BRITISH STANDARD

BS EN ISO 11734:1999

BS 6068-5.21: 1996

Incorporating Amendment No.1 to BS 6068-5.21:1996 (renumbers the BS as BS EN ISO 11734:1999)

Water quality — Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge — Method by measurement of the biogas production

The European Standard EN ISO 11734:1998 has the status of a British Standard

ICS 07.100.20

Confirmed July 2008

Committees responsible for this British Standard

The preparation of this British Standard was entrusted by Technical Committee EH/3, Water quality, to Subcommittee EH/3/5, Biological methods, upon which the following bodies were represented:

BLWA Ltd. (The Association of the Laboratory Supply Industry)
Department of Economic Development (Northern Ireland)
Department of Health
Department of the Environment (Her Majesty's Inspectorate of Pollution)
Department of the Environment (Water Directorate)
Freshwater Biological Association
Institution of Water and Environmental Management
Ministry of Agriculture, Fisheries and Food
National Rivers Authority
Royal Society of Chemistry
Scottish Natural Heritage
Soap and Detergent Industry Association
Water Research Centre

This British Standard, having been prepared under the direction of the Health and Environment Sector Board, was published under the authority of the Standards Board and comes into effect on 15 July 1996

The following BSI references relate to the work on this standard:
Committee reference EH/3/5
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Amendments issued since publication

Amd. No.	Date	Comments
10321	January 1999	Indicated by a sideline in the margin

Contents

National foreword

This British Standard is the English language version of EN ISO 11734:1998. It retains a dual identifier as BS 6068-5.21:1996.

It is identical with ISO 11734:1995 published by the International Organization for Standardization (ISO).

The international standard was prepared by ISO Technical Committee 147, Water quality, with the active participation and approval of the UK.

BS 6068 is being published in a series of Parts subdivided into Sections that will generally correspond to particular international standards. Sections are being, or will be, published in Parts 1 to 7, which, together with Part 0, are listed below:

— *Part 0: Introduction;*

— *Part 1: Glossary*;

— *Part 2: Physical, chemical and biochemical methods*;

— *Part 3: Radiological methods*;

— *Part 4: Microbiological methods*;

— *Part 5: Biological methods*;

— *Part 6: Sampling*;

— *Part 7: Precision and accuracy*.

NOTE The tests described in this Section of BS 6068 should only be carried out by suitably qualified persons with an appropriate level of biological expertise. Standard biological procedures should be followed throughout.

Textual errors. When adopting the text of the international standard, the textual errors listed below were discovered. They have been marked in the text and have been reported to ISO in a proposal to amend the text of the international standard.

In **6.7**, line 4, after "(e.g." insert "producing about".

In **8.1**, in line 5 of the last paragraph delete "other ingredients" and substitute "sludge inoculum (6.7)".

In **9.1**, in both definitions of symbol *n* after "gas" insert "in the given volume".

NOTE *Typographical errors*. In Annex B, The title should read "cumulative" and not "cumulated".

Cross references. The Technical Committee has reviewed the provisions of ISO 10634:1995 and of the draft of ISO/DIS 11923, to which normative reference is made in the text, and has decided that they are acceptable for use in conjunction with this standard.

A British Standard does not purport to include all the necessary provisions of a contract. Users of British Standards are responsible for their correct application.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, the EN ISO title page, pages 2 to 16, an inside back cover and a back cover,

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 11734

July 1998

ICS 13.060.40

Descriptors: Water, quality, sewage, sewage treatment sludges, organic compounds, tests, water tests, determination, biodegradability

English version

Water quality — Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge — Method by measurement of the biogas production

(ISO 11734:1995)

Qualité de l'eau — Evaluation de la biodégradabilité anaérobie "ultime" des composés organiques dans les boues de digesteurs — Méthode par mesurage de la production de biogaz
(ISO 11734:1995)

Wasserbeschaffenheit — Bestimmung der vollständigen anaeroben biologischen Abbaubarkeit organischer Verbindungen im Faulschlamm — Verfahren durch Messung der Biogasproduktion
(ISO 11734:1995)

This European Standard was approved by CEN on 21 June 1998.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart 36, B-1050 Brussels

Ref. No. EN ISO 11734:1998 E

Foreword

The text of the International Standard from Technical Committee ISO/TC 147, Water quality, of the International Organization for Standardization (ISO) has been taken over as a European Standard by Technical Committee CEN/TC 230, Water analysis, the Secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by January 1999, and conflicting national standards shall be withdrawn at the latest by January 1999.

According to CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of the International Standard ISO 11734:1995 has been approved by CEN as a European Standard without any modification.

NOTE Normative references to International Standards are listed in Annex ZA (normative).

WARNING — Sewage sludges may contain potentially pathogenic organisms. Therefore appropriate precautions must be taken when handling such sludges. Digesting sewage sludge produces flammable gases which present fire and explosion risks. Care must be taken when transporting and storing quantities of digesting sludge. Toxic test chemicals and those whose properties are not known must be handled with care. The pressure meter and microsyringes must be handled carefully to avoid injuries caused by needles. Contaminated syringe needles must be disposed of in a safe manner.

1 Scope

This International Standard specifies a screening method for the evaluation of the biodegradability of organic compounds at a given concentration by anaerobic microorganisms. The conditions described in this test do not necessarily correspond to the optimal conditions allowing the maximum value of biodegradation to occur, since a dilute sludge is used with a relatively high concentration of test chemical. The test allows exposure of sludge to the chemical for a period of up to 60 d, which is longer than the normal sludge retention time (25 d to 30 d) in anaerobic digesters, though digesters at industrial sites can have much longer retention times.

The method applies to organic compounds with a known carbon content and which are

— soluble in water;

— poorly soluble in water, provided that a method of exact dosing is applicable;

— not inhibitory to the test microorganisms at the concentration chosen for the test; inhibitory effects can be determined in separate tests or by an additional inhibition assay.

For volatile substances a case by case decision is necessary. Some can be tested if handled with special care, for example no release of gas during the test.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 10634:1995, *Water quality — Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium.*

ISO 11923:—, *Water quality — Determination of suspended solids by filtration through glass-fibre filters*[1)].

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1
ultimate anaerobic biodegradation

the level of degradation achieved when a test compound is utilized by anaerobic microorganisms resulting in the production of carbon dioxide, methane, mineral salts and new microbial cellular constituents (biomass)

3.2
primary anaerobic biodegradation

the level of degradation achieved when a test compound undergoes any structural change, other than complete mineralization, as a result of anaerobic microbial action

3.3
digested sludge

a mixture of the settled phases of sewage and activated sludge, which have been incubated in an anaerobic digester at about 35 °C to reduce biomass and odour problems and to improve the dewaterability of the sludge. Digested sludge consists of an association of anaerobic fermentative and methanogenic bacteria producing carbon dioxide and methane

3.4
concentration of total solids

the amount of solids obtained by drying a known volume of sludge under specified conditions at about 105 °C to constant mass

[1)] To be published.

4 Principle

Washed digested sludge, containing very low amounts of inorganic carbon (IC), is diluted to total solids concentration of 1 g/l to 3 g/l and incubated at 35 °C ± 2 °C in sealed vessels with a test chemical at an organic carbon (OC) concentration of 20 mg/l to 100 mg/l for up to about 60 d.

The increase in headspace pressure in the test vessels resulting from the production of carbon dioxide (CO_2) and methane (CH_4) is measured. A considerable amount of carbon dioxide will be dissolved in water or transformed to hydrogen carbonate or carbonate under the conditions of the test. This inorganic carbon (IC) is measured at the end of the test.

The amount of microbiologically produced carbon is calculated from the net gas production and the net IC formation in excess over blank values. The percentage biodegradation is calculated from the total IC formed and the measured or calculated amount of carbon added as test compound. The course of biodegradation can be followed by taking intermediate measurements of gas production only

As additional information, the primary biodegradation can be determined by specific analyses at the beginning and end of the test.

5 Test environment

Incubation shall take place in sealed vessels at a constant temperature of 35 °C ± 2 °C, a normal temperature for an anaerobic digester, in the absence of oxygen, initially in an atmosphere of pure nitrogen.

6 Reagents

6.1 *Distilled* or *deionized water*, containing less than 2 mg/l DOC.

6.2 *Test medium*

6.2.1 *Medium*

Use only reagents of recognized analytical grade. Prepare the dilution medium to contain the following constituents at the stated amounts:

Anhydrous potassium dihydrogenphosphate (KH_2PO_4)	0,27 g
Disodium hydrogenphosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	1,12 g
Ammonium chloride (NH_4Cl)	0,53 g
Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)	0,075 g
Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$)	0,10 g
Iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$)	0,02 g
Resazurin (oxygen indicator)	0,001 g
Sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$) (see note 1)	0,1 g
Stock solution of trace elements (optional)	10 ml
Add de-oxygenated water (**6.1**)	to 1 litre

To achieve anoxic conditions, sparge the medium with nitrogen for about 20 min immediately before use to remove oxygen.

Adjust the pH of the medium with dilute mineral acid or alkali, if necessary, to 7 ± 0,2.

NOTE 1 Freshly supplied sodium sulfide should be used or it should be washed and dried before use, to ensure sufficient reductive capacity.

6.2.2 *Stock solution of trace elements (optional)*

It is recommended to supply the test medium with the following trace elements to improve anaerobic degradation processes, especially if low inoculum concentrations are used.

Manganese chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$)	0,05 g
Boric acid (H_3BO_3)	0,005 g
Zinc chloride ($ZnCl_2$)	0,005 g
Copper(II) chloride ($CuCl_2$)	0,003 g
Disodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$)	0,001 g
Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$)	0,1 g
Nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$)	0,01 g
Disodium selenite (Na_2SeO_3)	0,005 g
Add water (**6.1**)	to 1 litre

6.3 *Test compound*

Add the test compound as a stock solution, supension, emulsion, or directly as solid or liquid to give a test concentration of 100 mg/l organic carbon. If stock solutions are used, prepare a suitable solution with water (**6.1**) of such strength that the volume added is less than 5 % of the total volume of reaction mixture. For test compounds which are insufficiently soluble in water, see ISO 10634, but do not use an organic solvent known to inhibit methane production such as chloroform or carbon tetrachloride.

NOTE 2 If solvents are used, a control with the solvent only is recommended.

6.4 *Reference substances*

Reference substances such as sodium benzoate, phenol or polyethyleneglycol 400 are permissible. These substances would be expected to have a biodegradation degree greater than 60 %. Prepare a stock solution in the same way as for the test compound.

6.5 *Inhibition control (optional)*

Add the test compound and reference substance to a vessel containing the test medium (**6.2**) each at the same concentrations as added, respectively, in **6.3** and **6.4**.

6.6 *Digested sludge*

Collect digested sludge from a digester at a sewage treatment plant treating predominantly domestic sewage. Use wide-necked bottles constructed from high density polyethylene or a similar material which can expand.

WARNING — For safety reasons, glass must not be used.

Fill the bottles to within 1 cm of the top and seal tightly. After transport to the laboratory, use directly or place in a laboratory-scale digester. Release excess biogas.

Alternatively, use a laboratory-grown anaerobic sludge as a source of inoculum.

Consider pre-digestion of the sludge to reduce back-ground gas production and to decrease the influence of the blanks. Allow the sludge to digest, without the addition of any nutrients or substrates, at 35 °C ± 2 °C for up to 7 d.

NOTE 3 It has been shown that pre-digestion for about 5 d gives an optimal decrease in gas production of the blank without unacceptable increases in either lag or incubation periods during the test phase.

For test compounds which are expected to be poorly biodegradable, consider pre-exposure of the sludge with the test substance to obtain an inoculum that is better adapted. In such a case, add the test substance at an OC concentration of 5 mg/l to 20 mg/l to the digested sludge. Wash the pre-digested sludge carefully before use. Indicate a pre-exposure in the test report.

6.7 *Inoculum*

Wash the sludge (**6.6**) just prior to use, to reduce the IC concentration to less than 10 mg/l in the final test solution, by first centrifuging in sealed tubes at a relatively low speed (e.g. 3 000 *g*) for up to 5 min. Suspend[2)] the pellet in oxygen-free test medium (**6.2**), centrifuge and discard the washings. If the IC has not been sufficiently lowered, wash the sludge up to twice more. Finally, suspend the pellet in the requisite volume of test medium and determine the concentration of total solids (**3.4**). The final concentration of total solids in the test vessels shall be in the range 1 g/l to 3 g/l. Conduct the above operations in such a way that the sludge has minimal contact with oxygen (e.g. use a nitrogen atmosphere).

7 Apparatus

Usual laboratory equipment and the following are required.

7.1 *Incubator* or *water or sand bath*, thermostatically controlled at 35 °C ± 2 °C.

7.2 *Pressure-resistant glass test vessels*, nominal size 0,1 litre to 1 litre, each fitted with a gastight septum, capable of withstanding about 2 bar (see example in Annex A). The headspace volume shall be about 10 % to 30 % of the total volume. If biogas is regularly released, about 10 % headspace volume is appropriate but if the gas release happens only at the end of the test, 30 % is appropriate.

NOTE 4 From a practical point of view, the use of serum bottles sealed with butyl rubber serum caps and crimped aluminium rings is recommended.

7.3 *Pressure-measuring device*, for example, a pressure meter connected to a suitable syringe needle; a 3-way gastight valve facilitates the release of excess pressure. The device shall be used and calibrated according to the manufacturer's instructions.

It is necessary to keep the internal volume of the pressure transducer tubing and valve as low as possible, so that errors introduced by neglecting the volume of the equipment are insignificant.

7.4 *Carbon analyser*, suitable for the direct determination of inorganic carbon in the range of 1 mg/l to 200 mg/l.

8 Procedure

Carry out the following initial procedures using techniques to keep the contact between digested sludge and oxygen as low as practicable, for example, work within a glove box in an atmosphere of nitrogen or purge the bottles with nitrogen.

[2)] See national foreword for details of textual error.

8.1 Preparation of test and control assays

Prepare test vessels (**7.2**) at least in triplicate for the test compound (**6.3**) and blank and at least one vessel each for reference substance (**6.4**) and inhibition control (**6.5**) (optional). Blank controls can be used for several test compounds in the same test. Prepare the diluted inoculum (**6.7**) before adding it to the vessels.

Add aliquots of the inoculum so that the concentration of total solids is between 1 g/l and 3 g/l and the same in all vessels. Add stock solutions of test compound and reference substance. The test concentration of carbon shall normally be 100 mg/l. In the case of toxic test substances, it may be reduced to 20 mg/l of organic carbon, or even less if only primary biodegradation with specific analyses is to be tested.

NOTE 5 The lower the test concentration, the higher the deviation of test results may be.

In the case of blank vessels, add equivalent amounts of de-oxygenated water (**6.1**). Prepare an extra replicate with test compound and measure the pH value. Adjust the pH to 7 ± 0,2, if necessary, with small amounts of dilute mineral acid or alkali. Add the same amount of neutralizing agents to all the test vessels (**7.2**). If primary degradation is to be measured, take an appropriate sample from the pH-control vessel or from an additional test mixture and measure the test compound concentration using specific analyses. Add covered magnets to the vessels if the reaction mixtures are to be stirred (optional). Ensure that the total volume of liquid V_l and the volume of headspace V_h are the same in all vessels. Note V_l and V_h (see clause **9**). If necessary, add anoxic test medium (**6.2**). Seal each vessel with a gas septum and put them into the incubator (**7.1**).

Add substances which are poorly soluble in water directly to the prepared vessels after weighing or dose them with the help of a solvent into the empty vessels. Evaporate the solvent by passing nitrogen gas through the vessels and then add the other ingredients[3)] Liquid test substances may be dosed with a syringe into the completely prepared sealed vessels, if it is expected that the pH will not exceed 7 ± 1, otherwise dose as described above.

8.2 Incubation and gas measurements

Incubate the prepared vessels at 35 °C ± 2 °C for about 1 h to allow equilibration and release excess gas to the atmosphere, for example, by shaking each vessel in turn, inserting the needle of the pressure meter (**7.3**) through the seal and opening the valve until the pressure meter reads zero. If at this stage or when making intermediate measurements, the headspace pressure is less than atmospheric, introduce nitrogen gas to re-establish atmospheric pressure. Close the valve (see **7.3**) and continue to incubate in the dark, ensuring that all parts of the vessels are maintained at the digestion temperature.

Observe the vessels after incubation for 24 h to 48 h. Reject vessels if their contents show a distinct pink coloration in the supernatant liquid, i.e. if resazurin (see **6.2.1**) has changed colour indicating the presence of oxygen. While small amounts of oxygen may be tolerated by the system, higher concentrations can seriously inhibit the course of anaerobic biodegradation.

Carefully mix the contents of each vessel by stirring, or by shaking for a few minutes at least two or three times per week and before each pressure measurement. Measure the gas pressure, for example, by inserting through the septum the syringe needle (see **7.3**) connected to the pressure-monitoring meter. Record the pressure in millibars (see **9.1**).

Shaking resuspends the inoculum and ensures gaseous equilibrium. While measuring pressure, maintain the gas in the headspread at the digestion temperature. Take care to prevent entry of water into the syringe needle. Should this occur, dry the wet parts and fit a new needle.

For readings of gas pressure, either measure the pressure in the vessels weekly, release excess gas to the atmosphere, or alternatively measure the pressure only at the end of the test to detect the amount of biogas produced.

NOTE 6 It is strongly recommended that intermediate readings of gas pressure be made, since the pressure increase provides guidance as to when the test may be terminated and allows the kinetics to be followed.

Finish the test after an incubation period of 60 d unless the biodegradation curve from the pressure measurement has reached a plateau phase, that is the phase in which the maximum degradation has been reached, and indicates a sufficient degree of biodegradation (> 50 %) for the test to be finished earlier. If at the end of the normal incubation period, a plateau phase is obviously not reached, the test should be prolonged until it is reached.

[3)] See national foreword for details of textual error.

8.3 Measurement of inorganic carbon

At the end of the test, after the last measurement of gas pressure, allow the sludge (**6.6**) to settle, open each vessel (**7.2**) and immediately determine the concentration, in milligrams per litre, of inorganic carbon (IC) in the supernatant. The supernatant shall not be centrifuged or filtered (see ISO 11923) at this stage (see note 7). After IC measurement record the pH. Carry out similar readings for the corresponding blank, reference substance (**6.4**) and inhibition control (**6.5**) vessels.

NOTE 7 Centrifugation or filtration would result in an unacceptable loss of dissolved carbon dioxide. If the supernatant sample cannot be analysed on being taken, it may be stored in a suitable sealed vial, without headspace, and cooled at about 4 °C for up to 2 d.

In some cases, especially if the same control vessels are used for several test compounds, consider measuring intermediate IC concentrations in test and control vessels. In this case use the following procedure.

After measuring the gas pressure without release of excess gas, take a known aliquot of the supernatant, which is as small as possible, with a syringe (see **7.3**) through the septum without opening the vessels and determine IC in the sample. After having taken the sample, the excess gas can be released from the incubation bottles or not (see **8.2**).

Take into account that even a small decrease in the supernatant volume (e.g. about 1 %) can yield a significant increase in the headspace gas volume. Correct the equations (**9.1**) by increasing V_h in equation (3) if necessary.

8.4 Specific analyses

If primary anaerobic degradation (**3.2**) is to be determined, take samples for specific analyses at the beginning (see **8.1**) and end of the test from the vessels containing the test compound. If this is done, note that the volumes of the headspace (V_h) and the liquid (V_l) will be changed and take this into account when calculating the results.

9 Calculation and expression of results

For practical reasons, the pressure of the gas is measured in millibars (1 mbar = 1 hPa = 10^2 Pa, 1 Pa = 1 N/m^2), the volume in litres and temperature in degrees Celsius.

9.1 Carbon in the headspace

1 mol of methane and 1 mol of carbon dioxide each contain 12 g of carbon. Calculate the mass of carbon in a given volume of evolved gas using equation (1):

$$m = 12 \times 10^3 \times n \qquad \text{... (1)}$$

where

m is the mass of carbon, in milligrams, in a given volume of evolved gas;

12 is the relative atomic mass of carbon;

n is the number of moles of gas.[a]

[a] See national foreword for details of textual error.

Calculate n from the gas laws using equation (2):

$$n = \frac{pV}{RT} \qquad \text{...(2)}$$

where

n[a] is the number of moles of gas;

p is the pressure, in pascals, of the gas;

V is the volume, in cubic metres, of the gas;

R is the molar gas constant [8,314 J/(mol·K)];

T is the incubation temperature, in kelvins.

[a] See national foreword for details of textual error.

Calculate the net mass of carbon (subtraction of the corresponding blank values) produced as gas in the headspace from the test compound using equation (3):

$$m_h = \frac{12\,000 \times (0,1(\Delta p \cdot V_h))}{RT} \qquad \text{... (3)}$$

where

m_h is the mass, in milligrams, of net carbon produced as gas in the headspace;

Δp is the mean of the differences between initial and final pressures, in millibars, in the test vessels minus those in the blank vessels;

V_h is the volume, in litres, of headspace in the vessel;

0,1 is the conversion factor for both newtons per square metre to millibars and cubic metres to litres.

For a normal incubation temperature of 35 °C (308 K) use equation (4):

$$m\text{h} = 0,468\ (\Delta_P \cdot V_h) \qquad \text{... (4)}$$

The course of biodegradation can be followed by plotting the cumulated pressure increase Δp, in millibars, against time, if appropriate. From this curve identify and record the lag-phase in days. The lag phase is the time from the start of the test until significant degradation starts (for example see Annex B).

9.2 Carbon in the liquid

Calculate the mass of carbon in the liquid of the test vessels using equation (5):

$$m_l = \rho_{IC,\,net} \times V_l \quad \text{... (5)}$$

where

m_l is the mass, in milligrams, of carbon, in the liquid;

$\rho_{IC,\,net}$ is the mean concentration of inorganic carbon, in milligrams per litre, in the test vessels minus that in the control vessels at the end of the test;

V_l is the volume, in litres, of liquid in the vessel.

9.3 Total gasified carbon

Calculate the total mass of gasified carbon in the vessel using equation (6):

$$m_t = m_h + m_l \quad \text{...(6)}$$

where

m_t is the total mass, in milligrams, of gasified carbon;

m_h and m_l are as defined in **9.1** and **9.2**.

9.4 Carbon of test substance

Calculate the mass of carbon in the test vessels, from the test concentration of added carbon, using equation (7):

$$m_v = \rho_{c,v} \times V_l \quad \text{... (7)}$$

where

m_v is the mass, in milligrams, of test compound carbon;

$\rho_{c,v}$ is the concentration, in milligrams per litre, of test compound carbon;

V_l is the volume, in litres, of liquid in the vessel.

9.5 Extent of biodegradation

Calculate the biodegradation from headspace gas using equation (8) and the total biodegradation using equation (9):

$$D_h = \frac{m_h \times 100}{m_v} \quad \text{... (8)}$$

$$D_t = \frac{m_t \times 100}{m_v} \quad \text{... (9)}$$

where

D_h is the biodegradation from headspace gas, expressed as a percentage;

D_t is the total biodegradation, expressed as a percentage.

m_h, m_v and m_t are as defined in **9.1**, **9.4** and **9.3**, respectively.

10 Validity of results

10.1 Maintenance of anaerobic conditions

Use only pressure readings from vessels which contain no oxygen, i.e. which do not show pink coloration. Contamination by oxygen is minimized by the use of proper anaerobic handling techniques.

10.2 Inhibition of degradation

Gas production in vessels containing both the test chemical and reference substance shall be at least equal to that in the vessel containing only reference substance; otherwise, inhibition of gas production is indicated. In the latter case, the test should be repeated using a lower concentration of test chemical but not less than 20 mg/l (see **8.1**).

10.3 Validity of the test

Consider the test to be valid if the reference sub stance has a plateau phase that represents > 60 % biodegradation. If the pH at the end of the test has exceeded the range 7 ± 1 and insufficient biodegradation has taken place, the test should be repeated using a test medium (**6.2**) with higher buffer capacity.

11 Test report

The test report shall contain at least the following information:

a) a reference to this International Standard;

b) identification of test compound and reference substance;

c) concentration of test compound and method of addition;

d) the main characteristics of biogas measurement (e.g. type of pressure meter) and of IC analyser;

e) all the measured data in the test, blank and control vessels and inhibition results, if determined, (e.g. pressure in millibars, concentration of inorganic carbon in milligrams per litre in tabular form; an example of data sheets is given in Annex C), statistical treatment of data and test duration;

f) the source, concentration and information of any pre-treatment of the inoculum (pre-digestion, pre-exposure);

g) the incubation temperature;

h) volumes of digester liquor (V_l) and headspace (V_h) in the vessels;

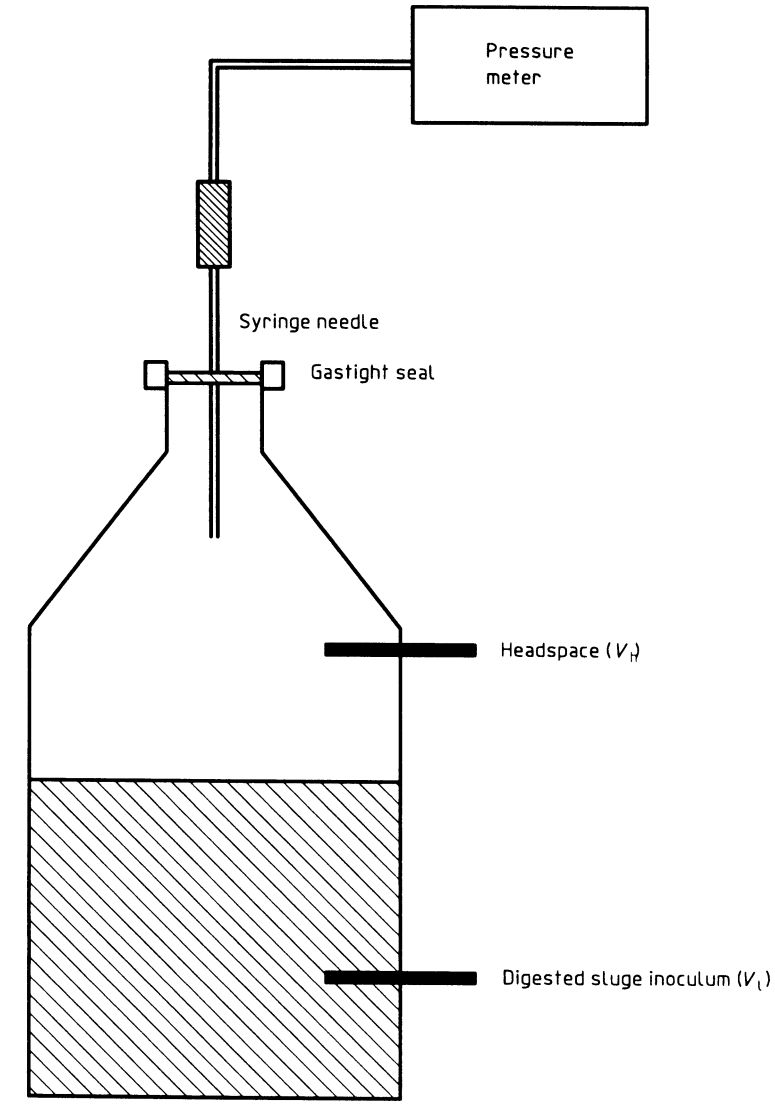
i) pH and IC values at the end of the test:

j) concentration of test compound at the beginning and end of the test if a specific measurement has been performed;

k) biodegradation curve plotted from headspace net gas production as shown in Annex B;

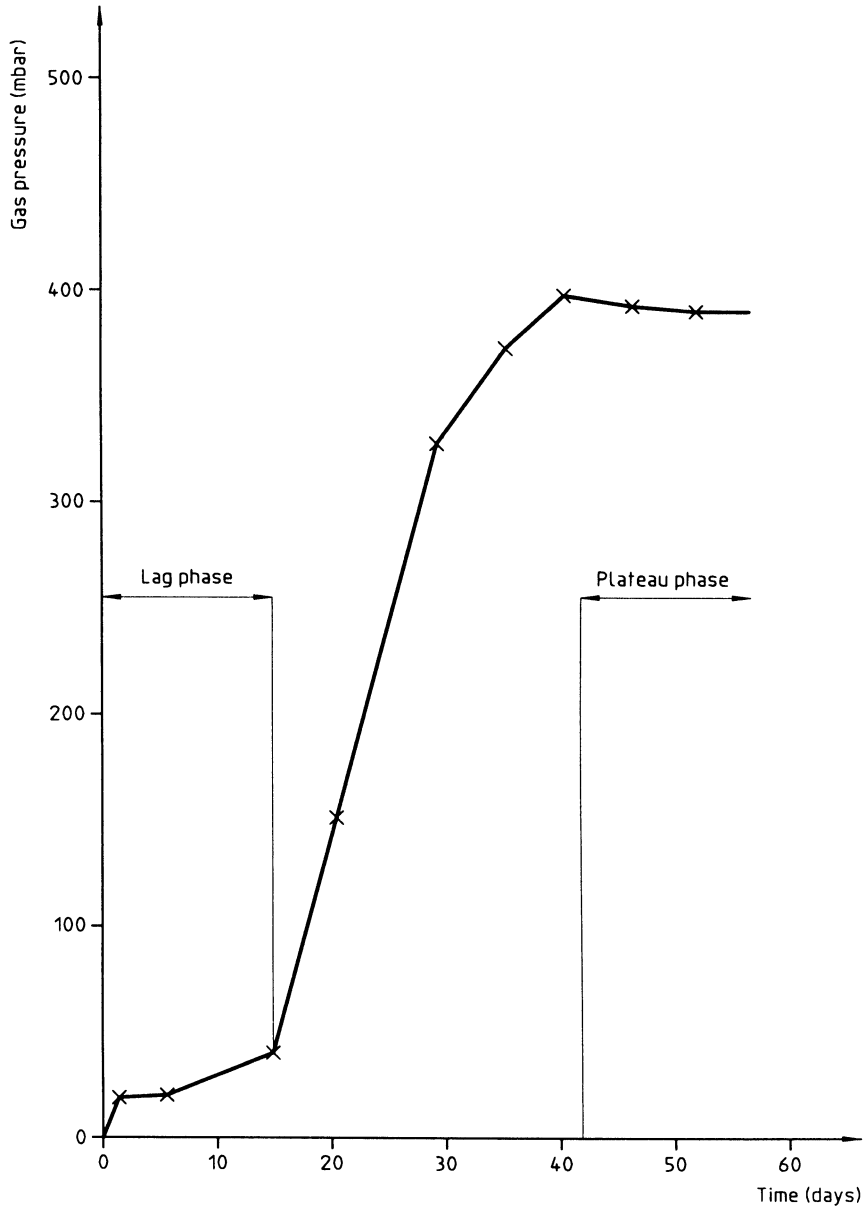
l) percentage of biodegradation of test compound and reference substance, the final test result should be indicated in ranges of 10 % (e.g. 20 % to 30 %).

Annex A (informative)
Example of an apparatus to measure biogas production by gas pressure



Test vessel in an environment of 35 °C ±2 °C

Annex B (informative)
Example of a degradation curve (cumulated net pressure increase)



Annex C (informative)
Examples of data sheets for the anaerobic biodegradation test

Anaerobic biodegradation test — Data sheet for the test compound

Laboratory: ..... Test compound: ..... Test No.: .....

Test temperature: ..... (°C) Volume of headspace (V_h): ..... (litres) Volume of liquid (V_l): ..... (litres) Carbon in test compound $\rho_{C,v}$: ..... (mg/l) m_v[3]: ..... (mg)

Day	p_1 (test) mbar	p_2 (test) mbar	p_3 (test) mbar	p (test) mean mbar	p_4 (blank) mbar	p_5 (blank) mbar	p_6 (blank) mbar	p (blank) *mean* mbar	p (net) test-blank mbar	Δp (net) cumulated mbar	m_h headspace C[1] mg	D_h biodegradation[2] %
	$\rho_{IC,1}$ test mg/l	$\rho_{IC,2}$ test mg/l	$\rho_{IC,3}$ test mg/l	ρ_{IC} test mean mg/l	$\rho_{IC,4}$ blank mg/l	$\rho_{IC,5}$, blank mg/l	$\rho_{IC,6}$ blank mg/l	ρ_{IC} blank mean mg/l	$\rho_{IC,net}$ test-blank mg/l	m_l liquid C[4] mg	m_t total C[5] mg	D_t biodegradation[6] %
IC (end)												
pH (end)												

1) Carbon in headspace, m_h (mg) at normal incubation temperature (35 °C)

$m_h = 0{,}468\ \Delta p \times V_h$

2) Biodegradation calculated from headspace gas D_h (%)

$$D_h = \frac{m_h \times 100}{m_v}$$

3) Carbon in test vessel, m_v (mg)

$m_v = \rho_{C,v} \times V_l$

4) Carbon in liquid, m_l (mg)

$m_l = \rho_{IC,net} \times V_l$

5) Total gasified carbon, m_t (mg)

$m_t = m_h + m_l$

6) Total biodegradation, D_t (%)

$$D_t = \frac{m_t \times 100}{m_v}$$

Anaerobic biodegradation test — Data sheet for the reference substance

Laboratory: ..... Reference substance: ..... Test No.: .....

Test temperature: ..... (°C) Volume of headspace (V_h): ..... (litres) Volume of liquid (V_l): .... (litres) Carbon in the reference substance[3]: $\rho_{C,v}$: .....(mg/l) m_v[3]: ..... (mg)

Day	p_1 (ref.) mbar	p_2(ref.) mbar	p_3 (ref.) mbar	p (ref.) mean mbar	p_4 (inhib.) mbar	p_5 (inhib.) mbar	p_6 (inhib.) mbar	p *(inhib.)* mean mbar	p (ref.) ref.-blank mbar	Δp (ref.) cumulated mbar	m_h headspace C[1] mg	D_h biodegradation[2] %
	$\rho_{IC,1}$ ref. mg/l	$\rho_{IC,2}$ ref. mg/l	$\rho_{IC,3}$ ref. mg/l	ρ_{IC} ref. mean mg/l	$\rho_{IC,4}$ inhib. mg/l	$\rho_{IC,5}$ inhib. mg/l	$\rho_{IC,6}$ inhib. mg/l	ρ_{IC} inhib. mean mg/l	$\rho_{IC,net}$ ref.-blank mg/l	m_l liquid C[4] mg	m_t total C[5] mg	D_t biodegradation[6] %
IC (end)												
pH (end)												

1) Carbon in headspace, m_h (mg) at normal incubation temperature (35 °C)

$m_h = 0{,}468\ \Delta p \times V_h$

2) Biodegradation calculated from headspace gas D_h (%)

$$D_h = \frac{m_h \times 100}{m_v}$$

3) Carbon in test vessel, m_v (mg)

$m_v = \rho_{C,v} \times V_l$

4) Carbon in liquid, m_l (mg)

$m_l = \rho_{Ic,net} \times V_l$

5) Total gasified carbon, m_t (mg)

$m_t = m_h + m_l$

6) Total biodegradation, D_t (%)

$$D_t = \frac{m_t \times 100}{m_v}$$

Annex D (informative)
Bibliography

[1] BIRCH, R. R., BIVER C., CAMPAGNA, R., GLEDHILL, W. E., PAGGA, U., STEBER, J., REUST, H. and BONTINCK, W. J. Screening of chemicals for anaerobic biodegradation. *Chemosphere* **19**, (1989), pp. 1527–1550. (Also published as ECETOC Technical Report No. 28, June 1988).

[2] BUSWELL, A. M. and MULLER, H. F. Mechanism of methane fermentation. *Ind. Eng. Chem*. **44**, (1952), pp. 550–552.

[3] PAGGA, U. and BEIMBORN, D. B. Anaerobic biodegradation test for organic compounds. *Chemosphere* **27**, (1993), pp. 1499–1509.

Annex ZA (normative)
Normative references to international publications with their relevant European publications

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

Publication	Year	Title	EN	Year
ISO 10634	1995	*Water quality — Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium*	EN ISO 10634	1995

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List of references

See national foreword.

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