INTERNATIONAL STANDARD

ISO

17556

Third edition
2019-05

Plastics — Determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved

Plastiques — Détermination de la biodégradabilité aérobie ultime des matériaux plastiques dans le sol par mesure de la demande en oxygène dans un respiromètre ou de la teneur en dioxyde de carbone libéré

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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).

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For an explanation on the voluntary nature of standards, 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.

This document was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 14, *Environmental aspects*.

This third edition cancels and replaces the second edition (ISO 17556:2012), which has been technically revised. The main changes compared to the previous edition are as follows:

a)   the unit for BOD, COD and DIC has been corrected (see Clause 3);

b)   the formula for calculating the percent biodegradation has been modified (see 9.1.1);

c)   the test period has been revised to two years at the longest (see Clause 4);

d)   the number of replicates has been corrected to three (see 9.2).

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

A number of plastic materials and products have been designed for applications ending up in or on soil. They have been developed for applications where biodegradation is beneficial from a technical, environmental, social or economic standpoint. Examples can be found in agriculture (e.g. mulching film), horticulture (e.g. twines and clips, flower pots, pins), funeral items (e.g. body bags), recreation (e.g. plastic "clay" pigeons for shooting, hunting cartridges), etc. In many cases, recovery and/or recycling of these plastic items is either difficult or not economically viable. Various types of biodegradable plastics have been developed which have been designed to biodegrade and disappear in situ at the end of their useful life. Several International Standards specify test methods for determining the ultimate aerobic or anaerobic biodegradation of plastic materials in aqueous or compost conditions. Considering the use and disposal of biodegradable plastics, it is important to establish a test method to determine the ultimate aerobic biodegradation of such plastic materials in soil.

Plastics — Determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved

WARNING — Appropriate precautions should be taken when handling soil because it might contain potentially pathogenic organisms. Toxic test compounds and those whose properties are unknown should be handled with care.

1 Scope

This document specifies a method for determining the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a closed respirometer or the amount of carbon dioxide evolved. The method is designed to yield an optimum degree of biodegradation by adjusting the humidity of the test soil.

If a non-adapted soil is used as an inoculum, the test simulates the biodegradation processes which take place in a natural environment; if a pre-exposed soil is used, the method can be used to investigate the potential biodegradability of a test material.

This method applies to the following materials:

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

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

— water-soluble polymers.

It does not necessarily apply to materials which, under the test conditions, inhibit the activity of the microorganisms present in the soil. Inhibitory effects can be measured using an inhibition control or by another suitable method. If the test material inhibits the microorganisms in the soil, a lower test material concentration, another type of soil or a pre-exposed soil can be used.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 10390, *Soil quality — Determination of pH*

ISO 10694, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis)*

ISO 11274, *Soil quality — Determination of the water-retention characteristic — Laboratory methods*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

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

3.2
biochemical oxygen demand
BOD
mass concentration of dissolved oxygen consumed under specified conditions by the aerobic biological oxidation of a chemical compound or organic matter

Note 1 to entry: It is expressed as milligrams of oxygen uptake per kilogram of test soil.

3.3
dissolved organic carbon
DOC
part of the organic carbon in water which cannot be removed by specified phase separation

Note 1 to entry: It is expressed as milligrams of carbon per litre.

Note 2 to entry: Typical means of separation are centrifugation at $40\,000\ m \cdot s^{-2}$ for 15 min or membrane filtration using membranes with pores of diameter $0,2\ \mu m$ to $0,45\ \mu m$.

3.4
theoretical oxygen demand
ThOD
maximum theoretical amount of oxygen required to oxidize a chemical compound completely, calculated from the molecular formula

Note 1 to entry: It is expressed as milligrams of oxygen uptake per milligram or gram of test compound.

3.5
theoretical amount of evolved carbon dioxide
$ThCO_2$
maximum theoretical amount of carbon dioxide evolved after completely oxidizing a chemical compound, calculated from the molecular formula

Note 1 to entry: It is expressed as milligrams of carbon dioxide evolved per milligram or gram of test compound.

3.6
lag phase
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* (3.8)

3.7
biodegradation phase
time, measured in days, from the end of the *lag phase* (3.6) of a test until about 90 % of the *maximum level of biodegradation* (3.8) has been reached

3.8
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

3.9
plateau phase
time from the end of the *biodegradation phase* (3.7) until the end of the test

Note 1 to entry: It is measured in days.

3.10
pre-conditioning
pre-incubation of soil under the conditions of the subsequent test in the absence of the chemical compound or organic matter under test, with the aim of improving the performance of the test by acclimatization of the microorganisms to the test conditions

3.11
pre-exposure
pre-incubation of soil in the presence of the chemical compound or organic matter under test, with the aim of enhancing the ability of the soil to biodegrade the test material by adaptation and/or selection of the microorganisms

3.12
water content
mass of water which evaporates from the soil when the soil is dried to constant mass at 105 °C, divided by the dry mass of the soil

Note 1 to entry: This is simply the ratio between the mass of the water and that of the soil particles in a soil sample.

3.13
total water-holding capacity
mass of water which evaporates from soil saturated with water when the soil is dried to constant mass at 105 °C, divided by the dry mass of the soil

3.14
total organic carbon
TOC
amount of carbon bound in an organic compound

Note 1 to entry: It is expressed as milligrams of carbon per 100 mg of the compound.

4   Principle

This method is designed to yield the optimum rate of biodegradation of a plastic material in a test soil by controlling the humidity of the soil, and to determine the ultimate biodegradability of the material.

The plastic material, which is the sole source of carbon and energy, is mixed with the soil. The mixture is allowed to stand in a flask over a period of time during which the amount of oxygen consumed (BOD) or the amount of carbon dioxide evolved is determined. Provided the CO_2 evolved is absorbed, the BOD can be determined, for example, by measuring the amount of oxygen required to maintain a constant gas volume in a respirometer flask, or by measuring either automatically or manually the change in volume or pressure (or a combination of the two). An example of a suitable respirometer is shown in Annex A. The amount of carbon dioxide evolved is measured at intervals dependent on the biodegradation kinetics of the test substance by passing carbon-dioxide-free air over the soil and then determining the carbon dioxide content of the air by a suitable method. Examples of suitable methods are given in Annexes B and C.

The level of biodegradation, expressed as a percentage, is determined by comparing the BOD with the theoretical oxygen demand (ThOD) or by comparing the amount of carbon dioxide evolved with the theoretical amount ($ThCO_2$). The influence of possible nitrification processes on the BOD has to be considered. The normal test period is six months. The test may be shortened or extended until the plateau phase (see 3.9) is reached, but the total test period shall not exceed two years.

Unlike ISO 11266, which is used for a variety of organic compounds, this document is specially designed to determine the biodegradability of plastic materials.

5   Test environment

Incubation shall take place in the dark or in diffused light in an enclosure which is free from vapours toxic to microorganisms and is maintained at a temperature constant to within ±2 °C in the range between 20 °C and 28 °C, preferably 25 °C.

6   Materials

6.1   Distilled water, containing less than 2 mg of DOC per litre.

6.2   Carbon dioxide absorber, preferably soda lime pellets.

7   Apparatus

Ensure that all glassware is thoroughly cleaned and, in particular, free from organic or toxic matter.

7.1   Closed respirometer, including test flasks and all other necessary equipment, located in a constant-temperature enclosure or in a thermostatically controlled apparatus (e.g. a water-bath). An example is described in Annex A.

Any respirometer capable of determining with sufficient accuracy the biochemical oxygen demand is suitable, preferably an apparatus which measures and automatically replaces the oxygen consumed so that no oxygen deficiency and no inhibition of the microbial activity occurs during the degradation process.

7.2   Apparatus for measuring the amount of carbon dioxide evolved

7.2.1   Test flasks: glass vessels (e.g. conical flasks or bottles), fitted with tubing impermeable to carbon dioxide to allow purging with gas, and located in a constant-temperature enclosure or in a thermostatically controlled apparatus (e.g. a water-bath).

7.2.2 CO_2-free-air production system, capable of supplying CO_2-free air at a flow rate of several ml/min to each test flask, held constant to within ±10 % (see example of system, including test vessels, in Annex B). Alternatively, the incubation apparatus shown in ASTM D5988 may be used.

7.2.3   Analytical equipment for accurately determining carbon dioxide. Typical examples are a carbon dioxide IR analyser, a dissolved inorganic carbon (DIC) analyser, apparatus for titrimetric determination after complete absorption in a basic solution (see Annex C), and apparatus for the gravimetric determination of carbon dioxide in accordance with ISO 14855-2.

7.3   Analytical balance.

7.4   pH-meter.

8   Procedure

8.1   Preparation of the test material

The test material shall be of known mass and contain sufficient carbon to yield a BOD or a quantity of carbon dioxide that can be adequately measured by the analytical equipment used. Calculate the TOC

b) all information necessary to identify the test and reference material, including name, chemical composition and formula (if known), ThOD, $ThCO_2$ (including the method of calculation), form, particle shape, amount/concentration in the samples tested, and content of additives (if possible);

c) the history of the test material (virgin granules, converted final plastic product or aged samples), detailing the pre-treatment conditions, if pre-treatment was carried out;

d) complete information on the soil, including source, date of collection, characteristics, amount used in the test, storage conditions, handling and details of any pre-exposure;

e) the main test conditions, including the amount of test material used, the incubation temperature and the duration of incubation;

f) the analytical techniques used, including the principle of the respirometer and the method used to measure the amount of carbon dioxide evolved;

g) all other operations carried out, including any addition of water to the test mixture during the test, and the results of analyses of the test mixture, including the water content, at the end of the test;

h) all the test results obtained for the test and reference materials (in tabular and graphical form), including the measured cumulative BOD or evolved carbon dioxide, the percentage biodegradation values and the curves of these parameters against time;

i) the duration of the lag phase and degradation phase, the maximum level of biodegradation, as well as the total test duration;

and, optionally, if run or determined:

j) the residual amount of test material or the percentage biodegradation calculated from the residual amount of test material;

k) the colony-forming units (cfu/g) in the soil;

l) details of the methods used during an extended test period (>six months) in order to support microbial diversity or to avoid nutrient deficiency;

m) all available information on the test material and the amount used if the test has been performed at a reduced test material concentration in order to avoid toxic effects;

n) any other relevant data (e.g. initial molecular mass of the sample, molecular mass of the residual polymer);

o) any deviation from the test method specified.

Annex A
(informative)

Principle of a manometric respirometer (example)

The respirometer, as shown in Figure A.1, is set up in a temperature-controlled environment (e.g. a water-bath) and contains test vessels each fitted with a CO_2 absorber in the headspace, a coulometric oxygen production unit, a manometer and an external monitoring device and recorder (printer, plotter or computer). The test vessels are filled to about one third of their volume with the test mixture. If biodegradation takes place, the microorganisms consume oxygen and produce carbon dioxide which is totally absorbed. The total pressure in the vessels decreases. The pressure drop is detected by a manometer and used to initiate the electrolytic generation of oxygen. When the original pressure is re-established, electrolysis is stopped and the quantity of electricity used, which is proportional to the oxygen consumption, is continuously measured and used to indicate the oxygen consumption in milligrams per litre BOD on the recorder.
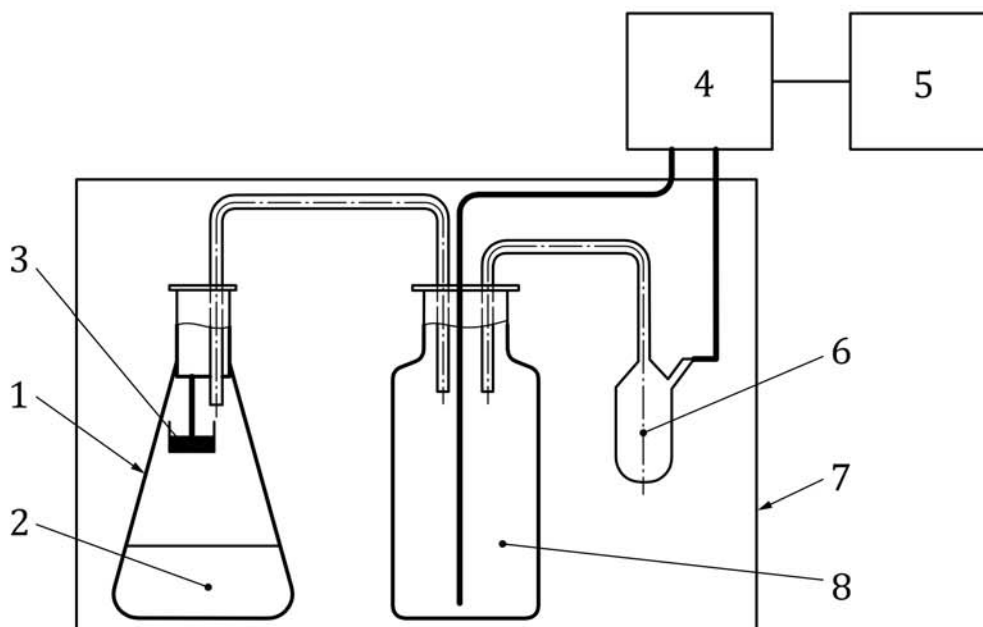
Key

1   test flask

2   test mixture

3   CO_2 absorber

4   monitor

5   printer, plotter or computer

6   manometer

7   thermostatically controlled enclosure

8   oxygen-generating unit

Figure A.1 — Schematic diagram of a manometric respirometer

Annex B
(informative)

Example of a system for measuring the amount of carbon dioxide evolved

Set up the flasks in series as shown in Figure B.1 and connect them with gas-impermeable tubing. Aerate the test system with several millilitres per minute of CO_2-free air at a constant low pressure (preferably at least a volume of air equivalent to the volume of three flasks per hour when using a high test material concentration, for example 2 500 mg of test material for 200 g of soil). Count air bubbles or use a suitable air-flow controller (2) to check the air-flow rate. Use synthetic CO_2-free air or compressed air. In the latter case, remove CO_2 by passing the air through a bottle (3) containing dry soda lime or through at least two gas-washing bottles containing e.g. 500 ml of a 10 mol/l aqueous potassium hydroxide solution. An additional flask containing e.g. 100 ml of 0,012 5 mol/l barium hydroxide solution and an empty flask can be used to indicate the presence of any CO_2 in the air by turbidity and to prevent carry-over of liquid to the test flask. If necessary, a humidifier (4) may be inserted before the test flask (5) to humidify the air so as to avoid evaporation of moisture from the test soil. This can be done for example by bubbling the air through a constant-humidity solution such as a saturated aqueous solution of sodium phosphate. If biodegradation takes place, CO_2 is produced in the test flask and absorbed in the subsequent absorption bottles (6) as described in Annex C. In order to maintain the test soil (flask 5) wet and the conditions aerobic, adjust the flow rate at the air inlet.

To ensure complete aeration and avoid the formation of gas concentration gradients within the test flask(s), the air inlet tubing should preferably be extended into the soil, in order to ensure that air passes through the soil mass.
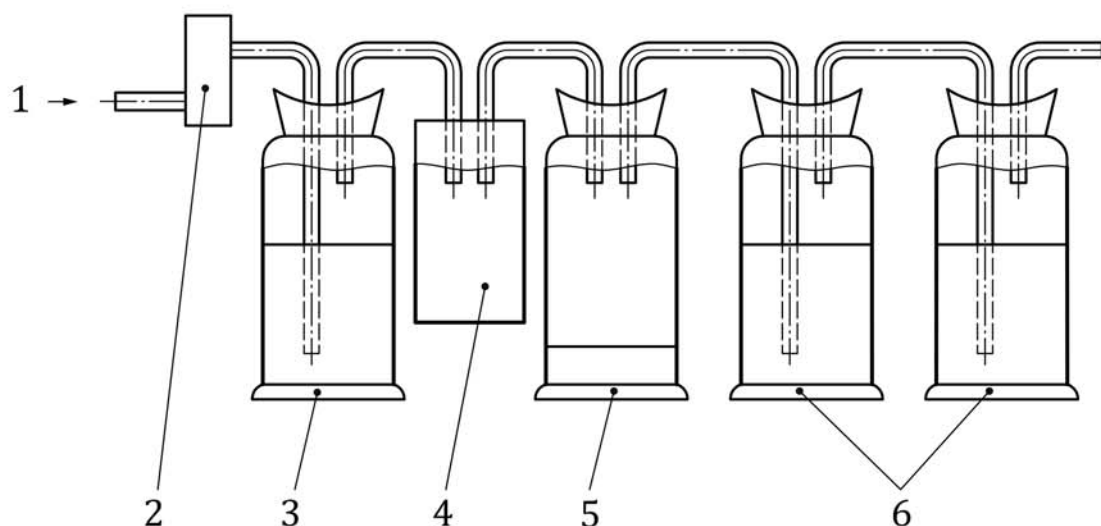
Key

1   air in
2   air-flow controller
3   CO_2 absorber
4   humidifier
5   test flask
6   CO_2-absorption bottles

Figure B.1 — Schematic diagram of a system for measuring the amount of carbon dioxide evolved

Annex C
(informative)

Examples of methods for the determination of evolved carbon dioxide

C.1 CO_2 determination by DIC measurement

The carbon dioxide evolved is absorbed in sodium hydroxide (NaOH) solution and determined as dissolved inorganic carbon (DIC) using, for example, a DOC analyser without incineration.

Prepare a solution of 0,05 mol/l NaOH in deionized water. Measure the DIC of this solution and use this blank value when calculating the CO_2 production. Connect in series with the test flask two absorption bottles, each containing 100 ml of the NaOH solution. Close the outlet of the last bottle with a small syphon to prevent CO_2 from the air from entering the NaOH solution. On the days when the CO_2 is determined, remove the absorption bottle next to the test flask and take a sample large enough for DIC measurement (e.g. 10 ml). Replace the bottle by the second and add a new one with freshly prepared NaOH solution. On the last day, after acidification of the test solution, measure the DIC in both bottles.

Calculate the CO_2 produced using Formula (C.1):

$$\left(CO_2\right)_T = \frac{\left(DIC_T - DIC_B\right) \times 3{,}67}{10}$$ (C.1)

where

$\left(CO_2\right)_T$ is the mass of CO_2 evolved, in milligrams;

DIC_T is the DIC of test material measured for the NaOH solution, in milligrams per litre;

DIC_B is the blank DIC measured for the NaOH solution, in milligrams per litre;

3,67   is the ratio of the molecular mass of CO_2 (44) to the atomic mass of carbon (12);

10   is a correction factor to allow for the fact that 100 ml of NaOH solution was used.

C.2   Titrimetric method using a barium hydroxide solution

The carbon dioxide evolved is determined in the process shown by the following two reaction formulas. The CO_2 produced reacts with the barium hydroxide $Ba(OH)_2$ and is precipitated as barium carbonate ($BaCO_3$). The amount of CO_2 evolved is determined by titrating the remaining $Ba(OH)_2$ with hydrochloric acid (HCl).

$CO_2 + Ba(OH)_2 \rightarrow BaCO_3 + H_2O$

$Ba(OH)_2 + 2HCl \rightarrow BaCl_2 + 2H_2O$
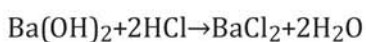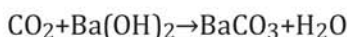
Dissolve 4,0 g of $Ba(OH)_2 \cdot 8H_2O$ in deionized or distilled water and make up to 1 000 ml to obtain a 0,012 5 mol/l solution. It is recommended that a sufficient amount, e.g. five litres, is prepared at a time when running a series of tests. Filter free of solid material and determine the exact concentration by titration with a standard HCl solution. Use phenolphthalein as indicator or an automatic titrator to

determine the end-point. Store as a clear solution in a sealed flask to prevent absorption of CO_2 from the air.

Dilute 50 ml of a 1 mol/l HCl solution (36,5 g/l) to 1 000 ml with deionized or distilled water to obtain a 0,05 mol/l solution.

At the start of the test, dispense 100 ml of $Ba(OH)_2$ solution into each of three absorption bottles. Depending on the character and amount of the test material, use modifications of the trapping volumes. Periodically remove the bottle nearest the test vessel for titration. This should take place as needed, for example when the first bottle is turbid and before any precipitation of $BaCO_3$ can be observed in the second bottle. At the beginning of the test, titration might be required every other day, and then every fifth day when the plateau phase is reached. After removing the absorption bottle, immediately seal it with a plug to avoid CO_2 entering from the air. Move the remaining two bottles one position closer to the test bottle and place at the end of the series a new bottle filled with fresh $Ba(OH)_2$ solution. Especially if longer test periods are used, determine the exact concentration of the solution. Handle all flasks containing test material, reference material, blank, inhibition control and inoculum control in exactly the same way.

Immediately after removing the bottle, titrate two or three aliquot portions of the $Ba(OH)_2$ solution with the HCl solution. Note the volumes of the HCl solution needed for neutralization.

Calculate the mass of CO_2 trapped in the absorption bottle using Formula (C.2):

$$m = \left(\frac{2c_B \times V_{B0}}{c_A} - V_A \times \frac{V_{Bt}}{V_{BZ}} \right) \times c_A \times 22 \tag{C.2}$$

where

m is the mass of CO_2 trapped in the absorption bottle, in milligrams;

c_A is the exact concentration of the HCl solution, in moles per litre;

c_B is the exact concentration of the $Ba(OH)_2$ solution, in moles per litre;

V_{B0} is the volume of the $Ba(OH)_2$ solution at the beginning of the test, in millilitres;

V_{Bt} is the volume of the $Ba(OH)_2$ solution at time t, before titration, in millilitres;

V_{BZ} is the volume of the aliquots of $Ba(OH)_2$ solution used for titration, in millilitres;

V_A is the volume of the HCl solution used for titration, in millilitres;

22   is half of the molecular mass of CO_2.

When the following conditions apply:

— the volume of the $Ba(OH)_2$ solution before and after absorption is exactly 100 ml;

— the complete solution is used for titration (V_{B0} = V_{Bt} = V_{BZ});

— the concentration c_B of the $Ba(OH)_2$ solution is exactly 0,012 5 mol/l;

— the concentration c_A of the HCl solution is exactly 0,05 mol/l;

use Formula (C.3):

$$m = 1,1 \times (50 - V_A) \tag{C.3}$$

Annex D
(informative)

Theoretical oxygen demand (ThOD)

D.1 Calculation of ThOD

The theoretical oxygen demand (ThOD) of the substance $C_cH_hCl_{cl}N_nS_sP_pNa_{na}O_o$ of relative molecular mass M_r, can be calculated if the elemental composition is known or can be determined by elemental analysis, using Formula (D.1):

$$ThOD = \frac{16\left[2c+0,5\left(h-cl-3n\right)+3s+2,5p+0,5na-o\right]}{M_r} \tag{D.1}$$

This calculation assumes that carbon is converted to CO_2, hydrogen to H_2O, phosphorus to P_2O_5 and sulfur to an oxidation state of +6 and that halogens are eliminated as hydrogen halides. The oxidation of N, P and S has to be checked by analysis. The calculation also assumes that nitrogen is released as ammonium. Express the ThOD in milligrams per gram of substance or in milligrams per milligram of substance.

D.2 Example: poly-(R)-3-hydroxybutyrate [(R)-PHB]

Summary formula[1)]: $C_4H_6O_2$, $c = 4$, $h = 6$, $o = 2$; relative molecular mass $M_r = 86$.

$$ThOD = \frac{16\left[2\times4+0,5\times6-2\right]}{86}$$

ThOD = 1,674 4 mg/mg of PHB = 1 674,4 mg/g of PHB

D.3 Example: blend of polyethylene/starch/glycerol

Component	Formula	ThOD mg/g	Amount of component %	Amount of component mg/flask	ThOD mg/flask
Polyethylene	$(C_2H_4)_n$	3 400	50	500	1 700
Starch	$(C_6H_{10}O_5)_n$	1 190	40	400	476
Glycerol	$C_3H_8O_3$	1 200	10	100	120
Total blend			100	1 000	2 296

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1)   PHB is a polymer of the (R)-3-hydroxybutyrate monomer. For polymerization (ester formation), water is removed, so that the summary formula for PHB is equivalent to that of the monomer minus one H_2O, which is eliminated in the chemical reaction.

Annex E
(informative)

Example of a determination of the amount and the molecular mass of water-insoluble polymer remaining at the end of a biodegradation test

It might be helpful to use a procedure for measuring the amount and the molecular mass of polymer remaining at the end of a biodegradation study. The following method or another appropriate one can be used to analyse water-insoluble polymers that dissolve in organic solvents which are not miscible with water.

a) Transfer the test mixture to a separate funnel, add a suitable organic solvent and shake for 10 min to 20 min to extract the remaining polymers. Separate the organic solvent layer from the aqueous layer. Add fresh solvent and repeat the procedure.

b) Combine the organic extracts and evaporate the solvent until dry. Dissolve the solid sample in an appropriate volume of a suitable eluent.

c) Using a microsyringe, inject a suitable amount into a high-performance liquid chromatography (HPLC) apparatus having a column packed with a size-exclusion chromatographic gel. Start the analysis and record the chromatogram.

d) Determine the amount of polymer present using a calibration curve.

e) Determine the molecular mass of the polymer by injecting into the chromatograph the same polymer, or polymers of structure similar to that of the test polymer whose molecular masses are known. The relationship between the retention time and the molecular mass is obtained from the resulting chromatogram. Calculate the molecular mass using this relationship.

The absolute molecular mass of the test polymer can also be determined by HPLC with a combined low-angle laser light scattering (LALLS) and differential refractive index (RI) detector.

Annex F
(informative)

Examples of long-term tests

F.1 Evolution of the biodegradation of cellulose, wheat gluten, flax fibres and broom fibres in soil

NOTE   Data taken from Reference [8].

Inoculum: agricultural soil (500 g, < 2 mm)

Materials: cellulose (5 g), wheat gluten (5 g), flax fibres (2 g), broom fibres (2 g)

Duration: cellulose: two years

                      wheat gluten, flax fibres, broom fibres: one year

Temperature: 20 °C ± 2 °C

Results (two replicates): see Table F.1 and Figure F.1

Table F.1 — Percentage biodegradation (first example)

Test material	Percentage biodegradation %		
	One year		Two years
	Average	Standard deviation	1 replicate
Cellulose	108,3	2,8	108,5
Wheat gluten	89,2	2,6	—
Flax fibres	94,8	6,8	—
Broom fibres	91,8	5,6	—

Key
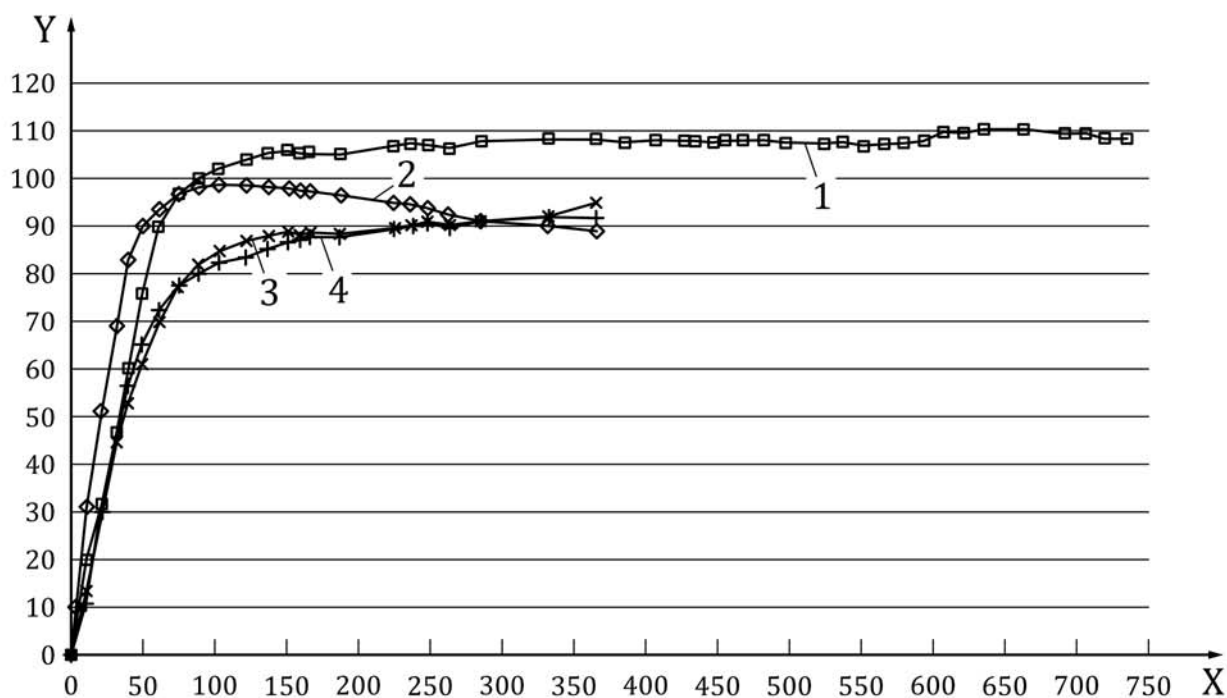
X   time (days)

Y   percentage biodegradation

1   cellulose

2   wheat gluten

3   flax fibres

4   broom fibres

Figure F.1 — Evolution of the biodegradation of cellulose, wheat gluten, flax fibres and broom fibres in soil

F.2 Evolution of the biodegradation of cellulose, birch leaves, oak leaves and pine needles in soil

NOTE   Data taken from Reference [9].

Inoculum: agricultural soil (500 g, < 2 mm)

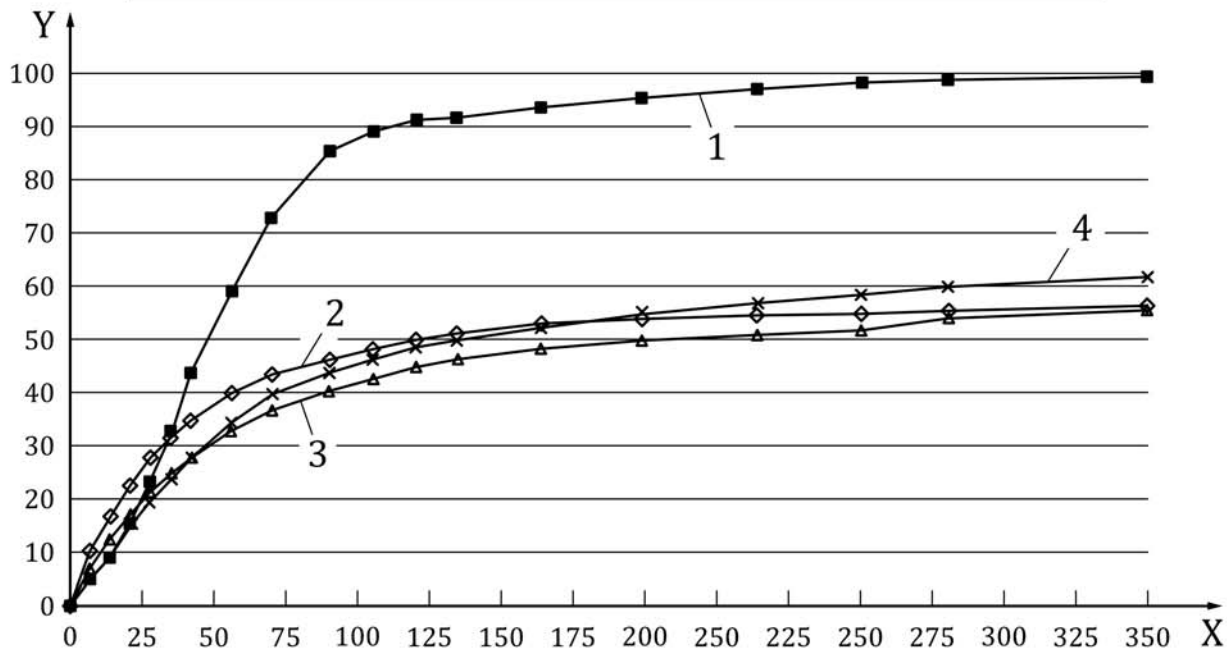Materials: cellulose (1 g), birch leaves (1 g), oak leaves (1 g), pine needles (1 g)

Duration: one year

Temperature: 20 °C ± 2 °C

Results: see Table F.2 and Figure F.2.

Table F.2 — Percentage biodegradation (second example)

Test material	Percentage biodegradation %	
	Absolute	**Relative to cellulose**
Cellulose	99,7	100,0
Birch leaves	56,2	56,4
Oak leaves	55,8	56,0
Pine needles	61,9	62,1

Key

X   time (days)

Y   percentage biodegradation

1   cellulose

2   birch leaves

3   oak leaves

4   pine needles

Figure F.2 — Evolution of the biodegradation of cellulose, birch leaves, oak leaves and pine needles in soil

F.3   Evolution of the biodegradation of cellulose and straw

Inoculum: mixture of one part agricultural soil and two parts forest soil (500 g, < 2 mm)

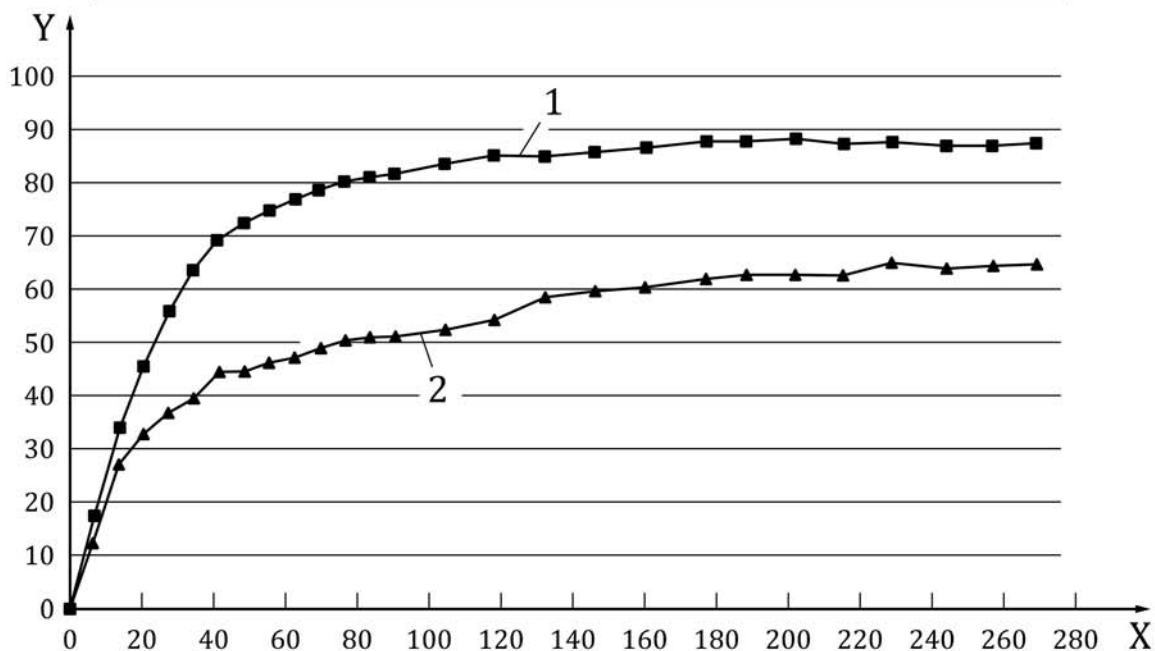Materials: cellulose (1 g), straw (1 g)

Duration: 270 days

Temperature: 20 °C ± 2 °C

Results (two replicates): (see Table F.3 and Figure F.3.

Table F.3 — Percentage biodegradation (third example)

Test material	Percentage biodegradation %		
	Absolute		Relative to cellulose
	Average	Standard deviation	
Cellulose	89,8	1,9	100,0
Straw	**66,4**	**0,0**	**74,0**

Key

X   time (days)

Y   percentage biodegradation

1   cellulose

2   straw

Figure F.3 — Evolution of the biodegradation of cellulose and straw

Annex G
(informative)

Interlaboratory test

An interlaboratory test was carried out in 2009 to validate the use of standard soil instead of a natural soil. The composition of the standard soil is given in 8.3.2. The sand and clay in this standard soil give the soil its texture, the natural soil provides microorganisms and the mature compost provides organic matter and additional microorganisms. Six different laboratories were involved in the round-robin test. Each laboratory independently collected a soil for use as the "natural soil", i.e. as the inoculum for the "standard soil". The test materials were microcrystalline cellulose reference material (RM) and a starch/poly(butylene adipate-co-butylene terephthalate) blend test material (TM). For the purposes of the round-robin test, participants were allowed to use a commercial soil, a natural soil or a mixture of different soils (e.g. a mixture of forest soil, one pasture soil and one garden soil). When using the materials in high concentration (10 g in 800 g of wet soil), the natural soil was fertilized using the mixture of salts listed in Table 2.

All the participants determined the biodegradation in soil measuring the amount of evolved carbon dioxide using either: an IR analyser, titration and, in one case, the gravimetric determination of CO_2 in accordance with ISO 14855-2. The temperature was between 20 °C and 28 °C. Two participants used a mix of two or three kinds of soil, two participants used a soil collected from a field and the other two used a commercial soil.

The final percentage biodegradation values are given in Table G.1.

Table G.1 — Results of interlaboratory test

Partici-pant	Inoculum	Apparatus	Time days	Biodeg-radation % TM	Standard deviation	Biodegra-dation % RM	Standard deviation	Relative biodegration of TM[a]
1	Natural soil	CO_2-free-air flow system	120	33,0[b]	2,16	70,3	0,31	47,0
1	Standard soil	CO_2-free-air flow system	120	11,8	1,17	59,0	0,5	20,1
2	Natural soil	ASTM D5988-03[d]	120	16,9[b]	2,9	63,8[b]	1,4	26,5
2	Standard soil	ASTM D5988-03[d]	120	23,8[b]	0,9	70,4[b]	3,4	33,8
2	Natural soil	CO_2-free-air flow system	120	21,9[b]	0,9	69,9[b]	1,6	31,3
2	Natural soil	ASTM D5988-03[d]	120	18,8[b]	0,2	61,1[b]	0,8	30,8
2	Natural soil	ASTM D5988-03[d]	120	19,9[b]	1,1	81,6[b]	2,8	24,4
3	Natural soil	CO_2-free-air flow system	182	61	1	72[c]	3,5	83,6

[a]	Relative biodegradation of TM = (% biodegradation of TM/% biodegradation of RM) × 100.
[b]	Had not yet reached plateau phase.
[c]	Still in a high CO_2 evolution phase.
[d]	See subclause 7.2.2.

Table G.1 *(continued)*

| Partici-pant	Inoculum	Apparatus	Time days	Biodeg-radation % TM	Standard deviation	Biodegra-dation % RM	Standard deviation	Relative biodegration of TM[a]
3	Standard soil	CO_2-free-air flow system	182	26	4	46	4,5	56,5
4	Natural soil	CO_2-free-air flow system	134	59,3	2,3	60,9	1,9	97,3
4	Standard soil	CO_2-free-air flow system	134	70,8	1,78	70,1	0,0	101,0
4	Natural soil	CO_2-free-air flow system	115	31,1	4,5	56,6	4,3	54,9
4	Standard soil	CO_2-free-air flow system	115	37,2	6,9	64,5	5,1	57,7
5	Natural soil	CO_2-free-air flow system	70	92,6	3,2	86,5	9,0	107,1
5	Standard soil	CO_2-free-air flow system	60	74,1[b]	0,5	77,0[b]	6,6	96,2
6	Natural soil	CO_2-free-air flow system	118	21,5[b]	4,9	104[b]	2,6	20,7
6	Standard soil	CO_2-free-air flow system	118	26[b]	4,2	107[b]	1,4	24,3

[a] Relative biodegradation of TM = (% biodegradation of TM/% biodegradation of RM) × 100.

[b] Had not yet reached plateau phase.

[c] Still in a high CO_2 evolution phase.

[d] See subclause 7.2.2.

Averages of the biodegradation values obtained in the different laboratories for natural soil and for standard soil are shown in Table G.2. Values from participant No. Six were not considered because the biodegradation of the reference material exceeded 100 % in both cases and a plateau phase was not reached.

**Table G.2 — Average percentage biodegradation in natural soil and in standard soil**

Material	Natural soil	Standard soil
Test material	39,39 ± 26,03	40,62 ± 25,96
Reference material	69,19 ± 9,91	64,50 ± 10,91

Figures G.1 and G.2 show the biodegradation curves for the various participants for the reference material in natural soil and in standard soil, respectively.

Key
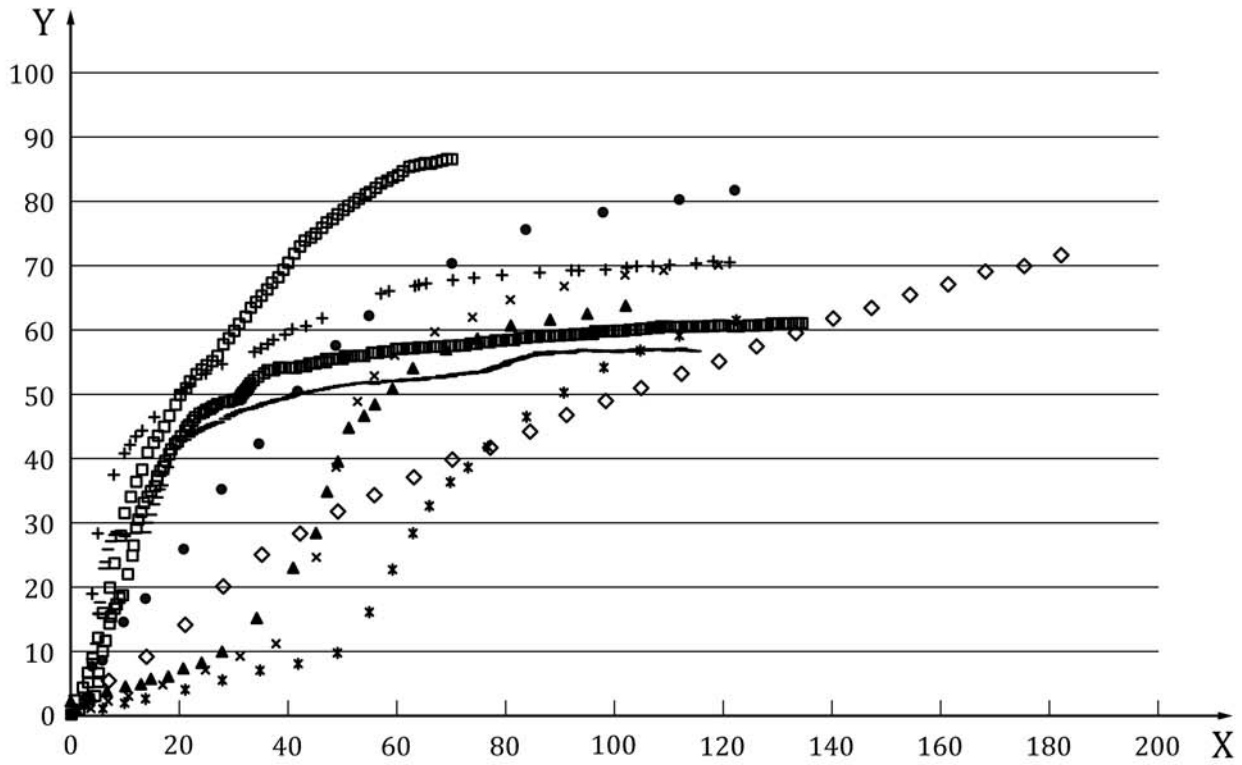
X   time (days)

Y   percentage biodegradation

Figure G.1 — Evolution of the biodegradation of microcrystalline cellulose in natural soil

Key
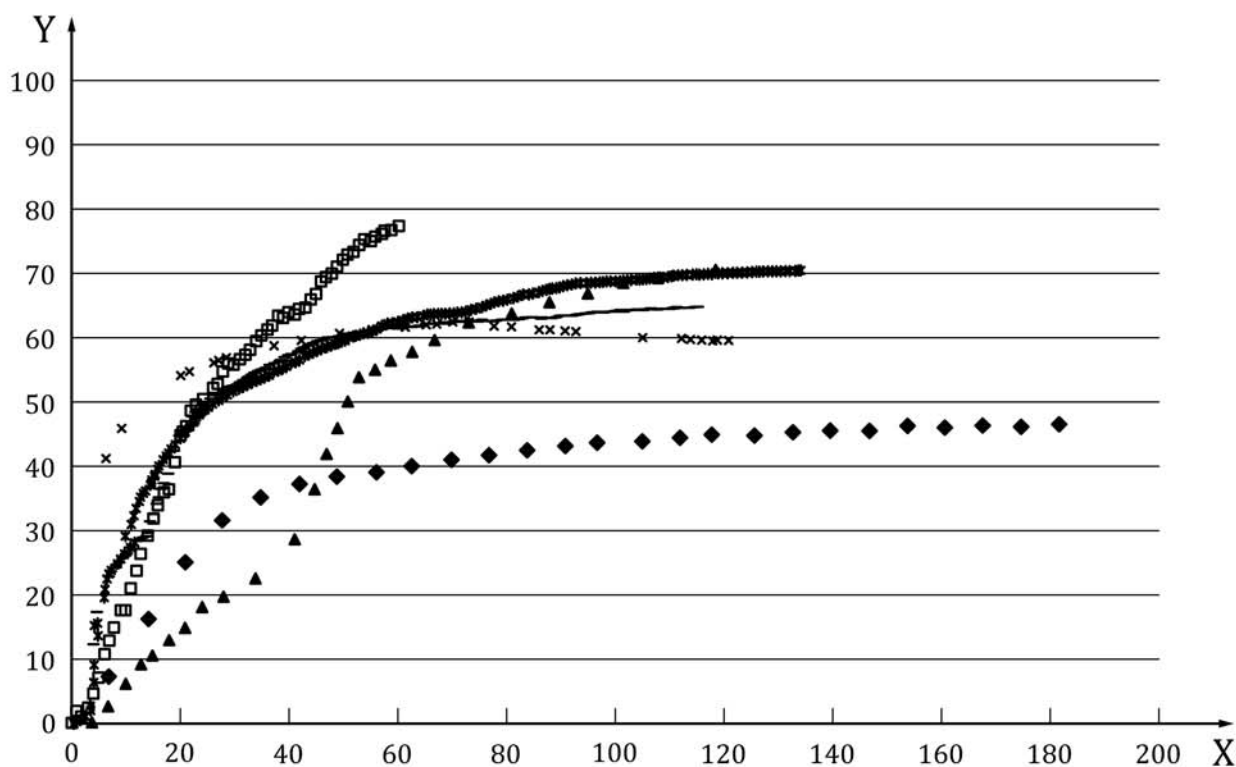
X   time (days)

Y   percentage biodegradation

Figure G.2 — Evolution of the biodegradation of microcrystalline cellulose in standard soil

The variability of the results at the interlaboratory level for natural soils is not high when testing cellulose. Cellulose seems to be a material whose biodegradation is not significantly affected by the test conditions. Biodegradation of the test material, on the other hand, appears to be more sensitive to the test conditions, resulting in greater variation in the test results. It is difficult to know whether this is the consequence of using different soils or whether other variables dominate, such as laboratories using different test conditions (i.e. different temperatures, aeration rates and material concentrations).

The preparation of a standard soil inoculated with natural soil does not seem to affect the interlaboratory variability, as can be seen from the standard deviation (see Table G.2).

The average values for the standard soil do not substantially differ from those for the natural soil. This reinforces the idea that the biological factor (i.e. the microbial population) of the natural soil is a relevant factor which is also important when the soil is used as an ingredient in the standard soil.

In order to make it possible to retrieve compounds at the end of the test, for the purpose of carrying out a final mass balance determination as described in Annex E, it seems necessary to start with a large amount of test material.

Generally speaking, standard soil can help in standardizing the test procedure, as it makes use of a standard matrix, with a standard texture and particle size. In particular, this seems to be helpful when using bulky soils.

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ICS 83.080.01

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