INTERNATIONAL STANDARD

ISO 14853

Second edition
2016-07-15

Plastics — Determination of the ultimate anaerobic biodegradation of plastic materials in an aqueous system — Method by measurement of biogas production

Plastiques — Évaluation de la biodégradabilité anaérobie ultime des matériaux plastiques en milieu aqueux — Méthode par détermination de la production de biogaz

Reference number
ISO 14853:2016(E)

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

The committee responsible for this document is ISO/TC 61, *Plastics*, Subcommittee SC 5, *Physical-chemical properties*.

This second edition cancels and replaces the first edition (ISO 14853:2005), which has been technically revised. It also incorporates the Technical Corrigendum ISO 14853:2005/Cor.1:2009.

Introduction

With the increasing use of plastics, their recovery and disposal have become a major issue. As a first priority, recovery should be promoted. For example, plastic litter, which originates mainly from consumers, is difficult to recover completely. Additional examples of materials difficult to recover are found in the disposal of fishing tackle, agricultural mulch films and water-soluble polymers. These plastic materials tend to leak from closed waste management infrastructures into natural environments. Biodegradable plastics are now emerging as one of the available options to solve such environmental issues. Plastic materials, such as products or packaging, which are sent to anaerobic treatment facilities should be potentially biodegradable. Therefore, it is very important to determine the potential biodegradability of such materials and to obtain a quantitative measure of their biodegradability in anaerobic environments.

Plastics — Determination of the ultimate anaerobic biodegradation of plastic materials in an aqueous system — Method by measurement of biogas production

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

1 Scope

This International Standard specifies a method for the determination of the ultimate anaerobic biodegradability of plastics by anaerobic microorganisms. The conditions described in this International Standard do not necessarily correspond to the optimum conditions for the maximum degree of biodegradation to occur. The test calls for exposure of the test material to sludge for a period of up to 90 d, which is longer than the normal sludge retention time (25 to 30 d) in anaerobic digesters, although digesters at industrial sites can have much longer retention times.

The method applies to the following materials:

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

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

— water-soluble polymers;

— materials which, under the test conditions, do not inhibit the microorganisms present in the inoculum. Inhibitory effects can be determined using an inhibition control or by another appropriate method (see e.g. ISO 13641). If the test material is inhibitory to the inoculum, a lower test concentration, another inoculum or a pre-exposed inoculum can be used.

2 Normative references

There are no normative references in this document.

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 to carbon dioxide, methane, water and mineral salts of any other elements present (mineralization) plus new biomass

3.2
primary anaerobic biodegradation
structural change (transformation) of a chemical compound by microorganisms, resulting in the loss of a specific property

3.3
digested sludge
mixture of settled sewage and activated sludge which have been incubated in an anaerobic digester at about 35 °C 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 bacteria producing carbon dioxide and methane.

3.4
concentration of suspended solids in digested sludge
amount of solids obtained by filtration or centrifugation of a known volume of activated sludge and drying at about 105 °C to constant mass

3.5
dissolved organic carbon
DOC
organic carbon in the water phase which cannot be removed by specified phase separation, for example, by centrifugation at 40 000 $m \cdot s^{-2}$ for 15 min or by membrane filtration using membranes with pores of 0,2 µm to 0,45 µm diameter

3.6
inorganic carbon
IC
inorganic carbon which is dissolved or dispersed in the aqueous phase of a liquid and is recoverable from the supernatant liquid after the sludge has been allowed to settle

3.7
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

3.8
theoretical amount of evolved biogas
Thbiogas
maximum theoretical amount of biogas (CH_4 + CO_2) evolved after complete biodegradation of an organic material under anaerobic conditions, calculated from the molecular formula and expressed as millilitres of biogas evolved per milligram of test material under standard conditions

3.9
theoretical amount of evolved carbon dioxide
$ThCO_2$
maximum theoretical amount of carbon dioxide evolved after complete oxidation of an organic material, calculated from the molecular formula and expressed as milligrams of carbon dioxide per milligram of test material

3.10
theoretical amount of evolved methane
$ThCH_4$
maximum theoretical amount of methane evolved after complete reduction of an organic material, calculated from the molecular formula and expressed as milligrams of methane evolved per milligram of test material

3.11
lag phase
lag period

time, measured in days, from the start of a 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

3.12
plateau phase
time, measured in days, from the end of the biodegradation phase until the end of the test

3.13
biodegradation phase
time, measured in days, from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached

3.14
maximum level of biodegradation
degree of biodegradation, measured in percent, of a chemical compound or organic matter in a test, above which no further biodegradation takes place during the test

4 Principle

The biodegradability of a plastic material is determined using anaerobic conditions in an aqueous system. Test material with a concentration of 20 mg/l to 200 mg/l organic carbon (OC) is incubated at (35 ± 2) °C in sealed vessels together with digested sludge for a period normally not exceeding 90 d. Before use, the digested sludge is washed so that it contains very low amounts of inorganic carbon (IC) and diluted to 1 g/l to 3 g/l total solids concentration. The increase in headspace pressure or the volumetric increase (depending on the method used for measuring biogas evolution) in the test vessels resulting from the production of carbon dioxide (CO_2) and methane (CH_4) is measured. A considerable amount of CO_2 will be dissolved in water or transformed to bicarbonate 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 biogas carbon is calculated from the net biogas production and the net IC formation in excess of blank values. The percentage biodegradation is calculated from the total amount of carbon transformed to biogas and IC and the measured or calculated amount of carbon added as test material. The course of biodegradation can be followed by making intermediate measurements of biogas production. As additional information, the primary biodegradability can be determined by specific analyses at the beginning and end of the test.

This test method is designed to determine the biodegradability of plastic materials under anaerobic conditions. Optionally, the assessment of the recovery rate may also be of interest (see Annex G).

5 Reagents and materials

5.1 Distilled or deionized water, free of toxic substances, containing less than 2 mg/l of DOC.

5.2 Test medium, prepared using only reagents of recognized analytical grade.

Prepare the test medium to contain the following constituents in the stated amounts:

Anhydrous potassium dihydrogen phosphate	KH_2PO_4	0,27 g
Disodium hydrogen phosphate 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
Disodium sulfide nonahydrate[a]	$Na_2S \cdot 9H_2O$	0,1 g
Stock solution of trace elements (optional)		10 ml
Stock solutions of vitamins (optional)	Vitamin solution No. 1	0,5 ml

Anhydrous potassium dihydrogen phosphate	KH_2PO_4	0,27 g
	Vitamin solution No. 2	0,5 ml
Add water (5.1) (oxygen-free) to		1 l

[a] Use freshly prepared sodium sulfide, or wash and dry it before use, to ensure sufficient reductive capacity. In order to ensure strictly anaerobic conditions, it is recommended that a small amount of sodium dithionite be added to the medium after it has been prepared until it becomes colourless. Do not use more than 10 mg/l because higher concentrations may produce inhibitory effects.

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

To ensure oxygen-free conditions, purge the water with nitrogen for about 20 min immediately before use.

5.3 **Trace-element solution** (optional).

It is recommended that the test medium be supplemented with the following trace elements to improve the anaerobic degradation process, 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
Disodium tungstate dihydrate	$Na_2WO_4 \cdot 2H_2O$	0,002 g
Add water (5.1) (oxygen free) to		1 l

Use 10 ml of trace-element solution per litre of test medium.

5.4 **Vitamin solutions** (optional).

5.4.1 Vitamin solution No. 1

4-Aminobenzoic acid	40 mg
D-Biotin	10 mg
Dissolve in hot water (5.1)	500 ml

Allow to cool and add:

D-Pantothenic acid, calcium salt	50 mg
Pyridoxamine dihydrochloride	150 mg
Thiamine dichloride	100 mg

Filter the solution through a membrane filter (pore size 0,45 µm) that neither adsorbs nor releases organic carbon in significant amounts, and store in the dark at 4 °C.

Use 0,5 ml of vitamin solution per litre of test medium.

5.4.2 Vitamin solution No. 2

Cyanocobalamin (vitamin B12)	10 mg
Dissolve in water (5.1)	100 ml

Filter the solution through a membrane filter (pore size 0,45 µm) that neither adsorbs nor releases organic carbon in significant amounts, and store in the dark at 4 °C.

Use 0,5 ml of vitamin solution per litre of test medium.

5.5 Barrier solution.

NaCl	200 g
Dissolve in water (5.1)	1 000 ml
Acidify with citric acid	5 g

Add a pH indicator such as bromophenol blue or methyl orange in order to be able to verify that the solution remains acid during the test.

5.6 Test material.

The test material is usually added directly as solid to give a concentration of 20 mg/l to 200 mg/l organic carbon. The test material (plastic) should be used in powdered form, if possible.

The test material should preferably be used in powder form, but it may also be introduced as films, pieces, fragments or shaped articles. The form and shape of the test material may influence its biodegradability. Similar shapes should preferably be used if different kinds of plastic material are to be compared. If the test material is used in the form of a powder, particles of known, narrow size distribution should be used. A particle-size distribution with the maximum at 250 µm diameter is recommended. Also, the size of the test equipment used may depend on the form of the test material.

The biodegradability of plastic materials which are not inhibitory to microorganisms can be determined using concentrations higher than 200 mg/l organic carbon. In this case, ensure that the buffer capacity and mineral-salt content of the medium are sufficient.

5.7 Reference material.

Use a well-defined anaerobically biodegradable polymer, e.g. poly-β-hydroxybutyrate, cellulose or poly(ethylene glycol) 400 as a reference material. If possible, the form, size, solubility and concentration of the reference material should be comparable with that of the test material.

Prepare the reference material in the same way as the test material.

5.8 Inhibition control (optional).

Add both the test material and the reference material to a vessel containing test medium (5.2) to give the concentrations specified in 5.6 and 5.7, respectively.

6 Apparatus

6.1 Laboratory equipment

Required is usual laboratory equipment, plus the following:

6.1.1 Incubator or water or sand bath, thermostatically controlled at (35 ± 2) °C.

6.1.2 Carbon analyser (optional), suitable for the direct determination of inorganic carbon in the range 1 mg/l to 200 mg/l IC. Alternatively, the IC in the supernatant may be determined indirectly by release of the dissolved IC as carbon dioxide that can be measured in the headspace, as described in 7.7.

6.2 Apparatus for use when biogas is measured by a manometric method

6.2.1 Pressure-resistant glass test vessels, nominal size 0,1 l to 1 l, each fitted with a gastight septum capable of withstanding about 2 000 hPa (for an example, see Annex A). The headspace volume shall be about 10 % to 30 % of the total volume. If gas is released at regular intervals, about 10 % headspace volume is adequate, but if gas is released only at the end of the test, 30 % is more appropriate.

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

6.2.2 Pressure-measuring device, e.g. a manometer connected to a suitable syringe needle, with a gastight three-way valve to facilitate the release of excess pressure. Use and calibrate the device in accordance with the manufacturer's instructions.

It is necessary to keep the internal volume of the tubing and the valve as low as possible so that errors introduced by neglecting the volume of the device are not significant.

6.3 Apparatus for use when biogas is measured by a volumetric method

6.3.1 Glass test vessels (e.g. conical flasks or bottles), nominal size 0,1 l to 1 l, preferably 300 ml for every 250 ml of medium. If foaming is not expected to occur, a headspace volume of 10 % to 20 % is recommended. The vessels shall be equipped with a septum for gas sampling (see Annex B) and shall be connected via gastight tubing to a graduated glass gas-collection tube which is filled with acidified salt solution (barrier solution 5.5). This graduated glass tube shall be connected to an expansion tank which can be moved up and down to bring the surface of the acidified solution in the expansion tank to the same level as that in the gas-collection tube.

7 Procedure

7.1 General

Carry out the following initial operations using techniques which will ensure that the digested sludge comes into contact with oxygen as little as practicable, e.g. work in a glove-box in an atmosphere of nitrogen or purge the test vessels with nitrogen.

7.2 Digested sludge

Collect digested sludge from a digester at a sewage treatment plant treating predominantly domestic sewage. Be sure to collect active sludge. Use wide-necked bottles made of high-density polyethylene or a similar material which can expand. Glass is not recommended for safety reasons. Fill the bottles to within 1 cm of the top and seal. 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 the inoculum.

Consider pre-incubation 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 ± 2) °C for up to 7 d.

It has been shown that pre-incubation for about 5 d gives an optimum decrease in gas production by the blank without an unacceptable increase in either lag period or incubation period during the test. For test materials which are expected to be poorly biodegradable, consider pre-incubating the sludge with the test material to get a better adapted inoculum. In such a case, add test material with a concentration of 5 mg/l to 20 mg/l OC to the digested sludge. Wash the pre-incubated sludge carefully before use. Indicate in the test report that pre-incubation was carried out.

