SLOVENSKI STANDARD
SIST EN ISO 9439:2000

01-december-2000

Nadomeģ a:
SIST EN 29439:1997

Kakovost vode - Vrednotenje popolne aerobne biologke razgradljivosti organskih snovi v vodi - Preskus z merjenjem spoģ enega ogljikovega dioksida (ISO 9439:1999)

Water quality - Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium - Carbon dioxide evolution test (ISO 9439:1999)

Wasserbeschaffenheit - Bestimmung der vollständigen aeroben biologischen Abbaubarkeit organischer Stoffe im wäßrigen Medium - Verfahren mit Kohlenstoffdioxid-Messung (ISO 9439:1999)

Qualité de l'eau - Evaluation de la biodégradabilité aérobie ultime en milieu aqueux des composés organiques - Essai de dégagement de dioxyde de carbone (ISO 9439:1999)

Ta slovenski standard je istoveten z: **EN ISO 9439:2000**

ICS:

13.060.70	Preiskava bioloških lastnosti vode	Examination of biological properties of water

SIST EN ISO 9439:2000 **en**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 9439

April 2000

ICS 13.060.70

English version

Water quality - Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium - Carbon dioxide evolution test (ISO 9439:1999)

Qualité de l'eau - Evaluation de la biodégradabilité aérobie ultime en milieu aqueux des composés organiques - Essai de dégagement de dioxyde de carbone (ISO 9439:1999)

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This European Standard was approved by CEN on 11 March 2000.

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.

This European Standard exists 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.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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

Foreword

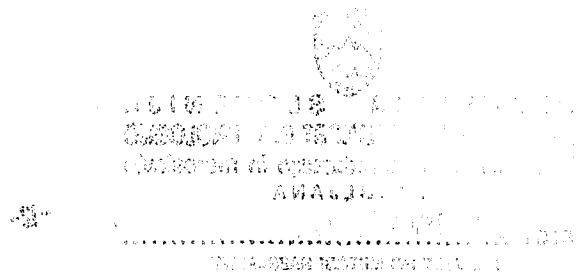
The text of the International Standard from Technical Committee ISO/TC 147 "Water quality" of the International Organization for Standardization (ISO) has been taken over as an European Standard by Technical Committee CEN/TC 230 "Water analysis", the secretariat of which is held by DIN.

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 October 2000, and conflicting national standards shall be withdrawn at the latest by October 2000.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of the International Standard ISO 9439:1999 has been approved by CEN as a European Standard without any modification.



INTERNATIONAL STANDARD

ISO 9439

Second edition
1999-03-01

Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Carbon dioxide evolution test

Qualité de l'eau — Évaluation de la biodégradabilité aérobie ultime en milieu aqueux des composés organiques — Essai de dégagement de dioxyde de carbone

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Contents

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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 3.

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 has been prepared by Technical Committee ISO/TC 147, *Water quality,* Subcommittee SC 5, *Biological methods.*

This second edition cancels and replaces the first edition (ISO 9439:1990), which has been technically revised.

Annexes A to D of this International Standard are for information only.

Introduction

The conditions described in this International Standard do not always correspond to the optimal conditions for allowing the maximum degree of biodegradation to occur. With this test system, the microbially derived carbon dioxide (CO_2) is measured in the traps through which gas exhausted from the test vessels is passed. Some of the CO_2 remains in the medium in the vessels as dissolved inorganic carbon (DIC), the concentration of which may increase as biodegradation proceeds. As the organic carbon approaches complete removal, the concentration of DIC gradually falls and tends to reach zero by the end of incubation. It is thus necessary to acidify the medium at the end of the test to measure the biogenically formed CO_2 completely. The measurement of CO_2 in the external traps may differ from the true production of CO_2 and the kinetic rate may also be lower than a rate based on DOC removal measurement. The consequence may be that the biodegradation curves based on the trapped CO_2 may not fully represent the true microbial kinetic rate. For alternative biodegradation methods, see ISO 15462 and in particular ISO 14593, which is based on CO_2 production as well but does not have this defect.

Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Carbon dioxide evolution test

WARNING — Activated sludge and sewage may contain potentially pathogenic organisms. 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, by determination of carbon dioxide (CO_2), for the evaluation in an aqueous medium of the ultimate biodegradability of organic compounds at a given concentration by aerobic microorganisms.

The method applies to organic compounds which are:

a) water-soluble under the conditions of the test, in which case removal of DOC may be determined as additional information (see annex D);

b) poorly water-soluble under the conditions of the test, in which case special measures may be necessary to achieve good dispersion of the compound (see, for example, ISO 10634);

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

NOTE For volatile substances use for example ISO 9408 or ISO 14593.

d) not inhibitory to the test microorganisms at the concentration chosen for the test.

NOTE 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 Terms and definitions

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

2.1
ultimate aerobic biodegradation
breakdown of a chemical compound or organic matter by microorganisms in the presence of oxygen to carbon dioxide, water and mineral salts of any other elements present (mineralization) and the production of new biomass

2.2
primary biodegradation
structural change (transformation) of a chemical compound by microorganisms resulting in the loss of a specific property

2.3
activated sludge
biomass produced in the aerobic treatment of wastewater by the growth of bacteria and other microorganisms in the presence of dissolved oxygen

2.4
concentration of suspended solids
<activated sludge> amount of solids obtained by filtration or centrifugation of a known volume of activated sludge and drying at about 105 °C to constant mass

2.5
dissolved organic carbon
DOC
that part of the organic carbon in a water sample which cannot be removed by specified phase separation

NOTE For example, by centrifugation at 40 000 m · s^{-2} for 15 min or by membrane filtration using membranes with pores of diameter 0,2 µm to 0,45 µm.

2.6
total inorganic carbon
TIC
all that inorganic carbon in the water deriving from carbon dioxide and carbonate

2.7
dissolved inorganic carbon
DIC
that part of the inorganic carbon in water which cannot be removed by specified phase separation

NOTE For example, by centrifugation at 40 000 m · s^{-2} for 15 min or by membrane filtration using membranes with pores of diameter 0,2 µm to 0,45 µm.

2.8
theoretical amount of formed carbon dioxide
$ThCO_2$

theoretical maximum amount of carbon dioxide formed after oxidizing a chemical compound completely

NOTE It is calculated from the molecular formula and expressed in this case as milligrams carbon dioxide per milligram (or gram) test compound.

2.9
lag phase
time from the start of a test until adaptation and/or selection of the degrading microorganisms are achieved and the biodegradation degree of a chemical compound or organic matter has increased to about 10 % of the maximum level of biodegradation

NOTE It is normally recorded in days.

2.10
maximum level of biodegradation
maximum biodegradation degree of a chemical compound or organic matter in a test, above which no further biodegradation takes place during the test

NOTE It is normally recorded in percent.

2.11
biodegradation phase
time from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached

NOTE It is normally recorded in days.

2.12
plateau phase
time from the end of the biodegradation phase until the end of the test

NOTE It is normally recorded in days.

2.13
pre-exposure
pre-incubation of an inoculum in the presence of the test chemical compound or organic matter, with the aim of enhancing the ability of this inoculum to biodegrade the test material by adaptation and/or selection of the microorganisms

2.14
preconditioning
pre-incubation of an inoculum under the conditions of the subsequent test in the absence of the test chemical compound or organic matter, with the aim of improving the performance of the test by acclimatization of the microorganisms to the test conditions

3 Principle

The biodegradability of organic compounds by aerobic microorganisms is determined using a static aqueous test system. The test mixture contains an inorganic medium, the organic compound as the nominal sole source of carbon and energy at a concentration of 10 mg/l to 40 mg/l organic carbon and a mixed inoculum obtained from a wastewater treatment plant or from another source in the environment. The mixture is agitated in test vessels and aerated with CO_2-free air normally up to 28 d (for example see annex A). The CO_2 formed during the microbial degradation is trapped in external vessels, determined by an appropriate analytical method (for examples see annex B), compared with the theoretical amount ($ThCO_2$) and expressed as a percentage.

For sufficiently water-soluble compounds, removal of DOC may optionally be measured to obtain additional information on the ultimate biodegradability. This can be done in the method given, but a convenient procedure is described in annex D which allows the use of higher concentrations of the test compound and the inoculum, thus improving the biodegradation potential of the test. If a substance-specific analytical method is available, information on the primary degradability may also be obtained.

4 Test environment

Incubation shall take place in the dark or in diffused light, at a temperature within the range 20 °C to 25 °C which shall not vary by more than ± 2 °C during the test.

5 Reagents

Use only reagents of recognized analytical grade.

5.1 Water, distilled or deionized, containing less than 1 mg/l DOC.

5.2 Test medium.

5.2.1 Composition

a) **Solution a)**

Dissolve

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
in water (5.1), quantity necessary to make up to	1 000 ml