SIST

SLOVENSKI STANDARD
SIST EN 29408:1998

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Nadomešča:

SIST ISO 9408:1997

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Kakovost vode - Vrednotenje popolne aerobne biološke razgradljivosti organskih snovi v vodnem okolju - Metoda določanja potrebe po kisiku v zaprtem raspirometru (ISO 9408:1991)

Water quality - Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds - Method of determining the oxygen demand in a closed respirometer (ISO 9408:1991)

Wasserbeschaffenheit - Bestimmung der vollständigen aerobiologischen Abbaubarkeit eines organischen Stoffes in einem wäßrigen Medium über die Bestimmung des Sauerstoffbedarfs in einem geschlossenen Respirometer (ISO 9408:1991)

Qualité de l'eau - Evaluation, en milieu aqueux, de la biodégradabilité aérobie "ultime" des composés organiques - Méthode par détermination de la demande en oxygene dans un respirometre fermé (ISO 9408:1991)

Ta slovenski standard je istoveten z: **EN 29408:1993**

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English version

Water quality - Evaluation in an aqueous medium of the "ultimate" aerobic biodegradibility of organic compounds - Method of determining the oxygen demand in a closed respirometer (ISO 9408:1991)

Qualité de l'eau - Evaluation, en milieu aqueux, de la biodégradabilité aérobie "ultime" des composés organiques - Méthode par détermination de la demande en oxygène dans un respiromètre fermé (ISO 9408:1991)

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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 9408. Endorsement of ISO 9408 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 9408:1991 was approved by CEN as a European Standard without any modification.

INTERNATIONAL STANDARD

ISO 9408

First edition
1991-02-15

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

Qualité de l'eau — Évaluation, en milieu aqueux, de la biodégradabilité aérobie "ultime" des composés organiques — Méthode par détermination de la demande en oxygène dans un respiromètre fermé

Reference number
ISO 9408:1991(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 9408 was prepared by Technical Committee ISO/TC 147, *Water quality*.

Annexes A, B, C and D 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 determining the oxygen demand in a closed respirometer

1 Scope

This International Standard specifies a method, by determination of the oxygen demand in a closed respirometer, for the evaluation in an aqueous medium of the "ultimate" biodegradability of organic compounds at a given concentration by aerobic micro-organisms.

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

The method applies to organic compounds which

a) are soluble in the test conditions;

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

c) do not reach and react with the CO_2 absorbant;

d) are volatile, provided that a suitable respirometer is used;

e) are not inhibitory to the test micro-organisms at the concentration chosen for the test. The presence of inhibitory effects 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 6060:1989, *Water quality — Determination of the chemical oxygen demand.*

ISO 6107-2:1989, *Water quality — Vocabulary — Part 2.*

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 biochemical oxygen demand (BOD): The mass concentration of dissolved oxygen consumed under specified conditions by the biological oxidation of organic and/or inorganic matter in water (see ISO 6107-2).

3.3 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.

3.4 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.

3.5 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 concentration of the test compound is normally 100 mg/l, but its theoretical oxygen demand (ThOD) shall be at least 100 mg/l.

The inoculated medium is stirred in a closed flask and the consumption of oxygen is determined either by measuring the amount of oxygen required to maintain a constant gas volume in the respirometer flask, or by measuring the change in volume or pressure (or a combination of the two) in the apparatus.

Evolved carbon dioxide is absorbed in a suitable substance in the test vessel.

The degradation is followed over a period of 28 days, or longer if necessary, by determining the consumption of oxygen either automatically or manually. The amount of oxygen consumed by the organic compound (after correction by comparison with the blank test) is expressed as a percentage of the theoretical oxygen demand (ThOD) calculated from the formula of the compound or the chemical oxygen demand (COD). Additionally, the degree of biodegradation may also be calculated from supplemental chemical analyses, for example, dissolved organic carbon (DOC) for sufficiently water-soluble compounds or specific analysis (concerning only primary biodegradation), made at the beginning and the end of incubation. Evaluation of the biodegradability of the test compound is made on the basis of these data.

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, just before use add 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).

6.3 Carbon dioxide absorber, Potassium hydroxide solution (about 10 mol/l), sodium carbonate pellets or another suitable absorbant.

7 Apparatus

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

Usual laboratory equipment, and

7.1 Closed respirometer.

The principle of a closed respirometer is given in annex D. When testing volatile compounds, the apparatus used shall be appropriate or adapted to this particular purpose. Care shall be taken that there is no loss of compound due to the apparatus.

7.2 Water-bath or constant temperature room (to comply with clause 5).

7.3 Equipment for measurement of dissolved organic carbon, instrument of sufficient sensitivity for the measurement of dissolved organic carbon (DOC).

7.4 Device for determining chemical oxygen demand (COD).

7.5 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 (see note 3 to 8.3).

7.6 Centrifuge.

7.7 pH-meter.

8 Procedure

8.1 Preparation of the test solutions

Prepare the following solutions:

a) solution of the test compound in the test medium (6.2) to obtain a concentration of 100 mg/l, but at least 100 mg/l ThOD;

b) solution of a known organic compound ("reference" compound), for example, sodium acetate, sodium benzoate, aniline, in the test medium (6.2) to obtain a concentration of 100 mg/l;

c) solution containing, in the test medium (6.2), the same concentrations of the test compound and the reference compound as used in a) and b).

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.

8.2 Preparation of the inoculum

Take a sample of activated sludge from the aeration tank of a biological wastewater treatment plant, or a laboratory unit, dealing with predominantly domestic sewage. If the sludge is actively respiring on external substrate, bring it to the "endogenous" phase (i.e. having no external substrate remaining) as follows:

— either aerate for a few hours before use, or

— centrifuge, wash with medium (6.2), recentrifuge and resuspend in the medium (this treatment is recommended if it is suspected that the sludge contains inhibiting matter).

When the sludge is judged to be in the "endogenous" phase, or free from inhibiting matter, mix well, maintain in an aerobic state by stirring or aeration at the required temperature, and use on the day of collection or one day later. Just before use, determine the concentration of suspended solids. If required, concentrate the sludge by settling, so that the volume of sludge added to obtain 30 mg/l of dry matter in the reaction mixture is less than or equal to 1 % of the mixture, that is, the sludge should contain at least 3 g/l of dry solids.

NOTES

3 A concentration of 30 mg/l of suspended solids in the final medium has been found suitable for concentrations of test compound in the range of 50 mg/l to 150 mg/l. The oxygen consumption of the blank solution shall not be greater than 60 mg/l in 28 days and should normally be in the range of 20 mg/l to 30 mg/l. In order to reduce the influence of the blank, the sludge may be pre-conditioned (see 3.5) by aeration for up to one week before it is used.

4 Secondary effluent and surface water may also be used as inoculum, but these inocula may have to be concentrated by filtration or centrifugation to get more biomass.

5 Pre-exposed inocula may be used for certain purposes. When such inocula are used, this should be clearly stated in the test results (e.g. percentage 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 run 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 identical compounds, contaminated areas, etc.).