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Plastics — Methods for the preparation of samples for biodegradation testing of plastic materials

Plastiques — Méthodes de préparation des échantillons pour les essais de biodégradation des matériaux plastiques

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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 10210 was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 5, *Physical-chemical properties*.

Introduction

Plastics recovery technology includes material recycling, organic recycling and energy recovery. The use of biodegradable plastics is one of the valuable recovery options in the field of organic recycling.

ISO standards for determining the ultimate aerobic and anaerobic biodegradability of plastic materials in an aqueous medium, activated sludge, compost, digesting sludge and soil have been published. These standards include ISO 14851, ISO 14852, ISO 14853, ISO 14855-1, ISO 14855-2, ISO 15985 and ISO 17556. For the user of these standards, it might be difficult to compare biodegradation changes during a test, even when using the same samples, due to differences in the test conditions. These differences might arise from the compost preparation, the test preparation methodology, the shape and/or size of the test sample, etc. Accurate comparison of biodegradability data for the same plastic material can be difficult to achieve unless the conditions specified in the standards are accurately followed.

A unified approach to test sample preparation is important in achieving consistency within the standards mentioned above. The methods described in this document help to provide a consistent approach to sample preparation techniques for biodegradation testing of plastic materials.

Plastics — Methods for the preparation of samples for biodegradation testing of plastic materials

WARNING — The use of this International Standard might involve hazardous materials, operations and equipment. This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to use.

1 Scope

This International Standard describes methods for the preparation of test samples used in the determination of the ultimate aerobic and anaerobic biodegradability of plastic materials in an aqueous medium, soil, controlled compost or anaerobic digesting sludge. The methods described are designed to provide dimensional consistency of test samples, resulting in improved reproducibility of test results during the determination of the ultimate biodegradability of the product.

These methods apply to the following materials:

- natural and/or synthetic polymers, copolymers or mixtures of these;
- plastic materials that contain additives, such as plasticizers or colorants;
- plastic composite materials that contain organic or inorganic fillers;
- products made from the above materials.

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 472, *Plastics — Vocabulary*

ISO 3310-1, *Test sieves — Technical requirements and testing — Part 1: Test sieves of metal wire cloth*

ISO 14851, *Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by measuring the oxygen demand in a closed respirometer*

ISO 14852, *Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by analysis of evolved carbon dioxide*

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

ISO 14855-1, *Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions — Method by analysis of evolved carbon dioxide — Part 1: General method*

ISO 14855-2, *Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions — Method by analysis of evolved carbon dioxide — Part 2: Gravimetric measurement of carbon dioxide evolved in a laboratory-scale test*

ISO 15985, *Plastics — Determination of the ultimate anaerobic biodegradation and disintegration under high-solids anaerobic-digestion conditions — Method by analysis of released biogas*

ISO 17088, *Specifications for compostable plastics*

ISO 17556, *Determination of the ultimate aerobic biodegradability of plastics materials in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 472 and the following apply.

3.1
sieve
wire mesh of specified aperture size

3.2
bulk material
test material taken from a polymer product or part of a product

NOTE The size of the bulk polymeric test sample is approximately 1 cm × 1 cm × 1 cm.

3.3
sheet
planar product of arbitrarily limited maximum thickness in which the thickness is small compared to the length and width

NOTE The thickness of sheets is typically 0,5 mm to 3 mm.

3.4
film
thin planar product of arbitrarily limited maximum thickness in which the thickness is very small compared to the length and width and which is generally supplied in roll form

NOTE 1 The arbitrary thickness limit can differ between countries and often between materials.

NOTE 2 The thickness of films is typically 0,01 mm to 0.3 mm.

3.5
pellet
small mass of preformed moulding material, having relatively uniform dimensions in any given batch and used as feedstock in moulding and extrusion operations

NOTE The average diameter of pellets can range from 1 mm to 5 mm.

3.6
granule
relatively small particle produced in various sizes and shapes in operations such as cutting, grinding, crushing, precipitation and polymerization

NOTE 1 These operations can also yield material in the form of powder and, in some precipitation and polymerization processes, material in the form of beads can be produced

NOTE 2 The average diameter of granules can range from 0,1 mm to 3 mm.

3.7
powder
very fine particulate material smaller in size than granules

NOTE The average diameter of polymeric powder particles can range from 0,01 mm to 0,1 mm.

3.8
test material
product from which a test sample is taken and used to assess the biodegradability of a polymeric item by means of standardized biodegradation tests

4 Principle

This International Standard describes methods for the preparation of test samples from polymer materials for biodegradation testing in the following environments:

- an aqueous medium as used in ISO 14851 and ISO 14852;
- mature compost as used in ISO 14855-1 and ISO 14855-2;
- digestion sludge as used in ISO 14853 and ISO 15985;
- simulated soil-burial conditions on a laboratory scale as used in ISO 17556.

The methods described provide a level of control over the test sample preparation from polymer pellets through to end-products, minimizing the effect of sample shape on the biodegradation test results.

Biodegradation data from test materials need to be as accurate and reproducible as possible. An important factor in establishing consistent test data is to use test samples with a regular surface area, produced by utilizing a defined preparation method. This will promote a high degree of homogeneity when the test sample is mixed with an aqueous medium, controlled compost or digestion sludge. The preparation methods described below use low-temperature mechanical milling or cutting without changing certain physical properties of the test materials. Any change in properties such as crystallinity, thermal history or thermal decomposition should be avoided or at least minimized during the sample preparation process. It is generally accepted that the crystallinity of a polymeric test material will not change during low-temperature mechanical milling/cutting at temperatures below the glass transition temperature of the polymer.

The surface area per unit mass of powder samples may be defined by regulation of the particle size, which shall be a parameter specified in the biodegradation test procedure. Very fine particles, such as nanospheres, might have properties different from those of microspheres, which have a significantly greater particle size. This might affect the biodegradation rates of the samples and make comparisons of test data unreliable. The relevant methods described minimize the variable effects of sample surface area in biodegradation testing by controlling the particle size.

5 Reagents

5.1 Solid carbon dioxide

Solid carbon dioxide used for cooling and maintaining the sample materials at low temperatures during mechanical size reduction is not required to be analytical grade.

Crushed solid carbon dioxide with a particle size of 1 mm to 10 mm is recommended.

NOTE Solid carbon dioxide is commonly known as dry ice.

5.2 Liquid nitrogen

Liquid nitrogen used for cooling and maintaining the sample materials at low temperature during mechanical size reduction is not required to be analytical grade.

6 Apparatus

All apparatus shall be thoroughly cleaned and free from organic or toxic matter.

6.1 Sieves

The particle size of sample powders is controlled by using sieves of different sizes to remove the oversize and undersize fractions. For the purposes of this International Standard, sieve sizes of 60 mesh and 120 mesh as specified in ISO 3310-1 shall be used.

6.2 Rotor mill

This type of mill has rotating, blunt blades and a ring sieve to mechanically reduce polymer pellets, polymer products or other samples to powders.

The recommended minimum aperture size of the ring sieve is > 0,5 mm to avoid blockage of the cutter. Accurate reproducibility of the sample powders strongly depends upon the sieve size.

6.3 Rotating mechanical mixer

This type of mixer has mechanically rotating blades to reduce polymer pellets, polymer products or other samples to powders.

A rotating mechanical mixer with titanium blades is recommended, since the blades do not fragment and contaminate the sample powders. A rotating mechanical mixer with stainless-steel blades may also be used.

6.4 Ball mill

This type of mill has a rotating chamber which contains a number of metal or ceramic balls used to grind a product to a reduced size.

When using liquid nitrogen added internally as a refrigerant, a non-vented test chamber should be cooled externally to eliminate build-up of pressure within the apparatus.

6.5 Sieve shaker

An automatic sieve shaker is recommended for the separation of the milled test sample powders. An automatic sieve vibrator can accommodate more than two sieves and will produce more consistent results than will the manual use of sieves.

6.6 Microscope

This can be used to measure the particle size distribution of the milled test samples (see 7.2.3). An optical or scanning-electron microscope may be used, but for ease of use an optical microscope with a digital camera attachment is recommended.

7 Procedure

7.1 Preparation and dimensional control of test material prior to size reduction

The test material shall be homogeneous and free from any contaminants. The test material shall be cooled for 5 min with solid carbon dioxide or liquid nitrogen prior to mechanical reduction. If any component of the original test material is too large for the mechanical process, reduce it in size to approximately 1 cm × 1 cm × 1 cm.

7.2 Powder/pellet/granule test material

7.2.1 Powder test material produced by mechanical milling

Pre-prepared test material is mechanically milled using a rotor mill, rotating mechanical mixer or other type of cryostatic mill. A suitable amount of the test material is added to the milling equipment, with the refrigerant added internally in a vented system or used externally in a non-vented system.

Suitable care should be taken when handling refrigerants. Ensure that the required safety equipment is used at all times and that the workplace is adequately ventilated.

It is important that the test material be maintained at a temperature below its glass transition temperature in order to facilitate the production of powders, but also to minimize thermal-ageing effects.

It is important to monitor the pressure inside the equipment in order to detect any increase in pressure caused by refrigerant gas.

7.2.2 Sieving of powder test material after mechanical milling

The mechanically milled test material is dried and then a specific particle size fraction is separated out using two sieves of different mesh size. Sieves of 60 mesh (250 µm aperture) and 120 mesh (125 µm aperture) shall be used for the separation procedure. The powdered test material passing through the 60 mesh sieve is collected and then sieved with the 120 mesh sieve. The fraction remaining on the 120 mesh sieve is retained as the test sample. The upper and lower fractions from this procedure are discarded.

NOTE Typical polymer powder samples produced in a rotating mechanical mill with titanium blades and using solid carbon dioxide as the refrigerant are shown as micrographs in Annex A.

7.2.3 Measurement of particle size distribution of milled test material

7.2.3.1 General

The particle size distribution of the milled test material obtained by the method described in 7.2.2 shall be determined from a minimum of 100 particles and the average particle size and particle size distribution recorded.

7.2.3.2 Particle size distribution measurement by microscopy

The particle size distribution of the milled test material can be measured using microscopy. The size of each particle is measured either by a digital microphotographic technique, possibly using suitable image analysis software, or by visual observation and measurement.

Microphotography is recommended as the measurement method, since the micrograph produced can be reviewed at any time.

7.3 Film and sheet test material

Film and sheet test samples are prepared by cutting them from the original film or sheet. The size of the pieces in the sample shall be 1 cm × 1 cm. The average thickness of the pieces in the sample shall be measured and recorded.

NOTE 1 Typical film samples with pieces measuring 1 cm × 1 cm are shown in Annex B.

If the pieces of film or sheet are too large for the test vessels, they can be reduced in size as described in 7.2.1. The required particle size fraction shall then be separated out from the resulting milled powder using the method described in 7.2.2. The particle size distribution of the separated powder sample shall be determined as described in 7.2.3.

NOTE 2 Typical biodegradation results for milled-film powders > 125 µm are shown in Annex B.

7.4 Test material in the form of products

Prepare test samples from products by cutting small blocks from the original product. The dimensions of these blocks shall be no greater than 1 cm × 1 cm × 1 cm, but at least two of the dimensions shall be > 0,5 cm.

Where powders (particle size 125 µm to 250 µm) are obtained, by mechanical milling (see 7.2), from blocks of material cut from products, their particle size distribution shall be measured and recorded as described in 7.2.3.

NOTE A typical test sample cut from a commercial plastic product and milled powder produced from this test sample are shown in Annex C.

7.5 Storage

If the test samples are not used in the biodegradation test directly after preparation, they shall be carefully stored. It is important to store the test samples below room temperature, in the absence of light and away from the influence of chemicals in the atmosphere.

Storage in a vacuum desiccator is recommended in order to keep the humidity low.

8 Calculation and expression of results

8.1 Particle size distribution

Where the particle size distribution of the test sample is measured, the mean particle size, the standard deviation from the mean and the particle size distribution curve shall be calculated and reported.

9 Validity of preparation

All test samples shall be examined visually. They are considered to be suitable for biodegradation testing provided the following criteria are met:

- no difference in colour between the prepared test sample and the original material;
- no changes within the sample due to thermal effects;
- no observed impurities or contamination of the sample.

If any of the above criteria are not met, a fresh test sample shall be prepared in accordance with the procedures described.

10 Sample preparation report

The sample preparation report shall provide all relevant information and contain as a minimum the following:

a) a reference to this International Standard;

b) all information necessary to identify and describe the test material, such as its organic-carbon content, the source, its age, its date of production, and details of storage, handling and stabilization plus, if available, the name, composition and characterization (e.g. molecular mass, crystallinity, melting point and density) of the material.

c) the shape and size of the pieces of test material, such as the average diameter (for pellets), the average thickness (for sheet or film), or the maximum and minimum particle size (for powders);

d) the milling apparatus used, the type and amount of refrigerant used and any other information about the milling apparatus, such as the capacity of the apparatus, the type of blade or the type of ring sieve used;

e) the type of sieve used, the number of shakes given to the sieves and the frequency of shaking;

f) any sensory observations concerning the test material, such as structure, colour or smell;

g) the conditions under which the test samples were stored, such as the temperature, humidity and length of storage;

h) for powders, the particle size distribution of the test sample and the method used to determine it.

Annex A
(informative)

Examples of the preparation and testing of powder test samples produced from pellets by milling using a rotating mechanical mixer

Pellets of two different kinds of polymer material, poly(lactic acid) (PLA) and polycaprolactone (PCL), were separately milled using a rotating mechanical mixer with titanium blades. In each case, the pellets were cooled using solid carbon dioxide. Milling was carried out for 15 cycles of 3 min each, each cycle being followed by a 5 min interval to prevent overheating of the motor in the mixer. The milled powders were then dried and separated into fractions using a sieve shaker with a 30 mesh sieve (mesh aperture of 500 µm), 60 mesh sieve (mesh aperture of 250 µm) and 120 mesh sieve (mesh aperture of 125 µm). The sizes of at least 100 particles were then measured by microphotography, .

The recovery and average particle diameter of the PLA powder and PCL powder are shown in Tables A.1 and A.2, respectively, the average particle diameter being calculated from measurements of the longest dimension of each particle. Small powder particles less than 10 µm in size present in the micrographs were ignored. The particle size distributions of the PLA powder and PCL powder are shown in Figures A.1 and A.2, respectively. Micrographs of the PLA powder and PCL powder are shown in Figures A.3 and A.4, respectively.

Table A.1 — Production of PLA powder from PLA pellets milled using a mixer for 45 min and separated into fractions using sieves (sieving time 15 min)

Particle size range (µm) [a]	**Recovery** (% by mass)	**Average particle diameter** (µm)	**Standard deviation** (µm)
0 to 125	25	60,8	39,7
125 to 250	25	214,2	64,7
250 to 500	31	303,9	97,2
> 500	19	—	—

[a] Fractionated using size of sieve indicated.

Table A.2 — Production of PCL powder from PCL pellets milled using a mixer for 45 min and separated into fractions using sieves (sieving time 15 min)

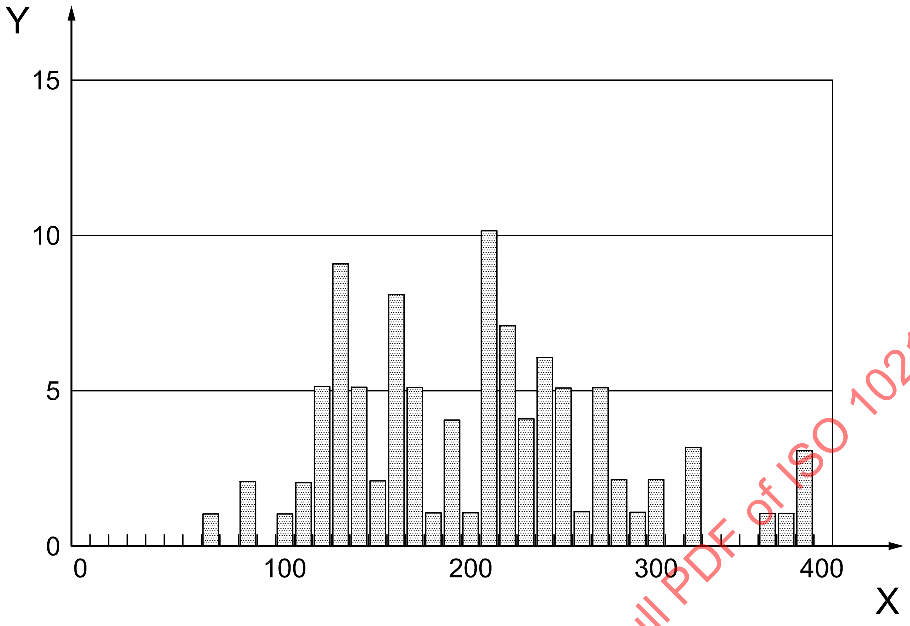
Particle size range (µm) [a]	**Recovery** (% by mass)	**Average particle diameter** (µm)	**Standard deviation** (µm)
0 to 125	35	75,7	41,4
125 to 250	30	180,7	76,8
250 to 500	20	297,6	86,5
> 500	15	—	—

[a] Fractionated using size of sieve indicated.

Biodegradation testing of the PLA powder and PCL powder in a controlled compost at 58 °C was carried out using the method specified in ISO 14855-2. A cellulose powder of thin-layer chromatography grade with a particle size of less than 20 µm was used as a positive control..

The results of the biodegradation testing of the PLA powder are shown in Figure A.5 and those for the PCL powder are shown in Figure A.6. The degree of biodegradation of the cellulose powder reached 70 % after 30 days and that of the PLA powder reached 90 % after 50 days, as shown in Figure A.5. As shown in Figure A.6, the degree of biodegradation of the PCL reached 70 % after 10 days.

In addition, biodegradation testing was carried out on PCL powder in an aqueous medium at 25 °C in accordance with ISO 14851. The test results are shown in Figure A.7. As shown in Figure A.7, the degree of biodegradation of the PCL reached 70 % after between 35 days and 60 days, depending on the test sample.

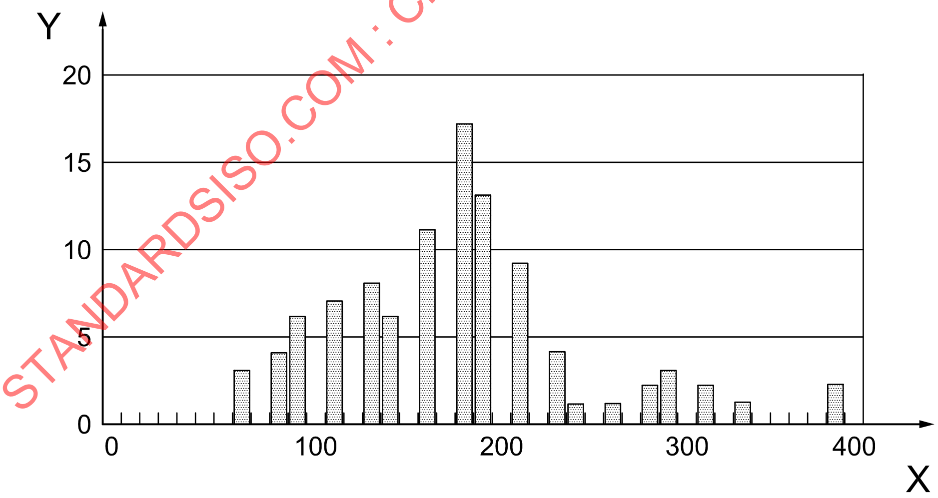


Key

X particle size (μm)

Y frequency (%)

Figure A.1 — Particle size distribution of PLA powder milled using a mixer and then sieved

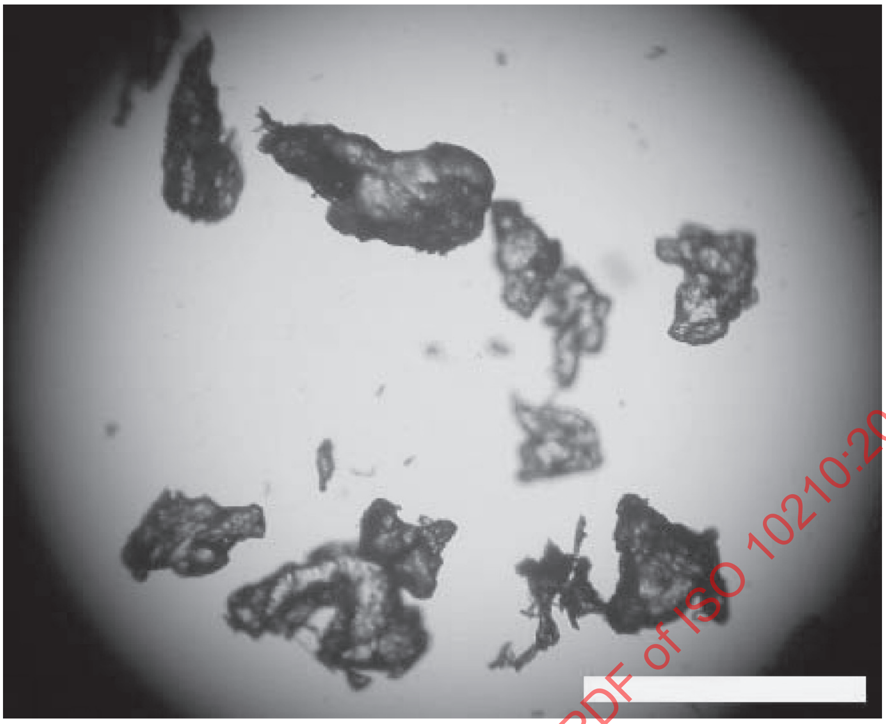


Key

X particle size (μm)

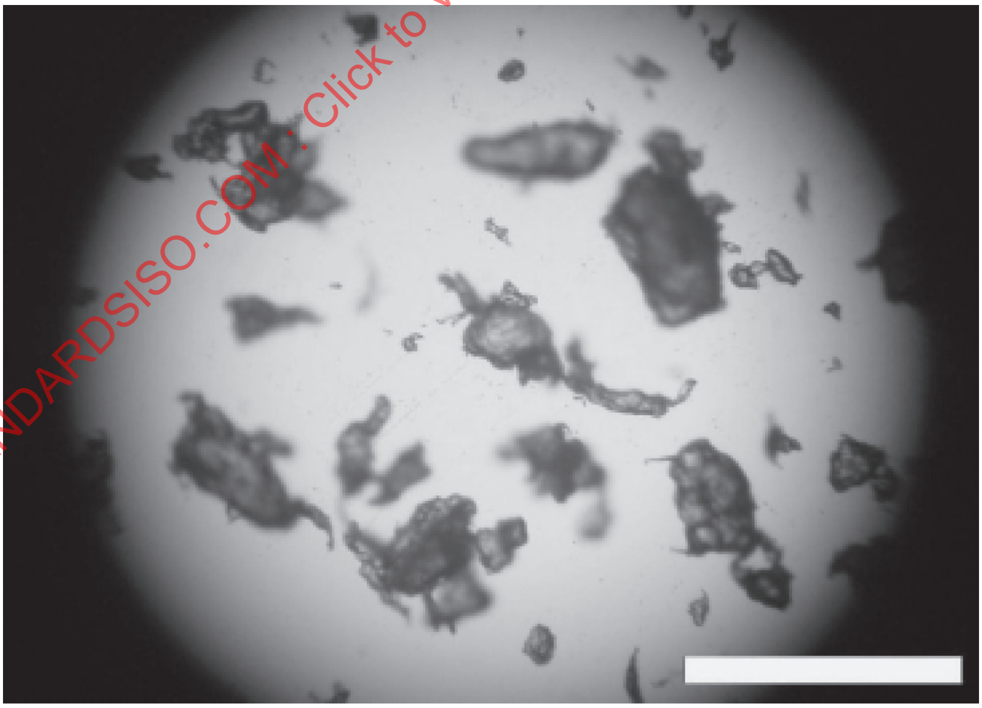
Y frequency (%)

Figure A.2 — Particle size distribution of PCL powder milled using a mixer and then sieved



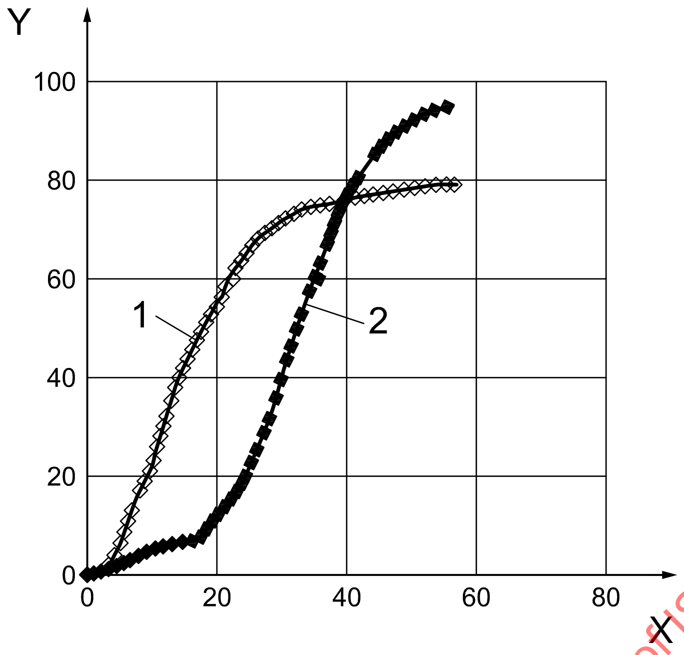
NOTE The scale bar indicates a distance of 500 µm.

Figure A.3 — Micrograph of PLA powder



NOTE The scale bar indicates a distance of 500 µm.

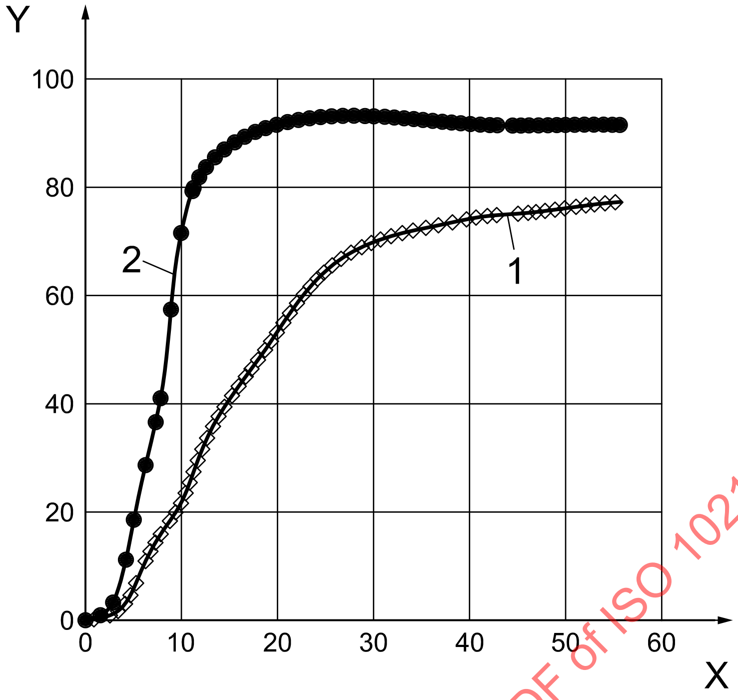
Figure A.4 — Micrograph of PCL powder



Key

X incubation time (days)
Y degree of biodegradation (%)
1 cellulose powder
2 PLA powder

Figure A.5 — Results of biodegradation testing of PLA powder (mean particle size 214,2 µm ± 64,7 µm) in controlled compost at 58 °C in accordance with ISO 14855-2
[cellulose powder (particle size < 20 µm) used as positive control]



Key

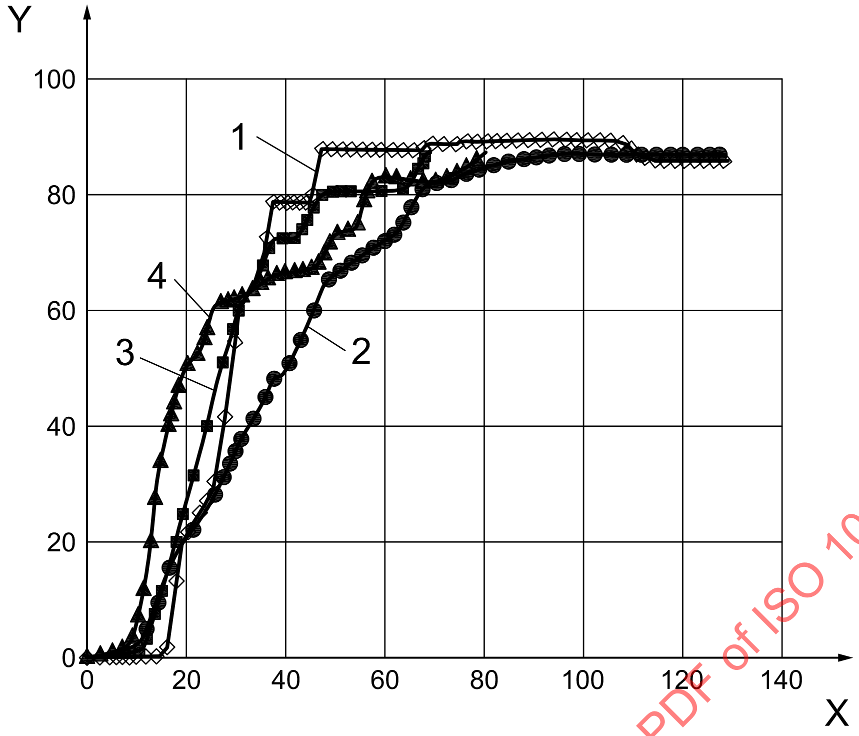
X incubation time (days)

Y degree of biodegradation (%)

1 cellulose powder

2 PCL powder

Figure A.6 — Results of biodegradation testing of PCL powder (mean particle size 180,7 µm ± 76,8 µm) in controlled compost at 58 °C in accordance with ISO 14855-2 [cellulose powder (particle size < 20 µm) used as positive control]



Key

X incubation time (days)

Y degree of biodegradation (%)

1 cellulose powder

2 PCL powder, test sample 1

3 PCL powder, test sample 2

4 PCL powder, test sample 3

Figure A.7 — Results of biodegradation testing of PCL powder (mean particle size 180,7 µm ± 76,8 µm) in triplicate using a closed respirometer at 25 °C, in accordance with ISO 14851
[cellulose powder (particle size < 20 µm) used as positive control]

Annex B
(informative)

Examples of the preparation and testing of film and powder test samples produced from PLA film and pellets

Two film test samples were cut from a composting bag (see Figure B.1) made of poly(lactic acid) (PLA) for biodegradation testing using ISO 14855-2. The dimensions of the pieces in one test sample were: length 1 cm, width 1 cm and thickness 25 µm. The dimensions of the pieces in the other sample were: length 5 cm, width 5 cm and thickness 25 µm. In addition, a powder test sample of particle size ≥ 125 µm was prepared from the film by milling and sieving with a 125 µm sieve. Another powder test sample was prepared from PLA pellets by milling and sieving to give particles in the range 125 µm to 250 µm. The biodegradation testing of the test samples was carried out in a controlled compost at 58 °C. Cellulose powder of thin-layer chromatography grade with a particle size of less than 20 µm was used as a positive control.

The results of the biodegradation testing are shown in Figure B.2.

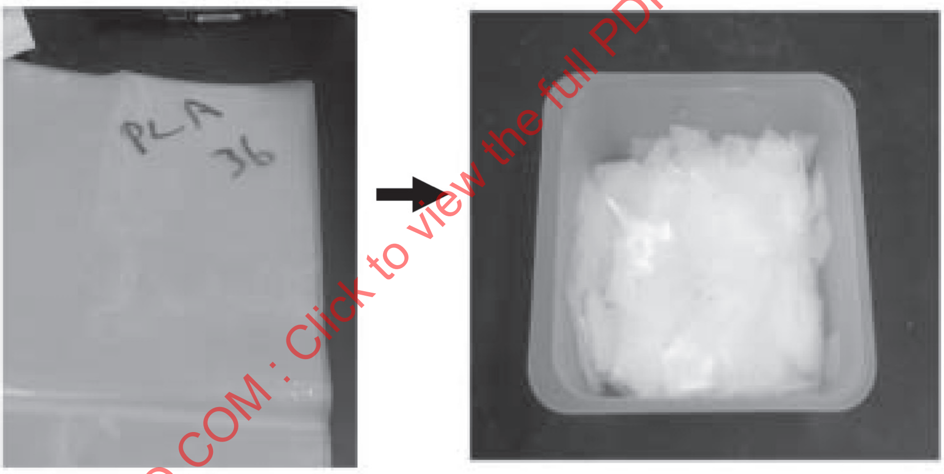
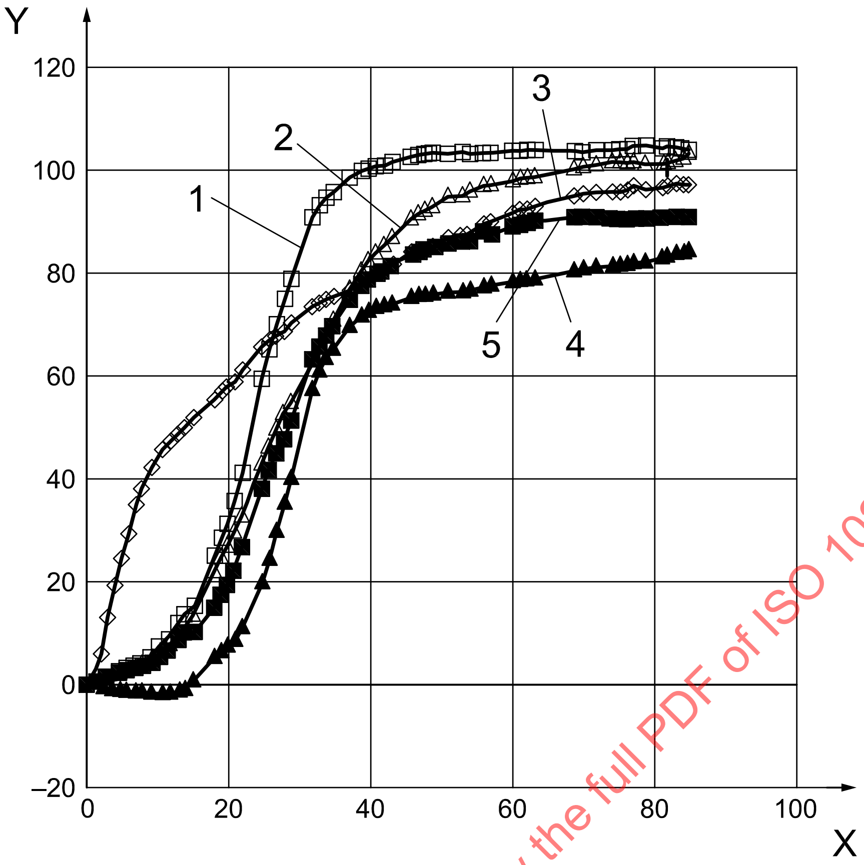


Figure B.1 — PLA composting bag (left) and film test sample cut from the bag (right)



Key

X incubation time (days)

Y degree of biodegradation (%)

1 PLA film (1 cm × 1 cm × 25 μm)

2 PLA powder from pellets (particle size 125 μm to 250 μm)

3 cellulose powder

4 PLA powder from 25 μm film (particles retained by a 125 μm sieve)

5 PLA film (5 cm × 5 cm × 25 μm)

Figure B.2 — Results of biodegradation testing of pieces of PLA film (1 cm × 1 cm × 25 μm), larger pieces of PLA film (5 cm × 5 cm × 25 μm), milled PLA film (fraction retained by a 125 μm sieve, mean particle size 183,2 μm ± 21,1 μm) and PLA powder from pellets (particle size 125 μm to 250 μm, mean particle size 214,2 μm ± 64,7 μm) in controlled compost at 58 °C in accordance with ISO 14855-2 [cellulose powder (particle size < 20 μm) used as positive control]