg/l spike
Acetic	5 Std[a]	0·58	0·84	1·02	4·02	0·5				
	2000 Std[b]	55·2	102·4	116·3	1984	56	47·1	3	102	97
	Sludge	2·2	2·8	3·6	35·9	1·7				
Propionic	5 Std	0·33	0·47	0·57	5·01	0·3				
	2000 Std	78·1	41·9	88·7	2016	43	62·4	2	106	98
	Sludge	4·7	8·5	9·7	194·7	4·7				
i-butyric	5 Std	0·15	0·43	0·45	4·74	0·2				
	2000 Std	49·4	34·4	60·2	2025	29	66·0	1	103	97
	Sludge	0·54	1·5	1·6	9·88	0·8				
n-butyric	5 Std	0·18	0·52	0·56	4·91	0·3				
	2000 Std	69·8	0	69·8	2004	34	56·4	1	102	97
	Sludge	0·14	0·45	0·47	4·30	0·2				
i-valeric	5 Std	0·16	0·18	0·24	4·88	0·1				
	2000 Std	81·2	0	81·2	2003	39	58·8	1	102	98
	Sludge	0·70	0·26	0·75	22·4	0·4				
n-valeric	5 Std	0·24	0·60	0·65	5·19	0·3				
	2000 Std	74·5	16·1	76·2	2009	37	41·9	1	106	98
	Sludge	0·30	1·4	1·4	8·42	0·7				
i-caproic	5 Std	0·20	0·75	0·78	5·77	0·4				
	2000 Std	99·4	19·3	100·9	2023	48	36·1	1	105	98
	Sludge	0	0·55	0·55	0·92	0·3				
n-caproic	5 Std	0·23	0·50	0·55	5·11	0·3				
	2000 Std	48·2	1·3	48·3	2006	23	30·3	1	105	99
	Sludge	0	0·67	0·67	1·10	0·3				

a. 5 mg/l in water.
b. 2000 mg/l in water.
c. Calculated from 5 mg/l standard, assuming $S_w = S_w$ blank. Limit $= 5 \cdot 12\, S_w$.
d. Samples stabilized with formic acid.
e. Degrees of freedom on all samples were $S_w^{(10)}$, $S_b^{(9)}$, $S_t^{(19)}$.

A2 Principle

After pretreatment of the samples by centrifugation, the separated liquor is treated with formic acid to minimize adsorption effects on the gas chromatographic column used subsequently (this also stabilizes the liquor and prevents further biological activity which would produce additional fatty acids). The individual acids present in the mixture are determined by gas chromatography using a primary column of FFAP on Chromosorb G and flame ionization detection. Confirmation of these determinations may be obtained using a column of polyethylene glycol adipate on acid washed diatomite, which alters the retention times of the acids, although the sequence of elution is the same.

A3 Interferences

Substances usually present in sludges do not normally interfere. However, any neutral volatile material, especially alcohols, can have an adverse effect on the determination if they have similar retention times to the volatile acids.

A4 Hazards

The solutions should be prepared in a fume cupboard. These acids give rise to lachrymatory and irritating vapours and can also cause skin burns, particularly formic acid. Sludge liquors should not be pipetted by mouth.

A5 Reagents

All reagents and standards should be stored in glass containers fitted with glass or plastic stoppers. Analytical grade reagents are to be preferred where available.

15.1 **Water**

Distilled or deionized water is suitable.

A5.2 **Volatile Fatty Acids**

Formic acid (90% or greater as HCOOH, low in acetic acid <0·01%)
Acetic acid (>99%)
n-Propionic acid (>99%)
2-Methyl-propionic acid (iso Butyric acid) (>99%)
n-Butyric acid (>99%)
3-Methyl-butanoic acid (iso-Valeric acid) (>99%)
Pentanoic acid (Valeric acid) (>99%)
Hexanoic acid (Caproic acid) (>99%)
4-Methyl-pentanoic acid (iso-Caproic acid) (98%) or better
Other acids, such as n-Heptanoic and n-Octanoic, as required.

A5.3 **Standard Acid Solutions (See footnote a)**

A5.3.1 *Solution A Formic Acid solution*

Fill a 100-ml calibrated flask with water up to the calibration mark. Add 10·0±0·1 ml formic acid as directed in Analytical Procedure (step A9.4).

A5.3.2 *Solution B* 1 ml containing 2 mg of each acid

Weigh 2000 ± 10 mg of each acid into separate stoppered glass vessels and transfer quantitatively to a one litre calibrated flask, and make up to the mark with water.

A5.3.3 *Solution C* 1 ml containing 0·5 mg of each acid

Transfer 25·0±0·1 ml *solution B* to a 100-ml calibrated flask and make up to the mark volume with water. Add 10·0±0·1 ml formic acid.

A5.4 **Reagent Storage and Replacement**

All solutions should be stored in a flame proof refrigerator
Replace *solution B* yearly.
Replace *solutions A and C* every two months.
Chromatograms obtained from *solution C* should be kept to provide a regular check on the composition of *solution B*.

A6 Apparatus

A6.1 A single column gas chromatograph fitted with an oven and a flame ionization detector. A chart recorder is a suitable form of read out.

A6.2 **Special Apparatus**

A6.2.1 *Primary Column*

A glass column of 1·75 – 2 m length and 4 mm bore (see Note a) and packed with 5% FFAP (Carbowax 20M terminated with 2-nitro terephthalic acid) on Chromosorb G 60/80 mesh AW DCMS. A column efficiency of approximately 2000 plates is most desirable. (See the publication in this series on Gas Chromatography).

A6.2.2 *Confirmatory Column*

For confirmation, a glass column of 1·5 m length and 4 mm bore (see footnote b) should be used and packed with 10% polyethylene glycol adipate (PEGA) on acid washed diatomite C 100/120 mesh.

A6.2.3 A 10 ul chromatographic syringe has been found to be most suitable.

Note a: The solutions should be prepared in a fume cupboard because the acids give rise to irritating and lachrymatory vapours.

Note b: Precise dimensions of the column may vary according to the make of instrument employed.

A7 Sample Collection and Preservation

Samples should be collected as described in the appropriate method in this series and in the general introduction. However, the liquor for analysis should be separated and pretreated as soon as possible.

A8 Sample Pretreatment

Sludge samples must be centrifuged and formic acid added to the separated liquor as soon as possible, in any event not later than 8 hours after sampling.

A9 Analytical Procedure

READ SECTION A4 ON HAZARDS BEFORE STARTING THIS PROCEDURE

Step	Experimental Procedure	Notes
	Preparation of the instrument (note a)	
A9.1	Set up the instrument according to the manufacturers' instructions. The detector oven should be set at 50°C above the ambient column oven temperature. The injection port if used, should be set at 50°C or nearest setting above the ambient column oven temperature. The carrier gas should be oxygen free nitrogen set for a flow of 30–40 ml/min. The column oven temperature should be set at 135°C for the primary column and 110°C for the confirmatory column.	(a) See a publication in this series.
A9.2	Equilibrate the column to obtain a steady base line (note b).	(b) The column must have been previously stabilized (see note a).
	Sample Pretreatment	
A9.3	Take sufficient of the sludge sample (usually 25 ml) to obtain at least 10 ml of clear liquor, and centrifuge. (note c).	(c) The liquor can also be separated by vacuum filtration of the sludge through a glass fibre paper.
A9.4	Fill a 10 ml calibrated flask to the calibration mark with liquor obtained in step 9.3. Add $1 \cdot 0 \pm 0 \cdot 01$ ml formic acid and mix thoroughly. (note d)	(d) Do not pipette the liquour by mouth.
	Gas Chromatographic Procedure	
A9.5	Inject ten 1µl samples of formic acid solution (solution A) in rapid succession on to the column to condition it and then allow the base line to restabilize.	
A9.6	Inject 1 ul of formic acid solution (solution A) and allow it to elute for about 30 minutes .Measure blank peak heights for the individual acids. Repeat this a further time and obtain mean peak heights H_b. (note e)	(e) These values can only be obtained once exact individual acid retention times have been obtained for Step 7.
A9.7	Inject 1 ul of the standard Solution C and allow the acids to elute. Measure the peak heights for the individual acids. Repeat this twice more and obtain obtain mean peak heights H_c. (note f)	(f) n-Hexanoic acid should elute in 20 minutes (see figure 1 for a typical trace).
A9.8	Inject 1 ul of the pretreated sample and allow acids to elute. Measure the peak height H_s for the individual acids. Inject all samples within a batch under examination. (note g)	(g) If a sample is found to contain exceptionally large concentrations of volatile fatty acids, ie. over 2000 mg/l, it should be followed by an injection of formic acid solution (Solution A).

Step	Experimental Procedure	Notes
	Calculation	
A9.9	The individual acids are identified by their retention times (see Figure 1). Calculate the concentration C of individual acids in the sample from: $$C = 500\left(\frac{H_s - H_b}{H_c - H_b}\right)$$	(h) All peak heights must be adjusted to allow for the attentuations employed.
	Confirmation Procedure	
A9.10	Occasional confirmation of identity and or quantity may be required. The confirmatory column must be employed repeating Step 1 at 110°C and steps 2 and 5–9 as set out above (note i).	(i) See figure 2 for typical trace.

1 – ACETIC ACID
2 – PROPIONIC ACID
3 – 2-METHYL PROPANOIC ACID (iso-BUTYRIC ACID)
4 – BUTANOIC ACID (n-BUTYRIC ACID)
5 – 3-METHYL BUTANOIC ACID (iso-VALERIC ACID)
6 – PENTANOIC ACID (n-VALERIC ACID)
7 – 4-METHYL PENTANOIC ACID (iso-CAPROIC ACID)
8 – HEXANOIC ACID (n-CAPROIC ACID)

Typical retention times, secs.
1 –201
2 –287
3 –329
4 –427
5 –516
6 –691
7 –925
8 –1111

NOTE: TRACE OBTAINED BY WATER RESEARCH CENTRE, STEVENAGE

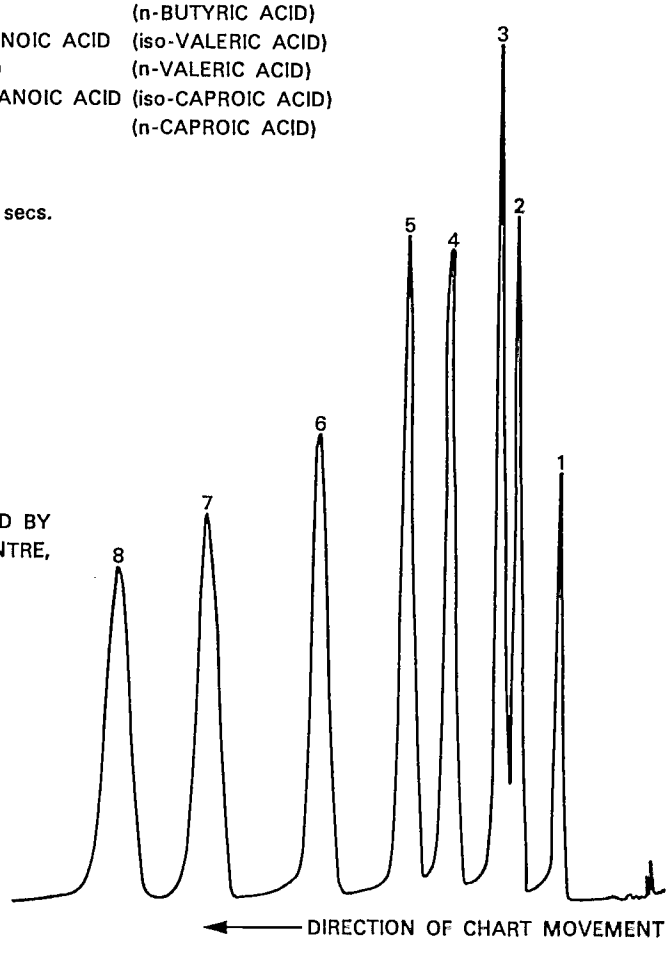

Figure 1 Typical trace on primary column

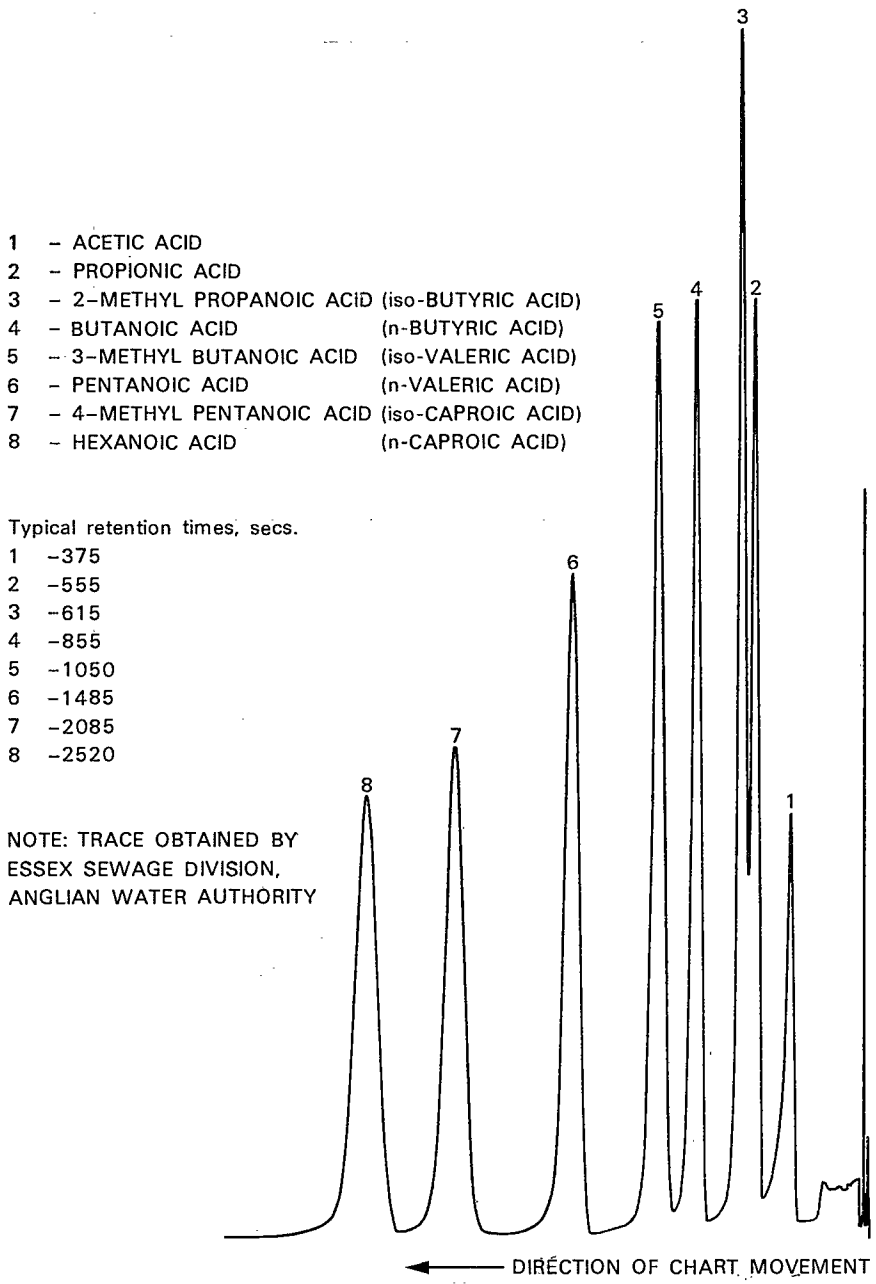

Figure 2 Typical trace on confirmatory column

A10 Checking the Linearity of the Calibration Curve

The procedure in this section must be carried out on at least two independent occasions before application of this method to any samples and occasionally thereafter.

To a series of 100 ml calibrated flasks pipette 0·10, 2·50, 5·00, 10·00, 25·00, 50·00 and 100·00 ml of standard *solution B* freshly prepared and dilute to the mark with water. These flasks contain respectively 2·0, 50, 100, 200, 500, 1000, and 2000 mg/l individual acids. Carry out the procedure given in section A8, steps 1, 5, 6, 7, 10 on each of these solutions. Plot the peak heights against mg/l for the individual acids.

The calibration curve should be linear up to at least 2000 mg/l for each individual acid.

A11 Sources of Error

The attention which it is necessary to pay to sources of error depends on the accuracy required of the analytical results. See section A3 for the effect of interfering substances. High concentrations of volatile fatty acids should always be followed by an injection of formic acid solution. Ghosting effects are minimized by this procedure.

A12 Checking the Validity of Analytical Results

Once the method has been put into normal routine operation many factors may subsequently affect the validity of the analytical results. It is recommended that experimental tests of the validity should be made regularly. As a minimum, however, it is suggested that a typical sample be analyzed in duplicate at the same time and in exactly the same way as normal samples. Such checks will be facilitated by plotting results obtained on quality control charts which will detect inadequate accuracy, and will also allow the standard deviation of routine analytical results to be estimated.

A13 References

(1) Cochrane, GC, J. Chromatog. Sci., 1975, **13**, 440.

Method B Spectrophotometric Determination

B1 Performance Characteristics of the Method

(For further information on the determination and definition of performance characteristics see a publication in this series)

B1.1	Substances determined	Carboxylic acids and some of their derivatives (mainly lower fatty acids, $C_2 - C_5$).	
B1.2	Types of sample	Raw, digesting and digested sewage sludge.	
B1.3	Basis of the method	Carboxylic acids are esterified with ethane diol and the resulting esters reacted with hydroxylamine to form hydroxamic acids. Reaction with ferric chloride results in the formation of purple coloured ferric hydroxamates which are determined spectrophotometrically.	
B1.4	Range of application (a)	Up to 5,000 mg/l as acetic acid.	
B1.5	Calibration curve (a)	Linear to 5,000 mg/l for acetic acid.	
B1.6	Standard deviation (b)	Mean concentration 10 samples (mg/l) 224	Standard deviation (within batch S_w) (mg/l) 8·6
B1.7	Limit of detection (c)	34 mg/l for acetic acid in distilled water.	
B1.8	Sensitivity (d)	100 mg/l acetic acid = 0·104 OD unit.	
B1.9	Bias	Reactivity decreases with increase of chain length.	
B1.10	Interferences	See Section 3.	
B1.11	Time required for (a) analysis	The total analytical and operator times are the same and for 10 samples are equal to 1·5 h excluding preparation of reagents and calibration curve.	

(a) Data taken from Montgomery *et al*[1].
(b) Because sludge itself degrades to volatile fatty acids, s_b cannot be obtained for a real sample. S_b for a 200 mg/l standard was zero (7 degrees of freedom) and S blank was 4·6 mg/l (9 degrees of freedom) (Lea Division, Thames Water Authority).
(c) Limit of Detection = $5 \cdot 185_w$ blank obtained by Lea Division, Thames Water Authority.
(d) Date obtained by Lea Division, Thames Water Authority.

B2 Principle

The method is based on an empirical method described by Montgomery *et al* [1]. Carboxylic acids and their salts are esterified with ethane diol and the resulting esters reacted with hydroxylamine to form hydroxamic acids. Reaction with ferric chloride results in the formation of purple coloured ferred hydroxamates. The intensity of colour is determined spectrophotometrically and is proportional to the concentration of volatile fatty acids in the sample.

B3 Interferences

The presence of substituted carboxylic acids, esters, amides, imides and other substances which form ferric hydroxamates under the conditions of the test will cause positive interference. A large number of such compounds have been described by Buckles and Thelen[2] and the effect of some specific substances have been investigated by Montgomery *et al*[1]. Colloidal suspended material and coloured material which absorb at 500 mm will also cause positive interference.

All carboxylic acids and some of their derivatives react to some extent but the most intense colours are given by the more volatile acids: acetic, propionic and butyric. The intensity of colour is compared with those intensities obtained from standard solutions of acetic acid and the concentration of volatile fatty acids present in the sample is calculated as acetic acid.

The significance of these interfering substances is in general likely to be low. Their concentration is unlikely to vary significantly from day to day in the digester and since the test is a routine control test which is looking for a sudden rise in the volatile fatty acids concentration from day to day, the effect of those interfering substances may be ignored.

B4 Hazards

Hydroxylamine and its salts are corrosive. They are also skin irritants and burn the eyes. Contact with the skin should be avoided; continued contact can cause dermatitis. Systemically, methaemoglobinaemia may occur. Eye protection and rubber gloves *must* be worn when handling these materials.

B5 Reagents

Analytical reagent grade chemicals are suitable and must be stored in glass containers with glass or plastic stoppers.

B5.1 Water

Distilled or deionized water is suitable.

B5.2 50% V/V Sulphuric acid

Add with care and constant stirring 100 ± 1 ml of sulphuric acid (d_{20} 1·84) to 100 ± 1 ml of water and cool.

B5.3 18% m/V Sodium hydroxide

Dissolve 90 ± 1 g of sodium hydroxide in about 400 ml of water. Cool and dilute to 500 ml with water.

B5.4 Acidic ethane diol reagent

Mix 30 ± 1 ml of ethane diol with $4{\cdot}0 \pm 0{\cdot}1$ ml of 50% V/V sulphuric acid. Prepare this reagent freshly each day. The ethane diol should not make a significant contribution to the blank. Replacement of the ethane diol every month is usually necessary.

B5.5 10% m/V Hydroxammonium sulphate (see Section B4)

Dissolve $10{\cdot}0 \pm 0{\cdot}1$ g of hydroxammonium sulphate in about 80 ml of water and dilute with water to 100 ml. Store in a refrigerator. Prepare fresh solutions monthly.

B5.6 Hydroxylamine reagent (see Section B4)

Mix $20{\cdot}0 \pm 0{\cdot}5$ ml of 18% m/V sodium hydroxide with $5{\cdot}0 \pm 0{\cdot}1$ ml of 10% m/V hydroxyammonium sulphate. Prepare this reagent immediately before it is required.

B5.7 **Acidic ferric chloride reagent**

Dissolve 20·0±0·1 g of ferric chloride hexaydrate in about 500 ml of water. Add 20·0±0·1 ml of sulphuric acid d_{20} 1·84, and dilute with water to 1 litre and filter if necessary. Store in refrigerator. Prepare fresh solutions monthly. This solution has a pH value of about 1·6 to avoid precipitation of ferric salts.

B5.8 **Standard acetic acid solutions**

B5.8.1 *Solution A* 1 ml contains 10 mg acetic acid

Weigh 10·00±0·01 g of glacial acetic acid (d_{20} 1·05) in a stoppered weighing bottle and transfer quantitatively to a 1 litre calibrated flask and dilute with water to 1 litre. Prepare fresh solutions monthly.

B5.8.2 *Solution B* 1 ml contains 0·5 mg acetic acid.

Dilute 5·00±0·005 ml of *solution A* with water to 100 ml in a calibrated flask. Prepare fresh solutions monthly. Store in a refrigerator.

B6 Apparatus

Spectrophotometer (visible range)
Test tubes; 125 mm × 15 mm are suitable.

B7 Sample Collection and Preservation

Samples should be collected as described in the appropriate method in this series and in the general introduction. No pretreatment is recommended.

B8 Analytical Procedure

Step	Experimental Procedure	Notes
	Analysis of sample	
B8.1	Filter. With the use of a filter aid (diatomaceous earth) about 10 ml of the sample to obtain a clear solution (note a)	(a) In some cases adequate clarification may be obtained by centrifugation.
B8.2	Pipette 0·50±0·01 ml of the clear filtrate (or centrate) into a dry test tube. Add from a microburette 1·70±0·05 ml of acidic ethane diol reagent and mix thoroughly. Heat in a boiling water bath for 3 min±10s (note b). Immediately cool the test tube in cold water.	(b) Avoid direct contact of the test tube with the heating element and the sides of the water bath.
B8.3	Add 2·5±0·1 ml of the hydroxylamine reagent and mix thoroughly. Set aside for 1 min ± 10s (note c).	(c) Batch analysis may be facilitated by the use of reagent dispensers.
B8.4	Add 10·0±0·1 ml of acid ferric chloride reagent into a 25 ml calibrated flask. Quantitatively transfer the solution in the test tube to the calibrated flask (note d). Make up to the mark with water and shake the flask vigorously. Allow to stand for at least 5 mins with the stopper removed (note e).	(d) Use a little water to assist in transferring the last traces. (e) This facilitates the escape of dissolved gases.
B8.5	Set up the spectrophotometer according to the manufactuer's instructions. Measure the absorbance, A_s, of the solution at 500 nm against distilled water using 40 mm cells within 1 h of carrying out step 4 (notes f and g).	(f) Take care to avoid the formation of gas bubbles in the cell. (g) Other sized cells may be used.

Step	Experimental Procedure	Notes
	Blank determination	
B8.6	A blank must be run with each batch of determinations using the same reagents as for the samples. Repeat steps 2 to 5 inclusive using 0·50±0·01 ml of water instead of the sample. Let the absorbance of the blank be A_b.	
	Calibration Standard	
B8.7	Duplicate calibration standards must be run with each batch of determinations, using the same reagents as for the samples. Repeat steps 2 to 5 inclusive using two separate 0·50±0·01 ml aliquots of standard acetic acid *solution B* instead of the sample. Let the absorbances of the calibration standards be at A_c1 and A_c2. If A_c1 and A_c2 are acceptable, calculate the mean A_c.	
	Calculation of the result	
B8.8	Calculate the concentration of volatile fatty acids, C, in the sample from $C = 500\left(\frac{A_b - A_b}{A_c - A_b}\right)$ mg/l	(h) This calculation assumes a linear calibration. Linearity must be checked. (See section 9).

B9 Checking the Linearity of the Calibration Curve

The procedure in this section must be carried out on at least two independent occasions before application of this method to any samples and regularly thereafter (at least monthly).

To a series of 100 ml calibrated flasks pipette 0·00, 2·50, 5·00, 10·00, 25·00, 50·00 and 100·00 ml of standard acetic acid *solution A* and dilute to the mark with water. These flasks contain respectively 0, 250, 500, 1000, 2500, 5000 and 10,000 mg/l acetic acid. Carry out the procedure given in section B8, steps 2 to 5 inclusive on each of these solutions. Plot the absorbance against mg/l acetic acid.

The calibration curve is normally linear to 10·000 mg/l acetic acid, however, the linearity of the curve may depend on the instrument used and therefore linearity must be checked.

B10 Sources of Error

The attention which it is necessary to pay to sources of error depends on the accuracy required of the analytical results. (See section B3 for the effect of interfering substances). Particular care must be exercised over the ageing qualities of the reagents and the times employed.

B11 Checking the Validity of Analytical Results

Once the method has been put into normal routine operation many factors may subsequently effect the validity of the analytical results. It is recommended that experimental tests of the validity should be made regularly. As a minimum, however, it is suggested that a typical sample be analysed in duplicate at the same time and in exactly the same way as normal samples, such checks will be facilitated by plotting results obtained on quality control charts which will detect inadequate accuracy, and will also allow the standard deviation of routine analytical results to be estimated.

B12 References

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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 Technical Secretary
The Standing Committee of Analysts
The Department of the Environment
2 Marsham Street
LONDON SW1P 3EB
England

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Members of the Committee Responsible for this Method:

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