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Water quality — Evaluation of the "ready", "ultimate" aerobic biodegradability of organic compounds in an aqueous medium — Method by analysis of dissolved organic carbon (DOC)

Qualité de l'eau — Évaluation de la biodégradabilité aérobie «facile», «ultime» des composés organiques en milieu aqueux — Méthode par analyse du carbone organique dissous (COD)

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Contents

Page

Foreword ... iv
1 Scope ... 1
2 Normative references ... 1
3 Terms and definitions ... 2
4 Principle ... 3
5 Test environment ... 3
6 Reagents ... 3
7 Apparatus ... 4
8 Procedure ... 5
9 Calculation and expression of results ... 7
10 Validity of the test ... 8
11 Test report ... 9
Annex A (informative) Typical degradation curve ... 10
Annex B (informative) Interpretation of results ... 11
Bibliography ... 13

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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 7827 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

This third edition cancels and replaces the second edition (ISO 7827:1994), which has been technically revised.

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Water quality — Evaluation of the "ready", "ultimate" aerobic biodegradability of organic compounds in an aqueous medium — Method by analysis of dissolved organic carbon (DOC)

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted according to this International Standard be carried out by suitably trained staff.

SAFETY PRECAUTIONS — Activated sludge and sewage contain potentially pathogenic organisms. Therefore take appropriate precautions when handling them. Handle toxic test compounds and those whose properties are unknown with care.

1 Scope

This International Standard specifies a method for the evaluation of the "ready" and "ultimate" biodegradability of organic compounds at a given range of concentrations by aerobic microorganisms. In this context, this International Standard also gives specific definitions for the terms "ready" and "ultimate".

The method applies to organic compounds which are:

a) soluble at the concentration used under the conditions of the test [dissolved organic carbon (DOC) concentrations of 10 mg/l to 40 mg/l];

b) non-volatile or having a negligible vapour pressure under the conditions of the test;

c) not significantly adsorbable on glass and activated sludge;

d) not inhibitory to the test microorganisms at the concentration chosen for the test.

The method is not suitable for waste waters, as they usually contain significant amounts of water-insoluble organic carbon, which is not included in DOC measurements.

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 8245, *Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)*

ISO 9408, *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium by determination of oxygen demand in a closed respirometer*

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ISO 9439, *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Carbon dioxide evolution test*

3 Terms and definitions

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

3.1
degradation time
t_2
time from the end of the lag time, t_1, until the time that about 90 % of the maximum level of biodegradation has been reached

NOTE Degradation time is expressed in days.

3.2
inherent biodegradation
level of biodegradation achieved which indicates the test compound is unlikely to be persistent in the environment

NOTE See Annex B.

3.3
lag time
t_1
time from the start of the test until 10 % biodegradation has been reached

NOTE Lag time is expressed in days.

3.4
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

3.5
primary biodegradation
structural change (transformation) of a chemical compound by microorganisms resulting in the loss of a specific property of that compound

3.6
“ready” biodegradation
level of biodegradation achieved under defined conditions which indicates the test compound is considered likely to degrade rapidly and completely under aerobic aquatic environmental conditions

NOTE See Annex B.

3.7
suspended solids
⟨activated sludge⟩ solid material within activated sludge with a particle diameter of >45 µm

NOTE The concentration of suspended solids is obtained by filtration or centrifugation of a known volume of sludge under specified conditions, drying at 105 °C, and correcting for the volume of sample. The concentration of suspended solids is expressed in milligrams per litre.

3.8
“ultimate” biodegradation
breakdown of a chemical compound or organic matter by microorganisms to carbon dioxide, water and mineral salts of any other elements present (mineralization), and the production of new biomass

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4 Principle

The biodegradation of organic compounds by aerobic microorganisms in a mineral medium is determined by measurement of the DOC concentration. The organic compound is the sole source of carbon in the medium. The concentration of the compound used is such that the initial DOC in the medium is between 10 mg/l and 40 mg/l. If necessary, concentrations greater than 40 mg/l may be used. The test solution is aerated in the dark or diffuse light at 22 °C ± 2 °C.

Biodegradation is monitored by measurement of the DOC at the start (day 0), at the end of the test (day 28 or longer if necessary), and at a minimum of three intermediate time intervals. The percentage removal of DOC is calculated at each time interval, and the biodegradability of the organic compound based on these data. Specific analysis can give additional information on primary biodegradation.

The test is not suitable for compounds which are inhibitory at the concentration used in the test. Inhibitory effects can be determined as specified in 8.3 or by using any other method for determining the inhibitory effect on bacteria of a substance (e.g. ISO 8192[1]).

The conditions specified in this International Standard do not necessarily correspond to the optimal conditions allowing the maximum degree of biodegradation to occur. Tests for ready biodegradability have very stringent conditions and a substance which passes these is considered likely to be rapidly and completely degraded in any aerobic aquatic environmental compartment, especially in waste water treatment plants. For alternative biodegradation methods, see ISO/TR 15462[6].

See Annex B for information on interpretation of results.

5 Test environment

Incubation shall take place in the dark or in diffused light in an enclosure which is maintained at 22 °C ± 2 °C and which is free from vapours that are toxic to microorganisms.

6 Reagents

Use only reagents of recognized analytical grade (where applicable).

6.1 Water, distilled or demineralized, containing less than 10 % of the initial DOC content introduced by the compound to be tested to maintain acceptable precision.

6.2 Test medium

6.2.1 Composition

6.2.1.1 Solution A

Anhydrous potassium dihydrogenphosphate, KH_2PO_4	8,5 g
Anhydrous dipotassium hydrogenphosphate, K_2HPO_4	21,75 g
Disodium hydrogenphosphate dihydrate, $Na_2HPO_4 \cdot 2H_2O$	33,4 g
Ammonium chloride, NH_4Cl	0,5 g
Water (6.1), quantity necessary to make up to	1 000 ml

Measure the pH value of the solution, which should be 7,4 ± 0,2. If it is not, then prepare a new solution.

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6.2.1.2 Solution B

Dissolve 22,5 g of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) in water (6.1) and make up to 1 000 ml.

6.2.1.3 Solution C

Dissolve 27,5 g of anhydrous calcium chloride ($CaCl_2$) in water (6.1) and make up to 1 000 ml.

6.2.1.4 Solution D

Dissolve 0,25 g of iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in water (6.1) and make up to 1 000 ml. Prepare the solution freshly just before use.

The need to prepare this solution just before use can be avoided if a drop of concentrated hydrochloric acid (HCl) or 0,4 g/l of ethylenediaminetetraacetic acid (EDTA) is added.

6.2.2 Preparation

For 1 l of test medium, add to about 500 ml of water (6.1):

a) 1 ml of each of solutions B, C, and D;

b) 10 ml of solution A.

Make up to 1 000 ml with water (6.1). Solution A is added last, to avoid precipitation of salts. Prepare the test medium freshly before use. Solutions A to C can be stored for up to 6 months in the dark at room temperature and Solution D) (with preservative) for 3 months.

7 Apparatus and materials

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

Use usual laboratory apparatus and, in particular, the following.

7.1 Apparatus of sufficient sensitivity, for the measurement of DOC in the concentration range 0,5 mg/l to 40 mg/l determined in accordance with ISO 8245.

7.2 Centrifuge, capable of centrifuging samples at 40 000 m/s^2, for concentration of sludge solids and preparing samples for DOC analysis.

7.3 Shaking device or **stirring device**, for aeration and mixing.

7.4 Incubator, or **temperature-controlled environment**, capable of maintaining test solutions at a temperature of 22 °C ± 2 °C, in the dark or diffused light.

7.5 pH-meter.

7.6 Conical flasks, of appropriate capacity (e.g. 2 000 ml).

7.7 Device for filtration, with **filters** of suitable porosity (nominal aperture diameter of 0,2 µm to 0,45 µm) which adsorb or release organic carbon to a minimum degree.

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8 Procedure

8.1 Preparation of test solutions

8.1.1 Solution of the test compound

Prepare a stock solution of the test compound in water (6.1) or test medium (6.2). Dilute a suitable amount of this solution in the test medium in order to obtain a final organic carbon concentration between 10 mg/l and 40 mg/l. Substances of low solubility (10 mg/l to 100 mg/l) may be added directly to the contents of the test vessel, ensuring the substance dissolves completely. (F_T in 8.3.1).

8.1.2 Solution of the reference compound

Prepare a stock solution of the reference compound (an organic compound of known high biodegradability such as sodium acetate, sodium benzoate or aniline) in the same way as in 8.1.1, in order to obtain a final organic carbon concentration between 10 mg/l and 40 mg/l. (F_C in 8.3.1).

8.1.3 Solution to check inhibition

If necessary, prepare a solution containing, in the test medium (6.2), the test compound and the reference compound in the respective concentrations used for the preparation of solutions in 8.1.1 and 8.1.2. (F_I in 8.3.1).

8.2 Preparation of the inoculum

8.2.1 General

Prepare the inoculum using the sources specified in 8.2.2 to 8.2.4, or a mixture of them, to obtain a microbial population that offers sufficient biodegradative activity. Use a suitable volume for inoculation (see Note 2).

NOTE 1 Under certain circumstances, a pre-exposed inoculum can be used, provided that this is clearly stated in the test results (e.g. percentage biodegradation = w %, using pre-exposed inoculum) and the method of pre-exposure is detailed in the test report. Pre-exposed inocula can be obtained from laboratory biodegradation tests conducted under a variety of conditions [e.g. Zahn-Wellens test (ISO 9888[3]) and SCAS test (ISO 9887[2])] or from samples collected from locations where relevant environmental conditions exist (e.g. treatment plants dealing with similar compounds or contaminated areas). If a pre-exposed inoculum is used, the results are interpreted as demonstrating that the test compound is “inherently” biodegradable (see Annex B).

NOTE 2 “Suitable volume” means:

a) sufficient to give a population which offers enough biodegradative activity;

b) degrades the reference compound(s) by the stipulated percentage (see Annex B);

c) gives between 10^3 and 10^6 active cells per millilitre in the final mixture;

d) gives an activated sludge concentration not exceeding the equivalent of 30 mg/l in the final mixture;

e) contributes DOC to the test solution of less than 10 % of that introduced by the test compound (e.g. <4 mg/l at a test concentration of 40 mg/l).

8.2.2 Inoculum from a secondary effluent

Take a sample of a secondary effluent collected from a treatment plant or a laboratory plant dealing with predominantly domestic sewage. Mix well, keep the sample under aerobic conditions and use it on the day of collection.

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From this sample, prepare an inoculum by:

a) allowing the sample of effluent to settle for 1 h;

b) taking a suitable volume of the supernatant (see Note 2 to 8.2.1).

8.2.3 Inoculum from an activated sludge plant

Take a sample of activated sludge collected from the aeration stage or sludge return pipe of a treatment plant or a laboratory plant dealing with predominantly domestic sewage. Mix well, keep the sample under aerobic conditions, and use it on the day of collection. If necessary (see Note 2 to 8.2.1), wash the inoculum to reduce its DOC concentration by centrifuging and resuspending the sludge solids in mineral medium (6.2).

Before use, determine the concentration of suspended solids. If necessary, concentrate the sludge by settling so that the volume of sludge added to the test assay is minimal. Add a suitable volume to obtain no more than 30 mg/l of suspended solids in the final mixture.

A further alternative is to use homogenized sludge at 3 g to 5 g/l of suspended solids. Treat the sludge in a blender for up to 2 min, but do not allow the temperature to go above 25 °C. Allow the liquid to settle for 30 min and use the decanted supernatant as the inoculum, at 10 ml per litre of test medium.

8.2.4 Inoculum from surface water

Take a sample of an appropriate body of surface water. Keep the sample under aerobic conditions and use it on the day of collection.

Take a suitable volume as inoculum (see Note 2 to 8.2.1).

8.3 Test procedure

8.3.1 Preparation of test and control vessels

Set up a sufficient number of conical flasks (7.6) of a suitable volume (e.g. 2 000 ml, but other volumes and types of flasks are also possible) in order to have:

a) at least two test flasks (designated F_T) containing 1 000 ml of the test solution (8.1.1);

b) at least two blank test flasks (designated F_B) containing 1 000 ml of the test medium (6.2);

c) at least one flask for checking the procedure (designated F_C) containing 1 000 ml of the reference compound solution (8.1.2);

d) if needed, one flask for checking a possible inhibitory effect of the test compound (designated F_I) containing 1 000 ml of the solution to check inhibition (8.1.3);

e) if needed, one flask for checking a possible abiotic removal (designated F_S) containing 1 000 ml of the test solution (8.1.1) but no inoculum, sterilized by addition of, for example, 1 ml/l of a solution containing 10 g/l of mercury(II) chloride ($HgCl_2$) or another suitable inorganic toxic compound to prevent microbial activity, or by filter sterilization — if very easily degradable substances are analysed, it is recommended to add the same amount of the toxic substance 2 weeks after the test has started.

By comparing the percentage removal in flasks F_T and F_S, it can be determined whether the test compound undergoes removal caused by abiotic, physicochemical mechanisms like stripping or adsorption.

If activated sludge is used as inoculum, significant quantities of test compound can be adsorbed on to the sludge. This can be checked using the test as described for flask F_S, but adding inoculum (8.2). Normally, only pure or virtually pure compounds are tested, but if mixtures are tested, selective adsorption of different components can occur.

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Inoculate flasks F_T, F_B, F_C, and, if included, F_I with a suitable volume (see Note 2 to 8.2.1) of the inoculum (8.2). Mix the contents of the flasks. Generally a volume of 10 ml to 100 ml of inoculum is sufficient for 1 000 ml of test solution.

8.3.2 Incubation and sampling

During the test, maintain the flasks on the shaking or stirring device (7.3) at a temperature of 22 °C ± 2 °C.

In order to compensate for water losses by evaporation, check the volume of the medium in the flasks before taking each sample and, if necessary, make up with water (6.1) to the volume or the mass measured after taking the preceding sample.

At the beginning of the test (day 0), at the end of the test (normally after 28 d), and at a minimum of three intermediate time intervals (e.g. 7 d, 14 d, 21 d), take a minimum volume from flasks F_T, F_B, and F_C and, if included, also from F_I. If necessary, take measurements at shorter intervals or over a period longer than 28 d. If the 10 d window (see Annex B) is to be identified, more sampling points are needed near the start of the test. At the beginning of the test, take a sample from flask F_S. If flask F_S is inoculated (see 8.3.1), take a sample after 0 d and 1 d. Filter all these samples, or, especially if the material adsorbs onto the membrane, centrifuge them at about 40 000 m/s^2 for 25 min.

Measure the DOC concentrations in the samples in accordance with ISO 8245 at least twice for each period and each flask. For additional information on primary degradation, specific analyses of the substance can be performed. The concentration measured in the test solution at the beginning of the test (day 0) is used as the initial concentration in the final calculation.

If a sufficient degree (>80 %) and a constant level of degradation is attained before the end of the 28 d test period, consider that the test is finished. Extend the test by 1 week or 2 weeks if degradation has obviously started but has not reached a plateau.

When measurements of organic carbon have to be postponed up to 48 h, keep the samples at 4 °C in the dark and in tightly stoppered flasks. If the samples have to be stored for more than 48 h before measurement, store the samples at $\leqslant -18$ °C. Alternatively, add a suitable inorganic toxic substance, e.g. 20 ml/l of a solution of mercury(II) chloride ($HgCl_2$), to prevent microbial activity and store at 4 °C ± 1 °C.

9 Calculation and expression of results

9.1 Calculation of percentage biodegradation (DOC removal)

For each test flask, determine the percentage removal of DOC, $w_{\text{DOC},t}$, using Equation (1):

$$w_{\text{DOC},t} = \left(1 - \frac{\rho_t - \rho_{\text{B}t}}{\rho_0 - \rho_{\text{B}0}}\right) \times 100 \qquad (1)$$

where

ρ_0 is the average DOC concentration, in milligrams per litre, at time 0, in each test flask F_T;

$\rho_{\text{B}0}$ is the average DOC concentration, in milligrams per litre, at time 0, in the blank test flask F_B;

ρ_t is the average DOC concentration, in milligrams per litre, at time t, in each test flask F_T;

$\rho_{\text{B}t}$ is the average DOC concentration, in milligrams per litre, at time t, in the blank test flask F_B.

Round percentage results to the nearest whole number.

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9.2 Calculation of primary biodegradation

When specific analyses of the test compound are performed, calculate the percentage of the primary biodegradation, w_{DS}, of the test compound in vessel F_S at the end of the test using Equation (2):

$$w_{DS} = \frac{\rho_S - \rho_T}{\rho_S} \times 100 \quad (2)$$

where

ρ_S is the average test substance concentration, in milligrams per litre, at time t, in test flask F_S;

ρ_T is the average test substance concentration, in milligrams per litre, at time t, in test flask F_T.

Round percentage results to the nearest whole number.

The abiotic removal (flask F_S) can also be calculated according to Equation (2), without considering the blank values (if F_S is inoculated to check the degree of adsorption, take the blanks into consideration). If a significant loss of organic carbon is observed, no differentiation between biotic and abiotic removal is possible. In this case, use a test based on parameters clearly indicating biological processes, such as the respirometric test in accordance with ISO 9408 or the carbon dioxide production tests in accordance with ISO 9439.

Additionally, the degree of removal of the mixture of test compound and reference substance in the inhibition control (flask F_I) can be calculated according to Equation (2). If the removal from the mixture is less than 35 % in 14 d, the test compound has inhibited the biodegradation of the reference substance and is therefore assumed to be toxic. In this case, repeat the test with a lower concentration of the test compound or with a pre-exposed inoculum.

9.3 Expression of results

Compile a table of DOC percentage removal for each concentration interval and each test vessel. If comparable results are obtained for the duplicate test vessels (see Clause 10), plot a mean removal curve as a function of time (see example in Annex A).

Biodegradation parameters can be determined from this curve. In particular, if sufficient data are available, determine the lag time (3.3), the degradation time (3.1), and the maximum level of biodegradation (3.4). If the test substance is not significantly removed abiotically (e.g. by adsorption) and the removal curve has a typical shape with a lag and degradation phase, assign the measured DOC removal to biodegradation. Refer to Annex B for further information on interpretation of results.

10 Validity of the test

Consider a test to be valid if, in the test flasks with the same test concentration and inoculum, the difference between the degradation values at the end of the test is less than 20 % DOC removal. If this is not the case, repeat the test.

Consider the test results to be valid if, in the test with one of the proposed reference compounds, the percentage degradation after 14 d is more than 70 %. If this is not the case, repeat the test.

The DOC contributed to the test system by the inoculum is less than 10 % of that contributed by the test compound.

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11 Test report

This test report shall contain at least the following information:

a) the test method used, with reference to this International Standard (ISO 7827:2010);

b) all information necessary for the complete identification of the test compound;

c) all the data (e.g. in tabular form) obtained and the degradation curve;

d) the concentration of the test compound used and the DOC content of this concentration;

e) the name of the reference compound used and the degradation obtained with this compound;

f) the source, the characteristics, the concentration, and the volume of the inoculum used, as well as information on any pretreatment;

g) the main characteristics of the DOC analyser used;

h) the incubation temperature of the test;

i) if included, the percentage of degradation obtained in flask F_S (monitoring abiotic removal);

j) if included, the percentage of degradation in flask F_I (toxicity test) and a statement on the toxicity of the test compound;

k) the reasons, in the event of rejection of the test (see Clause 10);

l) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s).

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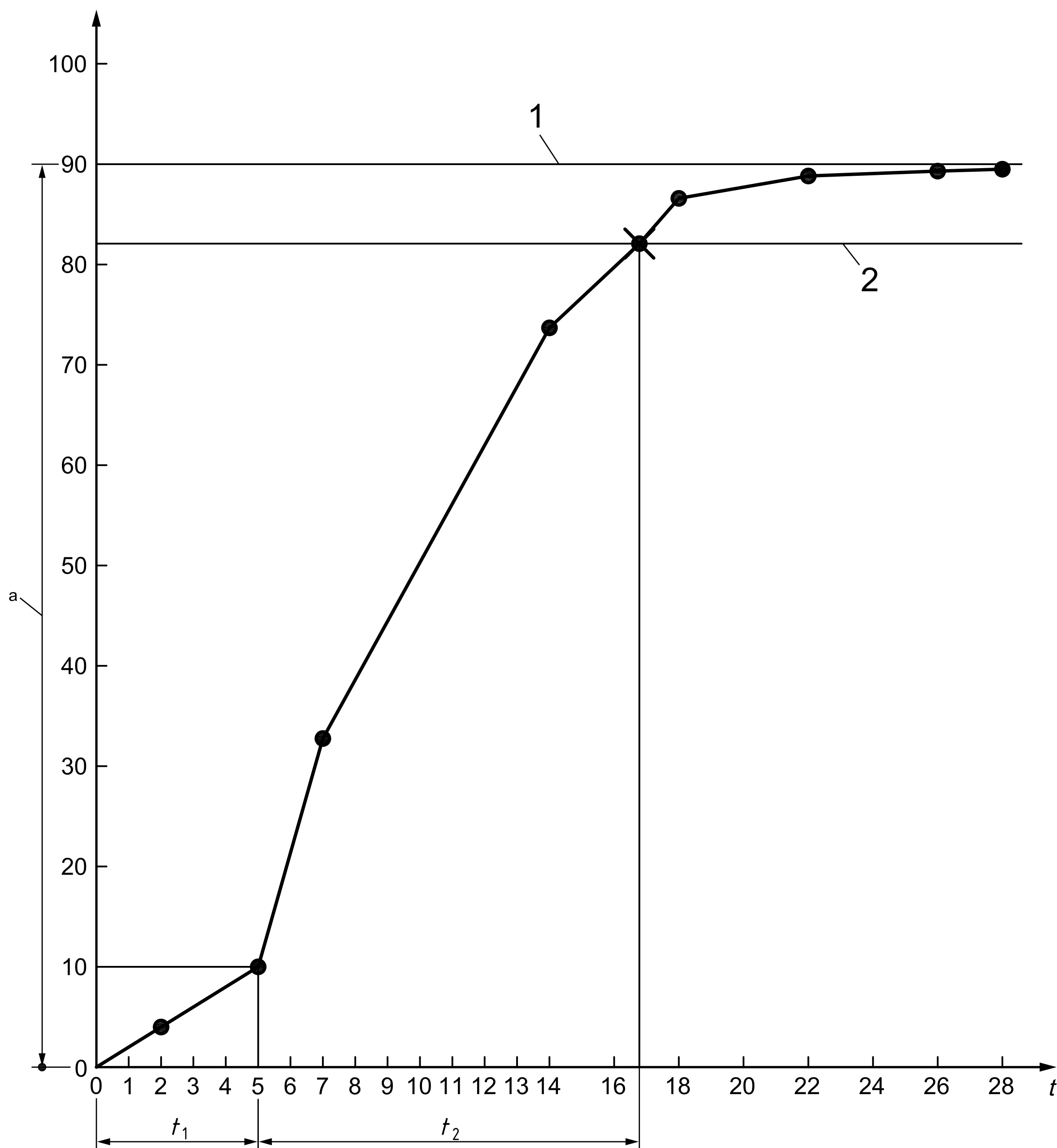
Annex A
(informative)

Typical degradation curve



Key

t time, in days

t_1 lag time

t_2 degradation time

1 maximum level of biodegradation

2 about 90 % of maximum level of biodegradation

a $w_{DOC,\,t}$ (DOC removal, in %).

Figure A.1

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Annex B
(informative)

Interpretation of results

B.1 General

The results from this biodegradation test can be used to classify substances according to their potential for biodegradation.

B.2 Ready biodegradability

Tests for ready biodegradability have very stringent conditions, and a substance which passes these is considered likely to be rapidly and completely degraded in any aerobic aquatic environmental compartment, especially in waste water treatment plants. Many tests can be used to determine if a substance is readily biodegradable (e.g. this International Standard, ISO 9408, ISO 9439, ISO 10707[4], ISO 14593[5]) but they all have a number of features in common:

a) the inoculum is at a relatively low concentration (up to 30 mg/l sludge solids, $<10^8$ cells/l);

b) the test substance is the only source of carbon available to the bacteria;

c) the inoculum has not been pre-exposed or adapted to the test substance in question.

Degradation can be calculated by measurement of one of a number of parameters, including carbon dioxide evolution, oxygen consumption or removal of DOC from the test solution.

The pass levels for ready biodegradability are 70 % removal of DOC and 60 % of theoretical oxygen demand (ThOD) or theoretical carbon dioxide ($ThCO_2$) production for respirometric methods. The pass levels are lower in the respirometric methods, as some of the carbon from the test chemical is incorporated into new cells. Therefore, the percentage of carbon dioxide produced is lower than the percentage of carbon being used. These pass values have to be reached in a 10 d window, within the 28 d test period. The 10 d window begins when the degree of biodegradation has reached 10 %, and shall end before day 28 of the test. Chemicals which reach the pass levels after the 28 d period are not deemed to be readily biodegradable.

B.3 Inherent biodegradability

Tests for inherent biodegradability have less stringent conditions than those for ready biodegradability. A substance which shows some mineralization in one of these tests is not considered to be persistent, and is likely to degrade in an aerobic aquatic environment in the medium to long term.

An inherent biodegradability test has one or more of the following features:

a) the inoculum is at a relatively high concentration (up to 1 000 mg/l sludge solids);

b) an easily metabolized food source is added to the test system, as well as the test material;

c) the inoculum is pre-exposed to the test substance so that the bacterial population has an opportunity to become adapted to the test substance.

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Tests commonly used for determination of inherent biodegradability (ISO 9887[2] and ISO 9888[3]) monitor degradation in terms of DOC removal. The more stringent biodegradation tests can also be used to determine inherent biodegradability by using a pre-exposed inoculum or simply by applying less stringent pass criteria.

Removal of 20 % of DOC, consumption of 20 % of ThOD or evolution of 20 % of $ThCO_2$ in any degradation test, with or without pre-exposure of inoculum, indicates that the test substance in question is inherently biodegradable and non-persistent.

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[4] ISO 10707, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by analysis of biochemical oxygen demand (closed bottle test)*

[5] ISO 14593, *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test)*

[6] ISO/TR 15462, *Water quality — Selection of tests for biodegradability*

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