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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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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.

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*

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

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.

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.

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.