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

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



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Foreword

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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.

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

This second edition cancels and replaces the first edition (ISO 7827:1984), which has been technically revised.

Annex A of this International Standard is for information only.

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

WARNING — Safety precautions — Activated sludge and sewage may contain potentially pathogenic organisms. Therefore appropriate precautions should be taken when handling them. Toxic test compounds and those whose properties are unknown should be handled with care.

1 Scope

This International Standard specifies a method for the evaluation of the “ultimate” biodegradability of organic compounds at a given concentration by aerobic microorganisms.

The conditions described in this International Standard do not necessarily always correspond to the optimal conditions allowing the maximum degree of biodegradation to occur.

The method applies to organic compounds which are

- soluble at the concentration used under the conditions of the test (10 mg/l to 40 mg/l DOC);
- non-volatile, or having a negligible vapour pressure under the conditions of the test (see note 5 in 8.3);
- not significantly adsorbable on glass and activated sludge (see note 6 in 8.3);
- not inhibitory to the test microorganisms at the concentration chosen for the test. Inhibitory effects can be determined as described in 8.3, or by using any other method for determining the inhibitory effect on bacteria of a substance (e.g. ISO 8192).

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 8192:1986, *Water quality — Test for inhibition of oxygen consumption by activated sludge*.

ISO 8245:1987, *Water quality — Guidelines for the determination of total organic carbon (TOC)*.

ISO 9408:1991, *Water quality — Evaluation in an aqueous medium of the “ultimate” aerobic biodegradability of organic compounds — Method by determining the oxygen demand in a closed respirometer*.

ISO 9439:1990, *Water quality — Evaluation in an aqueous medium of the “ultimate” aerobic biodegradability of organic compounds — Method by analysis of released carbon dioxide*.

ISO 9887:1992, *Water quality — Evaluation of the aerobic biodegradability of organic compounds in an*

aqueous medium — Semi-continuous activated sludge method (SCAS).

ISO 9888:1991, *Water quality — Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium — Static test (Zahn-Wellens method).*

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1 “ultimate” biodegradation: The level of degradation achieved when the test compound is totally utilized by microorganisms resulting in the production of carbon dioxide, water, mineral salts and new microbial cellular constituent (biomass).

3.2 primary biodegradation: The level of degradation achieved when the test compound undergoes any structural change, other than mineralization, as the result of microbial action.

3.3 concentration of suspended solids (of an activated sludge): The amount of solids obtained by filtration or centrifugation of a known volume of sludge under specified conditions and drying at 105 °C at constant weight.

4 Principle

Determination of the biodegradation of organic compounds by aerobic microorganisms using a test medium. The organic compound is the sole source of carbon and energy in the medium. The concentration of the compounds used is such that the initial concentration of organic carbon in the medium is between 10 mg/l and 40 mg/l.

If necessary, concentrations greater than 40 mg/l may be used.

Measurement of the dissolved organic carbon (DOC) at the start (day 0) and the end of the test (day 28 or longer if necessary) and at least at three regular, intermediate time intervals.

Determination of the percentage removal of DOC at each of these intervals. Evaluation of the biodegradability of the compounds used on the basis of these data.

Specific analysis may give additional information on primary biodegradation.

5 Test environment

Incubation shall take place in the dark or in diffused light in an enclosure which is maintained at 20 °C to 25 °C and which is free from vapours that are toxic to microorganisms.

6 Reagents

Use only reagents of recognized analytical grade.

6.1 Distilled or deionized water, containing less than 10 % of the initial DOC content introduced by the compound to be tested.

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 ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$)	33,4 g
Ammonium chloride (NH_4Cl)	0,5 g
Water (6.1), quantity necessary to make up to	1 000 ml

NOTE 1 The correct composition of the medium is checked by the measurement of the pH-value, which should be 7,4.

6.2.1.2 Solution b)

Dissolve 22,5 g of magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) in 1 000 ml of the water (6.1).

6.2.1.3 Solution c)

Dissolve 27,5 g of anhydrous calcium chloride (CaCl_2) in 1 000 ml of the water (6.1).

6.2.1.4 Solution d)

Dissolve 0,25 g of iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) in 1 000 ml of the water (6.1). Prepare the solution freshly just before use.

NOTE 2 It is not necessary to prepare this solution just before use 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 litre of test medium, add to about 500 ml of water (6.1):

- 10 ml of solution a);
- 1 ml of each of the solutions b) to d).

Make up 1 000 ml with the water.

7 Apparatus

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

Usual laboratory equipment and

7.1 Apparatus, of sufficient sensitivity for the measurement of dissolved organic carbon (see ISO 8245).

7.2 Centrifuge.

7.3 Shaking device, for aeration and mixing.

7.4 pH-meter.

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

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

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.

8.1.2 Solution of the reference compound

Prepare a stock solution of the reference compound (an organic compound of known 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 10mg/l and 40mg/l.

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.

8.2 Preparation of the inoculum

Prepare the inoculum using the following sources or a mixture of them, to obtain a microbial population that offers sufficient biodegradative activity.

NOTE 3 In certain circumstances, pre-exposed inocula may be used, provided that this is clearly stated in the test results (e.g. percent biodegradation = x %, using pre-exposed inocula) 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) and SCAS test (ISO 9887)] or from samples collected from locations where relevant environmental conditions exist (e.g. treatment plants dealing with similar compounds or contaminated areas).

Use a suitable volume for inoculation (see note 4).

NOTE 4 "Suitable volume" means

- sufficient to give a population which offers enough biodegradative activity;
- degrades the reference compound(s) by the stipulated percentage;
- gives between 10^3 and 10^6 active cells/ml;
- gives not more than the equivalent of 30 mg/l suspended solids of activated sludge in the final mixture.

The content of DOC in the inoculum shall be less than 10 % of the content of DOC introduced by the test compound (e.g. < 4 mg/l at a test concentration of 40 mg/l). If necessary and if possible, wash the inoculum.

8.2.1 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. If necessary, concentrate the sample by filtration or centrifugation. Mix well, keep the sample under aerobic conditions and use it on the day of collection.

From this sample, prepare an inoculum as follows:

- let the sample of effluent settle for 1 h;

- take a suitable volume of the supernatant, to be used as inoculum.

8.2.2 Inoculum from an activated sludge plant

Take a sample of activated sludge collected from the aeration tank 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.

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 30 mg/l of suspended solids in the final mixture.

8.2.3 Inoculum from a surface water

Take a sample of an appropriate surface water. If necessary, concentrate the sample by filtration or centrifugation. Keep the sample under aerobic conditions and use it on the day of collection.

Take a suitable volume as inoculum.

8.3 Test

Set up a sufficient number of conical flasks (7.5) of a suitable volume (e.g. 2 000 ml, but other volumes and types of flasks are also possible) in order to have

- at least two test flasks (symbol F_T) containing 1 000 ml of the test solution (8.1.1);
- at least two blank test flasks (symbol F_B) containing 1 000 ml of the test medium (6.2);
- at least one flask, for checking the procedure (symbol F_C) containing 1 000 ml of the reference compound solution (8.1.2);
- if needed, one flask for checking a possible inhibitory effect of the test compound (symbol F_I) containing 1 000 ml of a solution 8.1.3 ;
- if needed, one flask for checking a possible abiotic elimination (symbol F_S) containing 1 000 ml of 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. If very easily degradable substances are analysed, it is recommended to add the same amount of the toxic substance two weeks after the test was begun (see notes 5 and 6).

NOTES

5 By comparing the percentage elimination in flasks F_T and F_S , it can be determined whether or not the test compound is undergoing an elimination caused by abiotic, physicochemical mechanisms like stripping or adsorption. The results should be reported in the test report.

6 If activated sludge is used as inoculum, the test compound can be adsorbed significantly onto 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 should be analysed, but if mixtures are tested, selective adsorption of different components may occur.

Inoculate flasks F_T , F_B , F_C and, if included, flask F_I with an appropriate volume of the inoculum (8.2) and mix the content of the flasks (see note 4). Generally 1 ml to 10 ml of inoculum are sufficient for 1 000 ml of test solution.

During the test, maintain the flasks on the shaking device (7.3) and at a temperature of 20 °C to 25 °C.

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

At the beginning of the test (day 0), at the end of the test (normally after 28 d) and at least at three regular intermediate time intervals (e.g. 7 d, 14 d, 21 d), take a minimum volume from flasks F_T , F_B , F_C and, if included, also from F_I . If necessary, take measurements at shorter intervals and/or over a period longer than 28 d. At the beginning and the end of the test, take a sample from flask F_S . If flask F_S is inoculated (see notes 6 et 7), take a sample after 0 d and 1 d. Filter all these samples through a membrane filter (7.6), or, especially if the material adsorbs onto the membrane, centrifuge them at about 40 000 m/s² for 15 min.

Measure the DOC concentrations in the samples at least in duplicate 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 to 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 $-18\text{ }^{\circ}\text{C}$. Alternatively, add a suitable inorganic toxic substance, e.g. 20 ml/l of a solution containing 10 g/l of mercury(II) chloride (HgCl_2), to prevent microbial activity and store at $4\text{ }^{\circ}\text{C}$.

9 Calculation and expression of results

9.1 Calculation

For each test flask, determine the percentage elimination of dissolved organic carbon D_t using the equation

$$D_t = \left(1 - \frac{\rho_t - \rho_{Bt}}{\rho_0 - \rho_{B0}}\right) \times 100$$

where

- ρ_0 is the average DOC concentration, in milligrams per litre, at time 0, in each test flask F_T ;
- ρ_{B0} 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 ;
- ρ_{Bt} 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.

NOTES

7 The abiotic elimination (flask F_S) can be calculated according to the same equation, but without considering the blank values (if F_S is inoculated to check the degree of adsorption, consider the blanks). If a significant loss of organic carbon is observed, no differentiation between biotic and abiotic elimination is possible. In this case, other tests based on parameters clearly indicating biological processes should be performed, such as the respirometric test (ISO 9408) or the carbon dioxide production test (ISO 9439).

8 The degree of elimination of the mixture of test compound and reference substance in the inhibition control (flask F_I) can be calculated according to the same equation. If the percentage of elimination of 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, the test should be repeated with a lower concentration of the test compound or with a pre-exposed inoculum.

9.2 Expression of results

Compile a table of DOC percentage elimination for each concentration interval and each test vessel. If comparable results are obtained for the duplicate test vessels (see 10.1), plot a mean elimination curve as a function of time (see example in annex A).

Some parameters for the degradation can be determined from this curve: in particular, if sufficient data are available, the lag time, the degradation time and the maximum level of degradation as described in 9.2.1 to 9.2.3. If the test substance is not significantly eliminated abiotically (e.g. by adsorption) and the elimination curve has a typical shape with a lag and degradation phase, assign the measured DOC elimination to biodegradation.

9.2.1 Lag time t_1

In most of the degradation curves a so-called lag time can be observed. This is normally defined as the time from inoculation until the degradation percentage has increased to at least 10 % of the starting DOC content. This lag time is often highly variable and poorly reproducible. Note the lag time in days.

9.2.2 Maximum level of degradation

The maximum level of degradation is defined as the approximate level above which no further degradation takes place during the test.

9.2.3 Degradation time t_2

The degradation time t_2 is defined as the time from the end of the lag time t_1 till the time that about 90 % of the maximum level of degradation has been reached. Note the degradation time in days.

10 Validity of the test

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

10.2 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.

11 Test report

The test report shall contain at least the following information:

- a) a reference to this International Standard;
- b) all necessary information for the identification of the test compound;
- c) all the data (for example 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 or the volume of the inoculum used and 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 the abiotic elimination);
- 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) any alteration of the standard procedure or any other circumstance that may have affected the results.

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Annex A

(informative)

Typical degradation curve

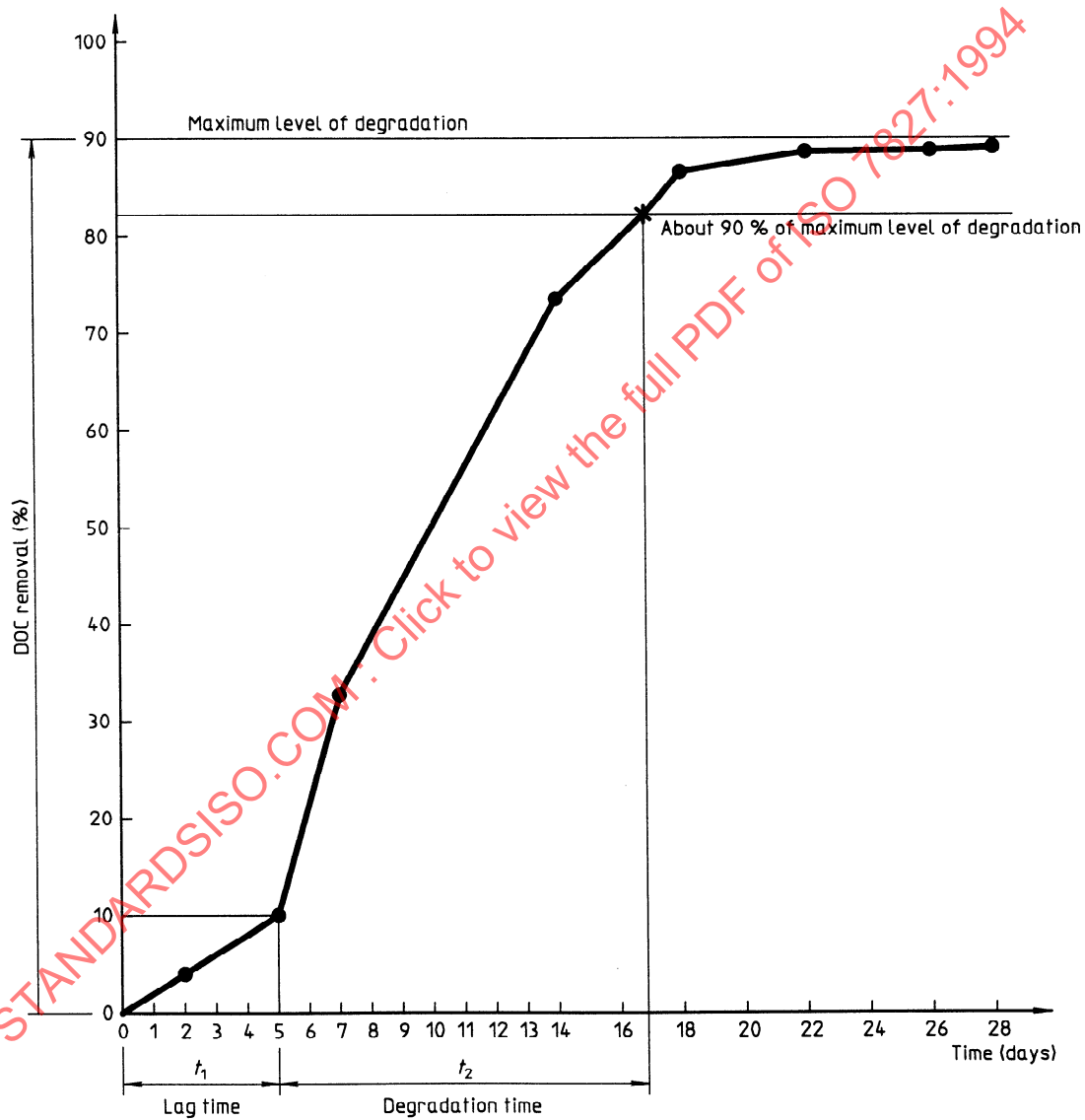


Figure A.1