SRI LANKA STANDARD ISO 11734: 2017
(ISO 11734: 1995)
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WATER QUALITY- EVALUATION OF THE "ULTIMATE" ANAEROBIC BIODEGRADABILITY OF ORGANIC COMPOUNDS IN DIGESTED SLUDGE - METHOD BY MEASUREMENT OF THE BIOGAS PRODUCTION

SRI LANKA STANDARDS INSTITUTION

Sri Lanka Standard
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NATIONAL FOREWORD

This Sri Lanka Standard was approved by the Sectoral Committee on Chemical and Polymer Technology and authorized for adoption and publication as a Sri Lanka Standard by the Council of the Sri Lanka Standards Institution on 2017-12-04.

The text of the International Standard **ISO 11734: 1995** Water quality- evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge- Method by measurement of the biogas production, has been accepted for adoption as a Sri Lanka Standard. This Standard specifies a screening method for the evaluation of the biodegradability of organic compounds at a given concentration by anaerobic microorganisms.

This Sri Lanka Standard is identical with **ISO 11734: 1995** Water quality- evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge- Method by measurement of the biogas production, published by the International Organization for Standardization (ISO).

TERMINOLOGY AND CONVENTIONS

The text of the International Standard has been accepted as suitable for publication, without deviation, as a Sri Lanka Standard. However, certain terminology and conventions are not identical with those used in Sri Lanka Standards. Attention is therefore drawn to the following:

a)   Wherever the words "International Standard" appear referring to a particular Standards they should be interpreted as "Sri Lanka Standard".

b)   Wherever page numbers are quoted, they are ISO page numbers.

Cross References

International Standard	**Corresponding Sri Lanka Standard**
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.	No corresponding Sri Lanka Standard
ISO 11923-1 Water quality — Determination of suspended solids by filtration through glass-fibre filters.	No corresponding Sri Lanka Standard

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INTERNATIONAL STANDARD

ISO
11734

First edition
1995-12-15

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

Qualité de l'eau — Évaluation de la biodégradabilité anaérobie «ultime» des composés organiques dans les boues de digesteurs — Méthode par mesurage de la production de biogaz

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 11734 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Annexes A, B, C and D of this International Standard are for information only.

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

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

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.

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 per-centage 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.

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1) To be published.

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 bio-degradation 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 background 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 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 $\pm$ 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.

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 $\pm$ 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. 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 $\pm$ 1, otherwise dose as described above.

8.2 Incubation and gas measurements

Incubate the prepared vessels at 35 °C $\pm$ 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.

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):

$$m = 12 \times 10^3 \times n \qquad \qquad \dots (1)$$

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.

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

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

where

n 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.

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):

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

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):

$$m_h = 0{,}468\ (\Delta p \cdot V_h) \qquad \ldots (4)$$

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):

$$m_l = \rho_{IC,net} \times V_l \qquad \ldots (5)$$

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):

$$m_t = m_h + m_l \qquad \ldots (6)$$

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):

$$m_v = \rho_{c,v} \times V_l \qquad \ldots (7)$$

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):

$$D_h = \frac{m_h \times 100}{m_v} \qquad \ldots (8)$$

$$D_t = \frac{m_t \times 100}{m_v} \qquad \ldots (9)$$

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 substance 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 biodegra-

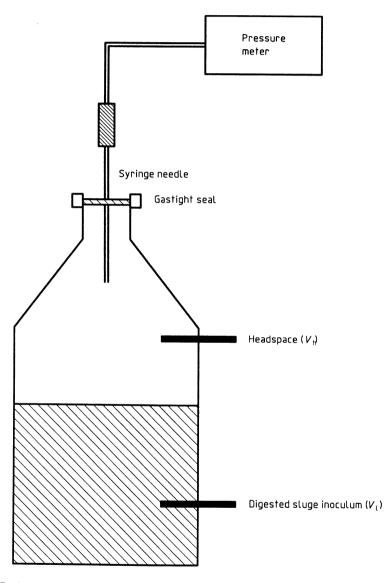dation has taken place, the test should be repeated using a test medium (6.2) with higher buffer capacity.

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 %).
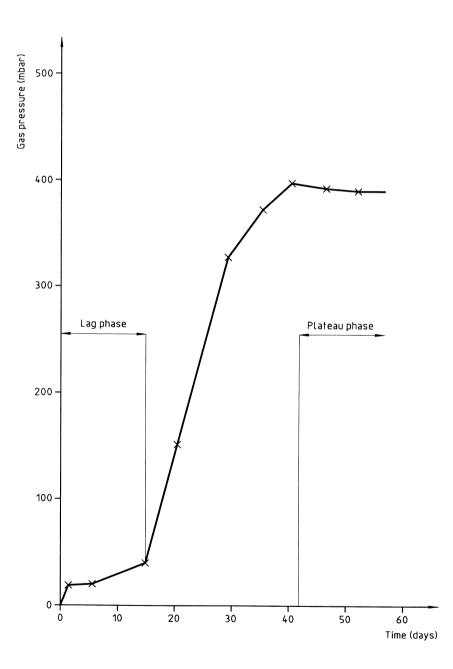
Annex A
(informative)

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 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)

Test compound: ....

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 (%) $D_h = \dfrac{m_h \times 100}{m_v}$	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 (%) $D_t = \dfrac{m_t \times 100}{m_v}$

Anaerobic biodegradation test — Data sheet for the reference substance

Laboratory: .... 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)

Reference substance: ....

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)

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

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