
International Standard



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

1 Scope

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

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

2 Field of application

The method applies to organic compounds which are

- soluble at the concentration used under the conditions of the test;
- non-volatile, or having a negligible vapour pressure under the conditions of the test;
- not significantly adsorbable on glass;
- not inhibitory to the test micro-organism at the concentration chosen for the test. Inhibitory effects can be determined as described in note 2 to 9.3, or by using any other method for determining the inhibitory effect on bacteria of a substance (see ISO 8192).

3 Reference

ISO 8192, *Water quality — Test for inhibition of oxygen consumption of activated sludge.*¹⁾

4 Definitions

For the purpose of this International Standard, the following definitions apply:

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

4.2 suspended solids (of an activated sludge): The amount of solids obtained by filtration or centrifuging of a known volume of sludge under specified conditions and drying at about 100 °C.

5 Principle

Determination of the biodegradation of organic compounds by aerobic micro-organisms using a test medium (7.2). The organic compounds are the sole source of carbon and energy in the medium. The concentration of the compounds used is such that the initial organic carbon content of the medium is normally between 10 and 40 mg/l.

If required, more than 40 mg/l may be used to give additional information.

Measurement of the dissolved organic carbon (DOC) at the start of the test (day 0), after 28 days (longer if necessary) and at least at three regular, intermediate time intervals (for example 7, 14 and 21 days). 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.

6 Test environment

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

7 Reagents

During the analysis use only reagents of recognized analytical grade.

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

1) At present at the stage of draft.

7.2 Test medium

7.2.1 Composition

7.2.1.1 Solution a)

Anhydrous potassium dihydrogenphosphate (KH_2PO_4)	8,5 g
Anhydrous potassium monohydrogenphosphate (K_2HPO_4)	21,75 g
Sodium monohydrogenphosphate dihydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$)	33,4 g
Ammonium chloride (NH_4Cl)	2,5 g
Water (7.1) (quantity necessary to make up to)	1 000 ml

The pH of this solution should be 7,2.

7.2.1.2 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 (7.1).

7.2.1.3 Solution c)

Dissolve 27,5 g of anhydrous calcium chloride (CaCl_2) in 1 000 ml of the water (7.1).

7.2.1.4 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 (7.1). This solution should be freshly prepared just before use.

7.2.1.5 Solution of micro-nutrients e)

Manganese sulfate tetrahydrate ($\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$) (or 30,23 mg of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$)	39,9 mg
Boric acid (H_3BO_3)	57,2 mg
Zinc sulfate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$)	42,8 mg
Anhydrous ammonium heptamolybdate ($\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ [or 36,85 mg of ($\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$]	34,7 mg
Iron chelate [$\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_8\text{Fe(III)Na}$]	100 mg
Water (7.1) (quantity necessary to make up to)	1 000 ml

NOTE — Iron chelate complex comprises an equivalent mixture of FeCl_3 and EDTA ($\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_8$).

7.2.1.6 Solution of yeast extract f)

Yeast extract	15 mg
Water (7.1) (quantity necessary to make up to)	100 ml

This solution should be prepared just before use. If the solution has to be stored, it shall be sterilized by filtration using a sterile filter assembly (8.6).

NOTE — Solution f) can be replaced by the following solution of growth factors (according to Schlegel).

Biotin	0,2 mg
Nicotinic acid	2,0 mg
Thiamine	1,0 mg
<i>p</i> -Aminobenzoic acid	1,0 mg
Pyridoxamine	5,0 mg
Pantothenic acid	1,0 mg
Folic acid	5,0 mg
Cyanocobalamine	2,0 mg
Water (7.1) (quantity necessary to make up to)	100 ml

7.2.2 Preparation

For 1 litre of test medium, add

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

Adjust to 1 000 ml with the water (7.1).

8 Apparatus

Ordinary laboratory equipment and

8.1 Apparatus, of sufficient sensitivity for the measurement of dissolved organic carbon.

8.2 Centrifuge.

8.3 Stirring device, for aeration and mixing.

8.4 pH-meter.

8.5 Conical flasks, of appropriate capacity (for example 250 ml).

8.6 Device for filtration, with membrane filters of suitable porosity which absorb organic compounds or release organic carbon to a minimum degree (see note 3 to 9.3).

The glassware shall be carefully cleaned and, in particular, free from any trace of organic or toxic matter.

9 Procedure

9.1 Preparation of test solutions

Prepare the following solutions.

9.1.1 Solution of the test compound in the test medium (7.2) to obtain an organic carbon concentration of between 10 and 40 mg/l (or more).

9.1.2 Solution of a known organic product ("reference" compound), for example sodium acetate, sodium benzoate, aniline, in the test medium (7.2) in order to obtain an organic carbon concentration of 40 mg/l.

9.1.3 Solution containing in the test medium (7.2) the same concentrations of the test compounds and the reference compound used as in 9.1.1 and 9.1.2.

9.2 Preparation of the inoculum

The inoculum can be prepared from the following sources or from a mixture of these sources in order to obtain a sufficiently varied and concentrated microbial flora for adequate biodegradative activity. This activity must be checked with the solution of the reference compound (9.1.2).

The content of DOC in the inoculum should be less than 10 % of the content of DOC introduced by the test compound.

9.2.1 Inoculum from a secondary effluent

Take a sample of a secondary effluent collected from a treatment plant dealing with a predominantly domestic sewage. Keep this sample under aerobic conditions and use on the day of collection.

From this sample, prepare an inoculum as follows:

- settle the sample of effluent for 1 h;
- take a suitable portion of the supernatant to be used as inoculum for tests carried out that day.

9.2.2 Inoculum from activated sludge

Take a sample of activated sludge from the aeration tank of a sewage works treating predominantly domestic sewage.

Mix well, keep aerobic and use on the day of collection.

Just before use the concentration of suspended solids is determined. If necessary the sludge should be concentrated by settling so that the volume of sludge added to obtain 30 mg/l of dry matter is minimal [$< 1\%$ (V/V) of the test medium].

9.2.3 Inoculum from a surface water

Take a sample of an appropriate surface water.

Take a suitable volume as inoculum. This inoculum, kept under aerobic conditions, shall be used on the day of preparation.

If necessary the inoculum can be concentrated by filtration or centrifugation.

9.3 Test

Set up a sufficient number of conical flasks (8.5) of a suitable volume (for example 250 ml) in order to have

- at least 2 test flasks (symbolized F_T) containing at least 100 ml of the test solution (9.1.1);
- 1 blank test flask (symbolized F_B) containing at least 100 ml of the test medium (7.2);
- 1 flask, if needed, for checking the activity of the inoculum (symbolized F_C) containing at least 100 ml of the reference compound solution (9.1.2);

- 1 flask (sterile) for checking a possible abiotic degradation or other non-biological removal (symbolized F_S) containing at least 100 ml of solution 9.1.1 sterilized by filtration or by addition of for example 0,1 ml of a concentrated solution of sodium hypochlorite, $c(\text{Cl}) = 4,28 \text{ mol/l}$, or 1 ml of a 1 g/l mercury(II) chloride (HgCl_2) solution (see note 1).

- If needed, one flask is used for checking the possible inhibitory effect of the test compound (symbolized F_I) (see note 2).

Inoculate flasks F_T , F_B , F_C and, if included, flask F_I with an appropriate volume of the inoculum (9.2). (Generally 0,1 to 1 ml of inoculum are sufficient for 100 ml of test solution.) Mix the contents of the flasks.

During the test, maintain the flasks on the stirring device (8.3) and at a temperature of 20 to 25 °C.

In order to compensate for water losses by evaporation, check before each sampling the volume of the medium in the flasks and, if necessary, make up with water (7.1) to the volume measured after the preceding sampling.

At the beginning of the test (day 0), at the end of the test (28 days or more) and at least at three intermediate time intervals (for example 7, 14 and 21 days), take from flasks F_T , F_B , F_C and, if included, also from F_I a minimum volume for DOC measurement. Filter these portions through a membrane filter of about 0,2 μm porosity or especially if the material adsorbs on the membrane, centrifuge them at a suitable speed (for example 40 000 m/s for 15 min).

At the end of the test, take a sample from flask F_S and measure the DOC concentration (see note 3).

Measure the DOC concentrations for each period and each flask at least in duplicate.

If a constant level of degradation is attained before the end of the 28 day test period, consider that the test is finished.

When measurements of organic carbon have to be postponed, keep the sample for DOC measurement at 4 °C in the dark and in tightly stoppered glass flasks. The acceptable storage time is normally 24 h. If it is not possible to carry out the analysis within 24 h, freeze the samples at a temperature below -18 °C.

NOTES

1 By comparing the percentage elimination in flasks F_T and F_S , it can be determined whether or not the test compound is undergoing a degradation process caused by physico-chemical mechanisms. Report the result in the test report.

2 In order to check whether or not the test compound has an inhibiting effect, flask F_I , containing at least 100 ml of solution 9.1.3, may be included.

3 Check the degree of adsorption of the tested compound after filtration or centrifugation (for example by measuring the organic carbon in the filtrate or supernatant).

The concentration measured in the tested solution at the beginning of the test (day 0) should be used as the initial concentration in the final calculation.

When dealing with mixtures, one should be aware that selective adsorption of different components may occur.

10 Calculation and expression of results

10.1 Calculation

Determine for each test flask the percentage elimination of dissolved organic carbon D_t using the equation

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

where

ρ_0 is the average concentration of dissolved organic carbon, at time 0, in each test flask F_T ;

ρ_{B0} is the average concentration of dissolved organic carbon, at time 0, in the blank test flask F_B ;

ρ_t is the average concentration of dissolved organic carbon, at time t , in each test flask F_T ;

ρ_{Bt} is the average concentration of dissolved organic carbon, at time t , in the blank test flask F_B .

Round percentage results down to the nearest whole number.

10.2 Expression of results

The percentage elimination of DOC, D_t , for each flask is plotted versus time.

One average curve can be drawn if similar results in the parallel test flasks are obtained (see clause 11).

From this curve, some parameters for the degradation may be determined. In particular, if sufficient data are available, the lag time and the degradation time can be calculated as described below (see the figure in the annex).

10.2.1 Lag time t_1

In most of the degradation curves a so-called lag time can be observed. This is 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.

The lag time should be noted in days.

10.2.2 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.

10.2.3 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 90 % of the maximum level of degradation has been reached.

The degradation time should be noted in days.

11 Validity of the test

11.1 A test is 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 %.

If it is not the case, the test should be repeated.

11.2 If in the test with one of the proposed reference compounds, the percentage degradation after 14 days is less than 80 % the test results are invalid. The test series should be repeated.

11.3 If flask F_T (toxicity test) was included and if the test compound is not degraded in the flask F_S (abiotic degradation), the test material is assumed to be inhibitory if the degradation percentage in flask F_T is less than 40 % in 7 days.

In this case, the test series should be repeated with lower concentrations of the test material.

12 Test report

The test report shall contain at least the following information:

- a reference to this International Standard;
- all necessary information for the identification of the test compound;
- all the data (for example in tabular form) obtained and the degradation curve (see 10.1 and 10.2);
- the concentration of the test compound used and the DOC content of this concentration;
- the name of the reference compound used and the degradation percentage obtained with this compound;
- the source, the characteristics and the volume of the inoculum used;
- the main characteristics of the DOC analyser used;
- the incubation temperature of the test;
- the percentage of degradation obtained in flask F_S (monitoring the abiotic degradation);
- the percentage of degradation after 28 days in flask F_T (toxicity test) if it was included;
- the reasons in the event of rejection of the test (see clause 11);
- any alteration of the standard procedure or any other circumstance that may have affected the results.