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Plastics — Test methods for determination of degradation rate and disintegration degree of plastic materials exposed to marine environmental matrices under laboratory conditions

Plastiques — Méthodes d'essai pour l'évaluation de la vitesse de dégradation et du degré de désintégration des matériaux plastiques exposés aux matrices environnementales marines dans des conditions de laboratoire

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Contents

Page

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

Plastics are potentially susceptible to ultimate biodegradation, i.e. to be decomposed by the actions of microorganisms under aerobic conditions into CO_2, water and biomass as can be proven with specific test methods. In most cases, biodegradation occurs at the surface of the plastics materials, i.e. at the solid-liquid interface. Microbes and enzymes cannot penetrate the solid plastic item, thus only the exposed surface is generally available to biodegradation. The physical effect of biodegradation on a solid plastic item is erosion leading to a thinning and weakening of the item. This process leads the item to lose mass, physical properties, and ultimately physical integrity by fragmentation into biodegradable particles whose ultimate fate is to be biodegraded. The term disintegration is used when the degradation process is extended until a total fragmentation of the original item into particles below a defined size is reached. When microorganisms cause degradation processes *bio*degradation, *bio*fragmentation, *bio*disintegration are the proper terms, etc. as suggested by CEN/TR 15351. However, when the physical breakdown rather than the chemical breakdown is measured, the generic term "degradation" is preferably used, reserving the term "biodegradation" to the assessment of the ultimate biodegradation, i.e. the conversion into CO_2, H_2O and biomass.

The assessment of specific degradation rates occurring when plastics materials are exposed to marine matrices is needed for designing products intended for marine applications (e.g. biodegradable plastic fish and mussel farming, floating devices) and for assessment of the risk caused by leakage of products into the sea.

In this document three test methods for testing degradation are described. Plastics samples can be exposed to three different test conditions and different marine matrices:

- buried into a wet sandy marine sediment;
- at the interface between a marine sandy sediment and the water column;
- to seawater.

The conditions applied in these test methods are designed to determine the degradation rates of plastics materials and give an indication of their propensity to physical degradation and disintegration in natural environments.

Degradation rates considered in this document are mass loss rate, erosion rate, and mechanical properties loss. Disintegration, i.e. physical breakdown of a sample into very small fragments (<2mm), can also be assessed.

Plastics — Test methods for determination of degradation rate and disintegration degree of plastic materials exposed to marine environmental matrices under laboratory conditions

1 Scope

This document specifies test methods for the measurement of the physical degradation of samples made with plastics materials when exposed to marine environmental matrices under aerobic conditions at laboratory scale.

This document is not suitable for the assessment of degradation caused by heat (thermo-degradation) or light exposure (photo-degradation).

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 527-1, *Plastics — Determination of tensile properties — Part 1: General principles*

ISO 527-2, *Plastics — Determination of tensile properties — Part 2: Test conditions for moulding and extrusion plastics*

ISO 527-3, *Plastics — Determination of tensile properties — Part 3: Test conditions for films and sheets*

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

ISO 16012, *Plastics — Determination of linear dimensions of test specimens*

ASTM D 638-14, *Standard Test Method for Tensile Properties of Plastics*

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
biodegradation
degradation caused by biological activity, especially by enzymatic action, leading to a significant change in the chemical structure of a material

[SOURCE: ISO 472:2013, 2.1680]

3.2
degradation
irreversible process leading to a significant change in the structure of a material, typically characterized by a change of properties (e.g. integrity, molecular mass or structure, mechanical strength) and/or by fragmentation, affected by environmental conditions, proceeding over a period of time and comprising one or more steps

[SOURCE: ISO 472:2013, 2.262]

3.3
disintegration
physical breakdown of a material into very small fragments

[SOURCE: ISO 14855-1:2012, 3.3]

3.4
total dry solids
amount of solids obtained by taking a known volume of test material or inoculum and drying at about 105 °C to constant mass

[SOURCE: ISO 13975:2019, 3.5]

3.5
volatile solids
amount of solids obtained by subtracting the residues of a known volume of test material or inoculum after incineration at about 550 °C from the *total dry solids* (3.4) content of the same sample

Note 1 to entry: The volatile solids content is an indication of the amount of organic matter present

[SOURCE: ISO 13975:2019, 3.6]

4 Principle

This document describes three laboratory test methods:

a) Method A: Sand burial degradation test;

b) Method B: Sediment/seawater interface degradation test; and

c) Method C: Seawater degradation test.

These three test methods are based on the exposure of plastic samples to environmental matrices taken from the sea and on the measurement of physical degradation.

These three test methods differ for the exposure conditions.

In Method A, the plastic samples are buried in a wet sandy sediment (a condition similar to the sandy shoreline where beach is maintained wet by the waves and tides).

In Method B, the plastic samples are laid at the interface between a sandy sediment bed and a water column (a condition similar to the seabed where most debris sinks, accumulates, and undergoes degradation).

In Method C, the plastic samples are exposed to seawater.

The degradation rate of the plastic material can be measured as:

a) mass loss and/or

b) erosion and/or

c) tensile properties decay.

The three test methods can also be used to determine the time for disintegration, i.e. the time needed to get the plastic samples fragmented into pieces below 2 mm, as determined by the surface area loss and/or mass loss determination.

The three test methods can be performed together or independently.

Claims of performance shall be limited to the numerical result obtained in the test and not be used for unqualified "biodegradable in marine environment" claims and similar. The results obtained are solely referred to the propensity to physical degradation caused by exposure to environmental matrices. The results do not give information regarding the ultimate biodegradability in the marine environment.

The test design (i.e. the total number of tested samples, the number of replicates and of repeated measurements) of the test methods is flexible. The complexity of test design and the cost of testing can be modulated according to the requests and purposes of the client. For example, tests planned for results delivered under statistically optimal conditions can be arranged for certification purposes, while simpler tests can be arranged for screening purposes.

5 Reagents

5.1 Distilled or deionized water, free of toxic substances (copper in particular) and containing less than 2 mg/l of DOC.

5.2 Artificial seawater

Dissolve:

Sodium chloride (NaCl)	22 g
Magnesium chloride hexahydrate ($MgCl_2 . 6 H_2O$)	9,7 g
Sodium sulfate (Na_2SO_4)	3,7 g
Calcium chloride ($CaCl_2$)	1 g
Potassium chloride (KCl)	0,65 g
Sodium hydrogen carbonate ($NaHCO_3$)	0,20 g

in water (5.1) and make up to 1 000 ml

6 Environmental matrix

6.1 Sampling

Take a sample of a sandy sediment with a shovel beneath the low-water line at the shoreline and/or seawater with a bucket. Record location and date of sampling. The wet sediment together with seawater is transferred into sealed containers for transport and fast delivered to the laboratory. After delivery, conserve the sediment and seawater at low temperature (approximately 4 °C) until use. The seawater/sediment sample should preferably be used within 4 weeks after sampling. Record storage time and conditions. More detailed instructions about sampling, preservation, handling, transport and storage of marine matrices are given in ISO 5667-3.

Measure the total dry solids, total organic carbon [(TOC) or, as an alternative, ashes and volatile solids], pH, and nitrogen content of the sediment and of the natural seawater.

The pH can be measured by applying ISO 10523 with seawater or ISO 10390 with marine sediments. ISO 10694 can be applied to determine the TOC and ISO 11261 can be applied to determine nitrogen content. A description on how to measure total dry solids, volatile solids and ashes of a solid environmental matrix (e.g. marine sediment or compost) is given in ISO 20200 and ISO 16929.

6.2 Preparation of the sediment and seawater

Remove, manually or by sieving, stones, pebbles and other materials until a clean marine sediment is obtained .

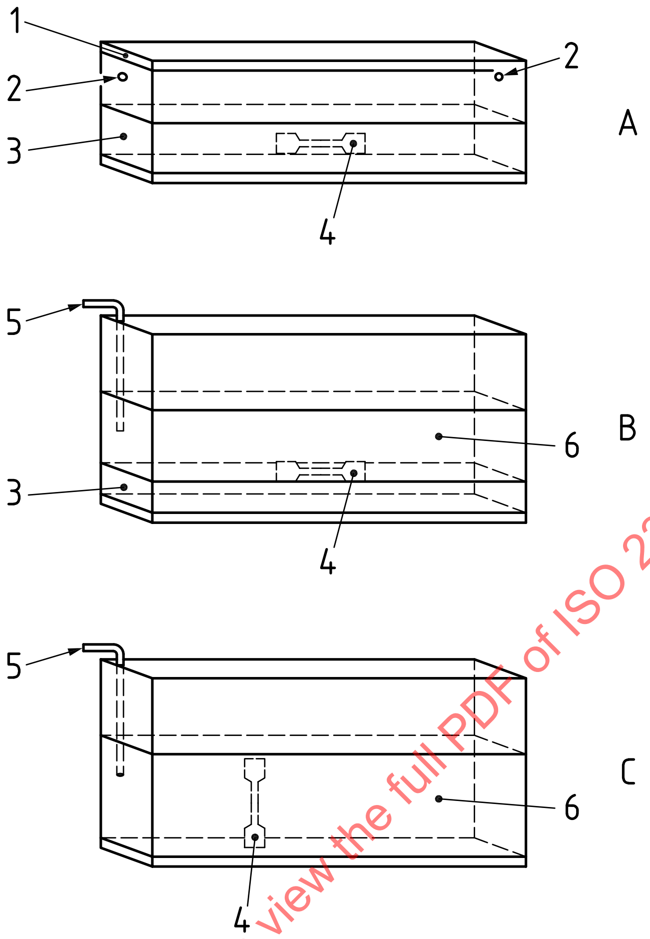
Filter the sediment in a funnel with a coarse filter paper to eliminate excess seawater. Sediment is ready for testing when seawater dripping stops. Sediment after filtering is named “wet sediment” hereafter and ready for Method A and Method B.

Natural seawater is directly used without filtration.

7 Apparatus

7.1 General

Polypropylene boxes or domestic aquariums (i.e. fish tanks for hobbyists) are suitable for the purposes of this document. However, if the test material is made with plastics expected to have degradation properties similar to the plastics used in the aquarium, glass should be used. See Figure 1 for a schematic representation of the tanks, which can be used to carry out the test methods.



Key

A	sand burial degradation test	3	wet sediment
B	sediment/seawater interface degradation test	4	plastic sample
C	seawater degradation test	5	aeration system
1	lid	6	seawater
2	5 mm hole for gas exchange		

Figure 1 — Apparatus

7.2 Test Method A (Sand burial degradation test)

Polypropylene (or other suitable material) boxes with a minimum dimension approximately of 30 cm × 20 cm × 10 cm (length, width, height) are appropriate. Alternatively, test devices as described in 7.3 can be used to perform the sand burial degradation test. Each box shall be provided with a lid assuring a tight closing to avoid an excessive vapour release. The closing between box and lid can be sealed with an adhesive tape to limit the water evaporation. In the middle of the two 20 cm wide sides, a hole of 5 mm of diameter shall be done at a height of about 6,5 cm from the bottom. The two holes provide gas exchange between the inner atmosphere and the outside environment. Attention shall be paid not to cover them with the adhesive tape, or in other way.

7.3 Test Method B (Sediment/seawater interface degradation test)

Tanks/aquariums (made with polypropylene or other suitable materials) with a minimum volume of 12 l (e.g. with dimension of 30 cm × 20 cm × 20 cm length, width, height) are appropriate. The dimension of the aquarium should be decided on the basis of the experimental design established by the operator, i.e. form and size of the plastic material to be tested and the number of replicates.

Aeration of seawater shall be applied with an appropriate aeration system. The typical systems used in domestic aquariums are appropriate.

7.4 Test Method C (Seawater degradation test)

See 7.3.

7.5 Analytical balance

7.6 pH meter

8 Materials

8.1 Test material

Test the material in the form of film, sheet or other forms. Prepare samples of adequate dimensions (e.g. 5 cm × 5 cm) from films and sheets. Measure the thickness of samples and register in final report (see Clause 11). Prepare the samples with a minimum thickness of 1 mm to 3 mm when thickness loss is measured. Standard "dumbbell-shaped" test samples made as reported in ISO 527-2 or ASTM D638 are suitable for the purpose.

When testing the degree of disintegration (see Clause 12), samples can be obtained from the product of interest or prepared from the plastic material of interest. Samples of 25 µm to 30 µm are suitable for testing the degree of disintegration.

8.2 Reference material

The following can be used as a reference material:

a) polycaprolactone (PCL)[1], or

b) ashless cellulose filter (e.g. filter paper Whatman® n. 42[2]), or

c) pure cotton (for example 100 % cotton gauze),

d) or poly-ß-hydroxybutyrate.

Prepare and characterize samples using the same procedure used for test materials (see 8.1). Form, size, and thickness of samples should be comparable to that of the test material. Report characteristics of reference material (density, etc.) in the test report.

8.3 Negative control

Optionally, a non-biodegradable polymer (e.g. polyethylene) can be tested as a negative control. Prepare samples using the same procedure used for test materials (see 8.1).

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[1] PCL products of the CAPA™ series (e.g. CAPA™ 6800) from Ingevity (USA) have been found satisfactory for this purpose. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

[2] Whatman® quantitative filter paper is the trade name of a product supplied by Aldrich. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

9 Sample preparation and measurements

9.1 Acclimatisation of samples

An acclimatisation phase of samples in seawater shall be carried out in order to simulate their initial exposure to seawater. This phase permits soluble components to leach out and not to concentrate in the close, static, and small-scale laboratory aquarium. Immerse the samples in seawater for at least 5 days at the same temperature that will be used for conducting the test prior to start the test.

9.2 Conditioning before measurements

Soak the exposed samples in freshwater/distilled water for 1 h, then rinse with distilled water to clean them, i.e. remove sediment particles, microbial biofilm, and sea salts. Condition the samples in an oven at (40 ± 2) °C until constant weight is reached. Then, measure and record the characteristic of interest (e.g. surface area, mass, thickness, tensile properties) of each individual sample. Apply conditioning before measurements to any sample both before acclimatisation and after exposure.

9.3 Marking of samples

When samples used for testing do not differ for more than 2 % for the characteristic of interest, i.e. the mass, or thickness or tensile properties or surface area, then all samples are considered as equal. Equal samples do not need to be individually identified because they are assumed to be equivalent, i.e. have the same original characteristic.

For practical reasons, it is suggested to prepare and test samples with equal physical characteristics (deviation < 2 %).

If samples are not equivalent, then mark each sample with appropriate means to ensure a clear permanent identification during the incubation time until final measurement and comparison with the initial measurement is performed.

9.4 Protective net

For assessment of disintegration degree, test each sample within a protective pocket made with a ≤2 mm mesh non-degradable net (e.g. a vinyl-coated fibreglass mosquito net or a polyester gauze). If degradation of the test material is expected to be similar to that of the net, then use alternative materials, more resistant to degradation. The purpose of the pocket is to cover the sample, preventing the loss of material with dimensions >2 mm during the exposure period, still allowing the exposure with the test environment. Particles with dimensions <2 mm can leak out. Duct tape can be used to close the pocket after introduction of the sample. Non-biodegradable plastic frames placed inside or outside the pocket can be used to prevent the pocket from folding back on itself, decreasing the exposure of the sample.

At sampling, the pocket is recovered and the plastic sample carefully removed.

NOTE The pocket made with the net can act as a barrier between the sample and the test environment and affect the degradation.

9.5 Tensile properties

Preferably, measure tensile strength at break. Other mechanical properties can be measured if deemed to be more suitable for the type of material and intended applications.

Apply ASTM D638 and/or ISO 527-1, ISO 527-2, ISO 527-3 for the determination of the tensile properties of the samples.

9.6 Thickness

Thickness of each sample shall be determined according to ISO 4593 (for film and sheeting) or ISO 16012 (for bulkier items).

10 Test set-up

10.1 Incubation

Incubation shall take place in the dark at a constant temperature, i.e. at a temperature between 15 °C to 25 °C, but not exceeding 28 °C with maximum seasonal variations within ±3 °C from the average temperature. For example, in a laboratory kept at an average room temperature of 21 °C, the temperature can fluctuate within 18 °C and 24 °C.

If the tests are conducted at different temperature, beyond the above specified temperature range, for example at more psychrophilic test conditions (<15 °C), then the different test temperature shall be indicated in the test report.

Test temperature may be adapted to the average temperature of seawater.

NOTE Test results are obtained for temperatures that can be different from real conditions in marine environment.

10.2 Sampling times and replicates

Degradation is determined as a change in a physical property occurred to the sample during the exposure time.

Measure rates (such as mass loss rate, erosion rate) by considering several exposure times and determining regression constants using suitable statistics programs. The statistical tools provided by the common commercial spreadsheet programs for data processing are sufficient for the purposes. The number of data points affects the quality of the regression constants.

The investigator in the beginning based on the desired statistical quality shall decide the experimental design, i.e. number of sampling times and replicates. However, at least three replicates shall be tested when determining the degree of disintegration at a given time.

Furthermore, based on the required statistical quality level of the results, more elaborated test designs can be devised, including for example two or more independent aquariums run in parallel.

Thickness, mass and tensile properties can be determined on the same samples with measurements carried out in series. Samples are then discarded after characterization.

Preferably, expose the samples of test material, reference material, and negative control tested at the same sampling time together in the same aquarium.

10.3 Start of the test

10.3.1 General

A general scheme of the test methods described in this document is shown in Table 1. In order to increase the statistical robustness of the results, all tests (Method A, Method B and Method C) can be performed in duplicate aquariums/tanks to which at least 3 test and reference material items per aquarium/tank are added.

Table 1 — Test methods

Test method	Method A Sand burial degradation test	Method B Sediment/seawater interface degradation test	Method C Seawater degradation test
Reactor (typical dimensions)	box with lid (30 cm × 20 cm × 10 cm)	aquarium/tank (about 30 cm × 20 cm × 20 cm)	aquarium/ tank (about 30 cm × 20 cm × 20 cm)
Inoculum	wet sediment (layer of 3 cm)	wet sediment (layer of about 3 cm) + artificial or natural seawater in a ratio 1:3- 1:5, v:v (sediment: water)	natural seawater
Forced aeration	no	yes	yes

10.3.2 Test Method A (sand burial degradation test)

Put the wet sediment on the bottom of the box and spread it in order to get a homogenous layer of about 3 cm, depending on the granulometry of the sediment. The wet sediment layer should not exceed half the height of the box.

Samples are buried at approximately 1 cm depth. Remove part of the sediment, smooth the surface of the residual sediment with a spatula but do not apply pressure. Place the sample on the sediment and cover it with the withdrawn sediment forming a homogenous layer.

The minimum horizontal distance between two samples shall be 2 cm.

Alternatively, samples in sheet or other forms can be inserted vertically or obliquely in the sediment. In any case, the complete coverage of the samples with the sediment shall be guaranteed.

Record the mass of the sediment and the sample introduced in each vessel.

Maintain moisture content constant by adding deionized water throughout the incubation period. Each box is weighed and, if needed, the initial mass restored by adding de-ionized or distilled water.

10.3.3 Test method B (sediment/seawater interface degradation test)

Put the wet sediment on the bottom of the aquarium and spread it in order to get a homogenous layer of about 3 cm, depending on the granulometry of the sediment. Carefully pour a volume of natural or artificial seawater in order to reach a volume ratio between 1:3 and 1:5 of wet sediment: seawater.

Place the samples on the sediment, at the sediment/seawater interface. The minimum horizontal distance between two samples shall be 2 cm.

A homogeneous contact between sample and marine sediment shall be guaranteed throughout the duration of the test by using suitable means, such as weights linked directly to the sample or to the plastic net.

Apply aeration of the system during the test. Introduce the air inlet tubing in the seawater column, in order to enable aeration of the system without disturbing the sediment. The same air flow rate should be used in each reactor by counting air bubbles/time or by using a suitable air-flow controller to check the air flow rate.

Maintain the seawater level/ volume constant by adding deionized water, in order to maintain constant the test conditions (e.g. salinity) during the test.

In case of slowdown of the biological activity as it can be deduced by the degradation course of the positive reference, change 1/3 of water volume every 3 months with fresh natural or artificial seawater.

It is possible to use artificial seawater supplemented with nutrients to support microbial diversity and to maintain the capacity to degrade the test material instead of natural seawater.

Test method B may be optionally performed under continuous flow conditions using natural seawater as incoming medium. Recommended constant supply of natural seawater is 1 reactor volume (liquid volume)/2 months.

10.3.4 Test method C (Seawater degradation test)

Fill the reactor (tank/ aquarium) with a volume of natural seawater in order to ensure submersion of the samples.

Suspend the samples in the water column by means of supports that allow hanging the samples.

During the test, apply the aeration of the seawater. See 10.3.3.

NOTE Salinity can be monitored with a simple aquarist's refractometer of by electric probes.

In case of slowdown of the biological activity as it can be deduced by the degradation course of the positive reference, change 1/3 of the water volume every 3 months with fresh natural or artificial seawater. It is possible to use artificial seawater supplemented with nutrients to support microbial diversity and to maintain the capacity to degrade the test material instead of natural seawater.

Maintain the seawater level/ volume constant by adding deionized water, in order to maintain constant the test conditions (e.g. salinity) during the test.

Test method C may be optionally performed under continuous flow conditions using natural seawater as incoming medium. Recommended constant supply of natural seawater is 1 reactor volume (liquid volume)/2 months.

10.4 End of the test

The duration of the tests should be set by the investigator and preferably be no longer than two years for technical reasons. If tests are performed under continuous flow conditions test duration can be longer than two years.

11 Degradation rate

Use values of thickness, mass, tensile strength at break of each sample at different sampling times throughout the incubation period to calculate the degradation rates of plastics materials.

NOTE 1 Tensile strength at break is not considered to be a parameter easy to follow during a degradation process because the shape of cross section changes in a biodegradation process. In addition, if a notch is formed during the degradation process, the sample is easily broken.

The investigator can decide to test all the characteristics or only some of them. For example, degradation can be followed by monitoring erosion, mass loss, and tensile strength decay all together or by monitoring just one or some parameters.

Erosion rate is the decrease of thickness per unit time (e.g. – µm/day).

Mass loss rate is the calculated as:

- decrease of mass per unit time (– µg/day);
- decrease of mass per unit time per unit surface area (– µg/day/cm^2).

Loss of tensile properties rate is the decrease of strength at break per unit time (– Pa/day).

NOTE 2 Tensile strength at break is valid only if ISO 527 requirements, especially test sample dimensions, are met.

Degradation rates are determined by linear regression of the plot of the measurements (mass, thickness or strength at break) referred to the respective sampling time for each sample. Use a suitable spreadsheet for data processing. The regression shall be determined considering a period of at least 6 months with a minimum of 3 measured data points. See Figure A.1 and Annex A as an example.

12 Degree of disintegration

12.1 General

Assessment of disintegration is carried out with samples protected within a pocket made with a <2 mm mesh non-degradable net (see 9.3). The net prevents the loss of particles with dimensions higher than 2 mm. The disintegration degree is followed examining samples at regular intervals and in any case determined at the end of the testing period (2 years). The final degree of disintegration is assessed on several (at least 3) replicates.

12.2 Surface area analysis

The possible plastic residues present inside the pocket are carefully collected and placed on a support inside a mask having the initial dimension of the sample (e.g. 5 cm × 5 cm), in order to measure the residual surface area. Measure the surface area of retrieved plastic item at sampling time (t_x) and compare it with the surface area at the beginning of the test (time = 0). Surface area can be determined analysing photographs of each sample with an image analysis software.

Calculate the degree of disintegration as percent of area loss, following Formula (1):

$$D_s = \frac{a_0 - a_x}{a_0} \times 100 \qquad (1)$$

where

D_s is the degree of disintegration of the test material, expressed in per cent (%) as obtained after a t_x time;

a_0 is the initial surface area of the test material (time t_0), expressed in square centimetre (cm^2);

a_x is the surface area of the retrieved test material at time t_x, expressed in square centimetre (cm^2).

Express the results as a mean of at least three replicates ± standard deviation. Disintegration degree shall be referred to the exposure time (t_x).

12.3 Mass loss

Measure the mass of the retrieved plastic item at sampling time (t_x) and compare it with the mass at the beginning (t_0). Weight the samples with an analytical balance to an accuracy of ±10 mg.

Calculate the degree of disintegration as percent of mass loss following Formula (2):

$$D_m = \frac{m_0 - m_x}{m_0} \times 100 \qquad (2)$$