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Plastics — Determination of the ultimate anaerobic biodegradation of plastic materials in controlled slurry digestion systems — Method by measurement of biogas production

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee PRI/21, Testing of plastics.

A list of organizations represented on this committee can be obtained on request to its secretary.

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INTERNATIONAL STANDARD

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Plastics — Determination of the ultimate anaerobic biodegradation of plastic materials in controlled slurry digestion systems — Method by measurement of biogas production

Plastiques — Évaluation de la biodégradabilité anaérobie ultime des matériaux plastiques dans des systèmes de digestion de boue contrôlés — Méthode par mesurage de la production de biogaz

Reference number
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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.

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The main task of technical committees is to prepare International Standards. 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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 13975 was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 5, *Physical-chemical properties*.

Introduction

Biological recycling (biorecycling) is a viable option, together with mechanical recycling and chemical recycling, for the recovery of plastic waste. This International Standard specifies a method of evaluating the anaerobic biodegradability of such waste in a controlled anaerobic slurry system. This is a representative anaerobic digestion test method and system for biodegradable plastic waste.

The production of a biogas is observed under anaerobic conditions suitable for the growth of thermophilic or mesophilic microorganisms. The biogas is collected in a bag under atmospheric pressure, and the biogas volume is measured with a syringe or a gas burette. The biodegradability of the test material is evaluated from the sum of the amount of carbon dioxide dissolved in the supernatant and the cumulative quantity of evolved biogas. This International Standard is a biodegradation test method for plastic materials in a controlled anaerobic slurry system, and differs from ISO 15985 which uses high-solids anaerobic digestion conditions and ISO 14853 which uses an aqueous system in an anaerobic environment.

INTERNATIONAL STANDARD ISO 13975:2012(E)

Plastics — Determination of the ultimate anaerobic biodegradation of plastic materials in controlled slurry digestion systems — Method by measurement of biogas production

WARNING — Sewage sludge and other organic waste might contain potentially pathogenic organisms. Therefore appropriate precautions should be taken when handling such materials. Digestion of organic materials produces flammable gases that present fire and explosion risks. These gases also contain toxic chemicals, including hydrogen sulfide and ammonia, in substantial concentrations. Appropriate safety measures, such as the use of a draft chamber, gas masks and/or well-ventilated laboratory facilities, should be taken. Toxic test chemicals and chemicals whose properties are not known should be handled with care and in accordance with safety instructions. Care should be taken when transporting and storing quantities of organic matter undergoing digestion.

1 Scope

This International Standard specifies a method of evaluating the ultimate anaerobic biodegradability of plastic materials in a controlled anaerobic slurry digestion system with a solids concentration not exceeding 15 %, which is often found for the treatment of sewage sludge, livestock faeces or garbage. The test method is designed to yield a percentage and rate of conversion of the organic carbon in the test materials to carbon dioxide and methane produced as biogas.

The method applies to the following materials, provided they have a known carbon content:

— natural and/or synthetic polymers, copolymers or mixtures;

— plastic materials that contain additives such as plasticizers, colorants, or other compounds;

— water-soluble polymers.

It does not apply to materials which exhibit inhibitory effects on the test microorganisms at the concentration chosen for the test.

NOTE Inhibitory effects can be determined by an inhibition test (e.g. ISO 13641-1 or ISO 13641-2).

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 14853:2005, *Plastics — Determination of the ultimate anaerobic biodegradation of plastic materials in an aqueous system — Method by measurement of biogas production*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
ultimate anaerobic biodegradation
breakdown of an organic compound by microorganisms in the absence of oxygen into carbon dioxide, methane, water and mineral salts of any other elements present (mineralization) plus new biomass

3.2
digested sludge
mixture of the settled sewage and activated sludge which has been incubated in a mesophilic or thermophilic anaerobic digester to reduce the biomass and odour and to improve the dewaterability of the sludge

NOTE 1 to entry: Digested sludge contains an association of anaerobic fermentative and methanogenic microorganisms producing carbon dioxide and methane.

3.3
slurry
watery mixture of insoluble matter

NOTE 1 to entry: The suspended-solids concentration of a slurry might be as high as around 15 %, but slurry is fluid and pumpable.

3.4
dissolved inorganic carbon
DIC
carbon dioxide dissolved in water or transformed into carbonic acid, hydrogen carbonate ion and carbonate ion

3.5
total dry solids
amount of solids obtained by taking a known volume of test material or inoculum and drying at about 105 °C to constant mass

3.6
volatile solids
amount of solids obtained by subtracting the residue of a known volume of test material or inoculum after incineration at about 550 °C from the total dry solids of the same test portion

NOTE 1 to entry: The volatile-solids content is an indication of the amount of organic matter present.

3.7
theoretical amount of evolved biogas
ThBiogas
maximum theoretical amount of biogas (CH_4 + CO_2) which will evolve after complete biodegradation of an organic compound under anaerobic conditions

NOTE 1 to entry: ThBiogas is calculated from the molecular formula and expressed as litres of biogas evolved per gram of test material under the standard conditions.

3.8
lag phase
time from the start of an anaerobic digestion test until adaptation and/or selection of the degrading microorganisms is achieved and the degree of biodegradation of a chemical compound or organic matter has increased to about 10 % of the maximum level of biodegradation

NOTE 1 to entry: It is measured in days.

3.9
biodegradation phase
time from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached

NOTE 1 to entry: It is measured in days.

3.10
plateau phase
time from the end of the biodegradation phase until the end of the test

NOTE 1 to entry: It is measured in days.

3.11
maximum level of biodegradation
degree of biodegradation of a chemical compound or organic matter in a test, above which no further biodegradation takes place during the test

NOTE 1 to entry: It is measured in percent.

4 Principle

This test method is designed to determine the biodegradability of plastic materials under anaerobic conditions in a slurry system. The methanogenic inoculum is obtained from an anaerobic digester operating on sewage sludge or, alternatively, on organic waste such as livestock faeces or garbage. The test material mixed with the inoculum is anaerobically incubated in a test vessel at a pre-selected temperature for a period normally of 60 days. The test period may be shortened or extended until a plateau phase is reached, but the total period shall not exceed 90 days. The digestion temperature shall be (55 ± 5) °C in order to simulate thermophilic anaerobic digestion. Alternatively, the digestion temperature may be set at (35 ± 3) °C in order to simulate mesophilic anaerobic digestion.

The volume of the gases produced in the test vessel, carbon dioxide (CO_2) and methane (CH_4), is measured. A considerable amount of CO_2 will also be dissolved in the digested sludge or dissociated to hydrogen carbonate ion and carbonate ion under the conditions of the test. This dissolved inorganic carbon (DIC) is measured at the end of the test. The amount of biogas produced is calculated from the volume of biogas collected and the amount of DIC formed in excess of blank values.

The percentage biodegradation is calculated as the ratio of the sum of the net increase of produced biogas and DIC to the theoretical amount of evolved biogas (ThBiogas). The biodegradation curve can be followed by making intermediate measurements of biogas production.

5 Test and reference materials

5.1 Test material: The test material is normally added directly as solids to give a concentration of volatile solids in the range of 7 g/l to 10 g/l. The test material should preferably be used in powder form or as film.

5.2 Reference material: TLC (thin-layer chromatography) grade microcrystalline cellulose with a particle size < 20 µm, for use as the reference material in the positive control.

6 Apparatus

Ensure that all glassware is thoroughly cleaned and, in particular, free from organic or toxic matter. Required is the usual laboratory equipment, plus the following:

6.1 Digestion vessel: Use conical or other suitable glass flasks with gastight connectors and gas-impermeable tubing. A minimum volume of 1,5 l is recommended in view of the requirements of 7.3.

6.2 Gas volume measurement system: Use a gas-sampling bag to collect the biogas evolved. A gastight syringe or a gas burette should preferably be used to measure the volume of the gas in the bag. The water in contact with the gas shall be at a pH < 2 to avoid CO_2 loss through dissolution in the water. All the connectors and tubing shall be gastight and gas-impermeable.

6.3 Dissolved inorganic carbon measurement system: Use a suitable carbon analyser for the direct detection of dissolved inorganic carbon in the supernatant in the digestion vessels. For example, measure the amount of CO_2 evolved by adding an excess quantity of diluted phosphoric acid (see Annex B).

6.4 Apparatus for gas analysis (optional): Use a gas chromatograph, or other apparatus, equipped with a suitable detector and column(s) for measuring the methane and carbon dioxide concentration in the evolved gases.

6.5 Analytical apparatus (optional), for determining volatile fatty acids, as well as total Kjeldahl nitrogen, ammonia nitrogen, dry solids (at 105 °C) and volatile solids (at 550 °C).

7 Procedure

7.1 General

Take all necessary precautions, as far as practically possible, to prevent the digested sludge from being exposed to air (oxygen), e.g. purge the digestion vessels with inert gas.

7.2 Preparation of inoculum

Collect digested sludge from a digester at a sewage treatment plant treating predominantly domestic sewage. Alternatively, digested sludge from a digester treating livestock faeces or garbage may be used. In either case, make sure that the digested sludge is collected from an active digester. Filter the digested sludge with a 2-mm-opening sieve. Use wide-necked bottles made of high-density polyethylene or a similar gas-impermeable but expandable material. Glass bottles are not recommended for safety reasons. Fill the bottles to within 1 cm of the top and seal tightly. After transport to the laboratory, use the digested sludge directly from the bottles or place it in a laboratory-scale digester. Release excess biogas to prevent the inside pressure from building up.

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

The final concentration of the total dry solids in the digested sludge in the test vessels shall not exceed 150 g/l. The pH of the digested sludge shall be between 7,5 and 8,5.

Consider pre-incubation of the digested sludge to reduce background gas production and to decrease the influence of the blanks. It has been shown that pre-incubation for about 5 days gives an adequate decrease in biogas production by the blank without an unacceptable extension of either the lag phase or the biodegradation phase during the test.

If a thermophilic digested sludge is prepared from a mesophilic digested sludge, the digested sludge can be adapted by raising the cultivation temperature, in steps, from 35 °C to 55 °C in about one month. Growth of thermophilic methanogens can be confirmed by an increase in the ratio of methane in the biogas.

An inoculum may be pre-conditioned, but normally no pre-exposed inoculum should be used, especially in the case of standard tests simulating biodegradation behaviour in natural environments. Depending on the purpose of the test, a pre-exposed inoculum may also be used, provided this is clearly stated in the test report.

If required, add any nutrient to the digested sludge during pre-incubation (see ISO 14853 for micronutrients). Indicate in the test report that pre-incubation was carried out.

7.3 Start-up of the test

Set up at least the following number of digestion vessels:

a) two vessels for the test material (V_T);

b) two vessels for the reference material (V_R);

c) two vessels for blank controls (V_B).

Pour 1,4 l of digested sludge (inoculum) into each digestion vessel. Add the test material or reference material containing 10 g to 15 g of volatile solids to each test vessel and purge the mixture with inert gas for 10 min. Prepare the two blank control vessels in the same manner, but without the test or reference material.

Place the vessels in an incubator or a water bath, and connect the digestion vessels to gas-collection bags. Use gas-impermeable tubing and gastight connectors. Weigh and record the mass of digested sludge in each vessel at the end of the digestion period to evaluate the concentration of inorganic carbon. Set the digestion temperature at (55 ± 5) °C for the simulation of thermophilic anaerobic digestion or at (35 ± 3) °C for the simulation of mesophilic anaerobic digestion.

If required, mix the test mixture by shaking the digestion vessel during the test.

7.4 Measurement of biogas produced (see Annex A)

The biogas produced is collected in a gas-collection bag and measured with a gastight syringe or a gas burette. Make a sufficient number of measurements of gas volume, pressure and temperature (normally every day) to determine the rate of gas production. In the early stages, more frequent readings might be required, with less frequent readings needed as time progresses.

7.5 Test duration

The normal test duration is 60 days. The test may be shortened or extended until the plateau phase (see 3.10) is reached, but the total test period shall not exceed 90 days.

7.6 Measurement of dissolved inorganic carbon (see Annex B)

At the end of the test period, after the last measurement of gas volume, allow the digested sludge to settle in the digestion vessels, open each digestion vessel and immediately determine the concentration of dissolved inorganic carbon (DIC) in the supernatant, in litres per litre, at the standard conditions. Do not centrifuge or filter the contents to obtain supernatant (see following paragraph). After the DIC measurement, record the pH. Carry out the DIC measurements on the blanks and on the reference material using the same procedure.

Centrifugation or filtration might result in an unacceptable loss of dissolved carbon dioxide. If the supernatant cannot be analysed immediately, it may be stored in a suitable sealed vial, without headspace, at about 4 °C for up to 2 days.

8 Calculation and expression of results

8.1 Amount of biogas produced

First, the volume under standard conditions (= STP) of biogas collected in the gas-collection bag from each digestion vessel is calculated. The biogas in the bag and the digested sludge in the vessel are in equilibrium, and the biogas in the bag contains the saturated water vapour at room temperature. Therefore, subtract the water vapour pressure at room temperature from the atmospheric pressure, and calculate the volume of biogas under the standard conditions of temperature and pressure using Equation (1):

$$V_0 = V \times (273{,}15 / T) \times (p - p_{\mathrm{w}}) / 1\ 013{,}25 \qquad (1)$$

where

p is the pressure, in hPa;

p_{w} is the water vapour pressure, in hPa, at pressure measurement (see Annex D for a table of water vapour pressures);

V is the volume measured with a syringe or gas burette, in litres;

V_0 is the volume of biogas, in litres, at standard conditions;

T is the room temperature, in K.

8.2 Amount of dissolved inorganic carbon

Calculate the volume of dissolved inorganic carbon in the liquid in the test vessels using Equation (2):

$$V_{0,\mathrm{L}} = V_{0,\mathrm{DIC}} \times V_{\mathrm{L}} \qquad (2)$$

where

$V_{0,L}$ is the STP volume of dissolved inorganic carbon in the liquid in the test vessel, in litres;

$V_{0,DIC}$ is the STP volume of dissolved inorganic carbon in the vessel at the end of the test, in litres per litre;

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

8.3 Calculation of percentage biodegradation

Calculate the percentage biodegradation using Equation (3):

$$\% \text{ Biodegradation} = \frac{\left[V_{0,g}(\text{test}) + V_{0,L}(\text{test})\right] - \left[\overline{V}_{0,g}(\text{blank}) + \overline{V}_{0,L}(\text{blank})\right]}{m_{C,i} / 12,0 \times 22,4} \times 100 \quad (3)$$

where

$V_{0,g}$(test) is the total volume of biogas evolved from the test vessel at STP, in litres;

$V_{0,L}$(test) is the STP volume of dissolved inorganic carbon in the liquid in the test vessel, in litres;

$\overline{V}_{0,g}$(blank) is the mean total volume of biogas evolved from the blank vessel at STP, in litres;

$\overline{V}_{0,L}$(blank) is the mean STP volume of dissolved inorganic carbon in the liquid in the blank vessel, in litres;

$m_{C,i}$ is the amount of carbon initially in the test material, in grams.

NOTE The denominator in Equation (3) corresponds to the ThBiogas production. The calculation of the ThBiogas production is described in ISO 14853:2005, Annex F.

Use the same equation to calculate the percentage biodegradation of the reference material.

9 Expression and interpretation of results

Compile tables containing the measured and calculated data on the test material, the reference material and the blank control.

Plot the cumulative amount of biogas evolved from each digestion vessel (blank, test material, and reference material) as a function of time. Plot a biodegradation curve (percentage of biodegradation as a function of time) for the test material and the reference material (see Annex C). Use mean values if the differences between the individual values are < 20 %. If this is not the case, plot biodegradation curves for each digestion vessel.

Calculate the mean degree of the percentage biodegradation at the end of test and report it as the final test result.

10 Validity of results

The test is considered valid if:

a) the percentage biodegradation of the reference material is > 70 % after 15 days;

b) the difference between the percentage biodegradation of the reference material in the different vessels is < 20 % at the end of the test.

11 Test report

The test report shall contain at least the following information:

a) a reference to this International Standard;

b) all information necessary to identify the test and reference materials, including their organic carbon content, ThBiogas, chemical composition and formula (if known), shape, form, and amount/concentration in the samples tested;

c) the concentration of the test material in the test vessels;

d) details of the way in which the amount of biogas produced was measured (e.g. type of volume-measuring system used) and of the carbon analyser used to measure the DIC;

e) information on the inoculum used, such as source, age, date of collection, storage, handling, any adaptation to the test material, any other pre-incubation, total dry solids, volatile solids, pH of the suspension, total nitrogen content and volatile fatty acids, as appropriate;

f) the results obtained for the biogas evolution and percentage of biodegradation for each digestion vessel and the averages, in tabular form and graphically, as well as the final percentage of biodegradation for the test material and the reference material and the activity of the inoculum;

g) the incubation temperature;

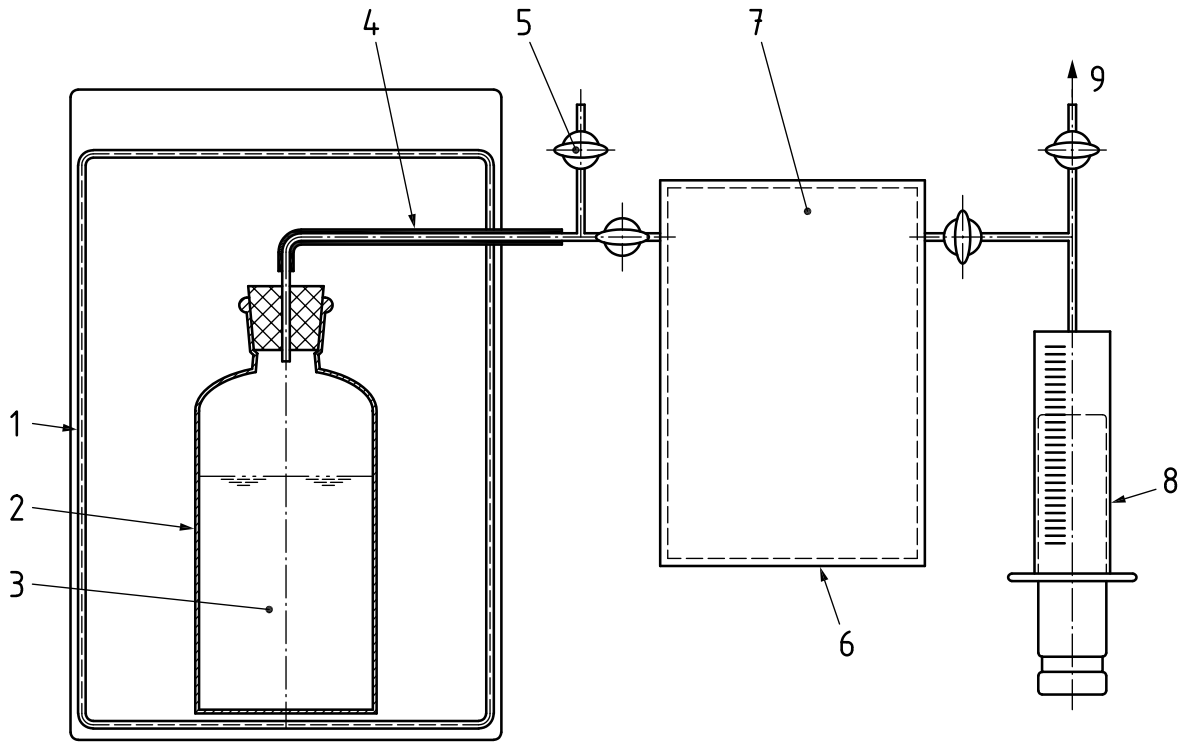
h) the pH and DIC of the test suspensions at the end of the test;

i) the duration of the lag phase and the degradation phase, and the duration of the test;

j) the reasons for rejection of any test results.

Annex A
(informative)

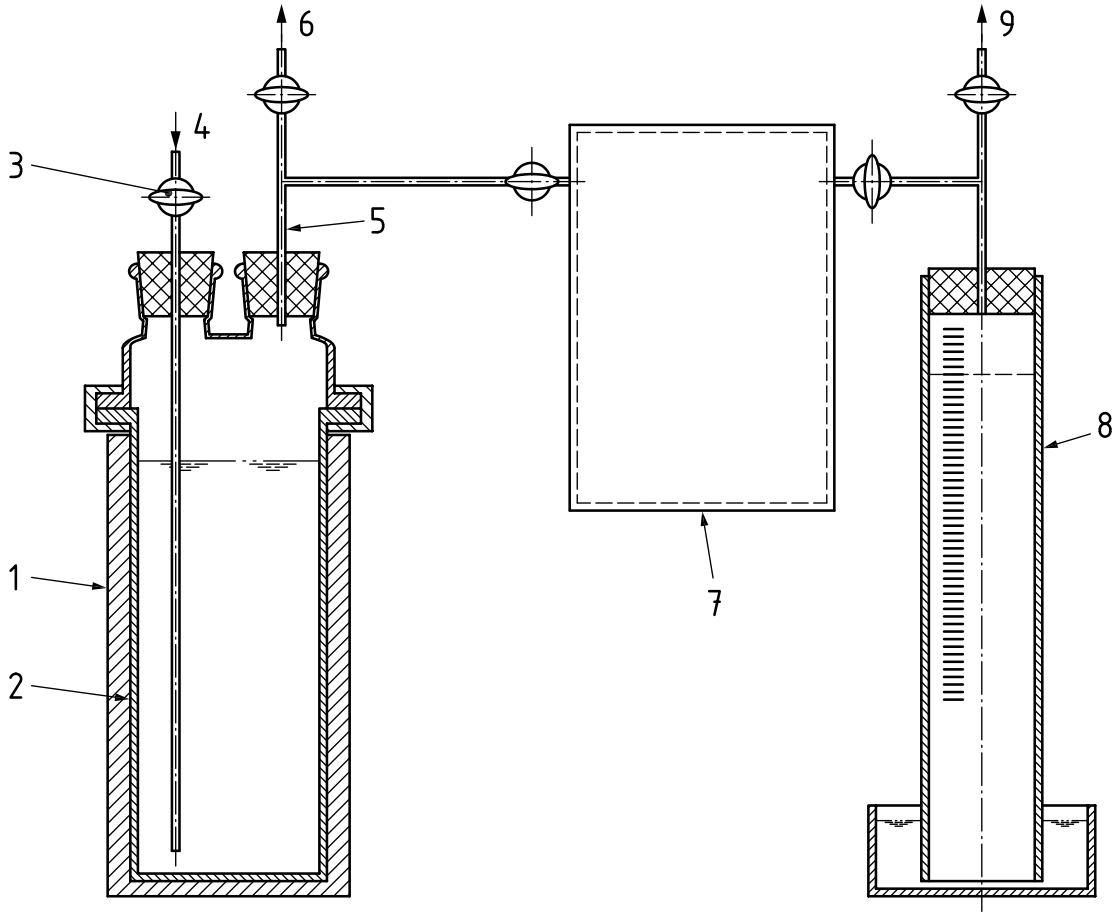
Examples of test systems



Key

1 incubator
2 digester
3 inoculum
4 gas outlet
5 leak valve
6 bag for gas collection
7 biogas
8 syringe
9 discharge

Figure A.1 — Example of a test system using a syringe to measure the volume of biogas produced



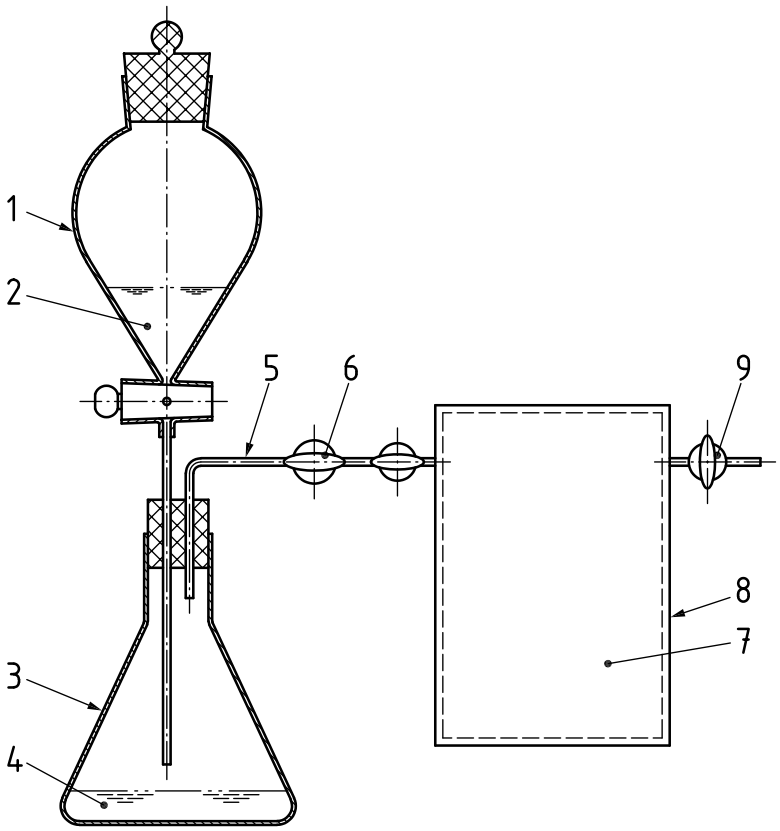
Key

1 thermostatically controlled bath
2 digester
3 valve
4 N_2 gas inlet
5 gas outlet
6 N_2 gas discharge
7 bag for gas collection
8 gas burette
9 discharge

Figure A.2 — Example of a test system using a gas burette to measure the volume of biogas produced

Annex B
(informative)

Example of apparatus for measurement of biogas dissolved in slurry



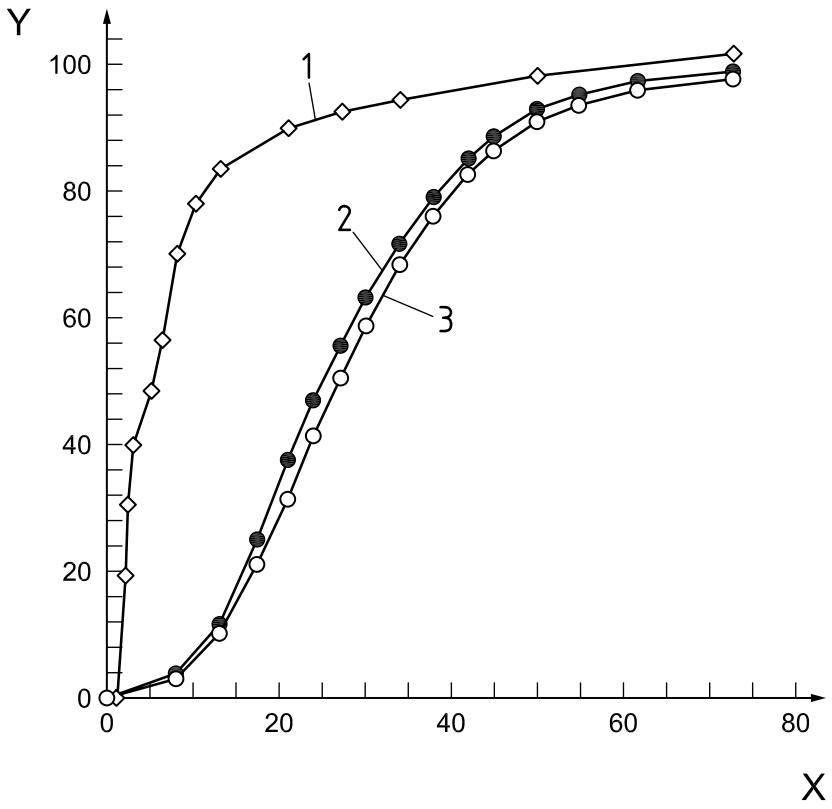
Key

1 separating funnel
2 1 M H_3PO_4 (50 ml)
3 flask (500 ml)
4 slurry (100 g)
5 gas outlet
6 stopcock
7 biogas
8 bag for gas collection (2 litres)
9 leak valve

Figure B.1 — Example of apparatus for the measurement under atmospheric pressure of the amount of biogas dissolved in the slurry

Annex C
(informative)

Example of a biodegradation curve



Key

X time (days)
Y degree of biodegradation (%)
1 cellulose
2 PLA-1
3 PLA-2
PLA: poly(lactic acid)

Figure C.1 — Biodegradation curve for anaerobic digestion of PLA and cellulose in a slurry system at 55 °C

Annex D
(informative)

Table of water vapour pressures at various temperatures

T °C	p kPa		T °C	p kPa
20	2,338 8		31	4,495 3
21	2,487 7		32	4,757 8
22	2,644 7		33	5,033 5
23	2,810 4		34	5,322 9
24	2,985 0		35	5,626 7
25	3,169 0		36	5,945 3
26	3,362 9		37	6,279 5
27	3,567 0		38	6,629 8
28	3,781 8		39	6,996 9
29	4,007 8		40	7,381 4
30	4,245 5		41	7,784 0

Data taken from the CRC Handbook of Chemistry and Physics[3]

NOTE The relationship between the temperature, in °C, and the water vapour pressure, in hPa, is given by the Clausius-Clapeyron equation:

$$p = \exp[-5\,267,6 / (273,15 + T) + 21,132] \quad \text{(E.1)}$$

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