SLOVENSKI STANDARD
SIST EN 29439:1997

01-avgust-1997

Kakovost vode - Vrednotenje popolne aerobne biološke razgradljivosti organskih snovi v vodnem okolju - Metoda z analiziranjem sproščenega ogljikovega dioksida (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 9439:1990)

Wasserbeschaffenheit - Bewertung der vollständigen aeroben biologischen Abbaubarkeit organischer Stoffe im wäßrigen Medium - Verfahren mittels Analyse des feigesetzten Kohlendioxids (ISO 9439:1990)

Qualité de l'eau - Evaluation, en milieu aqueux, de la biodégradabilité aérobie "ultime" des composés organiques - Méthode par dosage du dioxyde de carbone dégagé (ISO 9439:1990)

Ta slovenski standard je istoveten z: **EN 29439:1993**

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English version

Water quality - Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds - Method by analysis of released carbon dioxide (ISO 9439:1990)

Qualité de l'eau - Evaluation, en milieu aqueux, de la biodégradabilité aérobie "ultime" des composés organiques - Méthode par dosage du dioxyde de carbone dégagé (ISO 9439:1990)

Wasserbeschaffenheit - Bewertung der vollständigen aeroben biologischen Abbaubarkeit organischer Stoffe im wäßrigen Medium - Verfahren mittels Analyse des freigesetzten Kohlendioxids (ISO 9439:1990)

This European Standard was approved by CEN on 1993-01-20. CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

The European Standards exist in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart,36 B-1050 Brussels

Foreword

This European Standard is the endorsement of ISO 9439. Endorsement of ISO 9439 was recommended by Technical Committee CEN/TC 230 "Water analysis" under whose competence this European Standard will henceforth fall.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by July 1993, and conflicting national standards shall be withdrawn at the latest by July 1993.

The Standard was approved and in accordance with the CEN/CENELEC Internal Regulations, the following countries are bound to implement this European Standard : Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, United Kingdom.

Endorsement notice

The text of the International Standard ISO 9439:1990 was approved by CEN as a European Standard without any modification.

INTERNATIONAL STANDARD

ISO 9439

First edition
1990-12-01

Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by analysis of released carbon dioxide

Qualité de l'eau — Évaluation, en milieu aqueux, de la biodégradabilité aérobie "ultime" des composés organiques — Méthode par dosage du dioxyde de carbone dégagé

Reference number
ISO 9439:1990(E)

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.

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 9439 was prepared by Technical Committee ISO/TC 147, *Water quality*.

Annexes A, B and C of this International Standard are 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 released carbon dioxide

1 Scope

This International Standard specifies a method, by analysis of released carbon dioxide, for the evaluation in an aqueous medium of the "ultimate" biodegradability of organic compounds at a given concentration by aerobic micro-organisms.

The method applies to organic compounds which are

a) soluble in the test conditions;

b) insoluble in the test conditions, in which case special measures may be necessary to achieve good dispersion of the compound;

c) non-volatile or which have a negligible vapour pressure under the conditions of the test;

d) not inhibitory to the test micro-organisms at the concentration chosen for the test. The presence of an inhibitory effect can be determined as specified in 8.3 , or by using any other method for determining the inhibitory effect of a compound on bacteria (see, for example, 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 7827:1984, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by analysis of dissolved organic carbon (DOC).*

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

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 micro-organisms resulting in the production of carbon dioxide, water, mineral salts and new microbial cellular constituents (biomass).

3.2 suspended solids (of an activated sludge): Solids removed by filtration or centrifuging of a known volume of sludge under specified conditions, and, for the purpose of this International Standard, drying at about 100 °C.

3.3 pre-exposure (or pre-adaptation): The pre-incubation of an inoculum in the presence of the test compound, with the aim of enhancing the ability of the inoculum to degrade the test compound.

3.4 pre-conditioning (or pre-acclimatization): The pre-incubation of an inoculum under the conditions of the test in the absence of the test compound, to improve the performance of the test.

4 Principle

Determination of the biodegradation of organic compounds by aerobic micro-organisms, using a test medium.

The organic compound is the sole source of carbon and energy in the medium. The level of biodegradation is determined indirectly by measurement of the released carbon dioxide during the test time (generally 28 days). The concentration of the compounds used is such that the initial organic carbon content of the medium is normally between 10 mg/l and 40 mg/l. If required, more than 40 mg/l may be used to give additional information.

For sufficiently water-soluble compounds, DOC removal at the end of the test may be determined.

5 Test environment

Incubation shall take place in the dark or in diffused light, in an enclosure which is maintained at a constant temperature (within at least ± 1 °C) between 20 °C and 25 °C and which is free from toxic vapours.

6 Reagents

Use only reagents of recognized analytical grade.

6.1 Distilled or de-ionized water.

Containing less than 10 % of the initial DOC content introduced by the organic 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 ($Na_2HPO_4.2H_2O$)	33,4 g
Ammonium chloride (NH_4Cl)	0,5 g

Water (6.1) (quantity necessary to make up to) 1 000 ml

The pH of this solution should be about 7,4.

6.2.1.2 Solution b).

Dissolve 22,5 g of magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) in 1 000 ml of the water (6.1).

6.2.1.3 Solution c).

Dissolve 27,5 g of anhydrous calcium chloride ($CaCl_2$) or 36,4 g of calcium chloride dihydrate ($CaCl_2.2H_2O$) in 1 000 ml of the water (6.1).

6.2.1.4 Solution d).

Dissolve 0,25 g of iron(III) chloride hexahydrate ($FeCl_3.6H_2O$) in 1 000 ml of the water (6.1). Prepare this solution just before use.

NOTE 1 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 ethylenediamine-tetraacetic acid (EDTA) is added.

6.2.2 Preparation of the test medium.

For 1 litre of test medium, add just before use to 800 ml of the water (6.1)

— 10 ml of solution a);

— and then 1 ml of each solution b), c) and d), (to avoid the formation of turbidity in the final medium).

Make up to 1 000 ml with the water (6.1).

7 Apparatus

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

Usual laboratory equipment, and

7.1 Glass vessels, Erlenmeyer **flasks** or bottles allowing gas purge and magnetic stirring (see 8.3).

7.2 CO_2-free air production system, capable of supplying each test flask at a flow rate between 50 ml/min and 100 ml/min, held constant within 10 % (see example of assembly in annex A).

Check the absence of carbon dioxide in the air production system.

7.3 Apparatus for the determination of carbon dioxide, carbon dioxide analyser or device for titrimetric determination after complete absorption in a basic solution (see the principle of a test system with released carbon dioxide in annex A).

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

7.5 Instrument for the determination of dissolved organic carbon concentration

7.6 Centrifuge

7.7 pH-meter.

8 Procedure

8.1 Preparation of the test solutions

8.1.1 Solution of the test compound

Prepare a stock solution of the test compound in the test medium (6.2). Dilute a suitable amount of this solution in the previously aerated test medium (see 8.3) in order to obtain a final organic carbon concentration of between 10 mg/l and 40 mg/l.

NOTE 2 Compounds of low water solubility may be added directly, in solid or liquid form to the medium in the appropriate flask; an ISO International Standard will be prepared for guidance.

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, aniline) in the test medium (6.2) in the same way as in 8.1.1, in order to obtain a final organic carbon concentration of 20 mg/l.

8.1.3 Solution to check inhibition

If necessary, prepare the 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 using a mixture of these sources to obtain a microbial population that offers sufficient biodegradation activity. Check this activity by means of the reference compound (8.1.2).

The carbon dioxide production of the blank solution should be in the range of 30 mg/l to 40 mg/l but not greater than 70 mg/l. To reduce the influence of the blank, it may be helpful to precondition the sludge by aerating it up to one week before it is used [see clause 10, item b)].

The quantity of dissolved organic carbon provided by the inoculum shall be less than 10 % of the initial concentration of organic carbon introduced by the test compound.

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

8.2.1 Inoculum from a secondary effluent

Take a sample of secondary effluent collected from a treatment plant dealing with predominantly domestic sewage. Keep this sample under aerobic conditions and use 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[1] of the supernatant, to be used as inoculum for the test carried out that day.

8.2.2 Inoculum from an activated sludge plant

Take a suitable volume[1] of inoculum, for example, a sample of activated sludge from the aeration tank of a sewage works treating predominantly domestic sewage.

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

Just before use, determine the concentration of suspended solids[1]. If necessary, concentrate the sludge by settling so that the volume of sludge added to obtain 30 mg of dry matter per litre be minimum.

1) "Suitable" volume means

- sufficient to give a population which offers enough biodegradation activity;
- degrades the reference compound(s) by the stipulated percentage;
- gives between 10^3 and 10^5 active cells/ml;
- CO_2 production in the blank controls, must not be greater than 70 mg/l (normally 30 mg/l to 40 mg/l);
- gives not greater than the equivalent of 30 mg activated sludge solids per litre in the final reaction mixture.