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**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*

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# Contents

Page

Foreword	v
Introduction	vi
<b>1 Scope</b>	<b>1</b>
<b>2 Normative references</b>	<b>1</b>
<b>3 Terms and definitions</b>	<b>1</b>
<b>4 Principle</b>	<b>3</b>
<b>5 Reagents and materials</b>	<b>3</b>
<b>6 Apparatus</b>	<b>5</b>
6.1 Laboratory equipment	5
6.2 Apparatus for use when biogas is measured by a manometric method	6
6.3 Apparatus for use when biogas is measured by a volumetric method	6
<b>7 Procedure</b>	<b>6</b>
7.1 General	6
7.2 Digested sludge	6
7.3 Preparation of the inoculum	7
7.4 Preparation of test suspensions and controls	7
7.5 Incubation and gas measurement	8
7.6 Test duration	9
7.7 Measurement of inorganic carbon	9
7.8 Specific analyses	9
<b>8 Calculation and expression of results</b>	<b>9</b>
8.1 Amount of carbon in headspace	9
8.2 Calculation of amount of carbon in headspace when manometric measurement method is used	10
8.3 Calculation of amount of carbon in headspace when volumetric measurement method is used	11
8.4 Amount of inorganic carbon in the liquid	11
8.5 Total amount of carbon converted to gas	11
8.6 Amount of carbon in test material	12
8.7 Calculation of percentage biodegradation	12
<b>9 Validity of results</b>	<b>12</b>
9.1 Maintenance of anaerobic conditions	12
9.2 Inhibition of degradation	12
9.3 Validity of the test	12
<b>10 Test report</b>	<b>13</b>
<b>Annex A (informative) Example of apparatus for determining the amount of biogas produced by measuring the increase in gas pressure</b>	<b>14</b>
<b>Annex B (informative) Example of apparatus for determining volumetrically the amount of biogas produced</b>	<b>15</b>
<b>Annex C (informative) Example of a biodegradation curve</b>	<b>17</b>
<b>Annex D (informative) Examples of data sheets for anaerobic biodegradability tests</b>	<b>18</b>
<b>Annex E (informative) Table of water vapour pressures at various temperatures</b>	<b>21</b>
<b>Annex F (informative) Calculation of theoretical carbon dioxide (ThCO<sub>2</sub>) and theoretical methane (ThCH<sub>4</sub>) production</b>	<b>22</b>
<b>Annex G (informative) Example of determination of recovery rate</b>	<b>23</b>
<b>Annex H (informative) Example of a workflow scheme</b>	<b>26</b>

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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](http://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](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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](http://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.

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# 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·s<sup>-2</sup> 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<sub>4</sub> + CO<sub>2</sub>) 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<sub>2</sub>**

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<sub>4</sub>**

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  $(35 \pm 2)$  °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<sub>2</sub>) and methane (CH<sub>4</sub>) is measured. A considerable amount of CO<sub>2</sub> 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 <sub>2</sub> PO <sub>4</sub>	0,27 g
Disodium hydrogen phosphate dodecahydrate	Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	1,12 g
Ammonium chloride	NH <sub>4</sub> Cl	0,53 g
Calcium chloride dihydrate	CaCl <sub>2</sub> ·2H <sub>2</sub> O	0,075 g
Magnesium chloride hexahydrate	MgCl <sub>2</sub> ·6H <sub>2</sub> O	0,10 g
Iron (II) chloride tetrahydrate	FeCl <sub>2</sub> ·4H <sub>2</sub> O	0,02 g
Resazurin (oxygen indicator)		0,001 g
Disodium sulfide nonahydrate <sup>a</sup>	Na <sub>2</sub> S·9H <sub>2</sub> O	0,1 g
Stock solution of trace elements (optional)		10 ml
Stock solutions of vitamins (optional)	Vitamin solution No. 1	0,5 ml