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Plastics — Determination of specific aerobic biodegradation rate of solid plastic materials and disappearance time (DT50) under mesophilic laboratory test conditions

Plastiques — Détermination du taux de biodégradation aérobie spécifique des matières plastiques solides et du temps de disparition (DT50) dans des conditions d'essai de laboratoire mésophile

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Foreword

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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 of 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 www.iso.org/iso/foreword.html.

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

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

Several test methods have been developed by ISO to measure the biodegradation degree of plastics. Under aerobic conditions, the biodegradation reaction of a material is described by the following reaction:

$$C_M + O_2 \rightarrow C_{CO_2} + H_2O + C_B$$

where

C_M is the organic carbon present in the test material (e.g. a polymer or a plastic material);

C_{CO_2} is the carbon evolved as carbon dioxide;

C_B is the carbon assimilated by microorganism and incorporated in the microbial biochemistry.

The test methods follow the biodegradation reaction by measuring either the oxygen uptake (disappearance of the reactant) or the CO_2 evolution (formation of the product). The test methods return a biodegradation percentage (which, strictly speaking, is a "mineralization" percentage). This value is the reaction yield percentage, i.e. the mass of carbon oxidised to CO_2 during the reaction (actual yield) in comparison with the maximum possible yield (theoretical yield). This is expressed as evolved CO_2/theoretical CO_2, the latter value being the amount of CO_2 obtained in case of total oxidation of the original carbon present in the test substance.

A reliable test method for the determination of the C_B, i.e. the amount of C_M that has been assimilated in the biomass is not available at the date of publication.

The test methods are suitable for measuring the final degree of biodegradation but are not suitable for measuring the biodegradation rate, because they do not take into account the surface area of the tested sample. On the other hand, this document provides a guidance on how to measure the biodegradation rate using existing test methods.

Biodegradation of solid, non-water soluble polymers and plastics is a heterogeneous reaction because the polymer is in the solid state while microbes and enzymes are in the liquid phase. Even when the tested material is exposed to solid matrices (e.g. compost, soil, marine sediment) the microbes are in the liquid phase present within the solid matrix (e.g. micropores, macropores). Thus, the reaction of biodegradation happens in the liquid/solid interphase and the available surface area can become a limiting factor. It is a common knowledge that milling increases the biodegradation rate of a plastic sample. The biodegradation speed, i.e. the CO_2 evolution and the O_2 uptake rates, is controlled by the surface area of the tested sample. Therefore, biodegradation rate must be expressed as a function of the available surface area, otherwise the information is pointless and paradoxical results can be obtained.

There is an increasing interest in determining the biodegradation rate and related parameters (such as the disappearance time 50, DT50, i.e. the time within which the initial concentration of the test substance is reduced by 50 %) in order to assess the risk in the case of accidental or deliberate leakage of biodegradable plastics into the environment. Degradation of organic substances in the environment influences exposure and, hence, it is a key parameter for estimating the risk of long-term adverse effects on biota.

This document enables to determine the specific aerobic biodegradation rate i.e. the amount of carbon mineralized per unit time per unit surface area, under the conditions defined by the applied test method.

The approach showed in this document is aimed to measure mineralization rate. It differs from ISO 23832 that describes a test method for the determination of the physical degradation rate and disintegration degree of plastic materials. On the other hand, ISO 22403 identifies the plastic materials that show intrinsic biodegradability when exposed to marine inocula under mesophilic aerobic laboratory conditions.

Plastics — Determination of specific aerobic biodegradation rate of solid plastic materials and disappearance time (DT50) under mesophilic laboratory test conditions

1 Scope

This document specifies a method to determine the specific aerobic biodegradation rate of solid, non-water soluble plastic materials under mesophilic conditions.

NOTE The specific aerobic biodegradation rate (which, strictly speaking, is a specific mineralization rate, implying the assessment of the conversion of organic carbon into CO_2 but neglecting biomass formation) is expressed as amount of carbon mineralized into CO_2, per unit time, per unit area.

The method described in this document does not provide information on the ultimate aerobic biodegradability of the tested samples. Biodegradability criteria for plastic materials under mesophilic conditions are provided for example by ISO 23517 and ISO 22403. The method described in this document shall be used to determine the DT50 only when the plastic material is proven to be intrinsically biodegradable using suitable standard specifications such as ISO 23517 and ISO 22403. Furthermore, the biodegradation rate determined on plastic materials whose ultimate biodegradation has not been proven, shall not be considered as a specific characteristic of the whole material.

This document only considers the evolution of CO_2 as direct measurement of mineralization of the tested sample.

The method described in this document may be applied also to solid materials used as a reference.

This document is not applicable for “marine, soil and freshwater biodegradable” claims of biodegradable plastic materials. For such purposes, see relevant product standards if available.

NOTE Although results can indicate that the tested plastic materials and polymers will biodegrade under the specified test conditions at a certain specific aerobic biodegradation rate or DT50, the results of any laboratory exposure are not directly applicable to environmental compartments including soil, marine environments and limnic areas at the actual site of use or leakage.

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 4591, *Plastics — Film and sheeting — Determination of average thickness of a sample, and average thickness and yield of a roll, by gravimetric techniques (gravimetric thickness)*

ISO 4593, *Plastics — Film and sheeting — Determination of thickness by mechanical scanning*

3 Terms and definitions

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

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

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

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

3.1
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 to entry: The arbitrary thickness limit can differ between countries and often between materials.

Note 2 to entry: The thickness of films is typically 0,01 mm to 0,3 mm.

[SOURCE: ISO 10210: 2012, 3.4]

3.2
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 1 to entry: The average diameter of pellets can range from 1 mm to 5 mm.

[SOURCE: ISO 10210:2012, 3.5]

3.3
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

[SOURCE: ISO 10210:2012, 3.8]

3.4
specific surface area
SSA
ratio of the surface area of a sample of a solid material to its mass

Note 1 to entry: It is expressed in square centimetres per gram ($cm^2 \times g^{-1}$).

3.5
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

[SOURCE: ISO 17556:2019, 3.6]

3.6
specific aerobic biodegradation rate
r_{SAB}
ratio of the mineralization rate coefficient, k, to the *specific surface area, SSA* (3.4)

Note 1 to entry: It is generally expressed in mmol C × $day^{-1} \times cm^{-2}$.

Note 2 to entry: This is an assessment of the conversion rate of organic carbon into CO2 which neglects biomass formation

3.7
disappearance time 50
DT50
time required for the concentration of the organic carbon of a sample of a solid material to decrease by 50 % of its original value

Note 1 to entry: It is generally expressed in days.

Note 2 to entry: DT50 is also called "dissipation time".

3.8
mineralisation rate coefficient

k

slope of the regression line representing the mineralization of a *test material* (3.3) versus time

Note 1 to entry: It is expressed as millimoles of C that are mineralized into CO_2 per day (mmol C × day^{-1}).

4 Principle

The present document describes a method to determine the specific aerobic biodegradation rate and disappearance time DT50 of solid, non-water soluble plastic materials.

The method described in this document can be considered as an additional extension ("add-on") of the existing ISO standard test methods for measuring biodegradation of plastic materials in order to determine the specific aerobic biodegradation rate.

This document only considers the evolution of CO_2 as direct measurement of mineralization of the tested sample. The specific aerobic biodegradation rate is intended as specific mineralization rate, and it implies the assessment of the conversion of organic carbon of material into CO_2 but neglecting biomass formation. This rate is expressed as amount of carbon mineralized into CO_2, per unit time, per unit area. It is measured as millimoles C× day^{-1} × cm^{-2}.

Thus, the DT50 as determined following this document is an underestimation of the true dissipation of the tested materials, because conversion into biomass is not accounted for.

The method described in this document can be considered as an additional extension ("add-on"), i.e. a method for the elaboration and expression of results, of the existing ISO and ASTM standard test methods for measuring biodegradation of plastic materials under different test conditions:

- ISO 14852;
- ISO 17556;
- ASTM D5988;
- ISO 22404;
- ISO 19679;
- ISO 23977-1;
- ASTM D6691;
- ASTM D7991;

The present method makes it possible to determine the specific aerobic biodegradation rate and the disappearance time 50 (DT50) of a test material when tested using existing ISO test methods for biodegradation under mesophilic conditions in relation to surface area of test material.

5 Apparatus

5.1 Sieves

Sample powders with a specific particle size are obtained by using sieves of different sizes, to remove the oversize and undersize fractions. The recommended sieve sizes are of 60 and 120 mesh (250 µm and 125 µm) as specified in ISO 10210.

5.2 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 the manual use of sieves.

5.3 Laser particle size analyser (i.e. laser diffraction instrument)

Laser diffraction particle size analysers can be used to measure the sizes of particles of powder/granule of test material, both in wet or dry dispersion. The laser diffraction method assumes a spherical particle shape in its optical model. Particle size is calculated by measuring the angle of light scattered by the particles as they pass through a laser beam. The applicable size range is approximately between 0,1 µm to 3 mm, following ISO 13320.

5.4 Micropore and chemisorption analyser

The total surface can also be measured with instruments based on the BET method for the determination of the specific surface area of solids by gas adsorption, according to ISO 9277.

6 Determination of the specific surface area (SSA) of test sample

6.1 General

The present method shall be applied to pellets of the plastics material under study, either milled (see 6.2) or converted in films (see 6.3) depending on the test method used for measuring biodegradation.

The specific surface area of a plastic sample (SSA) is the surface area per unit mass, it is an average and it is expressed as $cm^2 \times g^{-1}$ of sample.

The density of test material shall be known in order to calculate the SSA of a sample in powder form.

If a film/sheet is used as a sample, its thickness shall be known for the determination of its SSA.

6.2 Powder test sample

6.2.1 Sample preparation

Refer to ISO 10210:2012, 7.2, for milling of plastic pellets and preparation of a specific size fraction from the powder.

The powder obtained through milling is sieved using two sieves of different mesh size. The powder passing through the sieve with the higher mesh size is collected and then sieved with the sieve of lower mesh size. The fraction remaining on the sieve with the lower mesh size is retained and used as the test sample. The upper and lower fractions from this procedure are discarded. The sieves to be used shall be within the range 125 µm and 700 µm. The maximum difference between the sizes of the used sieves shall be 200 µm.

6.2.2 Specific surface area determination

6.2.2.1 General

The specific surface area (SSA) of powder obtained in 6.2.1, is estimated either with a theoretical approach (see 6.2.2.2) or by using an instrumental determination (see 6.2.2.3).

The application of different methodologies can give rise to different SSA values depending on the ability to measure the surface originating from porosity at the nanometric level. For this reason, it is necessary that the results are always accompanied by the declaration of the SSA measurement methodology used, as indicated in Clause 9. There is currently insufficient evidence to recommend a specific methodology for SSA measurement. This may be done in the future when more data obtained by applying the method described in this document are available.

6.2.2.2 Theoretical method for specific surface area determination

This approach assumes that particles of the powder sample are perfect spheres. The mean surface area of the spheres is calculated by using the mean diameter.

NOTE The sphere is the only shape that can be described by one unique number and it is generally used to describe particles. One way to get a single unique number to describe an irregular shaped particle is to compare some feature of the actual particle to an imaginary spherical particle. Typical methods of doing this are those, which determine the “equivalent surface area“, or the “equivalent volume” calculating the diameter of a theoretical sphere that has the same surface area or volume of the original particle.

Determine the mean diameter of particles of the fraction sieved with two sieves S_1 and S_2, by using Formula (1).

$$D = \frac{d_1 + d_2}{2} \tag{1}$$

where

D is the mean diameter, in cm, of the particles considered as spheres;

d_1 is the mesh size, in cm, of the sieve with the larger mesh size (S_1);

d_2 is the mesh size, in cm, of the sieve with the smaller mesh size (S_2).

EXAMPLE 1 If the fraction is obtained by sieving between 0,025 0 cm and 0,012 5 cm mesh size, then the mean diameter of the particles is estimated to be 0,018 75 cm.

From the mean diameter of particles (D) determine the surface area (S), using Formula (2).

$$S = 4 \times \pi \times \left(\frac{D}{2}\right)^2 \tag{2}$$

where

S is the mean surface area, in cm^2, of the particles;

D is the mean diameter, in cm, of the particles.

EXAMPLE 2 If the mean diameter is 0,018 75 cm, then the mean surface area of the particles is 0,001 104 47 cm^2.

Calculate the volume (V) using Formula (3).

$$V = \frac{4}{3} \times \pi \times \left(\frac{D}{2}\right)^3 \tag{3}$$

where

V is the mean volume, in cm^3, of the particles;

D is the mean diameter, in cm, of the particles.

EXAMPLE 3 If the mean diameter is 0,018 75 cm, then the mean volume is 3,45 × 10^{-6} cm^3.

Calculate the mass (m) using Formula (4).

$$m = V \times d \tag{4}$$

where

m is the mean mass, in g, of the particles;

V is the mean volume, in cm^3, of the particles;

d is the density, in g × cm^{-3}, of test material.

EXAMPLE 4 If the density (d) of the material is 1,12 g × cm^{-3} and the volume is 3,45 × 10^{-6} cm^3, then the mean mass of the particles is 3,87 × 10^{-6} g.

Determine the SSA using Formula (5).

$$A = \frac{S}{m} \tag{5}$$

where

A is the specific surface area (SSA), in $\text{cm}^2 \times \text{g}^{-1}$ of the fraction;

m is the mean mass, in g, of the particles;

S is the mean surface area, in cm^2, of the particles.

EXAMPLE 5 If the mean surface area is 0,001 104 47 cm^2 and the mean mass of the particles is 3,87 × 10^{-6} g, then SSA is 286 $\text{cm}^2 \times \text{g}^{-1}$.

6.2.2.3 Instrumental determination of specific surface area

The SSA can be measured with a particle size analyser throughout a laser diffraction technique. The analysis is based on the light-scattering properties of the particles and it can be performed according to ISO 13320.

The SSA can also be measured with a micropore and chemisorption analyser based on the BET method for the determination of the specific surface area of solids by gas adsorption, according to the ISO 9277. BET measurement can provide higher SSA because it is a technique that is able to determine also porosity at nanometric level including pores and cracks.

6.3 Film/sheet test sample

6.3.1 Sample preparation

Film and sheet samples can be prepared by cutting them in pieces of 1 cm × 1 cm (following ISO 10210:2012, 7.3), or tested as unique piece. The dimensions and average thickness of the samples subjected to testing shall be measured and recorded. Apply ISO 4591 and/or ISO 4593 for determination of thickness of film/sheet materials.

6.3.2 Specific surface area determination

For the determination of the SSA of film/sheet sample apply the specific geometric formula depending on the shape of the film/sheet. If test sample has a circular shape, apply the formulas for cylindrical solid (with the thickness as the height) for surface and volume calculation, as shown in Formula (6) and (7). For samples in rectangular-square shape, use the formula of parallelepiped, as reported in Formula (8) and (9). The thickness of test sample is considered as the height of the solid.

$$S = 2\pi rh + 2\pi r^2 \tag{6}$$

$$V = \pi \times r^2 \times h \tag{7}$$

where

S is the surface area, in cm^2, of test sample in cylindrical shape;

r is the radius, in cm, of the base of the test sample in cylindrical shape;

h is the thickness, in cm, of the test sample in cylindrical shape;

V is the volume, in cm^3, of the test sample in cylindrical shape.

$$S = 2ab + 2bc + 2ac \tag{8}$$

$$V = a \times b \times c \tag{9}$$

where

S is the surface area, in cm^2, of test sample in rectangular/square shape;

a, b, c are the length, width and height (thickness), in cm, of the test sample in rectangular/square shape;

V is the volume, in cm^3, of the test sample in rectangular/square shape.

Weigh the test sample and calculate the SSA as $\mathrm{cm}^2 \times \mathrm{g}^{-1}$.

7 Determination of the net CO_2 evolution

The CO_2 evolution is determined by applying one of the following test methods: ISO 14852; ISO 17556; ASTM D5988; ISO 22404; ISO 19679; ISO 23977-1; ASTM D6691; ASTM D7991. All the conditions and requirements specified by each test method apply. If the tests are conducted under different conditions, test deviations shall be indicated in the test report.

Calculate the net CO_2 evolution for each test reactor from the amount of carbon dioxide evolved during each measurement interval using Formula (10):

$$m_{\mathrm{CO_2,net}} = \Sigma_{m\mathrm{T}} - \Sigma_{m\mathrm{B}} \tag{10}$$

where

$m_{\mathrm{CO_2,net}}$ is mass of the net evolved CO_2 value, in mg, of each reactor;

$\Sigma_{m\mathrm{T}}$ is the amount of CO_2, in mg, evolved in the test reactor between the start of the test and time, t;

$\Sigma_{m\mathrm{B}}$ is the amount of CO_2, in mg, evolved in the blank control reactor between the start of the test and time, t.

8 Determination of the specific aerobic biodegradation rate and disappearance time

8.1 Determination of C_R disappearance

Convert the mass of the net evolved CO_2 values ($m_{CO_2,net}$) of each reactor, expressed in mg, in mmol of carbon using Formula (11). This is the mineralized carbon and it is called C_{CO_2}.

$$C_{CO_2} = \frac{m_{CO_2,net}}{44} \tag{11}$$

where

C_{CO_2} is the amount of carbon, in mmol, evolved as CO_2 during the biodegradation process between the start of the test and time *t* (mineralized carbon);

$m_{CO_2,net}$ is the mass of the net evolved CO_2 value, in mg, of each reactor;

44 is the relative molecular mass, in $g \times mol^{-1}$, of C_{CO_2}.

Calculate the amount of carbon originally present in the plastic sample introduced in each reactor at time 0, using Formula (12). It is derived from the total organic carbon. This amount is expressed as mmol of C.

$$C_M = \frac{m \times w_c}{12} \tag{12}$$

where

C_M is the amount of organic C, in mmol, of test material at time 0; it does not include inorganic C;

m is the mass of test material, in mg, introduced into the test system;

w_C is the organic C content of the test material, determined from the chemical formula or from elemental analysis, expressed as a mass fraction;

12 is the atomic mass, in $mg \times mmol^{-1}$, of C.

Determine the amount of residual carbon, i.e. the un-mineralized carbon still present in each reactor, by subtracting the evolved net C_{CO_2} from the C_M, using Formula (13). This value includes both the "material" carbon still bound in the plastic constituents, and the carbon progressively assimilated as biomass. For the purposes of this document, this value is called residual organic carbon (C_R), that is, carbon expressed in mmol, which has not been converted into inorganic carbon, i.e. mineralized into CO_2 yet.

$$C_R = C_M - C_{CO_2} \tag{13}$$

where

C_R is the fraction of the residual organic carbon C, in mmol, originally present in the material (C_M) which has not been mineralized yet at any given time;

C_M is the amount of organic C, in mmol, of test material at time 0;

C_{CO_2} is the amount of carbon, in mmol, evolved as CO_2 during the biodegradation process between the start of the test and time *t* (mineralized carbon).

8.2 Determination of specific aerobic biodegradation rate

Build up the "C_R disappearance curve", by plotting C_R against time.

Successively, plot C_R against time considering only the initial part of the linear curve after the ended lag phase (see Annex A, Figure A.1).

Estimate the rate constant k by linear regression analysis of C_R versus time, by using a suitable statistics program, such as Microsoft Excel. The rate constant k, i.e. the slope of the regression line, represents the mineralization rate of test material and it is expressed as mmol C_R day^{-1}.

For the determination of the mineralization rate constant k, only the part of the curve, included between the end of the lag phase (if any) and before approx. 50 % of C disappearance is reached but in any case including at least three measurement points, should normally be used.

Some plastic materials require an abiotic hydrolysis phase before biodegradation. In case of plastic materials with degradation processes requiring long lag phases (i.e. more than 50 days), the mineralization rate constant of the lag phase ($k_{lag\ phase}$) shall be determined and reported.

The mineralization rate per surface area unit k cm^{-2} (mmol $C_R \times \text{day}^{-1} \times \text{cm}^{-2}$), i.e. the specific aerobic biodegradation rate (r_{SAB}), shall be calculated by dividing the mineralization rate constant k by the SSA, as shown in Formula (14).

$$r_{SAB} = \frac{k}{A \times m} \tag{14}$$

where

r_{SAB} is the specific aerobic biodegradation rate, in mmol C $\times \text{day}^{-1} \times \text{cm}^{-2}$

k is the mineralization rate constant;

A is the specific surface area (SSA), in $\text{cm}^2 \times \text{g}^{-1}$, of the tested sample;

m is the mass of tested sample, in g.

This procedure is described in Annex A.

8.3 Determination of DT50

In this document, the disappearance time DT50 is referred as the time taken for the concentration of the organic carbon of material (C_M) to decrease by 50 % of its original value.

Determine the DT50 (in days) graphically from the "C_R disappearance curve". The value can be obtained with the usual techniques of graphical interpolation. In case of no or short lag phase the DT50 can be obtained from the formula of the regression line obtained in 8.2, by dividing the C_M corresponding to 50% of initial value (C_M 50 %) with k, following Formula (15):

$$\text{DT50} = \frac{C_M\ 50\ \%}{k} \tag{15}$$

Since the DT50 for solid material depends on its surface area, it should be always expressed indicating the SSA of the tested sample.

9 Test report

The test report shall provide all relevant information, particularly the following:

a) a reference to this document, i.e. ISO 5148:2022;

b) all information necessary to identify and describe the test material, the reference material, including trade or brand name (if available), TOC, the physical form (powder or film/sheet) and shape (film/sheet), the thickness, with details on the measurements and applied standard test method;

c) the standard method used to determine the biodegradability of test material (and relative test temperature) and for which the present method has been applied for the expression of results;

d) biodegradation level reached by the tested material at the end of the test applied to measure rate or DT50;

e) the test method used for the determination of the specific surface area with details on instruments (if apply), measurements, and quality of measurements;

f) a plot of C_R versus time;

g) specific aerobic biodegradation rate, including k lag phase, if applicable, and specific DT50;

h) any deviations from the test conditions described in this document;

i) details about the regression analysis applied to obtain the biodegradation rate.

Annex A
(informative)

Example for the determination of r_{SAB} and DT50

A.1 Determination of the specific aerobic biodegradation rate (r_{SAB})

In this example, results of the biodegradation test of a polymer performed following ASTM D5988 are reported. Pellets of the test material were mechanically milled, and the resulting powder was sieved using two sieves of different mesh size. Sieves of 200 µm and 355µm mesh size were used for the separation procedure. The powdered test material passing through the 355 µm sieve was collected and then sieved with the 200 µm sieve. The fraction remaining on the 200 µm sieve was retained as the test sample. The upper and lower fractions from this procedure were discarded.

The SSA of this fraction was estimated by theoretical approach and resulted to be 193 cm^2 × g^{-1} as follows.

The mean diameter of particles of the fraction sieved was calculated as following [(355 µm + 200 µm)/2]/10 000= 0,027 75 cm

The mean surface (S) = 4 × π × (0,027 75 cm/2)^2 = 0,002 4 cm^2

The mean volume of particles was (V) = 4/3 × π × (0,027 75 cm/2)^3 = 1,118 × 10 $^{-5}$ cm^3

The mean mass of particles was (m) = 1,12 g × cm^{-3} · 1,118 ×10 $^{-5}$ cm^3 = 1,253 × 10 $^{-5}$ g

From the mean surface and the mean mass of particles, the specific surface area (SSA) was estimated: SSA = 0,002 4 cm^2 /1,253×10^{-5} g = 193 cm^2 × g^{-1}

The amount of test material subjected to the biodegradation test was 1,002 99 g.

The carbon content (w_C) of test material, determined by elemental analysis, was 65,43 %.

The mean initial amount of organic carbon present in the material (C_M) was 54,69 mmol. This value was calculated as following: 1 002,99 mg × (65,43/100) / 12 [see Formula (12)].

The selected fraction was tested following the ASTM D5988 procedure.

The average of cumulative mineralized carbon (C_{CO_2}) against time was determined. From this parameter the residual organic carbon (C_R) was calculated by subtracting C_{CO_2} from the initial organic carbon of test material (C_M), as reported in Table A.1.

The C_R disappearance curve was built by plotting C_R versus time (Figure A.1).

The linear regression analysis was performed for the initial part of the curve, i.e. after ended lag phase (or 10 % of biodegradation) and before approx. 50 % C_R disappearance was reached. In Figure A.1 this part of the curve is shown with a black continuous line. This analysis was performed using the common statistic package available with the commercially available spreadsheets for data processing.

The linear regression analysis allows to determine the mineralization rate constant *k* (i.e. the slope of the regression line) from the resulting equation of the regression line, as shown in Figure A.1. In this example, the formula is y = - 2,02 × x + 58,8 and the coefficient of determination R^2 = 0,96.

Thus, the resulting *k* is 2,02 mmol C×day^{-1}.

From the SSA and the amount of the tested sample, the surface area of the tested sample is determined as 193 cm^2 × g^{-1} × 1,002 99 g = 193,577 07 cm^2

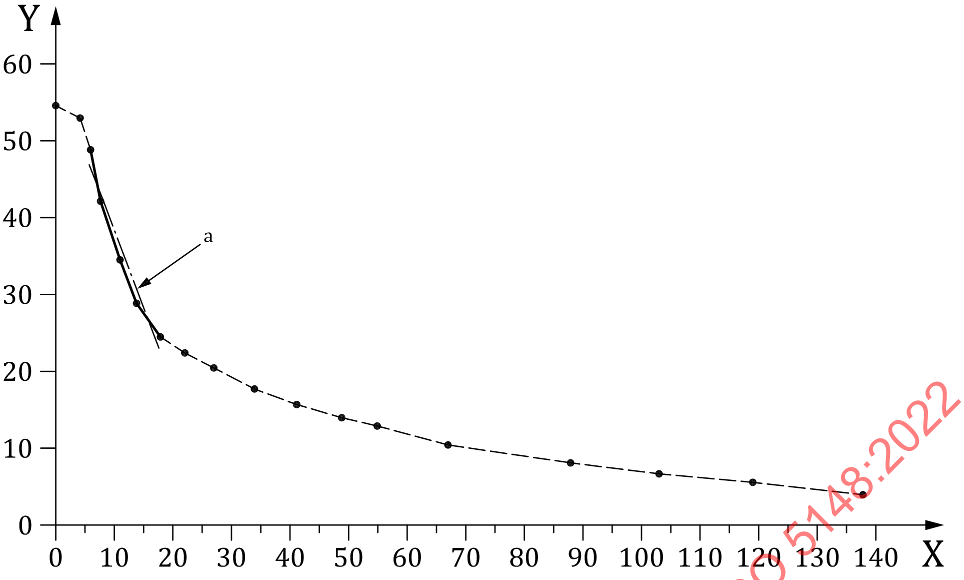
From the ratio of k and the surface area of the tested sample, it is possible to determine the specific aerobic biodegradation rate (r_{SAB}) of the material.

In this case, the r_{SAB} is 2,02 mmol C × day^{-1}/193,577 07 cm^2 = 0,010 43 mmol C × day^{-1} ×cm^{-2}.

Table A.1 — Data from biodegradation test

Time (days)	% C_R disappearance	Mineralized carbon (average) C_{CO_2} mmol	Residual organic carbon (average) C_R mmol
0	100,00	0,00	54,69
4	96,68	1,82	52,87
6	89,28	5,87	48,82[a]
8	77,07	12,54	42,15[a]
11	63,00	20,24	34,45[a]
14	52,85	25,79	28,90 [a]
18	44,65	30,27	24,42 [a]
22	40,89	32,33	22,36
27	37,44	34,22	20,47
34	32,28	37,04	17,65
41	28,74	38,97	15,72
49	25,53	40,73	13,96
55	23,72	41,72	12,97
67	19,17	44,21	10,48
88	14,92	46,53	8,16
103	12,31	47,96	6,73
119	10,09	49,17	5,52
138	7,46	50,61	4,08

[a] Data used for regression analysis, corresponding to 90 % to 50 % of C disappearance of test material.



Key

– – – –	C_R disappearance curve
—·—	regression line of the initial curve, i.e. from 90 % to 50 % of C_R
———	90 % to 50 % of C_R
X	*t,* time, in days
Y	$C_{R,}$ fraction of the residual organic carbon C, in mmol, originally present in the material (C_M) which has not been mineralized yet at any given time
a	In this example, the regression line is y = -2,02 x + 58,80, R^2 = 0,96.

Figure A.1 — C_R disappearance curve and regression line of the initial curve, i.e. from 90 % to 50 % of C_R

A.2 Determination of DT50

The DT50 is the time, in days, taken for the concentration of the residual organic carbon (C_R) to decrease by 50 % of its original value. The DT50 is determined by graphical interpolation (see Figure A.2). In this example, the 50 % C_R is 27,35 mmol C and the DT50 is 15,3 days. Figure A.3 shows the case of a material with a lag phase that shifts the DT50 to 35,3 days.